Scientific Research and Essays Vol. 6(8), pp. 1771-1779, 18 April, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE09.213
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Determination of carbofuran in fish and corn leaves by two types of commercial immunoassays

E. N. Wandan[1]*, M. J. Zabik[2] and E. F. Elleingand[3]

[1]Département Eaux, Forêts and Environnement, Institut National Polytechnique-HB BP 1313 Yamoussoukro, Côte d'Ivoire.
[2]Centre for Food Toxicology and Pesticide Research Centre, Michigan State University, 204 PRC 4888 MI, USA.
[3]Département Génie Chimique et Agroalimentaire, Institut National Polytechnique-HB BP 1313 Yamoussoukro, Côte d'Ivoire.

Accepted 12 August, 2010

We evaluated two commercially available immunoassay kits for the detection of carbofuran in fish and corn leaves. We observed strong matrix interference or inhibition due to the extraction solvent. This interference was corrected by either diluting or decolorizing and diluting the extract. The coefficient of variation of the recovery ranged from 3.9 - 11.0% for the coated tubes compared to 1.3 - 3.9% for magnetic particles bases kits. We detected carbofuran in the range of 0.33 - 0.51 ng/g in fish and 0.75 - 2.20 ng/g in corn leaf. The ELISA detection limits were 0.5 ng/g in corn leaf and 0.3 ng/g in fish fillet. The samples, which were positive for carbofuran (by kits) were found to contain carbofuran (by GLC) with a good coefficient of correlation (r^2 = 0.997). These results showed that ELISA can be used for the determination of pesticides in the environment, but observed cross reactivity with the metabolites may interfere with the detection of the parent compound and hinder the detection. The possibility of false positive or false negative detection may require the use of chromatography as confirmation technique. In any case, ELISA is advantageous to developing countries because of the low cost of material acquisition.

Key words: ELISA, kit, carbofuran, fish, leaf, polystyrene tube, magnetic particles.

INTRODUCTION

Carbamate insecticides are used increasingly in agriculture as a replacement for environmentally more persistent organochlorine insecticides for the control of insect pests. Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), a broad spectrum systemic insecticide-nematicide is one of the widely used carbamate insecticides in rice and corn production. The toxicity, metabolism, and degradation of carbofuran has been extensively studied (Eisler, 1985). At the recommended application rates and in a variety of formulations, carbofuran has been held responsible for sporadic kills of fish (Kanasawa, 1975). In plants, carbofuran is taken up by the root system and distributed throughout the entire plant and can remain in grains or plant materials after harvesting (for fourteen to twenty-one days). Carbofuran is metabolized by hydroxylation and hydrolysis in plants (Metcalf et al., 1968). An appreciable amount of carbofuran and its major metabolite, 3-hydroxycarbofuran, remain in the leaves of corn at silage stage as well as at harvest (Turner and Caro, 1973).

In the third world, rice paddy is use as forage or to build silos and fish are raised in paddy water to provide protein for local people. It is therefore, important to assess the level of this chemical in fish and plant materials.

Several chromatographic methods have been developed to determine residues of carbofuran. All GC methods are based on the original method of Cook et al. (1969). They consist of acid hydrolysis of the sample to release any conjugated 3-hydroxycarbofuran to the aglycone form followed by extraction of the aqueous phase with dichloromethane and column cleanup to remove possible interferences. These methods are lengthy and expensive. More recently, enzyme-linked immunosorbent assays (ELISA) have gained interest for environmental analysis because of their sensitivity,

*Corresponding author. E-mail: wandaneb@yahoo.fr.

selectivity, and cost effectiveness (Argarate et al., 2010; Sun et al., 2009; Chang et al., 2009; Tananuja et al., 2007; Watanabe et al., 2006; Brena et al, 2005; Conde et al., 2004; Gabaldon et al., 2002; Gabaldón et al., 2002; Danks et al., 2001; Sullivan and Goh, 2000; Hennion, 1998; Williams et al., 1997; 1996; Van Emon and Mumma, 1990; Hammock and Mumma, 1980). Commercial ELISA kits have been applied to the analysis of carbofuran in water (Bushway et al., 1992) and in meat and liver (Lehotay and Argauer, 1993).

The objectives of the present study were to (1) investigate a broader use of commercial kits as rapid detection systems for carbofuran in complex matrices such as fish and plant material; and to (2) compare the results with the detection by GLC.

MATERIALS AND METHODS

Instrumentation

ELISA

The RaPID® Assay kit, the magnetic separation rack, and the RaPID® Photometric analyzer were purchases from Ohmicron (Newton, Pennsylvania). The kits contained anti-carbofuran antibody coupled with magnetic particles units, as well as carbofuran coupled with peroxidase. Envirogard® Assay kits were purchased from Millipore (Bedford, MA). The kits contain antibody-coated test tubes.

Chromatography

Analyses were carried out by using a Hewlett Packard 5890 Series II gas chromatography equipped with a nitrogen specific detector (NPD) and a 10 m × 0.33 mm I.D. J&W DB-5MS (1.5 µm film thickness) fused silica capillary column. The injection of the samples was done automatically using a HP model 7673 automatic injector. A Hewlett Packard Data handling systems was used for data handling and processing. The samples were run under the following conditions (Cook et al., 1969): flow rate 1 ml/min helium, make-up gas flow was 45 L/min nitrogen. Temperature were 200, 250 and 250°C for the oven, injector, and detector, respectively.

