Bull. Environ. Contam. Toxicol. (1993) 51:260–267

Environmental Contamination and Toxicology

Validation of a Paramagnetic Particle-Based ELISA for the Quantitative Determination of Carbaryl in Water

Jeanne A. Itak,[1] Eric G. Olson,[1] James R. Fleeker,[2] and David P. Herzog[1]

[1]Ohmicron Corporation, 375 Pheasant Run, Newtown, Pennsylvania 18940, USA and [2]Department of Biochemistry, North Dakota State University, 310 Loftsgard Hall, P.O. Box 5516, University Station, Fargo, North Dakota 58105, USA

Pesticide residue monitoring has attracted enormous attention in the past several years as concern has focused on the environmental impact of widespread pesticide use. This has resulted in a growing demand for determining pesticide residues, accompanied by a need for rapid and simple testing procedures. Enzyme linked immunosorbant assays (ELISAs) are becoming increasingly popular as screening methodologies to meet the testing demand. They are capable of surveying larger numbers of samples than could be accomplished by conventional analysis, and, in many cases, they also exhibit lower detection limits.

Carbaryl was the first of the carbamate insecticides; it was introduced in 1958 as a broad-spectrum, contact insecticide with systemic properties (Baron 1991). The compound was rapidly accepted on the world market because of its efficacy, moderate mammalian toxicity and relatively low persistence in the environment (Dorough 1977). Today it is used for the control of over 150 major pests on more than 100 crops, on lawns and turfs and on domestic animals (Baron 1991). Carbaryl occurrence in the aquatic environment is due mainly to its widespread use (Fernandez Muino et al. 1991).

This paper describes the validation of a paramagnetic particle-based ELISA for the quantitation of carbaryl in environmental water samples. Magnetic particle-based ELISAs for the quantitation of other pesticide residues have been described previously (Itak et al. 1992; Lawruk et al. 1992; Rubio et al. 1991). In these systems, the antibody is covalently coupled to the paramagnetic-particle solid phase. Because the antibody is covalently bound, sensitivity and precision problems associated with passive adsorption to polystyrene surfaces are eliminated. The problems associated with passive adsorption of proteins include desorption or leaching from microtiter plates and coated tubes or from well-to-well variability within microtiter plates (Howell et al. 1981; Engvall et al. 1980; Lehtonen and Viljanen 1980; Harrison et al. 1989). With magnetic particle-based assays, the paramagnetic particles are uniformly dispersed throughout the reaction mixture and allow for precise addition of antibody and rapid reaction kinetics.

The carbaryl immunoassay presented takes less than one hour to perform and requires no sample preparation for the analysis of water samples. Photometric determination of the final colored product is done with a specially designed

Send reprint requests to Jeanne Itak at the above address.

microprocessor-controlled photometer with extensive data reduction capability that reports parts per billion (ppb) of carbaryl.

MATERIALS AND METHODS

The Carbaryl RaPID Assay®, Carbaryl Sample Diluent buffer (buffered saline containing preservative and stabilizers, pH 4), Magnetic Separation Unit, RPA-I Analyzer™ and Vortex mixer were obtained from Ohmicron Corporation, Newtown, PA. Additional apparatus used: adjustable precision pipette, Gilson P-200 (Rainin; Woburn, MA) and repeating pipette (Eppendorf; Hamburg, Germany). The pesticides and solvents used in this study were all analytical grade as supplied by commercial sources. All other chemicals were reagent grade. Environmental water samples were collected locally in Pennsylvania, New Jersey and Delaware; nationwide water samples came from sources in Florida, Idaho, Iowa, Illinois, Louisiana, Minnesota, Montana, Rhode Island, Vermont, Washington and Wisconsin. Water samples were characterized using Hach Test Kits for nitrate/nitrite (cat. # 14081-00), salinity (cat. # 24218-00) and chlorine, hardness, iron and pH (cat. # 2230-02).

