Comparison of a Time-Resolved Fluorescence Immunoassay and an Enzyme-Linked Immunosorbent Assay for the Analysis of Atrazine in Water

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Immunoassays for atrazine based on a time-resolved fluorescent label and an enzyme label were optimized and utilized to measure atrazine in water. The time-resolved fluorescent immunoassay (TRFIA) was based on a polyclonal antibody and a europium label, whereas the enzyme-linked immunosorbent assay (ELISA) utilized a monoclonal antibody and horseradish peroxidase as the label. Detection limits and IC_{50} values calculated from standard curves were 0.05 ± 0.03 and 0.17 ± 0.08 ng/mL ($n = 8$) for the TRFIA, respectively, and 0.05 ± 0.04 and 0.3 ± 0.2 ng/mL ($n = 17$) for the ELISA, respectively. Four different environmental water samples were fortified at various levels of atrazine. When these samples were analyzed, the % RSD for replicate fluorescence or absorbance readings was small (5 and 6%, respectively). The average accuracies for the TRFIA and ELISA were 1.4 ± 0.42 ($n = 13$) and 1.0 ± 0.38 ($n = 13$), respectively, reflecting the slight bias of the TRFIA. TRFIA offers an advantage over ELISA in that the fluorescent label is less susceptible to interferences that inhibit enzyme activity and reagents may be more stable than enzyme reagents.

Keywords: *Atrazine; triazine; herbicide; time-resolved fluorescence; immunoassay; enzyme-linked immunosorbent assay*

INTRODUCTION

Immunoassays have been successfully employed for the analysis of environmental contaminants and are becoming accepted as standard environmental analytical tools (Aga and Thurman, 1997). When compared to other instrumental methods, immunoassays have comparable detection limits (DLs). However, increasingly stringent regulations, for example, with 2,3,7,8-tetrachloro-*p*-dibenzodioxin, have driven the need for lower DLs (Environmental Protection Agency, 1997). In addition, pesticides such as sulfonylureas are being developed that are used at much lower application rates, yet even at these low rates, can still impact ecological health (Ghildyal and Kariofillis, 1995). Thus, even lower DLs are required to measure environmental residues. This paper describes the evaluation of a strategy to lower the DL for immunoassays.

The lower DL of an immunoassay is often determined by the relative affinity of the antibody for the analyte and the competing hapten−label conjugate (Tijssen, 1985). However, the DL also can be decreased with improved detection of the label, although this is ultimately limited by the experimental error of the assay (Ekins et al., 1968). Most immunoassays used for the detection of pesticides and other environmental contaminants are enzyme-linked immunosorbent assays (ELISAs). These assays utilize a substrate that is detected in the visible wavelength range. The disadvantage of this method is that the enzyme can be sensitive to matrix interferences. An advantage is that the enzyme reaction provides an amplification of the signal. Even if the amount of enzyme that is bound is low, the substrate reaction can be run for an extended period of time to improve the detectability. An assay that utilizes a fluorescent label eliminates the need for an enzyme, thus potentially making this method less susceptible to interferences. In addition, the use of lanthanide chelates and time-resolved technology has been developed to improve the detectability of the label over conventional fluorescence detection methods (Dickson et al., 1995).

In this paper a time-resolved fluorescence immunoassay (TRFIA) for atrazine is developed. A TRFIA has been previously reported for triazine herbicides using a europium-labeled streptavidin system (Wortberg and Cammann, 1993). The present work employed a streptavidin−thyroglobulin complex that has been shown to provide a substantial increase in europium loading (Diamandis et al., 1988). This, along with the time-resolved technology, should lead to a greater detectability of the label, resulting in a more sensitive assay.

The ELISA utilized in this study has been previously reported (Wortberg et al., 1995) and is a coated antigen format that uses a horseradish peroxidase labeled secondary antibody. The performances of the TRFIA and ELISA methods were compared. Sensitivity, precision, and accuracy were determined during the analysis of fortified water samples that were taken from four different geographical areas.

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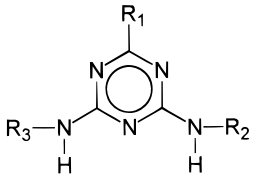

Compound	R1	R2	R3
1	$S(CH_2)_2COOH$	ethyl	isopropyl
2h	Cl	$(CH_2)_5COOH$	isopropyl
4a	$S(CH_2)_2COOH$	ethyl	ethyl

Figure 1. Structures of triazine haptens used in this study.