Reagents

All organic solvents used for sample extractions, cleanup, gas chromatography, and ELISA were distilled-in-glass grade. Analytical reference: Carbofuran (95%) and its metabolites 3-hydroxycarbofuran and 3-ketocarbofuran were provided by FMC Corp. Ag Div. (Princeton, NJ). The stock solution was prepared in methanol at a concentration of 100 ng/ml. Dilutions of this for enrichment and chromatographic purposes were made with methanol and stored in the refrigerator when not in used and were stable for at least one month. Formulated carbofuran for fish exposure was prepared by diluting 1 ml of the commercial formulation (Furadan 4F) in distilled water to reach 2.4 g/L.

Methods

Fish rearing

Sixty fish, bluegills (*Lepomis macrochirus*) size of 10 - 12.5 cm purchased from a commercial hatchery, were first acclimated in a 75-L tank, and 20 fish were later transferred into 40-L aquarium and exposed to the pesticide. The final concentration in the tanks was made in consideration of the 96 h LC_{50} for Bluegill. The experiment consisted of a control (not treated) and a treated (0.24 mg/l). To avoid ammonia build-up in the tank, 25% of the water was replaced each day and 25% of the initial amount of the pesticide was added in order to keep constant concentration. Each treatment was replicated. At the end of the exposure, the fish were removed from the tanks, killed, the scales removed, and filleted. The fillets were put in plastic bags, and kept in the freezer until analysis.

Corn plants

Field corn grown in the greenhouse, were treated with carbofuran. The experiment consisted of a control (non-treated) and a treated (2.2 kg a.i./ha). The leaves were sampled before Furadan application, the day of treatment, and at the harvest. The leaves were chopped in a Hobart chopper, thoroughly mixed and stored in jars at -10°C until analysis.

Extraction of fish for ELISA

Five grams of chopped fish fillet was ground twice its weight of Na_2SO_4, in a mortar. The ground sample was placed into an Erlenmeyer and homogenized for 10 min with 50 ml of the extraction solvent using a mechanical homogenizer. The homogenate was allowed to stand for 3 - 5 min, then an aliquot of the supernatant was collected and analyzed after dilution with the supplied buffered saline diluent (Ohmicron kits) or distilled water (Millipore kits).

Extraction of corn leaves for ELISA

50 g of chopped leaves were blended in a Sorvall mixer for 2 min at a medium speed with 50-ml methanol. The slurry was filtered with suction through a Buchner funnel containing 5 g of Celite 455 on Whatman N°1filter paper.

Extraction of fish for chromatograph

10 g of sample were extracted according to the method of Martin et al. (1991). The sample was mixed with 50 g of anhydrous sodium sulfate (Na2SO4), ground with a mortar and homogenized with acetonitrile. The homogenate was filtered, partitioned with hexane, then with 10% NaCl solution, and finally cleaned-up on a silicagel (grain size?) column and analyzed by GLC.

Extraction of corn leaves for chromatography

The extraction of carbofuran from corn leaves was done according to cook et al. (1969). Twenty-five g of chopped leaves were placed into a round-bottom flask and refluxed in 0.25 HCl for 1 h with continual stirring. After cooling, the homogenate was filtered under vacuum using Whatman N°1 paper. The filter cake was rinsed with 50-ml methylene chloride followed by 50 ml of 0.25N HCl solution. Each filtrate was transferred into a separatory funnel and after shaking, the methylene chloride layer was removed. The aqueous fraction was further extracted with 7 × 50-ml portion of methylene chloride. The combine methylene chloride layer was dried over anhydrous sodium sulfate (Na_2SO_4), discolored by adding 2 g of active charcoal, and filtered by gravity. The filtrate was evaporated to dryness, redissolved in 5 ml of 5% (v/v) acetone in hexane and

subjected to clean-up. A column filled with 7 g of silicagel and topped with 2 - 3 g of anhydrous sodium sulfate (Na_2SO_4) was washed with 50 ml 5% acetone in hexane, the column was eluted with 250 ml of 10% acetone in hexane. The eluate was evaporated to nearly dryness, redissolved in 5-ml hexane, and analyzed by GLC.

ELISA assays procedures

The assay as apply to corn leaves and fish extracts was done according to the procedures for samples specified in the kits by the manufacturers, except all the determination were done in duplicate. For both kits, the determination consists on the incubation of the sample with the enzyme conjugated followed by color development with a chromogen and reading of the absorbance at 450 nm with a photometer (the RaPID® photometric Analyzer). The amount of carbofuran in the sample was determined by reference to the standard curve.

The absorbance readings from the ELISA are inversely proportional to the concentration of the analytes in the sample extracts. The absorbance of the control (extract blank, Bo) at 450 nm divided by the absorbance of the sample extract (B) times 100, (%B/Bo) referred here as %Bo, which when plotted versus the log concentration is linear with a negative slope. These plots were used to approximate the concentration of the samples.

Immunoassay performance characteristics including sensitivity, precision, and recovery of diluted and spiked samples were evaluated on both within-day and between-days assays. Sensitivity is defined as the lowest measurable concentration of carbofuran that can be distinguished from the respective 3.28 ng/g calibrator (95% confidence interval) associated with the EIA (Hennion, 1998).

