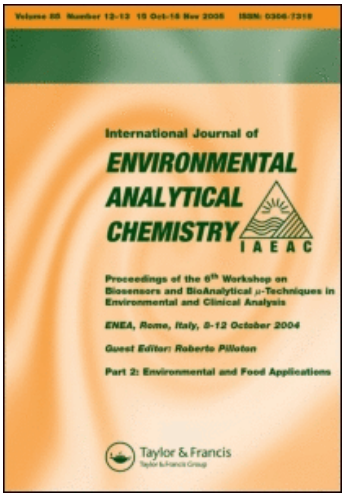

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Preliminary Screening Method for Dioxin Contamination Using Polarization Fluoroimmunoassay for Chlorinated Phenoxyacid Pesticides

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PRELIMINARY SCREENING METHOD FOR DIOXIN CONTAMINATION USING POLARIZATION FLUOROIMMUNOASSAY FOR CHLORINATED PHENOXYACID PESTICIDES

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Polarization fluoroimmunoassays (PFIA) were developed for the chlorinated pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). In order to optimize the PFIA procedures, a number of fluorescein-labeled 2,4-D and 2,4,5-T derivatives were synthesized and the influence of their structures on PFIA characteristics was studied. Also, several antisera were tested in developing the PFIA for 2,4,5-T. The assays were adapted for use with the Abbott TDx Analyzer and could be run in automatic mode by the adaptation of existing software and protocols. Dynamic ranges for 2,4-D and 2,4,5-T were 0.2–200 ng mL^{-1} and 30–10 000 ng mL^{-1}, respectively. Total time for the automated assay of 20 samples was about 22 min. PFIA provides a suitable means for screening of a large number of samples. The rapid determination of 2,4,5-T, which is one of the precursors of polychlorinated dibenzo-*p*-dioxins, one of the most toxic groups of pollutants, may potentially be used to provide preliminary evidence of dioxin contamination.

Keywords: Pesticides; 2,4-D; 2,4,5-T; Dioxin; Polarization fluoroimmunoassay; Environmental monitoring

INTRODUCTION

Chlorinated phenoxyacetic acids are a widely used class of herbicides. 2,4-Dichlorophenoxyacetic acid (2,4-D) is applied as a post-emergency herbicide for broadleaf weed control. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) was used previously for eradication of bushy and woody plants. About 25 years ago the usage of 2,4,5-T was banned but up to the present day trace amounts of this pesticide are found in natural substrates, mainly because of its presence in commercial 2,4-D preparations. The danger of 2,4,5-T is its capacity to form polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F). It is reported [1,2] that PCDD/F form under natural conditions in substrates such as compost and sewage sludge. Also, toxic PCDD/F congeners are

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formed during the manufacture of chlorinated phenoxyacids and are therefore present at varying levels in commercial herbicide preparations [3]. Some authors report another interesting tendency: dioxins can be formed in living organisms including man by a peroxidase-catalyzed process from the pesticides and related compounds [4]. The most toxic dioxin – 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) – is a main product of 2,4,5-T transformation in all cases. Thus, there is a great necessity for regular monitoring of the phenoxyacetic acid pesticides in the environment, and their detection may provide circumstantial evidence for the presence of PCDD/F.

Chromatographic methods have been the most used techniques for the detection of polychlorinated phenoxyacids, although they are expensive and require special procedures for sample preparation. These facts limit their usage for routine screening of pesticides in large numbers of samples. Alternative methods developed during the last decade are capillary electrophoresis [5], supported liquid membrane technique [6], photochemically induced fluorescence detection [7] and micellar electrokinetic chromatography with laser-induced fluorescence detection [8]. Biosensors [9,10] and immunosensors [11–13] have also been described. All of these methods are sensitive and precise, but need complicated and expensive instruments.

The most appropriate technique for pesticide detection from our point of view is the immunoassay, which is based on the specific antigen–antibody interaction. Several enzyme immunoassay systems for detection of these pesticides have been developed during the last few years. 2,4-D has often been used as a model analyte for the development of new assays, while only a few immunoassays have been developed for 2,4,5-T [14,15]. Reported methods are enzyme immunoassays (ELISA) on microtiter plates [16–18] or in a flow system with fluorescent [19], chemiluminescent [20] or electrochemical [21] detection of peroxidase as label, as well as immunosensors [22] and biosensors [23]. Moreover, new enzyme immunoassays for 2,4-D with higher sensitivity (up to 5 pg/L [24] and 2.7×10^{-11} M [25]) or faster detection (2 min [26]) have been published recently. Unfortunately the methods are mostly multi-step and need special immunoreagents and instruments.