MATERIALS AND METHODS

Chemicals. Buffer salts were of reagent grade and obtained from either Fisher Chemical Co. (Pittsburgh, PA) or Aldrich Chemical Co. (Milwaukee, WI). Tween 20 (Tw-20) was obtained from Aldrich or Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (BSA), goat anti-rabbit IgG, affinity-purified, 3,3′,5,5′-tetramethylbenzidine (TMB), and goat anti-mouse IgG conjugated to horseradish peroxidase were from Sigma. Tris(hydroxymethyl)aminomethane (Tris) and sodium azide were obtained from Aldrich.

Immunoreagents. The anti-triazine polyclonal antibody used in the TRFIA, designated antiserum 357, was raised in rabbits to the conalbumin conjugate of hapten **2h** (Figure 1) as described by Goodrow et al. (1990) and characterized by Harrison et al. (1991). The labeled analyte used in the TRFIA was prepared by biotinylating the conalbumin conjugate of hapten **4a** (Goodrow et al., 1990) according to a method similar to that of Khosravi and Diamandis (1987).

The ELISA monoclonal antibody used was designated AM7B2.1 and reported by Karu et al. (1991). It was raised to hapten **1**. The coating antigen was hapten **2h** conjugated to ovalbumin (Wortberg et al., 1995).

TFRIA Reagents. Wash solution was an aqueous solution of NaCl (0.15 M) and Tw-20, (0.5 mL/L). Assay buffer was an aqueous solution of 0.1 M Tris, 0.5% BSA, 0.13 M NaCl, 0.01% Tw-20, and 0.2 g/L NaN_3. Concentrated assay buffer was an aqueous solution of 0.1 M Tris, 5% BSA, 1.32 M NaCl, 0.1% Tw-20, and 0.031 M NaN_3. The pH was adjusted to 7.7 with concentrated HCl. Tris buffer was the same as assay buffer but without BSA and Tw-20. Trapping antibody solution was a 1 μg/mL solution of goat anti-rabbit IgG antibody (reconstituted to 1 mg/mL; Sigma, R-2004) in Tris buffer. Antibody solution was an 870-fold dilution of anti-triazine serum 357 in concentrated assay buffer. Labeled analyte solution was a 0.21 μg/mL solution of labeled analyte in concentrated assay buffer. Tracer solution was similar to that described by Diamandis et al. (1988), which contained a streptavidin−thyroglobulin−BCPDA−Eu complex (40 μM Eu) in 50 mM Tris, pH 7.8, buffer. BCPDA is 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid.

ELISA Reagents. Coating buffer was 0.1 M carbonate−bicarbonate buffer, pH 9.6. Assay buffer was normal strength phosphate-buffered saline (PBS; 8 g of NaCl, 0.8 g of KH_2PO_4, 1.2 g of Na_2HPO_4, and 0.2 g of KCl per liter of double-distilled water, pH to 7.5) containing 0.05% Tw-20. ELISA wash solution was $^1/_{10}$ normal strength PBS containing 0.05% Tw-20. The substrate was prepared by mixing 0.4 mL of a 6 mg/mL solution of TMB in dimethyl sulfoxide (DMSO), 0.1 mL of a 1% solution of hydrogen peroxide in water, and 25 mL of a 0.1 M citrate−acetate buffer, pH 5.5.

Sample Source. Water samples were collected from (1) near the mouth of the Fraser River at New Westminster Quay, BC, Canada (April 1993), (2) a freshwater marsh by Jericho beach in Vancouver, BC (April 1993), (3) Putah Creek (small oxbow lake of minimal flow, turbid with algae) in California, and (4) the south fork of the Big River in California (downstream from an area of geothermal activity). Water samples were filtered through coarse Whatman No. 541 paper and stored in amber glass bottles at 4 °C. Water samples collected in Canada were fortified at 0, 0.032, 0.063, 0.16, 1.61, and 16.2 μg/L, split, and sent to California. California water samples were fortified at 0, 0.05, 1, 0.25, 2.5, and 25 μg/L, split, and sent to Canada. Fortification levels were chosen to be near the expected limits of detection, near the IC_{50}, and at levels well above the IC_{50}. All samples were measured by using both methods, with Canadian samples measured in a blind fashion in the California laboratory and vice versa.