Recovery of diluted and spiked samples is a gauge of the linear relationship between carbofuran measured in diluted or spiked samples relative to the neat samples. Dilution recovery was measured by diluting fish or corn leaves sample extracts spiked with carbofuran in the range of 3.28 - 32.80 and 49.2 - 154.00 ppb, respectively. Dilution and spiked recovery was expressed as a percentage by dividing the measured concentration of the diluted or spiked sample by the theoretically expected concentration of the diluted or spiked sample, and the result was multiplied by 100.

Assay methods typically exhibit experimental variability at intra- and inter-assay levels. Both types of variation can arise from several sources. Precision refers to the within-run rand between-run reproducibility of carbofuran signal that is produced for a particular sample in an ELISA. We evaluated precision by calculating the percent coefficient of variation observed between duplicate measurements corresponding to the five calibrator solutions and spiked samples (6.6 - 312.0 ppb). The resulting % CV values were averaged to express precision.

Reliable quantitative determinations using the calibration curve can be obtained provided there is no cross-reactivity. Experimental measurement of the extent of cross-reactivity is performed by spiking samples with related compounds, recording the corresponding absorbance, and drawing the corresponding dose-response curves, similar to the standard curves. The percent of cross-reactivity (% CR) is usually defined as 100-fold the ratio between the IC50 values of the antigen and of the cross-reactant. Cross reactivity (%CR) was calculated by dividing the metabolite concentration of carbofuran that gave 50% Bo.

Corn leaves and fish samples were spiked with standard solution at 3.28 ng/g; extracted and assayed after dilution using Ohmicron test kits. The dose providing two or three times the standard deviation from the mean measurement is used to approximate the method limit of detection (MLD) or least detectable dose (LDD). The lower limit of detection (LLD) can also be calculated by determining the analyte concentration yielding 90% Bo.

Data analysis

The test of equality of adjusted means was used to assess the presence of significant differences in the linear part of the curve. Statistical release 7.1 (StatSoft, France) was used to generate the curve GLC vs. ELISA and statistical analysis. The average percentage error (ε%) and the coefficient of correlation (r^2) are the criteria that were used to compare the ELISA and the GLC determination of carbofuran in neat samples.

RESULTS

Determination of the range of linearity

Standard solutions prepared in 10% methanol in water were assayed during the study. The concentrations ranged from 0.08 -11.36 ppb. Three replicate assays (in duplicates) were performed only for the Ohmicron kits because the Millipore test kits were provided with only 2 standard solutions. According to the significance level in a two sided test, one group of similar data can be detected resulting in a curve (Figure 1); The Ohmicron kits present a linear range between 0.038 and 9.88 ppb. The regression coefficient r^2 was 0.99 and the least detectable dose (LLD) calculated as 90%Bo was determined to be 0.32 ppb for these kits.

Recovery study

For fish fillet, the preliminary assays, without dilution, gave a very high recovery (>177) (Table 1a). When the extracts were diluted 10 times with distillated water, the anomalously high recovery observed was markedly reduced (104 - 152%). Fifty times dilution eliminates the enhancement; the recovery obtained was 104 - 107% (RSD ≤ 13%).

Concerning corn leaves, the preliminary assays yielded recoveries in the range of 170 - 180%. The extracts were diluted 50 times with distilled water and assayed, the recoveries obtained ranged from 115 -137% with RDS ≤ 17% (Table 1b). When the discolored extracts were diluted 50 times, the recoveries ranged from 105 - 124%. Further dilution (1:100, v/v) yielded a recovery of 90 - 108% with RSD ≤ 13%.

Reproducibility

Standard solutions of carbofuran from both kits and enriched sample extracts were assayed to determine the within-day and between-day variations. The coefficient of variation of the recoveries for standard solutions ranged from 4.20 ± 0.67 to 8.90 ± 1.44% (within-day) to 5.7 ± 1.16 to 9.50 ± 1.85% (between-day) for the Millipore test kits and from 1.30 ± 10.20 to 7.0% ± 1.25% (within-day) to 5.70 ± 0.85 to 9.90 ± 1.23 (between-day) for the Ohmicron kits (Table 2). For the spiked sample, the Millipore kits gave a coefficient of variation of 3.90 ± 0.52

