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Evaluation of an Immunoassay Kit for the Detection of Certain Organochlorine (Cyclodiene) Pesticide Residues in Apple, Tomato, and Lettuce

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Currently, the conventional multiresidue methods (MRMs) (Anderson et al. 1986, Krause 1980) are used in this laboratory for the residue determinations of organochlorine (OC), organophosphate, (OP) and carbamate pesticides. MRMs are time - and solvent - consuming and also require the use of expensive analytical instruments, such as gas and liquid chromatographs which have to be operated by highly-trained analysts.

The enzyme-linked immunosorbent assay (ELISA) has been used extensively in clinical chemistry but has been introduced only recently in environmental chemistry (Wie et al. 1982, Newsome et al. 1987). Some of the commercially-available kits have been tested and used to screen herbicides in water, soil, and food (Bushway et al. 1988a, 1988b; Thurman et al. 1990).

This paper reports the results of an evaluation of a commercially-available ELISA kit (1) as a quick qualitative (yes-no) method to screen for residues of cyclodiene pesticides (endrin, endosulfan and dieldrin) in fruit (apple) and vegetables (lettuce and tomato), (2) as to the limits of detection (LODs) attainable and (3) as to the matrix effect observed by fortifying endosulfan in apple, lettuce and tomato extracts. The advantages and limitations of this kits in terms of the numbers of analytes determined per run and the LODs are compared with those of MRMs currently used in this laboratory.

MATERIAL AND METHODS

Res-I-Mune™ immunoassay kit (ImmunoSystem Inc., Scarborough, ME) was used for the evaluation.

All organic solvents were of high purity and suitable for use in residue analysts. All standards of pesticides: endrin (99.0%), endosulfan (98.7% with 64-67% alpha - stereoisomer and 29-32% beta - stereoisomer) and dieldrin (99.0%) were obtained from the Pesticide Standards Bank in Agriculture Canada and were used

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without further purification. Tween-20 (polyoxyethylenesorbitan monolaurate) was purchased from Sigma (St. Louis, MO).

Two stock solutions were prepared: (1) containing monobasic sodium phosphate ($NaH_2PO_4.H_2O$, 1.38 g) and sodium chloride (8.5 g) in deionized or distilled water (1 L), and (2) containing dibasic phosphate (anhydrous Na_2HPO_4, 1.42 g) and sodium chloride (8.5 g) in deionized or distilled water (1 L).

Buffer solution was prepared by mixing sodium azide (NaN_3, 10 mg) with stock solutions 1 (280 mL) and 2 (720 mL) and Tween-20 (1 mL). The pH of the mixture was adjusted to 7.2 with 0.1M HCl or NaOH solution if necessary. The solution was stored in a sealed glass container.

A chopped crop sample (1 kg) was blended in a food processor for 2 min. A portion of this sample (50 $\pm$ 0.1 g) and acetone (150 mL) were placed in a 500-mL Mason jar. The mixture was blended for 5 min with an Omni mixer and filtered through a Buchner funnel containing a Whatman No. 1 filter paper. The filtrate was transferred into a 250-mL volumetric flask. The Mason jar was further rinsed with acetone (3 x 10 mL). The rinses were filtered, and the filtrates were transferred to the same volumetric flask. The combined filtrate was adjusted to 250 mL by addition of acetone and then mixed. An aliquot (125 mL, 25 g) of the filtrate was transferred to a separatory funnel (500 mL) containing hexane and dichloromethane (210 mL, 1:1). After mixing and separating the two phases, the aqueous phase was extracted twice with dichloromethane (2 x 75 mL). The organic extract and two CH_2Cl_2 washes were combined and filtered through Na_2SO_4 into a round-bottom flask (500 mL) and rotary evaporated to near dryness. The residuum was transferred to a volumetric flask (25 mL) with buffer/tween (99.9/0.1) solution and the extract was diluted to volume to give a final concentration of 25 g/25 mL (1 g/mL). This extract was analysed and found to be OC-free (LOD = 0.02-0.03 ppm) using the MRM method (Anderson et al. 1986).

The fortified crop extracts were obtained by adding appropriate aliquots of standard solutions of endosulfan in acetone (1.1, 0.11 or 0.022 ug/uL) to apple, lettuce or tomato extracts (5 mL) to give the respective levels of 0.5, 0.05 and 0.01 ppm. Similarly and separately, appropriate aliquots of standard solutions of endrin in acetone (2.0, 0.2 or 0.01 ug/uL) were added to apple extracts to give the respective fortification levels of 0.5, 0.05 and 0.01 ppm and separately dieldrin in acetone (0.86, 0.43 and 0.09 ug/uL) to apple extracts to give 1.0, 0.1 and 0.02 ppm.