All samples were assayed according to the RaPID Assay package insert. The procedure required adding 200 μL of standard or sample to a disposable test tube with 250 μL of horseradish peroxidase labeled carbaryl analog and 500 μL of carbaryl antibody coupled paramagnetic particles. The tubes were vortexed and incubated for 20 min at room temperature. The reaction mixture was separated by placing the tube rack into the magnetic base and the supernatent was decanted. Particles were washed twice with 1 mL washing solution (preserved deionized water). The substrate solution, peroxide/TMB (3,3',5,5'-tetramethylbenzidine) was added, 500 μL per tube, and allowed to develop for 20 min. The color reaction was stopped with 500 μL of stopping solution (2M sulfuric acid). The concentrations of carbaryl for each sample were determined using the RPA-I Analyzer set at 450 nm. The RPA-I compares the observed sample absorbances to a regression line using a log-linear standard curve derived from the calibrator absorbances and reports parts per billion carbaryl in the sample.

RESULTS AND DISCUSSION

The carbaryl immunoassay described uses a competitive assay format. Since the enzyme-labeled carbaryl competes with the unlabeled (sample) carbaryl for the antibody sites, the color developed is inversely proportional to the concentration of carbaryl in the sample. It is common to report displacement in terms of a B/Bo measurement to describe color inhibition. B/Bo is defined as the absorbance observed for a sample or standard divided by the absorbance at a zero analyte concentration.

Figure 1 illustrates the dose-response data for the carbaryl calibrators collected over 55 runs of the assay during a 3 month period. A 10% inhibition of signal was considered to be significantly different than the zero analyte concentration and was used to estimate the sensitivity of the assay. This corresponded to a B/Bo of 90% which calculated to 0.25 ppb carbaryl. The 90% B/Bo sensitivity estimation was supported by a second study in which three sets of 20 replicates of the zero standard concentration were assayed and the mean B/Bo value and standard deviation were calculated for each run. The mean Bo

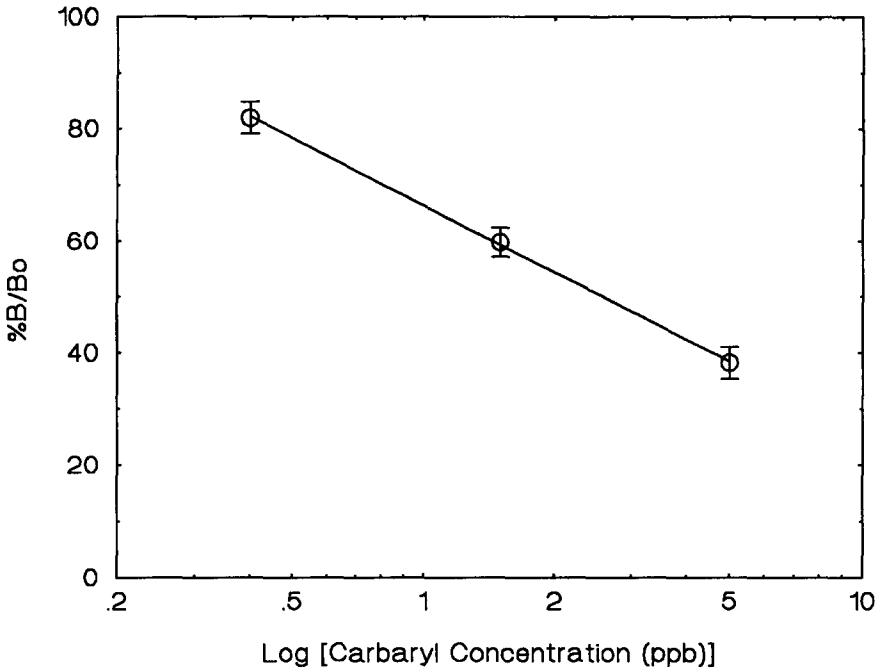

Figure 1. Dose-response curve for carbaryl. Each point represents the mean of 55 determinations. Vertical bars indicate +/- 2 SD about the mean.

Table 1. Precision of carbaryl measurement by immunoassay

	Concentration level (ppb)			
	0.5	1	2	4
N	25	25	25	25
Mean ppb	0.46	0.85	2.04	3.78
%CV within assay	7.5	9.8	8.0	6.9
%CV between assay	<0.01	1.8	5.1	14.1
%CV total	7.2	9.9	9.2	14.6

absorbance value minus 3 and 4 standard deviations was equivalent to 93% and 90% B/Bo corresponding to 0.22 and 0.25 ppb carbaryl, respectively. EPA method 531.1 for the determination of N-methylcarbamates has an estimated detection limit (EDL) of 2 ppb (μg/L).