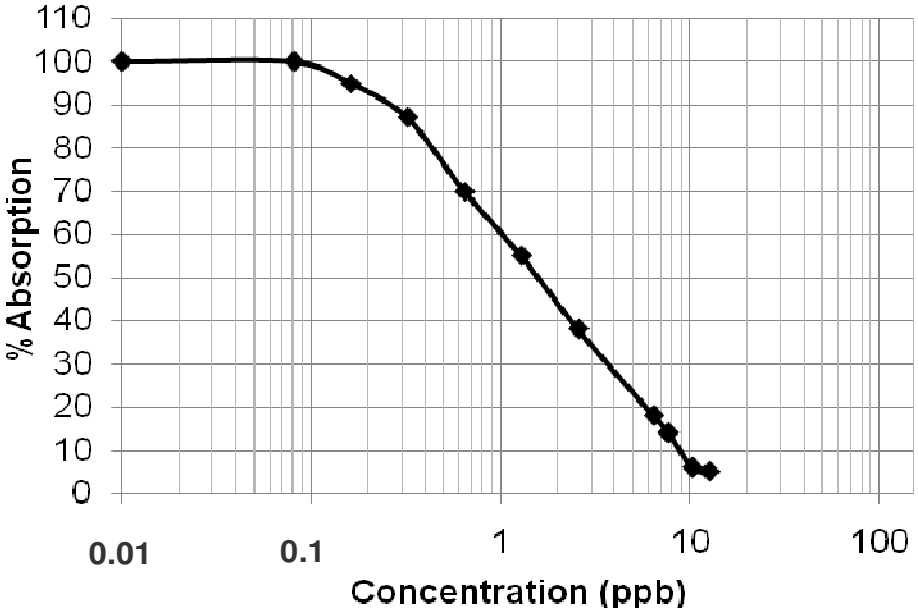

Figure 1. Plot of the standard curve using the Ohmicron kit. Each point represents the means of three replicate assays.

Table 1a. Recovery (%) of carbofuran in fish using the Ohmicron test kits.

Spiking levels (ppb)	Dilution levels	
	01:10	01:50
3.28	152 ± 90	nd
5.62	150 ± 11	98.3 ± 13
16.40	134 ± 70	94.2 ± 12
32.80	106 ± 11	102.6 ± 11

Tabulated values are Mean ± SD Mean of 5 assays per spiking level. nd = not found.

Table 1b. Recovery (%) of carbofuran in corn leaves using the Ohmicron test kits.

Spiking levels (ppb)	Dilution levels		
	No discoloration	Discoloration	
	01:50	01:50	1:100
49.2	138 ± 10	112 ± 14	90 ± 17
65.6	122 ± 8	115 ± 5	103 ± 13
98.4	120 ± 17	105 ± 16	91 ± 16
131.2	115 ± 11	113 ± 13	108 ± 16
164.0	120 ± 17	101 ± 10	94 ± 15

Tabulated values are Mean ± SD of 5 assays per spiking level.

to 10.80 ± 1.47 and 7.60 ± 1.15 to 11.00 ± 1.87 for within-day and between-day analyses, respectively. The Ohmicron kits yielded a within-day coefficient of variation of 3.00 ± 0.44 to 7.70 ± 0.65 and a coefficient of variation of 6.90 ±1.25 to 9.90 ± 1.44 for between-day determinations.

Sensitivity

The method detection limit (MDL) of the test kits were found to be 0.326 and 0.200 ng/g for corn leaves and fish samples, respectively using 2σ (Table 3).

Table 2. Assay of reproducibility study expressed by the coefficient of variation (% +RSD) for test kits in corn leaves extracts.

	Level of carbofuran (ppb)	Ohmicron test kit		Millipore test kit	
		Intra-assay#	Inter-assay*	Intra-assay#	Inter-assay*
Standard solutions	0.0	1.30 ± 0.20	5.70 ± 0.85	5.00 ± 0.54	5.70 ± 1.16
	0.1	3.10 ± 0.77	5.90±1.10	nd	nd
	0.2	nd	nd	4.20 ± 0.67	5.90± 1.77
	1.0	4.00 ± 0.67	nd	nd	nd
	5.0	7.60 ± 1.25	9.50 ± 1.23	8.90 ± 1.44	9.50 ± 1.85
	Mean	4.00 ± 0.45	7.03 ± 0.55	6.03 ± 0.65	7.03 ± 0.71
Spiked samples	6.6	7.70 ±0.65	8.50 ± 0.55	3.90 ± 0.52	7.60 ± 1.15
	32.8	4.20 ±0.37	6.90 ± 1.25	7.40 ± 0.84	7.90 ± 1.22
	65.6	3.00 ±0.44	8.10 ± 1.24	10.80 ± 1.47	11.00 ± 1.87
	312.0	6.10 ±0.57	9.90 ± 1.44	7.40 ± 0.69	8.80 ± 1.05
	Mean	4.97 ±0.75	7.83 ± 0.81	7.37 ± 0.85	8.83 ± 0.77

Tabulated values are Mean ± SD of 5 assays per standard solution or spiking level. nd = not detected.

Table 3. Method determination limit (MDL) calculated from standard deviation (2σ) using the Ohmicron test kits. Six replicate determination per assays.

Assay	Corn leaf		Fish fillet	
	% Bo	Concentration (ppb)	% Bo	Concentration (ppb)
1	99	0.067 ± 0.02	90	0.121 ± 0.05
2	93	0.108 ± 0.03	88	0.138 ± 0.02
3	84	0.176 ± 0.05	74	0.282 ± 0.03
4	72	0.317 ± 0.06	90	0.122 ± 0.13
5	83	0.185 ± 0.05	71	0.333 ± 0.07
6	63	0.512 ± 0.07	95	0.095 ± 0.04
Mean	82.3	0.2275 ± 0.05	84.7	0.1818 ± 0.07
Stdv	13.23	0.163	9.75	0.100
2σ	39.69	0.326	29.25	0.200

Tabulated values are Mean ± SD of six replicate determinations per assay.