One of the simplest and most rapid amongst immunochemical methods is polarization fluoroimmunoassay (PFIA). PFIA is a competitive assay and is based on detection of the difference of fluorescence polarization between a small fluorescent-labeled antigen (tracer) and its immunocomplex with specific antibody. The performance of PFIA involves sequential addition of the sample (or standard), tracer and antibody solution. After a brief incubation (a few minutes or even seconds) the signal is measured and the concentration of analyte in the sample is calculated. The kinetics of immune reaction in solution are so fast that the equilibrium in the reaction mixture is reached in minutes or even seconds. Previously we showed the feasibility of PFIA for 2,4-D and 2,4,5-T determination [27–30]. As described in our previous publications for several drugs [31–33], the method can be adapted to a widely available automated instrument, the Abbott TDx Analyzer, for effective rapid screening of large sample numbers. In the present studies, PFIAs were developed and adapted for use for the detection of 2,4-D and 2,4,5-T.

EXPERIMENTAL

Reagents

Chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Organic solvents and inorganic salts were supplied by Reakhim (Moscow, Russia). Monoclonal anti-2,4-D antibodies (Lot E2/G2) were obtained from the Veterinary Research Institute (Brno, Czech Republic). Polyclonal 2,4,5-T antisera were prepared by immunizing rabbits with 2,4,5-T conjugated to keyhole limpet hemocyanin (KLH), a mollusc protein. Polyclonal antisera against 2,4-D and 2-(3,5,6-trichloropyridyl)-oxyacetic acid (trichlopyr) were prepared by immunizing rabbits with conjugates of these pesticides to bovine serum albumin (BSA) (AOOT Immunotekh, Moscow, Russia). Polyclonal antisera against BSA conjugates of 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP) and 2,4,6-TP were supplied by Abuknesha (King's College, London, UK).

For thin-layer chromatography (TLC), Silufol pre-coated silica gel aluminium sheets were used (Kavalier, Czech Republic). All solutions for PFIA were prepared in borate buffer: $1\,g\,L^{-1}$ $Na_2B_4O_7 \times 10H_2O$, $1\,g\,L^{-1}$ NaN_3, pH 8.0. A stock solution of each pesticide ($1\,mg\,mL^{-1}$) was prepared in methanol.

Apparatus

The Abbott TDx Analyzer was used. Disposable sample cartridges and glass cuvettes were purchased from Sigma.

Synthesis of Fluorescent Tracers

Synthesis of 2,4-D-EDF; MCPA-EDF; 2,4-DBr-EDF; 2,4,5-T-EDF; 2,4,5-TP-EDF and trichlopyr-EDF tracers. Fluoresceinthiocarbamyl ethylenediamine (EDF) was synthesized as previously described from fluorescein isothiocyanate isomer I and ethylenediamine [34]. The EDF tracers were prepared using the *N*-hydroxysuccinimide ester method. Eight mg (80 μmol) of *N*-hydroxysuccinimide and 8 mg (40 μmol) of *N*,*N*′-dicyclohexylcarbodiimide were added to a solution of 20 μmol of the corresponding pesticide or derivative (2,4-D, 2-chloro-4-methylphenoxyacetic acid (MCPA), 2,4-dibromophenoxyacetic acid (2,4-DBr), 2,4,5-T, 2,4,5-TP or trichlopyr) in 0.2 mL of dimethylformamide. After 2 h stirring at room temperature the solution was added to 5 mg (10 μmol) of EDF. Then the reaction mixture was stirred at room temperature for 3 h. Small portions of reaction mixture (50 μL) were separated by TLC using chloroformmethanol (4/1, v/v) as the eluent. The main yellow band at R_f 0.9 was eluted and stored in methanol at 4°C. Tracer concentration was estimated spectrophotometrically at 492 nm, assuming an extinction coefficient in the borate buffer of $8.78 \times 10^4\,M^{-1}\,cm^{-1}$ [34]. The tracer 2,4-D-NH$(CH_2)_6$NH-FITC was prepared by the same method from 2,4-D and fluoresceinthiocarbamyl hexylenediamine, which was synthesized from fluorescein isothiocyanate isomer I and hexylenediamine [34].