Table 1 summarizes a precision study that was conducted with four concentrations of carbaryl in distilled water. Carbaryl was added at 0.5, 1, 2 and 4 ppb. Each level was assayed five times per day in singlicate over 5 days. The within and between day and total variation was determined by the method of Bookbinder and Panosian (1986) using Statistical Analysis Software (SAS Institute, Inc. 1988). Coefficients of variation (CV) were very good, especially at the lower concentration levels.

Table 2. Characterization of waters used in accuracy and method correlation experiments

Sample	iron, mg/L	nitrate, mg/L	nitrite, mg/L	sodium chloride (M)	pH	hardness $CaCO_3$, g/L
1	<0.1	na	<0.03	na	7.7	17
2	<0.1	<4.4	na	<0.03	7.5	51
3	0.2	4.4	<0.03	0.17	7.5	2054
4	na	7.0	na	na	7.7	na
5	<0.1	30.8	0.07	na	7.6	171
6	<0.1	11.0	<0.03	na	7.6	102
7	na	na	na	na	7.0	102
8	<0.1	8.8	<0.03	na	7.8	154
9	na	na	na	na	7.9	188

na = information not available

Table 3. Accuracy of carbaryl measurement by immunoassay (n = 8)

Expected conc (ppb)	Mean (ppb)	SD (ppb)	% Recovery
1.0	0.87	0.07	87
2.0	2.03	0.17	102
3.0	2.96	0.21	99
4.0	4.09	0.30	102
Average			98

The accuracy of the assay was assessed by evaluating four water samples each spiked with known amounts of carbaryl at four levels. These water samples are described in Table 2, samples 1 through 4. Each sample was assayed twice to verify reproducibility. Table 3 summarizes the accuracy of the spike recoveries.

A study was conducted to compare results of the ELISA method with an established HPLC method. Five local water sources were used to prepare 19 samples with known levels of added carbaryl. The water sources are characterized in Table 2, designated as samples 5 through 9. HPLC analysis was performed by ABC Research (Gainsville, FL) using EPA method 531.1 using direct injection and determination with a modified mobile phase. This modified procedure had an EDL of 10 ppb, slightly higher than the 2 ppb EDL reported for the EPA procedure. Samples were prepared at concentrations greater than the limit of the immunoassay (5 ppb) to accomodate the less sensitive HPLC method. Therefore for the immunoassay analysis, samples were diluted in Carbaryl Sample Diluent buffer. Correlation of the 19 samples by the ELISA method (y) and the HPLC method (x) are illustrated in Figure 2. A good correlation ($r = 0.965$) and slope (0.988) between methods was obtained.

Table 4 summarizes the cross-reactivity data with the major carbaryl metabolite 1-naphthol, a variety of carbamates and many other pesticides in the carbaryl assay. As with the earlier carbaryl sensitivity estimations, the