The ELISA procedure was performed according the manufacturer's instruction. Briefly, an aliquot (160 uL) of negative control or fortified sample extract followed by enzyme conjugate (4 drops) was added to an antibody-coated tube with gentle swirling to mix the reactants. After 5 min, the tube was rinsed 4 times with tap water to remove the excess unreacted sample and enzyme

conjugate. Immunoassay substrate (4 drops) and chromogen (4 drops) were sequentially added to each tube followed by gentle swirling for 2-3 sec. After 2 min the colour was fixed with a stop solution (1 drop of 2.5N sulfuric acid) and diluted with deionized water (0.5 mL).

The absorbance readings (A450) of the negative control and fortified samples were measured at 450 nm using a UV/visible spectrometer (Spectronic 1201 from Milton Roy, Rochester, NY). The % control (total binding) was calculated as the absorbance of sample (B) at 450 nm divided by A450 of the negative control (extract blank, Bo) and times 100.

RESULTS AND DISCUSSION

The absorbance readings from ELISA are inversely proportional to the concentration of analytes fortified in the sample extracts. In this assay, a negative control sample was run with each set of fortified extracts. Calculating the % control normalized the absorbance reading of each fortified sample against the control sample and reduced the coefficient of variation (CV) (see Tables 1 and 2).

Table 1. Absorbance (A450)[a] of tomato extracts fortified with endosulfan (ppm)

	0 ppm	0.01ppm		0.5 ppm		0.5 ppm	
	Bo	B	% cont.[b]	B	% cont.	B	% cont
	0.36	0.29	80.6	0.21	58.3	0.09	25.0
		0.29	80.6	0.17	47.2	0.08	22.2
	0.26	0.22	84.6	0.14	53.9	0.05	19.2
		0.19	73.1	0.14	53.9	0.05	19.2
	0.35	0.27	77.1	0.18	51.4	0.08	22.9
		0.25	71.4	0.21	60.0	0.08	22.9
	0.34			0.19	55.9		
	0.35			0.19	54.3		
$\bar{X}$	0.33	90.25	77.9	0.18	54.4	0.07	21.9
S.D.	0.04	0.04	5.0	0.03	4.0	0.02	2.3
C.V.	10.8	16.0	6.4	15.3	7.3	28.6	10.5

[a]A450 = Absorbance units of control (Bo) and fortified samples (B) measured at 450 nm.

[b]% cont. = % control which was (B/Bo) x 100

When the % total binding was plotted against the concentration of analyte (in ppb on a log scale) the curves were linear in the concentration range of 0.01 to 0.5 ppm for endosulfan in tomato, lettuce and apple and for endrin in apple; for dieldrin in apple, it was 0.02 to 1.00 ppm (see Figures 1A to 1D).

The LOD is defined as 3 times the standard deviation (SD) obtained from the extracts at the lowest fortification level (0.01 ppm) (ACS Committee, 1980). For

Table 2. Absorbance of crop extracts fortified with endosulfan or endrin (ppm)

Crop	OC	0 ppm Bo	0.01 ppm A450 (B)	% cont.	0.05 ppm A450 (B)	% cont.	0.5 ppm A450 B)	% control
Lettuce	Endosufan	0.30	0.23	76.7	0.19	63.3	0.09	30.0
			0.27	90.0	0.20	66.7	0.10	33.3
		0.27	0.24	88.8	0.17	63.0	0.10	37.0
			0.24	70.6	0.19	70.4	0.08	29.6
		0.34	0.26	76.5	0.20	58.8	0.09	26.5
			0.27	79.4	0.21	61.8	0.10	37.0
	$\bar{X}$	0.30	0.25	80.3	0.19	64.0	0.10	32.2
	S.D.	0.04	0.02	7.6	0.01	4.0	0.01	4.3
	C.V.	11.7	6.9	9.5	7.2	6.3	10.0	13.3
Apple	Endosulfan	0.36			0.22	61.1	0.08	22.2
		0.47	0.36	76.6	0.25	53.2	0.10	21.3
	$\bar{X}$					57.2		21.8
Apple	Endrin	0.36	0.29	80.6	0.20	55.6	0.08	22.2
		0.47	0.39	83.0	0.28	59.6	0.11	23.4
	$\bar{X}$	0.42		81.8		57.6		22.8

Table 3. Absorbance of apple extracts fortified with dieldrin (ppm)