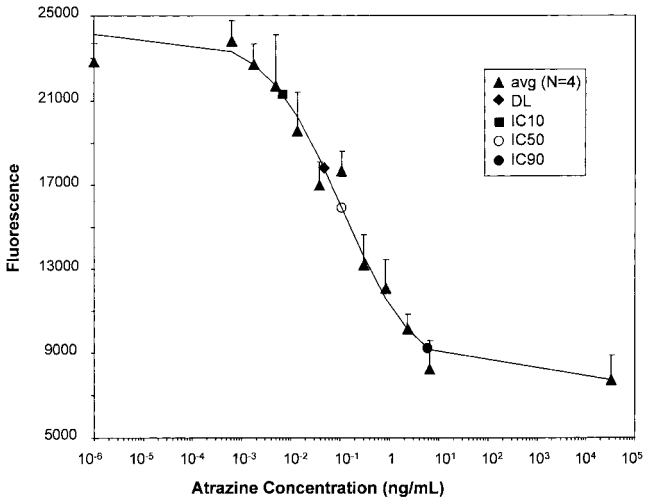

Figure 2. Standard curve for the TRFIA. The assay was conducted as described under Materials and Methods. Each point is the mean and SD for four well replicates. IC_{10}, IC_{50}, and IC_{90} indicate concentrations at which the maximum signal was inhibited by 10, 50, and 90%, respectively.

TRFIA Method. Microtiter plates were (1) coated with anti-rabbit trapping antibody (100 μL/well of trapping antibody solution; 16 h, 4 °C), (2) washed once with wash solution, (3) blocked with BSA (200 μL/well of 1% BSA in Tris Buffer; 1 h, 24 °C), (4) washed twice with wash solution, tapped dry, and (5) stored in a sealed plastic bag at −20 °C.

Twelve serially diluted atrazine standard solutions (600 μL) were prepared in distilled water (in glass minitubes; 1.2 cm × 7.5 cm o.d.). Twelve water samples (unknowns) were diluted if necessary, and 600 μL was aliquoted into minitubes. Aliquots (34 μL) of antibody solution and labeled analyte solution were added to tubes containing standards and unknowns, resulting in a 17100-fold dilution of antibody, and 0.01 μg/mL of labeled analyte. These solutions were then aliquoted (100 μL/well) to the coated plate using a Biomek 1000 workstation (Beckman Instruments Corp., Palo Alto, CA). Following a 16-h incubation at 4 °C, the plate was washed three times and tracer solution was added (100 μL/well). The plate was shaken for 30 min (Easyshaker EAS 2/4, SLT Instruments, Grodin, Austria), washed three times, and dried in a convection oven at 30 °C for 10 min. The time-resolved fluorescence of the dry wells was measured using a Cyberfluor 615 immunoanalyzer (Nordion, Kanata, ON, Canada) at 615 nm after excitation at 295−340 nm followed by a 200-μs decay period.

Standards and unknowns were applied to 96-well plates in quadruplicate. Each plate contained a 12-point standard curve. A typical TRFIA standard curve is shown in Figure 2. The detection limit, IC_{10}, IC_{50}, and IC_{90} are shown. The useable concentration range of each standard curve was defined as the region between IC_{10} and IC_{90}.

ELISA Method. The ELISA method used is similar to that described previously (Wortberg et al., 1995). Briefly, 96-well microtiter plate wells (Nunc, Roskilde, Denmark) were coated with a 1/10000 dilution of coating antigen (ovalbumin conjugate of **2h)** in coating buffer overnight at 4 °C. The following day the plates were washed five times with ELISA wash solution to remove unbound coating antigen. A 50-μL aliquot of standard or water sample was added to each well, followed immediately by 50 μL of anti-triazine antibody AM7B2.1

diluted in assay buffer (1/2000). The plates were covered with an acetate plate sealer and incubated at room temperature for 40 min. Following another wash step (five times), 0.1 mL of goat anti-mouse IgG conjugated to horseradish peroxidase (1/3000 dilution in assay buffer) was added to each well. The plate was covered and incubated for 40 min at room temperature. Plates were again washed five times. The substrate preparation was then added to each well (0.1 mL). After ≈30 min of incubation at room temperature, the enzyme reaction was stopped using 50 μL of 4 N sulfuric acid. Microtiter plates were then placed in a UVmax plate reader (Molecular Devices, Sunnyvale, CA) and read at 450 minus 650 nm. The absorbance data were collected and analyzed using the software program Softmax (ver. 2.2, Molecular Devices). Standards and samples were run in four-well replicates.