Synthesis of 2,4-D-NHF; MCPA-NHF; -2,4,5-T-NHF; 2,4-D-5-F-NHF and 2,4,5-T-GAF tracers. *N*,*N*′-Dicyclohexylcarbodiimide (41 mg, 200 μmol) and aminofluorescein (NHF) (35 mg, 200 μmol) were added to a solution of 100 μmol of the corresponding pesticide or derivative (2,4-D, MCPA, 2,4,5-T or 2,4-dichloro-5-fluorophenoxyacetic

acid (2,4-D-5-F)) in 3 mL of ethanol. The reaction mixture was stirred at room temperature for 2 days and the organic solvent evaporated in vacuum. The residue was dissolved in 0.5 mL of methanol and chromatographed by TLC using chloroform/methanol (4/1, v/v) as the eluent. The main yellow band at R_f 0.9 was isolated and stored in methanol at 4°C. Concentration was estimated spectrophotometrically as above. The tracer 2,4,5-T-GAF was prepared by the same method from 2,4,5-T and glycylaminofluorescein (GAF).

Polarization Fluoroimmunoassay (PFIA) Procedure

Dilution curves were constructed as follows. The immunoglobulin G (IgG) fraction of antiserum and monoclonal antibodies were diluted 1/50, 1/100, 1/200, ... 1/51200 and incubated with the tracer solution in a total volume of 1 mL for 1 min at room temperature, followed by measurement of fluorescence polarization in 10 TDx glass cuvettes loaded into the "Photo Check" carousel. The optimal antibody dilution for construction of PFIA calibration curves was determined as that at which 70% tracer binding was observed.

Competitive calibration curves were constructed using the 1 mg mL^{-1} analyte stock solutions diluted with borate buffer to give 0.1, 1, 10, 100, 1000, 10 000 and 1 00 000 ng mL^{-1}. These standards (50 µL) were vortex mixed with tracer solution (0.5 mL) and an appropriate dilution of antiserum (0.5 mL). After 1 min incubation, fluorescence polarization values were measured. The calibration curves were analyzed using a four-parameter equation using Origin 6.0 for Windows.

Sample Screening Procedures in Automatic Mode of the TDx Analyzer

For 2,4-D screening, monoclonal anti-2,4-D antibodies (lot E2/G2) were used with tracer MCPA-EDF. For 2,4,5-T screening, polyclonal anti-2,4,5-T-KLH serum was used with tracer 2,4,5-T-NHF. Spare TDx reagent vials were washed thoroughly. For 2,4-D assay, vial S was refilled with antibody diluted 8-fold in borate buffer; vial T with 80 nM tracer in borate buffer; and vial P (pretreatment) with borate buffer. For 2,4,5-T assay, vial S was refilled with antiserum diluted 45-fold in borate buffer; vial T with 100 nM tracer in borate buffer; and vial P with borate buffer. These replacement vials were placed in spare TDx reagent packs: a Digoxin II pack for 2,4-D and a Cortisol pack for 2,4,5-T. The barcode reader accepted the reagent pack and the protocol was edited to run the given assay in automated mode.

Aliquots (100 µL) of each standard or sample were pipetted into the sample well of TDx cartridges. These cartridges and empty glass fluorimeter cuvettes were loaded onto a carousel for calibration or for running an assay. The carousel was placed in the analyzer and from then on the assay was performed entirely automatically. Each sample was first dispensed with pretreatment solution and dilution buffer into a glass cuvette and blank signals (vertically and horizontally polarized fluorescence components) were measured. Next the analyzer dispensed additional aliquots of the samples into the cuvettes along with aliquots of tracer and antiserum plus more pretreatment solution and dilution buffer. After a short incubation period to allow immunoassay binding reactions, the polarized fluorescence components were again measured, polarization was calculated from the blank-corrected signals, and results were printed

out in polarization and concentration units. Total time for the assay of 20 samples was about 22 min.

Testing of Water Samples

Natural waters were used for testing the applicability of the method. Mineral water was bottled water from S. Antonio well (''Piano di Cadorago'', Caslino, Italy). Surface water was collected from the Volga river (Dubna, Moscow region, Russia) in June 2001. Snow samples were collected in Moscow city, Russia. All water samples were analyzed without any pretreatment.