Cross reactivity

The metabolites of carbofuran (3-hdroxy-carbofuran and 3-keto-carbofuran) may cross-react with the detection by ELISA kits. For this reason, fish samples were spiked separately and in a mixture with standard of carbofuran, 3-keto and 3-hydroxy-carbofuran. The responses of the metabolites to the Ohmicron kit were found to be significantly higher than the response for the parent compound carbofuran, with the 3-keto metabolite producing more than twice the increase due to the 3-hydroxy metabolite (Figure 2). The LDD were found to be 0.3, 1.7 and 20 ppb, respectively for carbofuran, 3-keto carbofuran and 3-hydroxy carbofuran, When the metabolites were mixed with carbofuran, the cross reactivity was found to be 8.9% for the 3-ketocarbofuran and 1.45% for the hydroxycarbofuran.

Incurred samples

Since the Millipore kits contain only two standard solutions, it was used qualitatively, that means the sample contains carbofuran (> 2 ppb) or not (< 2 ppb). The results indicated 33% of false negative and 50% of false positive (Tables 4a and b).

The Ohmicron was used for the quantitative comparison with gas chromatography. Most of the samples found positive for carbofuran were found also positive using GC determinations; only in one sample carbofuran was detected by the Ohmicron kit, but was not detected by chromatography.

Plotting the ELISA results against GLC results gave a regression line of 0.967 slop (r^2 = 0.992) (Figure 3). This result showed a good correlation between the ELISA and gas chromatography.

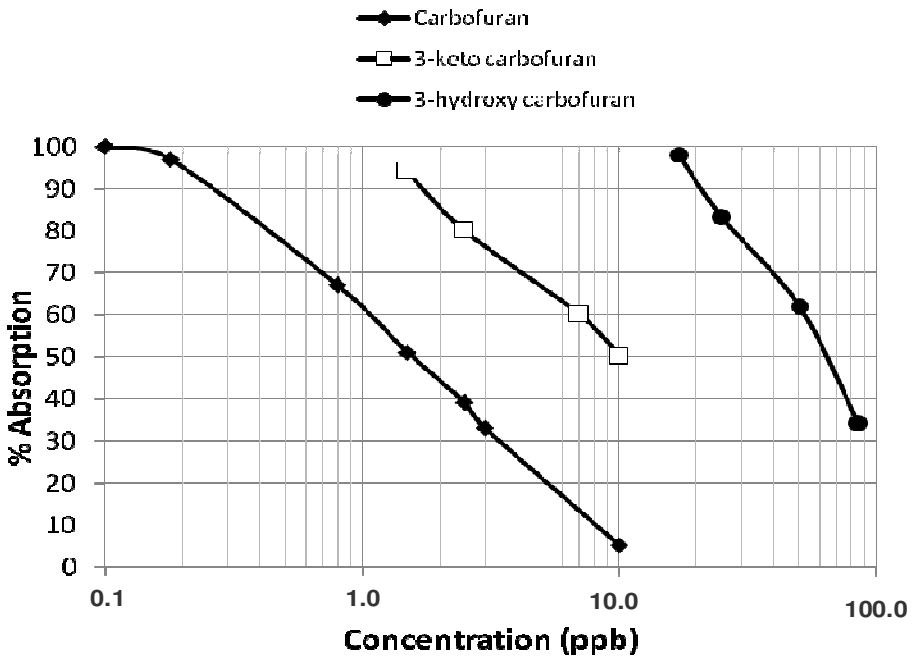

Figure 2. Curve of cross reactivity. Each point represents the mean of 5 replicate determinations.

Table 4a. Carbofuran concentration in incurred corn leaves by both ELISA kits and GLC.

Assay	Method	DBT	DOT	DOH
1	Millipore	< 2 ppb	>2ppb	>2ppb
	Ohmicron	nd	nd	nd
	GLC	nd	nd	nd
2	Millipore	< 2 ppb	<2ppb	> 2 ppb
	Ohmicron	nd	1.70 ± 0.02	nd
	GLC	nd	1.69 ± 0.07	nd
3	Millipore	< 2 ppb	>2ppb	<2 pbp
	Ohmicron	nd	2.00 ± 0.06	0.75 ± 0.07
	GLC	nd	1.88 ± 0.06	0.71 ± 0.06
4	Millipore	> 2 ppb	>2ppb	< 2 ppb
	Ohmicron	nd	2.20 ± 0.05	0.82 ± 0.11
	GLC	nd	2.13 ± 0.10	0.86 ± 0.05

Tabulated values are Mean ± SD of triplicate determinations for each assay.
nd = not found.

DISCUSSION

The ELISA kits yielded a very high recovery of carbofuran when the extract was not diluted; > 177% for fish extracts and 170 - 180% for corn leaves extracts. These observed 70 - 80% overestimation of the readings can be explained by matrix effects, which is a non-specific reactions between one or more ELISA components (e.g., antibody, conjugate, target analyte) and constituents (e.g., fat, pigments and other components) from fish or leaves. This may lead to inferior test results (Seiden et al., 2000) This anomalously high recovery observed were corrected by diluting 10 times fish extract or discolored and diluting 100 times. Corn leaves extracts.

Dilutions gave recoveries ranging from 104 - 107% (RSDs < 14%) and 90 - 108% (RSDs ≤ 17%) for fish and

Table 4b. Carbofuran concentration (ng/kg) in incurred fish fillet by both ELISA kits and GLC.