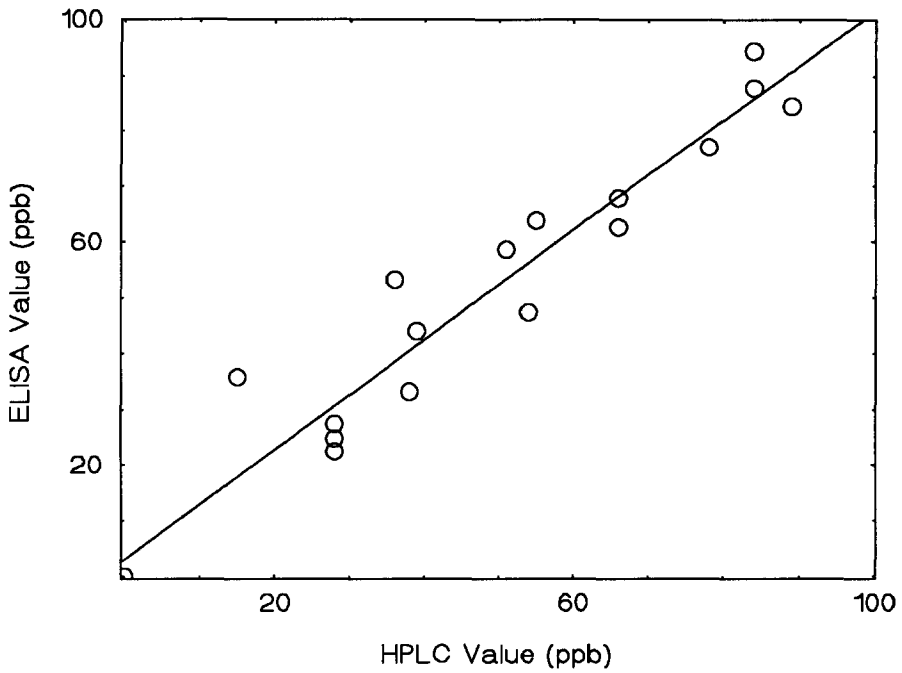

Figure 2. Correlation between carbaryl concentrations as determined by ELISA and HPLC methods. n = 19, r = 0.967, y = 0.988x + 2.97.

least detectable dose (LDD) for each compound was approximated at 90% B/Bo. The percent cross reactivity was determined by estimating the amount of compound required to displace 50% of the enzyme conjugate compared to the amount of carbaryl required for 50% displacement. Several observations suggest that the antiserum is most reactive with the [ring-O-C(O)-N(CH_2)-R] portion of the carbaryl molecule. First, low reactivity was seen with 1-naphthol, carbaryl's major metabolite. 1-naphthol retains the double cyclic ring structure of carbaryl but lacks any side chain. Second, no reactivity was seen with aldicarb and methomyl. They both lack any ring structures but possess the R-O-C(O)-N(CH_2)-R chain basic to the carbamate group of pesticides. Lastly, some reactivity is seen with carbofuran, benomyl and bendiocarb, all of which contain the specified side chain attached to a cyclic structure.

Sample pH and substances that could potentially be found in water were evaluated for interferences in the carbaryl assay. No interferences were seen up to the concentrations listed in Table 5. The concentrations chosen would most likely exceed levels found in groundwater samples (American Public Health Association 1989; Wolfe et al. 1990). Sample pH also had no adverse effect on blank results or spike recoveries from pH 3 to 11.

Results from the previous experiment suggest that the assay is robust and free from interferences from commonly found groundwater components. To examine this, 443 water samples from around the United States were evaluated as blanks and spiked with 2.5 ppb carbaryl to determine recovery and possible interferences. Twelve samples (2.7%) contained flocculent material or

Table 4. Cross reactivity of pesticides and related agricultural compounds in the carbaryl immunoassay

Compound	LDD[a] (ppb)	% Cross Reactivity[b]
Carbaryl	0.25	100.00
Carbofuran	7	0.32
Bendiocarb	8	1.06
Pentachlorophenol	8	0.10
Iprodione	12	0.35
Dichlorophenol	25	0.04
MCPA	53	0.24
2,4-D	56	0.20
Procymidone	298	0.03
1-Naphthol	495	0.05
Dinoseb	524	0.05
Benomyl	931	<0.01

[a] LDD is the least detectable dose calculated at 90% B/Bo.

[b] % Cross reactivity is determined by estimating the amount of compound required to displace 50% of the enzyme conjugate compared to the amount of carbaryl required for 50% displacement.

No reactivity was seen with the following compounds up to 10,000 ppb: alachlor, aldicarb, aldicarb sulfone, aldicarb sulfoxide, ametryn, atrazine, butylate, captan, captafol, carbendazim, cyanazine, de-ethylatrazine, dicamba, dichlorprop, 1,3-dichloropropene, folpet, MCPB, metalaxyl, methomyl, mecoprop, metribuzin, metolachlor, phosphamidon, picloram, prometon, prometryn, propachlor, propazine, silvex, simazine, terbufos, terbutryn, terbutylazine, THPI, thiophanat methyl, triclopyr and vinclozolin.