RESULTS AND DISCUSSION

Characterization of Tracer Binding and Assay Sensitivity for Different Antisera

The successful development of a PFIA requires a suitable combination of antibody and tracer reagents, to ensure both satisfactory binding and sensitive displacement by analyte. As reported earlier [30], slight changes in the specificity and structure of the immunochemical reagents may significantly influence the assay characteristics. Thus in the present work, five different antisera were assessed for the development of the PFIA for 2,4,5-T. Polyclonal antibodies were obtained by immunization of rabbits with conjugates of the analyte or its analogues and a carrier protein (immunogen). In addition to the homologous immunogen 2,4,5-T-KLH, other antisera were obtained with the heterologous immunogens 2,4-D-BSA, 2,4,5-TP-BSA, 2,4,6-TP-BSA and triclopyr-BSA in order to study the influence of immunogen structure on the assay characteristics. The antibodies' binding to the tracers is characterized by their titer (dilution giving 50% binding of tracer). The antibodies to 2,4,6-TP-BSA gave no binding, while the other three heterologous antibodies had titers from 1 : 700 to 1 : 1100. However, calibration curves obtained using any of the heterologous antisera had unsatisfactory IC_{50} values (IC_{50} being defined as the analyte concentration causing a 50% decrease in the assay signal as compared to the uninhibited signal). Therefore only anti-2,4,5-T-KLH was used for the further development of the 2,4,5-T assay. The titer of anti-2,4,5-T-KLH with the tracer 2,4,5-T-NHF was 1 : 800 (Table I).

TABLE I Values of antibody titer* and PFIA calibration curve IC_{50}** for different tracers. Monoclonal anti-2,4-D antibodies (lot E2/G2) were used for 2,4-D tracers and polyclonal anti-2,4,5-T-KLH antiserum for 2,4,5-T tracers

2,4-D			*2,4,5-T*		
Tracer	*Titer*	*IC_{50}* ($ng\,mL^{-1}$)	*Tracer*	*Titer*	*IC_{50}* ($ng\,mL^{-1}$)
2,4-D-EDF	1 : 3300	80	2,4,5-T-EDF	1 : 800	650
2,4-D-NHF	1 : 2100	70	2,4,5-T-GAF	1 : 700	500
2,4-D-NH$(CH_2)_6$NH-FITC	1 : 6000	520	2,4,5-T-NHF	1 : 800	550
MCPA-EDF	1 : 1600	10	2,4-D,5-F-NHF	1 : 200	1000
MCPA-NHF	1 : 1400	10	2,4,5-TP-EDF	1 : 100	570
2,4-DBr-EDF	1 : 2400	75	Trichlopyr-EDF	1 : 800	1100
2,4,5-T-EDF	1 : 1800	10	2,4-D-NHF	1 : 500	1500

*The antibody dilution that gives 50% tracer binding; **the analyte concentration causing a 50% decrease in the assay signal as compared to the uninhibited signal.

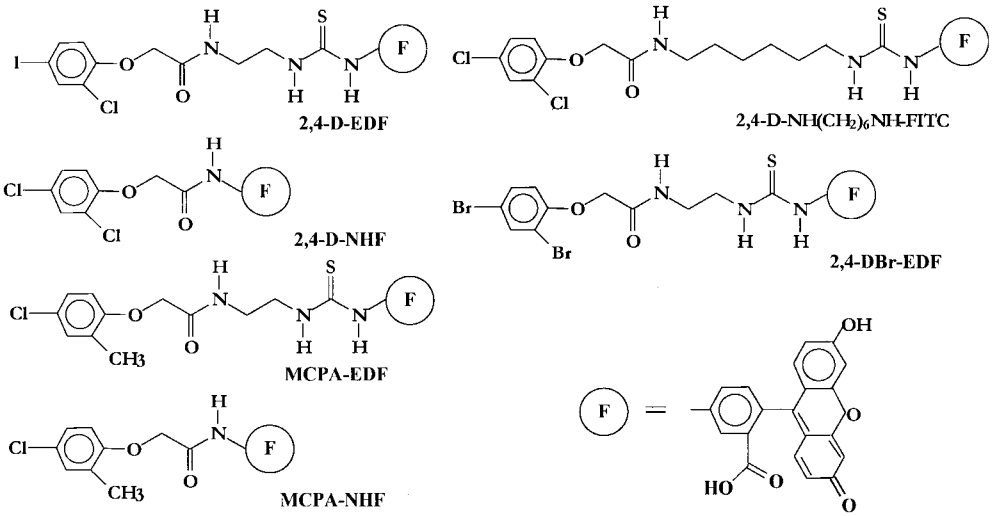

FIGURE 1 Structures of labeled antigens (tracers) in PFIA for 2,4-D.