Assay	Method	Treated	Non treated
Fish 1	Millipore	nd	nd
	Ohmicron	nd	0.510 ± 0.06
	GLC	nd	0.540 ± 0.07
Fish 2	Millipore	>2 ppb	> 2 ppb
	Ohmicron	nd	0.327 ± 0.01
	GLC	nd	nd
Fish 3	Millipore	nd	> 2 ppb
	Ohmicron	nd	nd
	GLC	nd	nd
Fish 4	Millipore	>2 ppb	> 2 ppb
	Ohmicron	nd	nd
	GLC	nd	nd
Fish 5	Millipore	nd	> 2ppb
	Ohmicron	nd	nd
	GLC	nd	Nd

Tabulated values are Mean ± SD of triplicate determinations in each fish sample. nd = not detected.

corn leaves, respectively indicating that the ELISA kits were well standardized for the analysis of both samples after dilution. The same results were reported in the determination of alachlor in water and vegetable samples (Gabaldón et al., 2002) and in the determination of metsulfuron-methyl in soil samples (Seiden et al., 2000)

These results indicated that the Ohmicron kits could quantitatively be used to determine carbofuran in fish and plant material provided that matrices and extraction solvent interference is eliminated.

The results of reproducibility tests showed that the variations as percent of coefficient of variation (% CV) in the ELISA readings were more pronounced with the spiked samples (4.97 ± 0.97 - 8.83 ± 0.77%) compared to the standard solutions (4.00 ± 0.45% - 7.03 ± 0.71%). This can be explained by variations introduced during dilution steps or by the effects of coextracts (Manclús and Montoya, 1995). The overall results showed a mean coefficient of variation of the assay of less than 12%, which is very good for qualitative determination.

There is a general consensus in favor of selecting the dose, which inhibits 10% of the binding of the antibody with the enzyme tracer at 90% *B*/*B*0 for MDL determination. The obtained MDLs (0.25 ng/g) was close to the one determined with the 2 σ method (0.200 – 0.326 ng/g) (Table 3). These values fall in the range of linearity of the standard solutions provided with the test kits. Similar sensitivity was observed using ELISA for the detection of carbofuran in water (0.96 - 5 ng/ml) (Hennion 1998) and trace analysis of alachlor in vegetable samples.

The overall results in the cross-reactivity study indicated that the metabolites particularly the 3-keto exhibit a non significant cross-reactivity with the kits (8.9%) This value is twice higher than the specificity found in the determination of alachlor in water and vegetable samples using ELISA. Practically 3-hydro-carbofuran was undetectable by the Ohmicron kits in the range of determination. This indicates that the Ohmicron kit is specific and that it can be successively used for the quantitative determination of carbofuran in plant materials.

The Millipore kit, which was used for qualitative determination, presented more bias (33% of false negative and 48% of false positive) compared with the Ohmicron kit, which is used for quantitative determination that gave only 4.5% of false positive. The results obtained with the Millipore kit may be explained by reversible binding of protein to polystyrene coated tube, which may hinder the detection (Howell et al., 1981; Lehtonen and Viljanen, 1980). The false positive observed with the Ohmicron kits may be explained by the expression of the sensitivity of ELISA compared to the chromatographic determinations.

Figure 3 shows the Comparison of data obtained with the ELISA determinations with those obtained with GLC determination. The average percentage errors (ε%) between the ELISA determinations and the values