A working solution of atrazine standard (50 μg/mL) was prepared in DMSO from a serial dilution of a 1 mg/mL stock solution. Standard curves were prepared by serial dilution (in microtiter plates from Dynatech, Chantilly, VA) of the working solution in assay buffer to yield standards in the range of 0−250 ng/mL containing 0.25% or less DMSO final concentration in the well. All water samples were analyzed directly by ELISA. The only sample treatment used was dilution of the samples in the assay buffer.

Calculations. Fluorescence and absorbance readings of the standards were fit to a four-parameter equation (Rodbard, 1981) using Excel 5 Solver and Softmax software, respectively. The concentration of atrazine in the water samples was determined by interpolation of the signal of the sample. The ELISA value reported for each sample was that determined from the dilution of the sample that resulted in an absorbance that was closest to the absorbance determined for the IC_{50} of the standard curve. Absorbance values for samples that did not inhibit sufficiently were reported from analysis of the undiluted sample. Samples analyzed by TRFIA were diluted such that their fluorescence values were greater than IC_{90} (for example, as marked in Figure 2).

The detection limit was based on assay precision (Rodbard, 1981) and was calculated at the 95% confidence level and included false positive (type α) and false negative (type β) errors (Grant, 1991). The calculation was as follows:

$$\mathrm{DL} = C\left(\frac{A - D}{S_{\mathrm{dl}} - D} - 1\right)^{1/B} \quad (1)$$

$$S_{\mathrm{dl}} = S_{\mathrm{max}} - 2t(\mathrm{SD}) \quad (2)$$

where $A-D$ are the standard curve parameters, t is the one-sided Student t value for the 95% confidence level, SD is the pooled standard deviation (Taylor, 1990) of quadruplicate absorbance and fluorescence readings of the four and six most dilute standards, respectively, and S_{max} is the average ($n = 4$) signal (fluorescence or absorbance) of the blank standard (atrazine concentration = 0).

RESULTS AND DISCUSSION

Optimization. For the TRFIA a checkerboard titration was used to determine the optimum dilution of anti-triazines serum and concentration of labeled analyte. Antibody dilution was varied by row, and concentration of labeled analyte was varied by column in the 96-well microtiter plate. The "elbow" of each fluorescence versus labeled analyte concentration curve (Figure 3) defined an antibody dilution and labeled analyte concentration at which the numbers of available antibody binding sites and labeled analyte molecules were approximately equal. These elbows were better defined in plots of dy/dx versus concentration of labeled analyte, as shown in Figure 4 for the 24100-fold antibody dilution curve. From the family of curves in Figure 3, an elbow was chosen that had the lowest fluorescence value which was judged to be acceptably precise as the maximum fluorescence in a standard curve. Therefore, the antibody dilution of 24100-fold was chosen (Figure 4), and the corresponding labeled analyte concentration at the elbow was ≈0.01 μg/mL (Figure 4). This labeled analyte concentration and a slightly lower antibody dilution (17100-fold) were used for subsequent TRFIA assays. A similar optimization experiment for the ELISA resulted in the use of a 1/10000 dilution of coating antigen and a 1/2000 dilution of antibody. Such optimizations should be carried out each time a new batch of reagents is used to maintain the quality of the assay. At fixed antibody concentrations the lowest concentration of labeled analyte, which retains acceptable precision of the signal, will result in the most sensitive assays (Tijssen, 1985).

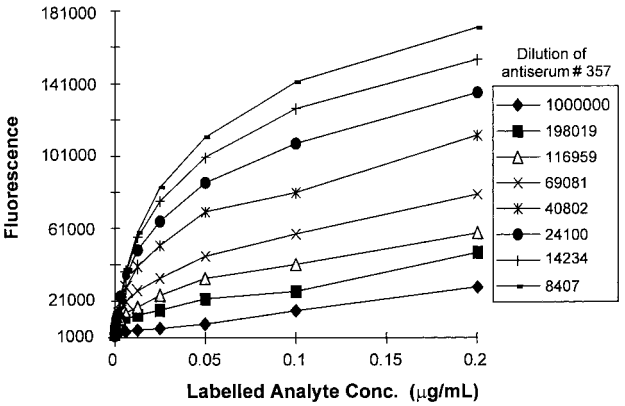

Figure 3. Checkerboard titration results for the TRFIA. The assay was conducted as described under Materials and Methods except that no analyte was present. Each point represents the value from a single well.