In the PFIA for 2,4-D only one type of antibody was used – monoclonal anti-2,4-D-BSA – owing to its proven quality for immunoassays [17]. This antibody showed good binding with the various 2,4-D tracers (titers from 1 : 1800 to 1 : 6000) and also gave satisfactory IC_{50} values (Table I).

Influence of Labeled Antigens on Assay Sensitivity

To study the influence of tracer structure on assay sensitivity, seven different tracers were synthesized for the 2,4-D PFIA (Fig. 1). Tracers that differed in the length of the bridge between the 2,4-D residue and the fluorescent part were 2,4-D-NHF (with the shortest bridge), 2,4-D-EDF and 2,4-D-NH$(CH_2)_6$NH-FITC (with a long, 6-carbon bridge). The structure of the target molecule was varied in the tracers MCPA-EDF and MCPA-NHF, where a CH_3-group is present instead of one of the chlorine atoms of 2,4-D, and in 2,4-DBr-EDF and 2,4,5-T-EDF.

Table I shows the serum titers and IC_{50}s of calibration curves obtained with these tracers. The short bridge tracers, 2,4-D-EDF and 2,4-D-NHF, revealed lower titers than the long bridge tracer 2,4-D-NH$(CH_2)_6$NH-FITC, but their IC_{50} values showed they were much better in terms of assay sensitivity. This tendency was also observed in our earlier studies [29]. The heterologous tracers (MCPA-EDF, MCPA-NHF and 2,4,5-TEDF) have lower titers, which may be explained by their lower resemblance to the immunogen. However, the new tendency found was that all these heterologous tracers revealed much better assay sensitivity (by 7–8 times) than the homologous ones, enabling the limit of detection to be decreased. The only exception was 2,4-DBr-EDF, which gave about the same sensitivity as 2,4-D-EDF and 2,4-D-NHF, which may be explained by its closer structural similarity to them. The detailed characterization of the PFIA for 2,4-D was carried out using MCPA-EDF.

The fact that heterologous tracers give better assay sensitivity can be explained as follows. The lowest detection limit in a competitive immunoassay is obtained by achieving the most effective competition between the analyte and the tracer. This

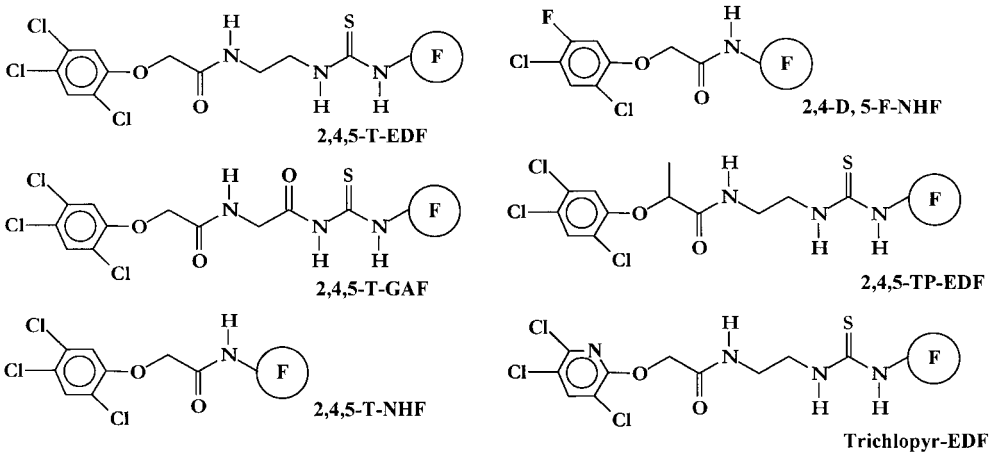

FIGURE 2 Structures of labeled antigens (tracers) in PFIA for 2,4,5-T.

