J. Agric. Food Chem. 1993, 41, 2329–2332 

# Determination of Benomyl (as Carbendazim) and Carbendazim in Water, Soil, and Fruit Juice by a Magnetic Particle-Based Immunoassay

Jeanne A. Itak,*,† Michele Y. Selisker,† Scott W. Jourdan,† James R. Fleeker,‡ and David P. Herzog†

Ohmicron Corporation, 375 Pheasant Run, Newtown, Pennsylvania 18940, and Biochemistry Department, North Dakota State University, P.O. Box 5516, University Station, Fargo, North Dakota 58105

A competitive enzyme-linked immunosorbent assay (ELISA) for the quantiation of benomyl (as carbendazim) and carbendazim in water was developed. This assay utilizes magnetic particles as the solid phase to attach polyclonal rabbit anti-carbendazim antibodies. The ELISA has an estimated detection limit of 0.1 part per billion [ppb (nanograms per milliliter)] of carbendazim in water. It compares favorably with HPLC measurements in the analysis of water samples ($r$ = 0.920). Studies indicate that this immunoassay can also be used for the determination of carbendazim in fruit juice and soil. Fortified juice samples, diluted 1:300 for the ELISA, averaged 103% recovery with a detection limit of 300 ppb. A fortified soil sample recovered greater than 85% in the immunoassay with an estimated detection limit of 37.5 ppb of carbendazim using a 0.5–3-h extraction with methanol/0.5 N sodium hydroxide (3:1 v/v).

## INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate] and carbendazim [methyl 2-benzimidazole-carbamate (MBC)] are widely used systemic fungicides for control of a broad range of fungi affecting fruits, nuts, vegetables, turf, and field crops. Carbendazim, although not used in the United States, has been registered in over 15 countries (Watterson, 1988). As systemic fungicides, benomyl and carbendazim can be adsorbed through the roots, leaves, and green tissues of plants along with water (Ben-Aziz and Aharonson, 1974). Determination of benomyl and carbendazim in water, soil, and various crops is frequently required because of their widespread use and to assure compliance with federally regulated tolerances.

Benomyl readily decomposes to form carbendazim in acidic solution and in the presence of organic solvents (Clemens and Sisler, 1969). It has also been shown to decompose in water and plants (Tang et al., 1992; Singh et al., 1990; Peterson and Edgington, 1971). Residue analysis is complicated by the instability of benomyl in organic solvents (Calmon and Sayag, 1976; Kilgore and White, 1970). Therefore, it has become accepted methodology to determine benomyl as the stable degradation product carbendazim (Kirkland et al., 1973; Bardalaye and Wheeler, 1985; Zweig and Gao, 1983). This is due to the fact that carbendazim is also fungitoxic, and it is suspected that the fungitoxicity of benomyl is in fact due to carbendazim (Clemens and Sisler, 1969).

Enzyme-linked immunosorbent assays (ELISAs) have been shown to be sensitive analytical methods ideal for meeting the growing demand for reliable, rapid, and cost-effective residue testing (Van Emon and Lopez-Avila, 1992). The ELISA reported here utilizes magnetic particles as the solid phase to covalently couple antibody. This format eliminates imprecision problems associated with microtiter plates and polystyrene tubes (Howell et al., 1981; Engvall, 1980; Lehtonen and Viljanen, 1980; Harrison et al., 1989). The benefits and use of magnetic particle-based immunoassays for pesticide detection in water and soil have been described previously (Lawruk et al., 1993; Itak et al., 1992; Rubio et al., 1991).

The assay presented takes less than 1 h to perform and requires no sample preparation for the analysis of water samples. Simple sample preparation procedures are also described to adapt the assay to the analysis of soil and fruit juice samples.

## MATERIALS AND METHODS

**Immunochemicals.** Rabbit anti-carbendazim antiserum was produced using immunization protocols similar to those described by Tijssen (1985). The immunogen hapten, 2-succinamidobenzimidazole, and the immunogen, 2-succinamidobenzimidazole conjugated to bovine serum albumin (BSA), were prepared according to the methods of Newsome and Collins (1987). Bovine serum albumin was purchased from Miles Inc. (Kankakee, IL). The procedure for coupling anti-carbendazim antiserum to magnetic particles was previously described (Itak et al., 1992; Rubio et al., 1991) using paramagnetic particles purchased from Advanced Magnetics Inc. (Cambridge, MA). Efficiency of antiserum coupling to the magnetic particles exceeded 90%. The prepared particle stocks were diluted 1:000 for use in the assay.