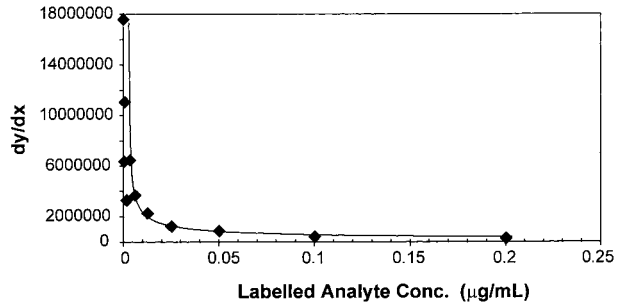

Figure 4. First-derivative plot for the 24100-fold antiserum dilution curve in Figure 3.

Table 1. Average Standard Curve Parameters[a]

		DL	IC_{10}[b]	slope[b] (B)	IC_{50}[b]
TRFIA					
	av ($n = 8$)	0.05	0.014	0.67	0.17
	SD	0.033	0.0084	0.095	0.076
	%RSD	72	62	14	45
ELISA					
	av ($n = 17$)	0.054	0.038	1.217	0.316
	SD	0.040	0.025	0.194	0.169
	%RSD	75	66	16	54

[a] Atrazine concentrations are ng/mL. [b] IC_{10}, IC_{50}, and slope determined from the curve fit.

DLs. TRFIA DLs were calculated from eight standard curves. The average DL was 0.05 ± 0.03 ng/mL ($n = 8$; in-well; Table 1). The DLs for the ELISA were similarly calculated for 17 standard curves. The average ELISA DL (0.05 ± 0.04 ng/mL) was the same as that for the TRFIA. These DLs make both of these assays suitable for monitoring atrazine in water as the

Table 2. DLs and Related Parameters for Published Atrazine Immunoassays

reference	DL (ng/mL)	DL criteria	IC_{50} (ng/mL)	assay	antibody
Wortberg et al. (1995)	*a*		0.64	ELISA	poly; 357
Harrison et al. (1991)			8	ELISA	poly; 357
Schneider and Hammock (1992)	0.03		0.24	ELISA	mono; AM7B2.1
Muldoon et al. (1993)			8.6	ELISA	mono; AM7B2.1
Wortberg and Cammann (1993)	0.1	IC_{10}	5	TRFIA	mono; K1F4
Karu et al. (1991)	<0.1	spike recovery studies	13	ELISA	mono; AM7B2.1
Rubio et al. (1991)	0.05	IC_5	1.1	ELISA	poly
Schlaeppi et al. (1989)	0.05	2 (SD)	0.45	ELISA	mono; 4063-21-1
Wittmann and Hock (1989)	<0.001		0.02	ELISA	poly; C193
Giersch (1993)	0.03	80% B/B_0	0.1	ELISA	mono; K4E7
Franek et al. (1995)	0.01	90% B_0	0.16	ELISA	poly
Wust and Hock (1992)	0.03		0.18	ELISA	poly; sheep 84

[a] Blanks indicate the information was not given.

Environmental Protection Agency limit for atrazine in drinking water is 3 ng/mL.

The parameter IC_{50} is also an indicator of assay DLs and is often used for the comparison of different immunoassays due to its relatively high precision. The IC_{50} values of the TRFIA (0.17 ± 0.08 ng/mL, $n = 8$) and the ELISA (0.3 ± 0.2 ng/mL, $n = 17$) were not significantly different. However, both of these assays had lower IC_{50} values than have been reported for 7 of 12 atrazine immunoassays (Table 2). The antibodies used for TRFIA and ELISA (polyclonal 357 and monoclonal AM7B2.1) probably had similar avidities for atrazine as judged from the published IC_{50} values using these antibodies, which were similar (although variable) (Table 2).

Precision. The TRFIA and ELISA were compared by analyzing four different environmental waters that were fortified with atrazine at various levels. The average %RSDs of quadruplicate fluorescence and absorbance readings for water samples (blank and fortified) were 5% ($n = 96$) and 6% ($n = 192$), respectively, indicating the reproducibilities of the TRFIA and ELISA were acceptable when the same sample was applied to replicate wells.