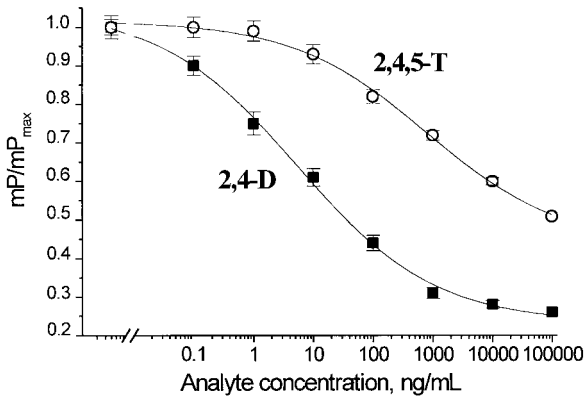

FIGURE 3 Optimal PFIA calibration curves for 2,4-D and 2,4,5-T.

generally implies that the tracer should have affinity for the antibody close to that of the analyte. In fact, homologous tracers such as 2,4-D-EDF and 2,4-D-NHF bind antibodies better than the free analyte (in this case 2,4-D) because their structure is closer to that of the immunogen. Heterologous tracers have lower binding affinity which may be closer to that of the analyte. Thus it may be beneficial if the structure of the analyte residue in the labeled antigen is changed so as to decrease the affinity constant and thereby attain higher assay sensitivity.

In development of the PFIA for 2,4,5-T, seven tracers were assessed (Fig. 2). As homologous tracers, 2,4,5-T-NHF, 2,4,5-T-EDF and 2,4,5-T-GAF were prepared. The two former were used in our previous investigations [30]. The difference in the structure of these three tracers is not very significant, and they demonstrated similar titers and IC_{50} values (Table I). The heterologous tracer 2,4,5-TP-EDF binds to the antibodies more poorly than the homologous tracers (serum titer is 1 : 100) but has a similar IC_{50}. The other heterologous tracers (2,4-D,5-F-NHF, trichlopyr-EDF and 2,4-D-EDF) showed better binding to the antibodies but in this assay system there

TABLE II Cross-reactivity study for PFIAs of 2,4-D (monoclonal anti-2,4-D antibodies and tracer MCPA-EDF) and of 2,4,5-T (polyclonal anti-2,4,5-T-KLH antiserum and tracer 2,4,5-T-NHF)

Cross-reactant	*%CR*	
	2,4-D	*2,4,5-T*
2,4-Dichlorophenoxyacetic acid	100	3.7
2,4-Dibromophenoxyacetic acid	165	ND*
2-Chloro-4-methylphenoxyacetic acid	11	0.7
2-Chloro-4-fluorophenoxyacetic acid	7.5	0.01
2-(2,4-Dichloro)-phenoxypropionic acid	1.6	0.3
2-(4-Chloro-4-methyl)-phenoxypropionic acid	0.3	ND*
2,5-Dichlorophenoxyacetic acid	2.7	0.8
3,5-Dichlorophenol	0.1	0.06
2,4,5-Trichlorophenol	0.6	1.3
2,4,5-Trichlorophenoxyacetic acid	6.8	100
2,4,5-Trichlorophenoxypropionic acid	0.1	1.2
2,4-Dichloro-5-fluorophenoxyacetic acid	19	5.5
Trichlopyr	ND*	8.5
2,3,6-Trichlorophenol	0.4	0.04
Pentachlorophenol	14	10.5
Pentachlorophenoxyacetic acid	0.6	0.6

* Not determined.

was no gain in sensitivity from the use of a heterologous tracer. The tracer selected for the routine assay was 2,4,5-T-NHF.

Optimal PFIA calibration curves for 2,4-D and 2,4,5-T are shown in Fig. 3. The specificity of the PFIAs was evaluated by testing various structural analogues of the pesticides, including different chlorinated aryloxycarboxyacids, their bromine and fluorine derivatives, and also polychlorophenols (Table II). High percentage crossreactivity (%CR) in the assay for 2,4-D was observed for three compounds: 2,4-DBr, 2,4-D,5-F and pentachlorophenol (PCP) (165, 19 and 14%, respectively). The two former compounds are not used in agriculture, so only the presence of PCP can interfere in the determination of 2,4-D in real samples. Noticeable %CR was also exhibited by MCPA (11%), 2-chloro-4-fluorophenoxyacetic acid (7.5%) and 2,4,5-T (6.8%). When one of the chlorine atoms in the 2,4-D molecule was "shifted" to another position in the benzene ring (2,5-dichlorophenoxyacetic acid), cross-reactivity fell more dramatically (down to 2.7%). Polychlorophenols demonstrated insignificant %CR, apart from PCP.