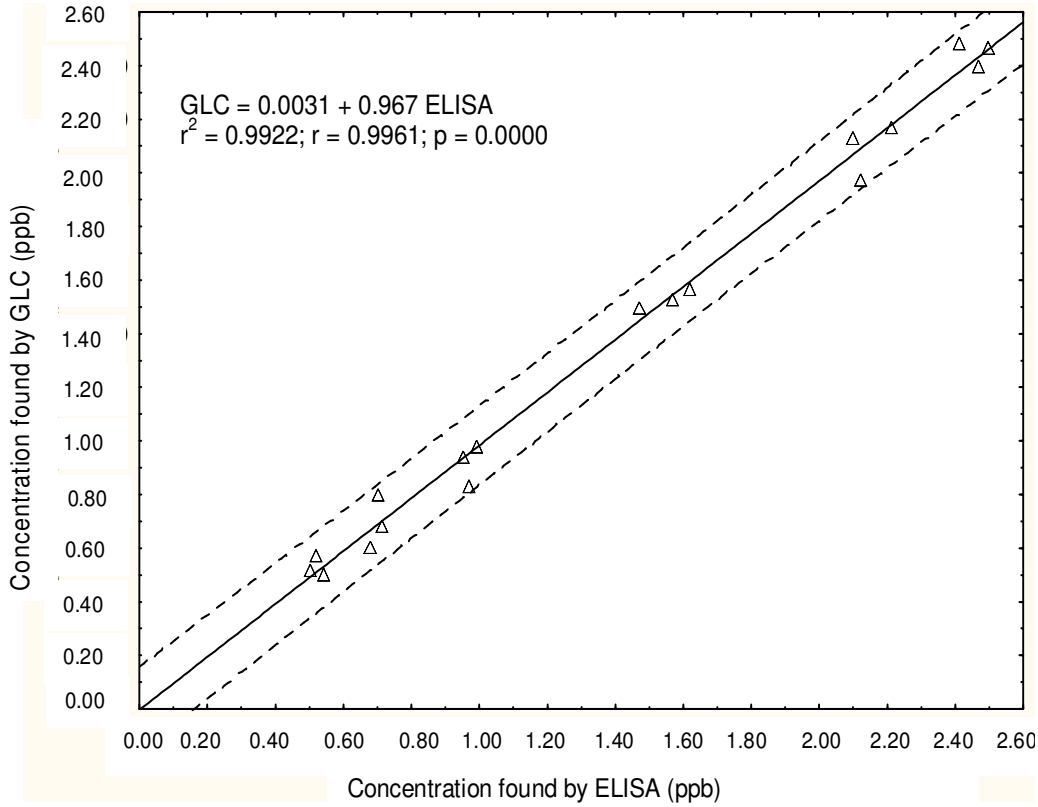

Figure 3. Curve of concentration found by the ELISA against concentration found by the GLC. Each point represents the mean of five assays.

obtained with GLC were evaluated using Equation (1):

$$\varepsilon^{\%} = \frac{\sum_{i=1}^{N} \left| \frac{C_{ELISA} - C_{GLC}}{C_{ELISA}} \right|}{N} \times 100 \quad (1)$$

The average percentage error (ε%) and the coefficient of correlation (r^2) are the criteria used to compare the two methods of determination. The correlation coefficient (r^2) and the average percentage errors ($\varepsilon^{\%}$) were found to be 0.992 and 1.34. These results indicated that the ELISA was suitable for the determination of carbofuran in fish or plant material compared to the traditional GLC determination because of the higher value of the coefficient of correlation and the smaller average percentage errors. A good correlation ($r > 0.85$) was also obtained when ELSA was compared with multiresidue extraction and GC–MS detection of alachlor in water and vegetable samples (Gabaldón et al., 2002)

The Ohmicron test kits used in this study is a unit packed with 80 test tubes. It allows the analysis of 80 samples at time in less than one hour. For GLC, apart from the lengthy extraction and column cleanup steps, it takes 20 min for the determination of carbofuran in one sample. If the GLC system is not equipped with an automatic injector, it will take 28 h or more than one day for the determination of the 80 samples.

Conclusion

Commercial enzyme immunoassay (EIA) kits were successively used to determine carbofuran in plant and fish samples. The comparison of the results showed good agreement between the immunoassays results as far as concern the Ohmicron kits, and gas chromatographic determination.

Our study showed that coextracts from fish (fat and proteins) and from corn leaves (pigments, proteins, and cellulose) strongly interfered with the assay detection. This was corrected by diluting the extracts with distilled water. But if the level of carbofuran in the sample is very low, the attempt to decrease the effects of background interference by diluting can be counterproductive because the kit may no longer be able to detect the analytes at these levels of dilution.

The results of the study indicated that the Ohmicron RaPID™ kits were more precise and reproducible than the Millipore Envirogard® kits. Desorption or leaching off of the antibodies, which have been passively adsorbed inside polystyrene tubes are major factors that adversely

affect assay precision and reproducibility of the Millipore kits (Howell et al., 1981; Engwall, 1980; Lehtonen and Viljanen, 1980). The Ohmicron RaPID® Assay kits was more sensitive and had in general, a lower LLD compared to the Millipore Envirogard® kits.

The results of the study have shown that the ELISA compares favorably to GLC although ELISA may give false positive or false negative results or certain metabolites may exhibit cross-reactivity with the parent compound. These biases may be overcome by using GLC as a confirmation tool when using ELISA.

The ELISA is less time consuming; it takes about 1 min to assay one sample, while it takes 20 min for the same determination. Besides that, the size of sample to analyze and the volume of solvent used in ELISA are very small compared to the large sample size (15 - 25 g) and the large volume of organic solvent (250 - 500 ml) used in GLC determinations. The real advantage of the ELISA compared to chromatographic techniques for developing countries, is the cost associated with instrumentation; the GC instrumentation used in this study cost more than $ 35,000. Starting-up, an ELISA will cost less than $ 4,000 (including the RaPID Analyzer® and the EnviroQuant™ photometer which can process the data). In developing countries another factor that might favor the ELISA over GLC is the cost associated with maintenance and training associated with the GC compared to that of the ELISA.

REFERENCES

Argarate N, Arestin M, Ramón-Azcón J, Alfaro B, Barranco A, Sánchez-Baeza F, Marco MP (2010). Evaluation of Immunoassays as an Alternative for the Rapid Determination of Pesticides in Wine and Grape Samples. J. AOAC Int., 93(1): 2-11.

Brena BM, Arellano L, Rufo C, Last MS, Montaño J, Cerni EE, Spienza GG, Last JA (2005). ELISA as an Affordable Methodology for Monitoring Groundwater Contamination by Pesticides in Low-Income Countries. Environ. Sci. Technol., 39(11): 3896-3903.

Bushway RJ, Hurt LH, Perkins LB; Tian J, Cabanillas GC, Young BES, Ferguson BS, Jennings HS. (1992). Atrazine, Alchlor, and carbofuran contamination of well water in central Maine. Bull. Environ. Contamin. Toxicol., 49: 1-9.

Chang NB, Mani S, Parvathinathan G, Kanth RS (2009). Pesticide Impact Assessment via Using Enzyme-linked Immunosorbent Assay (ELISA) Technique in the Lower Rio Grande River Basin, Texas. Water Quality, Exposure Health., 1(3-4): 145-158.