Table 5. Possible interfering substances in groundwater that showed no interference in the immunoassay up to the concentrations listed.

Compound	Maximum Concentration Tested
Calcium	250 ppm
Copper	250 ppm
Humic acids	50 ppm
Iron	250 ppm
Magnesium	200 ppm
Manganese	500 ppm
Mercury	500 ppm
Nickel	100 ppm
Nitrate	250 ppm
Peroxide	2000 ppm
Silicates	1000 ppm
Sodium chloride	1 M
Sulfate	1000 ppm
Sulfite	250 ppm
Zinc	100 ppm

precipitate that required filtration; a 1.2 μm syringe tip filter was used (Gelman Sciences; Ann Arbor, MI). Only one sample recovered outside the acceptable range of 100% +/- 20%. A mean recovery of 99% (SD = 9) was observed.

The performance characteristics of this ELISA have shown it to be ideal for the analysis of water samples. Recovery and precision results looked very good even at levels of detection below the current EPA HPLC method of analysis. The immunoassay is forty times more sensitive than the HPLC method and measurements compare favorably. The assay described was free from interferences over a wide pH range and with numerous compounds commonly found in water. The technology is simple enough that it does not require highly trained personnel. It is also inexpensive and quick, allowing larger numbers of samples to be screened than could be accomplished with conventional analysis. Future efforts will extend to the application of this technology to the analysis of soil and food samples.

REFERENCES

American Public Health Association (1989) Standard methods for examination of water and wastewater. American Public Health Association, Washington, DC.

Baron, RL (1991) Carbamate insecticides. In: Handbook of Pesticide Toxicology, vol. 3 (WH Hayes Jr and ER Laws Jr, Eds) Academic Press, SanDiego, CA, pp. 1125-1189.

Bookbinder, MJ, Panosian, KJ (1986) Correct and incorrect estimation of within-day and between day variation. Clinical Chemistry 32:1734-1737.

Dorough, HW (1977) Metabolism of carbamate insecticides PB-266 233. U.S. Environmental Protection Agency, Research Triangle Park, NC. pp. 3.

Engvall, B (1980) Enzyme immunoassay ELISA and EMIT. In: Methods in enzymology, vol. 70 (Van Vunakis, H and Langone, JJ, Eds) Academic Press USA, Inc., New York, pp. 419-439.

Fernando Muino, MA, Simal Gandara, J, Simal Lozana, J (1991) Simultaneous determination of pentachlorophenol and carbaryl in water. Chromatographia 32:238-240.

Harrison, RO, Braun, AL, Gee, SJ, O'Brien, DJ, Hammock, BD (1989) Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the direct analysis of molinate (Odram®) in rice field water. Food Agr Immunol 1:37-51.

Howell, EH, Nasser, J, Schray, KJ (1981) Coated tube enzyme immunoassay: factors affecting sensitivity and effects of reversible protein binding to polystyrene. J Immunoassay 2:205-225.

Itak, J, Selisker, MY, Herzog, DP (1992) Development and evaluation of a magnetic particle based enzyme immunoassay for aldicarb, aldicarb sulfone and aldicarb sulfoxide. Chemosphere 24:11-21.

Lawruk, TS, Hottenstein, CS, Herzog, DP, Rubio, FM (1992) Quantification of alachlor in water by a novel magnetic particle-based ELISA. Bull Environ Contam Toxicol 48:643-650.

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

Rubio, FM, Itak, JA, Scutellaro, AM, Selisker, MY, Herzog, DP (1991) Performance characteristics of a novel magnetic particle based ELISA for

the quantitative analysis of atrazine and related triazines in water samples. Food and Agricultural Immunology 3:113-125.
SAS Institute, Inc. (1988) SAS user's guide: Statistics, Version 6.03 Edition. SAS Institute, Inc., Cary, NC.
Wolfe, NL, Mingelgrin, U, Miller, GC (1990) Abiotic transformation in water, sediments and soil. In: Pesticides in the soil environment: Processes, impact and modeling (Cheng, HH, Ed) Soil Society of America, Inc., Madison, WI pp 106.

Received October 20, 1992; accepted January 17, 1993.