The enzyme-conjugate ligand, $N$-(1$H$-benzimidazol-2-yl)-2,2′-oxybis(acetic acid) monoamide, was prepared according to the method of Newsome and Shields (1991) using succinic anhydride and 2-aminobenzimidazole. Carbendazim hapten–horseradish peroxidase (HRP) enzyme conjugate was synthesized by active ester activation of the hapten followed by carbodiimide coupling of the enzyme (Langone and VanVanukis, 1975). Peroxidase from horseradish (HRP) was purchased from Sigma Chemical (St. Louis, MO). Tetramethylbenzidine and hydrogen peroxide were obtained from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, MD).

**Additional Chemicals.** 2-Aminobenzimidazole, 2-benzimidazolylurea, and benzimidazole were purchased from Aldrich Chemical Co. (Milwaukee, WI). Carbendazim, benomyl, and other pesticide standards were purchased from Crescent Chemical Company Inc. (Hauppauge, NY).

**Buffers.** Tris-buffered saline (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% BSA was used for diluting the rabbit anti-carbendazim magnetic particles and carbendazim hapten–HRP enzyme conjugate. Sodium acetate buffer (pH 4.0) with 1 mM EDTA and 0.1% gelatin was used to dilute the carbendazim standards.

**Equipment.** The magnetic separation rack and RPA-I Analyzer were supplied by Ohmicron Corp. (Newtown, PA).

**Competitive Immunoassay Procedure.** Two hundred microliters of the carbendazim standards, control, or samples to be

* Author to whom correspondence should be addressed.
† Ohmicron Corp.
‡ North Dakota State University.

0021-8561/93/1441-2329$04.00/0 © 1993 American Chemical Society

**Table I. Specificity (Cross-Reactivity) in the Immunoassay**[a]

| compound | ring structure | $R_1$ | $R_2$ | LDD[b] (ppb) | $IC_{50}$[c] (ppb) |
|---|---|---|---|---|---|
| carbendazim | A | H | $NHCO_2CH_3$ | 0.10 | 1.60 |
| benomyl | A | $CONH(CH_2)CH_3$ | $NHCO_2CH_3$ | 0.38 | 5.61 |
| 2-benzimidazolylurea | A | H | $NHCONH_2$ | 0.62 | 9.22 |
| thiabendazole | A | H | CCHSCN | 6.30 | 91.0 |
| thiophanate-methyl | B | $NHCSNHCO_2CH_3$ | $NHCSNHCO_2CH_3$ | 19.4 | 401 |
| 2-aminobenzimidazole | A | H | $NH_2$ | 120 | 2321 |
| benzimidazole | A | H | H | 681 | 10229 |

[a] No reactivity was seen for the following compounds up to 10 ppm: alachlor, aldicarb, aldicarb sulfone, ametryn, atrazine, butylate, captafol, captan, carbaryl, cyanazine, dicamba, dichlorophenol, dichloroprop, metalaxyl, mecoprop, metribuzin, metolachlor, pentachlorophenol, phosphamidon, picloram, procymidone, prometon, prometryn, propachlor, propazine, silvex, terbufos, terbutryn, terbutylazine, triclopyr, and vinclozolin. [b] LDD, least detectable dose (90% $B/B_0$). [c] 50% inhibition concentration (50% $B/B_0$).

tested was added to a disposable test tube along with 250 μL of carbendazim-HRP conjugate and 500 μL of paramagnetic particles with carbendazim specific antibodies attached. Tubes were vortexed and incubated for 20 min at room temperature. The reaction mixture was separated in the magnetic separation rack, and the particles were washed twice with 0.1% Tween in Tris-buffered saline. Five hundred microliters of color reagent (TMB and hydrogen peroxide mixed 1:1) was added per tube. The tubes were vortexed to resuspend particles and incubated for another 20 min at room temperature to allow color development. The color reaction was then stopped with 500 μL of 2 M sulfuric acid solution. The final concentrations of carbendazim for each sample were determined using the RPA-I photometric analyzer which records the absorbance at 450 nm and compares the observed sample results to a linear regression line using a log/logit standard curve prepared from calibrators containing 0, 0.25, 1.0, and 5.0 ppb of carbendazim. The functions of the RPA-I photometric analyzer have previously been described in detail (Rubio et al., 1991). Samples greater than 5 ppb were diluted in the zero standard for analysis.