The triplicate fortified water samples were analyzed by TRFIA on the same plate but on different plates using ELISA. The *intra*-assay precision of the atrazine concentrations (fortification level > 0.05 ng/mL) observed using TRFIA was 12%RSD ($n = 10$). The *inter*-assay precision using ELISA was 21%RSD ($n = 13$).

Accuracy. The accuracies (Table 3) of observed atrazine concentrations for the spiked waters are plotted in parts A and B of Figure 5 for ELISA and TRFIA, respectively. The accuracies inside the boxes in these graphs do not include results below the DL or outliers. The averages of these representative accuracies are 1.4 ± 0.42 (30%RSD; $n = 13$) and 1.0 ± 0.38 (37%RSD; $n = 13$) for TRFIA and ELISA, respectively. Although there is no significant difference between these values, the results suggest that there was a positive bias associated with the TRFIA, possibly due to error in the preparation of serially diluted standards. This bias was not caused by a matrix interference because the average fluorescence of the blank samples (22988, $n = 12$; excluding Putah Creek) was not significantly different from the average maximum fluorescence of the standard curves (22518, $n = 8$). In general, accuracies between 0.8 and 1.2 are acceptable. It can be seen from Table 3 that the accuracies for both assays improved as the spike concentration increased and was best near the IC_{50} of the assays. Neither assay provided reasonable accuracies for the Putah Creek matrix except at the 25 ng/mL spike level.

Table 3. Accuracies of Atrazine Concentrations Observed in Spiked Water Samples

		ELISA				TRFIA			
matrix	spike level (ng/mL)	accuracy	SD[a]	%RSD	*n*	accuracy	SD[a]	%RSD	*n*
Fraser R.	0.03	3.48	3.13	90	3	0.46	0.42	93	5
Fraser R.	0.06	1.19	1.19	100	2	0.65	0.07	10	3
Fraser R.	0.16	0.29	0.29	100	2	1.10	0.21	19	3
Fraser R.	1.60	0.90	0.11	12	3	1.50	0.21	14	3
Fraser R.	16.00	1.00	0.10	10	2	2.15	0.43	20	3
marsh	0.03	2.00	1.47	73	3	0.85	0.63	73	5
marsh	0.06	1.58	0.63	40	2	1.01	0.05	5	3
marsh	0.16	1.19	0.57	48	3	1.32	0.30	23	3
marsh	1.60	0.90	0.38	42	4	1.46	0.18	12	3
marsh	16.00	1.25	0.15	12	3	1.33	0.20	15	3
Big R.	0.05	0.70	0.70	100	2	1.51	0.47	32	5
Big R.	0.10	0.70	0.22	31	3	1.72	0.24	14	3
Big R.	0.25	1.06	0.22	21	2	4.37	2.73	62	3
Big R.	2.50	0.86	0.08	9	3	1.51	0.18	12	3
Big R.	25.00	0.76	0.07	10	3	1.44	0.13	9	3
Putah C.	0.05	52.60	8.11	15	3	12.05	2.58	21	6
Putah C.	0.10	33.10	5.14	16	3	9.03	6.64	74	6
Putah C.	0.25	10.57	1.18	11	3	3.45	0.54	16	3
Putah C.	2.50	1.72	0.23	14	3	2.00	1.16	58	3
Putah C.	25.00	0.89	0.10	11	3	0.92	0.05	6	3

[a] Population SD.

The correlation between TRFIA- and ELISA-observed atrazine concentrations in spiked waters is shown in Figure 6. The slope of 1.4 is consistent with the average positive bias observed for TRFIA. The high errors observed for TRFIA and ELISA average accuracies (average %RSD = 34%) were reflected in the relatively low correlation coefficient of 0.86.

The Putah Creek water showed a matrix affect for both immunoassays as indicated by the poorer accuracies for this water at the lower spiking levels (Table 3). When blank Putah Creek water samples were analyzed by TRFIA, the fluorescence did not change significantly after 2-fold dilution with distilled water. This suggested that the interfering substance was not a triazine but a structurally unrelated substance which was present at relatively high concentration and which exerted a weak interaction to somehow reduce the amount of bound label. An interference was also observed for Putah Creek water in another study using the same polyclonal antibody as was used in this TRFIA (Harrison et al., 1991).