In the PFIA of 2,4,5-T, maximum %CR (10.5%) was observed for PCP. Other structurally similar compounds exhibited little cross-reactivity: for trichlopyr 8.5%, for 2,4-D,5-F 5.5%, for 2,4-D 3.7%, for 2,4,5-TP 1.2% and for 2,4,5-trichlorophenol 1.3%. For the remaining compounds tested, the %CRs were low ($< 1\%$) and corresponded with the difference between their structure and that of 2,4,5-T.

These results show that the two PFIAs are selective to the individual pesticides, which may be determined in the presence of most of the structurally related compounds tested.

Applications

The applicability of the PFIAs was studied using natural water samples. Recovery tests were carried out in mineral and river waters and in snow. A sample volume of 50 μL

TABLE III Recovery tests in natural water samples ($n = 3$) for PFIAs of 2,4-D and 2,4,5-T

Added ($ng\,ML^{-1}$)	*Found* ($ng\,mL^{-1}$)					
	Mineral water		*River water*		*Snow*	
	$ng\,mL^{-1}$	%	$ng\,mL^{-1}$	%	$ng\,mL^{-1}$	%
2,4-D						
0.5	0.4 ± 0.2	89	0.4 ± 0.4	86	0.6 ± 0.4	110
2	1.7 ± 0.5	87	1.8 ± 0.6	91	2.1 ± 0.6	105
10	10.5 ± 1	105	9.8 ± 3.2	98	11.5 ± 1.9	115
50	51.5 ± 5	103	56 ± 2	112	51 ± 3	102
2,4,5-T						
50	45 ± 5	90	44 ± 10	88	56 ± 8	111
100	109 ± 7	109	90 ± 14	90	109 ± 6	109
200	218 ± 22	109	224 ± 28	112	214 ± 16	107
500	530 ± 44	106	545 ± 74	109	520 ± 49	104

was used in each analysis. None of the samples gave an analytical signal. Four different amounts of 2,4-D and then 2,4,5-T were added to each sample. Recoveries of the pesticides, presented in Table III, ranged from 86 to 115% for 2,4-D and from 88 to 112% for 2,4,5-T. The data show that matrix influences in river water and snow are greater than in mineral water.

The developed assays are very convenient for screening of large numbers of environmental samples. Though the assay sensitivity is lower than that of classical and ELISA methods, the main advantage of PFIA is its rapidity. Ten water samples can be screened in 7 min using the Photo Check mode of the TDx. Analyzer without any pretreatment procedure. If it is necessary to detect the pesticide at lower concentration levels, the PFIA can be used as a prescreening method for revealing heavily contaminated samples in order not to subject them to long pretreatment procedures and reduce the cost of the analysis.

For the analysis of samples with greater matrix effect, such as soil and foods, sample preparation is needed. Extraction with polar solvents (methanol, ethanol or dimethylsulphoxide) should be carried out and then the extract should be diluted in water or in buffer at least 1 : 10. Matrix effects in many cases are strong, and so further dilution up to 1 : 100 is usually needed. Thus in this case there are losses in sensitivity, but still the method has significant advantages for environmental monitoring.

Furthermore, the PFIA for 2,4,5-T may provide a means for the preliminary detection of dioxin contamination. As 2,3,7,8-TCDD is a main product of 2,4,5-T transformation, the presence of 2,4,5-T in a sample can be circumstantial evidence of dioxin presence. In such a way, sources of dioxin contamination may be revealed in order to prevent further environmental pollution.

CONCLUSIONS

In the development of PFIAs for 2,4-D and 2,4,5-T, we synthesized a number of tracers and tested several antisera. It was demonstrated that the PFIAs could be applied for pesticide detection in natural water sources.

The rapidity and simplicity of PFIA give it great promise for wide application in environmental monitoring. For instance, hormone determinations in the last century were performed mainly using chromatographic methods, while today hormones and drugs are generally measured by immunoassay methods for medical diagnostic purposes. We anticipate that the same will happen in environmental analysis in the near future, and routine detection of pesticides probably will be done mainly by immunoassay methods. Moreover, if therapeutic drug monitoring and drug abuse screening tests are today performed more by PFIA than by ELISA, the same trend may be expected for pesticide determination.

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