Crop	OC	Control A450(Bo)	0.02 ppm A450(B)	% cont.	0.10 ppm A450(B)	% cont.	1.00 ppm A450(B)	% cont.
Apple	Dieldrin	0.36	0.28	77.8	0.23	63.9	0.10	27.8

endosulfan, it was 15-23% reduction from the negative control sample which was equivalent to 0.01 ppm in apple and tomato, and 0.02 ppm in lettuce. Using the criterion of 15-23 % reduction from control (100%), the LOD for endrin in apple was 0.01 ppm, and for dieldrin in apple, it was 0.03 ppm. A comparison of % control of endosulfan in apple, lettuce and tomato extracts showed a slight matrix effect (Figures 1A-1C). The cross reactivity was measured as a 50% reduction of A450 from the negative control which was equivalent to 0.08 ppm for endrin in apple; 0.08 ppm for endosulfan in apple; 0.1 ppm for endosulfan in lettuce, and 0.06 ppm for endosulfan in tomato and 0.17 ppm for dieldrin in apple. The LODs and the cross reactivity of other cyclodiene pesticides (chlordane, heptachlor and aldrin) were not tested but were expected to be at a higher concentration than those for dieldrin based on the data provided for this kit by the manufacturer.

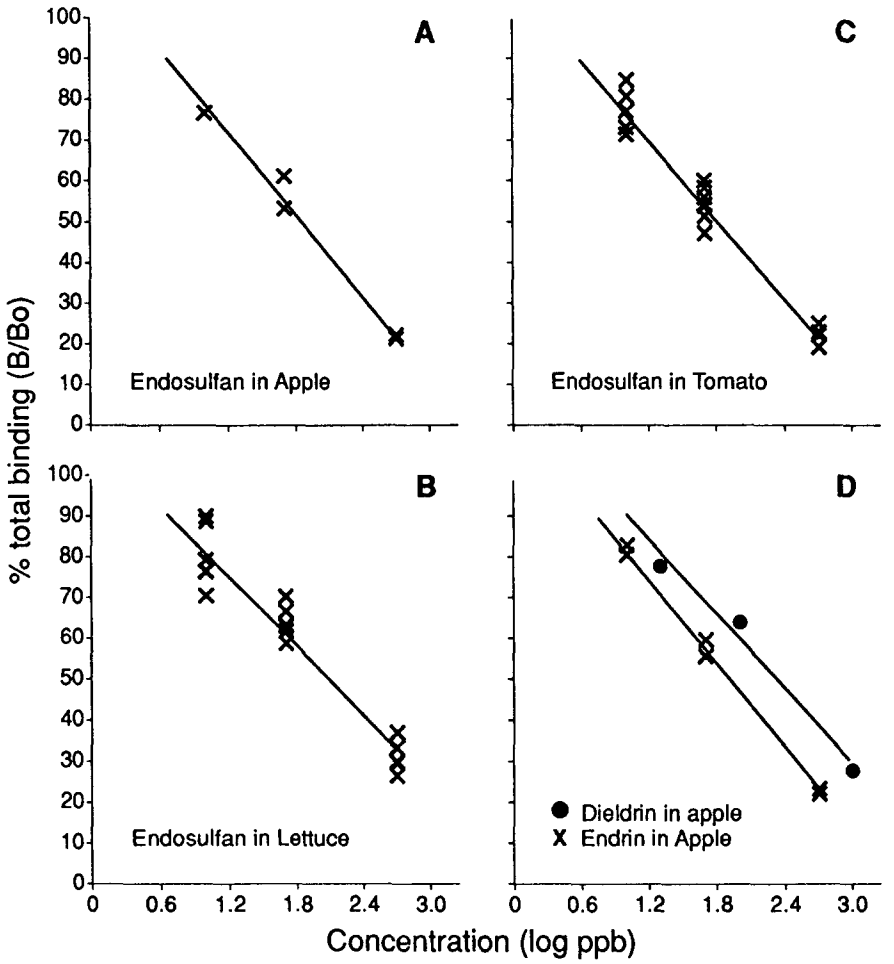

Figure 1. % total binding of: A, endosufan in apple (n=2), B, endosulfan in lettuce (n=6), C, endosulfan in tomato (n=6), and D, endrin in apple (n=2) and dieldrin in apple (n=1) plotted against concentration of analyte (log ppb).

After the preparation of the crop extracts, the actual assay took less than 20 min to complete and required only a UV spectrometer. Each set of assays should consist of a maximum of 4 samples, 3 fortified samples (1 at, 1 above, and 1 below the LOD) and 1 negative control. The number of samples to be assayed in each set was restricted by the time interval (2 min) required before a stop solution was added.

While MRMs can detect and quantitate over 20 OCs, 19 OPs and 9 carbamates including their metabolites at LODs of 0.01-0.08 ppm, this ELISA kit can detect 3 OCs (endrin, endosulfan and dieldrin) and possibly three others (chlordane, heptachlor and aldrin) at relatively higher LODs. In conclusion, this kit has demonstrated to be relatively simple, rapid and inexpensive as a screening procedure to detect endrin, endosulfan and dieldrin in fruit (apple) and vegetables (tomato and lettuce) with LODs of 0.01-0.03 ppm.

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