Conde SB, Suyama K, Itoh K, Ymamoto H (2004). Method assessment for the determination of pesticide residue in soil sample using commercially produced ELISA kit. Nippon Noyaku Gakkai Taikai Koen Yoshishu., 29: 114.

Cook RF, Stanovick RP, Cassil CC (1969). Determination of Carbofuran and its carbamate Metabolite Residues in Corn Leaves Using a Nitrogen Specific Gas Chromatographic Detector. J. Agric. Food Chem., 17(2): 277-282.

Danks C, Chaudhry MQ, Parker L, Barker I, Banks JN (2001). A Development and Validation of an Immunoassay for the Determination of Tebuconazole Residues in Cereal Crops. Food and Agricultural Immunol., 13(3): 151-159.

Eisler R (1985). Carbofuran Hazards to Fish, Wildlife, and Invertebrates. A Synoptic Review. U.S. Fish Wild. Serie. Biol. Rep., 85(1.3): 36

Gabaldón JA, Cascales JM, Maquieira A, Puchades R (2002). Rapid trace analysis of alachlor in water and vegetable samples. J. Chromatogaphy., 963(1-2): 125-136.

Hammock BD, Mumma RO (1980). In Recent Advances in Pesticide Analytical Methodology, Harvey JJr; Zweig G. (ed). American Chemical Society, Washington, DC,, ACS Symp. Ser., 136: 321-352.

Hennion MC (1998). Applications and validation of immunoassays for pesticides analysis. Anal. Mag., 26(6): 149-155.

Howell EH, Nasser J, Schray KJ (1981). Coated Tube Enzyme Immunoassay Factors Affecting Sensitivity and Effects of Reversible Protein Binding to Polystyrene. J. Immunoassay., 14(3): 346-352.

Kanasawa J (1975). Uptake and Excretion of Organophosphorus and Carbamate Insecticides by Freshwater Fish, Motsugo, pseudorasbora Parva. Bull. Environm. Contam. Toxicol., 14(3): 346-352.

Lehotay SJ, Argauer RJ (1993). Detection of Aldicarb Sulfone and Carbofuran in Fortified Meat and Liver with Commercial ELISA Kits after Rapid Extraction. J. Agric. Food Chem., 41: 2006-2010.

Lehtonen OP, Viljanen MK (1980). Antigen Attachement in ELISA. J. Immunol. Meth., 34: 61-70.

Martin PA, Solomon KR, Forsyth DJ, Boermans HJ, Westcott ND (1991). Effect of Exposure to carbofuran-sprayed vegetation on the behaviour, cholinesterase Activity and Growth of Mailard Ducklings (Apas platyrhychos). Environ. Toxicol. Chem., 35: 873-909.

Metcalf RL, Fukuto TR, Collin C, Bork K, Al-Aziz SA, Mumoz R, Cassil CC (1968). Metabolism of 2,2-Dimethyl-2,3-Dihydrobenzofluranyl-7,6-Methyl Carbamate (Furadan) in plants, Insects, and Mammals. J. Agric. Food Chem., 16(2): 300-311.

Seiden P, Bossi R, Streibig, JC (2000). Applicability of ELISA for determination of metsulfuron-methyl in soil samples. Pest. Manage. Sci., 56(7): 637-643.

Sullivan JJ, Goh KS (2000). Evaluation and Validation of a Commercial ELISA for Diazinon in Surface Waters, J. Agric. Food Chem., 48 (9): 4071-4078.

Sun J, Wang S, Dong T (2009). A Rapid and Sensitive Chemiluminescence Enzyme Linked Immunosorbent Assay for the Determination of Metolcarb Residue in Agricultural Products, 3rd International Conference on Bioinformatics and Biomedical Engineering, Beijing, China, pp.1-4.

Tananuja P, Venugopal N, Sashhidhar RB (2007). Development and evaluation of thin-layer chromatography-digital image-based analysis for the quantitation of the botanical pesticide azadirachtin in agricultural matrixes and commercial formulations: Comparison with ELISA. J. AOAC Int., 90(3): 859-865.

Turner BC, Caro JH (1973). Uptake and Distribution of Carbofuran and its Metabolites in Field-grown Corn Plants. J. Environ. Quality., 2: 245-247.

Van Emon JM, Mumma RO (1990). Immunological Methods for Environmental Analysis. ACS Symposium Series p. 442. American Chemical Society (ed), Washington, DC,

Williams KJ Thorpe SA, Reynolds SL (1996). The use of ELISA for the determination of pesticide residues in food. Symposium on Chemistry and Fate of Modern Pesticides N°5, Paris , France., 65(1-4): 149-152.

Williams KJ, James CR, Thorpe SA, Reynolds SL (1997). Two analytical methods for the measurement of 2,4-D in oranges : an ELISA screening procedure and a GC-MS confirmatory procedure, Pest. Sci., 50(2): 120-170.

Watanabe H, Nguyen MH, Komany S, Vu SH, Asami Y, Phong TK, Tournebiz J (2006). Applicability of ELISA in pesticide monitoring to control runoff of bensulfuron-methyl and simetryn from paddy fields. J. Pest. Sci., 31(1): 123-129.