The accuracy and robustness of these assays may be improved by using some sample preparation techniques. Buffering the water sample prior to analysis can eliminate some variability due to pH differences among samples. At very low analyte concentrations, using solid-phase extraction cleanup and concentration would

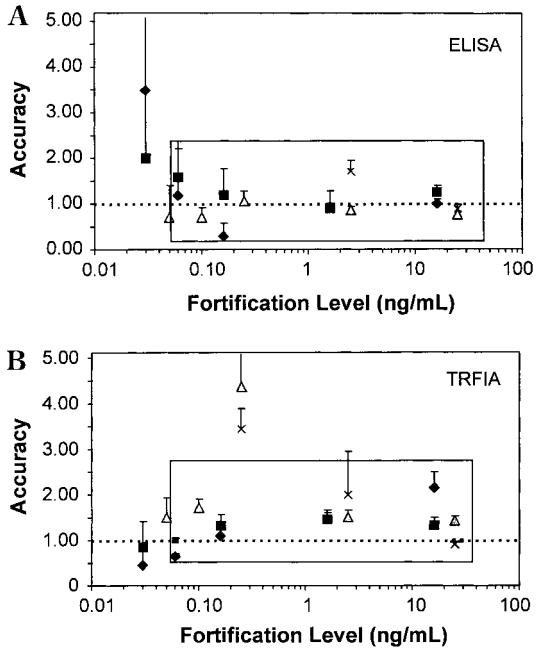

Figure 5. Accuracies of observed atrazine concentrations for spiked water samples. Error bars are SDs of triplicate results: (A) ELISA results and (B) TRFIA results; (◆) Fraser River; (■) marsh; (▲) Big River; (×) Putah Creek. The box contains representative accuracies, excluding outliers and results below the DL.

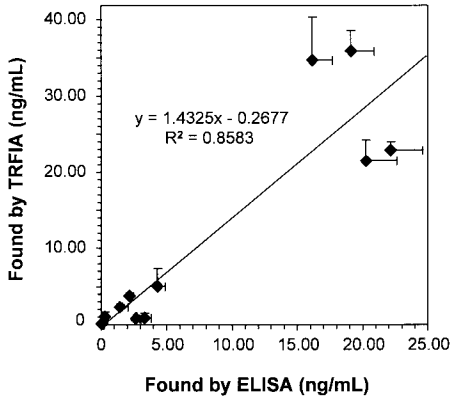

Figure 6. Correlation between TRFIA- and ELISA-observed atrazine concentrations in spiked waters. Results below DLs and Putah Creek results were not included.

also improve accuracy and contribute to a more robust assay at these low analyte levels.

Conclusion. The TRFIA and ELISA reported here are among the most sensitive immunoassays for triazines reported to date. These two methods proved to be similar in DL, suggesting that assay precision, as well as the ability to detect the label, determined the assay DL. Analysis of spiked water samples showed that the accuracies of the TRFIA and ELISA were not significantly different, but there appeared to be a positive bias associated with the TRFIA, which was probably due to method error and not to matrix interference. There was significant error (≈30%) in the accuracies of both assays, confirming the generally semiquantitative nature of immunoassays when employed at the extreme limit of their sensitivity. Because the methods did not significantly differ in assay parameters, they are both suitable for the analysis of samples at the levels tested.

The instrumentation and wet chemical reagents for both TRFIA and ELISA are evolving rapidly. Currently, ELISA dominates the environmental field primarily because of its precedent in clinical analysis and ease of use over radioisotope labels, but both technologies offer advantages now and for future developments. A very positive aspect is that most of the same reagents developed for ELISA are directly applicable to TRFIA as illustrated by this study. TRFIA methods reduce problems with background fluorescence and thus matrix effects. By avoiding the enzyme amplification used in ELISA, with TRFIA a reagent is eliminated, which is often the least stable component of kits and which often is more susceptible to matrix effects than the antibodies. The ability to read TRFIA in dry samples presents numerous advantages for high sample throughput and miniaturization of the assay.

ABBREVIATIONS USED

BSA, bovine serum albumin; DL, detection limit; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; RSD, relative SD; SD, standard deviation; TMB, 3,3′5,5′-tetramethylbenzidine; TRFIA, time-resolved fluorescence immunoassay; Tris, tris(hydroxymethyl)aminomethane; Tw-20, Tween 20.

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