**Determination of Cross-Reactivity.** The relative sensitivity of the immunoassay was determined by assaying a dilution series of each compound in sodium acetate buffer and comparing the $IC_{50}$ values (concentration of analyte that produces a 50% decrease in the maximum normalized response).

**HPLC Analysis for Correlation Study.** Two laboratories performed HPLC analysis on carbendazim-spiked water samples. Dr. Ralph O. Mumma at Pennsylvania State University, Pesticide Research laboratory, Department of Entomology, University Station, PA (lab A), performed the analysis on 15 of the 34 carbendazim spiked water samples. The other 19 natural water samples were analyzed by an independent research laboratory (lab B) according to the method of Kitada et al. (1982).

**Fruit Juice Analysis.** Fruit juices (100% pure) purchased at local markets were fortified with carbendazim at four known concentrations. For analysis, samples were diluted 1:300 in the zero standard. The samples were then assayed according to the standard protocol (using standards prepared in buffer) and the results multiplied by the dilution factor (300).

**Soil Sample Analysis.** A Plano loam soil (38% sand, 48% silt, 26% clay, 5% humus, pH 6.1) was mixed for 2 h with carbendazim-spiked solutions prepared in water to yield a concentration in soil of 0.1–1 ppm. Soils were then air-dried for 2 days and ground with a mortar and pestle. Ten grams of soil was extracted for 0.5, 3, or 16 h by agitating in 30 mL of extracting solution (see Tables VI and VII). An aliquot of the extract was filtered (0.45-μm glass fiber) and diluted 1:50 in zero standard to neutralize the extract and to eliminate solvent effects in the immunoassay. The diluted soil extract was assayed as above, and the results obtained were multiplied by the appropriate dilution factor to determine carbendazim concentration (i.e., multiply by 150 to correct for the initial 1:3 dilution of soil with extracting solution and the 1:50 extract dilution).

**Table II. Precision of Carbendazim Measurement in Water by Immunoassay**

| | concentration level (ppb) | | | |
|---|---|---|---|---|
| | 0.5 | 2.0 | 3.0 | 4.0 |
| N | 25 | 25 | 25 | 25 |
| mean ppb | 0.41 | 1.98 | 2.85 | 3.69 |
| % CV within-assay | 13.5 | 7.2 | 5.7 | 6.4 |
| % CV between-assay | 10.4 | 6.1 | 3.6 | 5.4 |
| % CV total | 16.5 | 9.1 | 6.6 | 8.1 |

## RESULTS AND DISCUSSION

**Sensitivity and Cross-Reactivity.** The immunoassay described uses a competitive assay format. Since the enzyme-labeled carbendazim competes with unlabeled (sample) carbendazim for the antibody sites, the color developed is inversely proportional to the concentration of carbendazim in the sample. It is common to report displacement in terms of a $B/B_0$ measurement to describe color inhibition. $B/B_0$ is defined as the absorbance observed for a sample or standard divided by the absorbance at a zero analyte concentration.

Three sets of 20 replicates of the zero standard were assayed, and the mean absorbance value and standard deviation were calculated for each run. The mean absorbance value minus four standard deviations was equivalent to a 90% $B/B_0$, which corresponded to an estimated detection limit of 0.10 ppb of carbendazim. Table I summarizes the immunoassay cross-reactivity with carbendazim, benomyl, a variety of other benzimidazoles, and nonstructurally related agricultural compounds. The least detectable dose (LDD) was determined as the amount of compound required to achieve 90% $B/B_0$. The antiserum is most reactive with carbendazim and benomyl but also shows a preference for benzimidazoles containing larger side chains at the $R_2$ position. It appears to be less affected by the size of the chain on the $R_1$ position.

**Precision.** Table II summarizes a precision study that was conducted with four concentrations of carbendazim in water. Carbendazim was added at 0.5, 2.0, 3.0, and 4.0 ppb. Each level was assayed five times per day in singlicate over 5 days. The within- and between-day and total variations were determined by the method of Bookbinder and Panosian (1986) using Statistical Analysis software (SAS Institute, 1988).

**Accuracy.** The accuracy of the assay in water was assessed by evaluating four water samples each spiked with known amounts of carbendazim at four levels. These water samples are described in Table III. Each sample was assayed three times in duplicate to verify reproducibility. Table IV summarizes the accuracy of the spike

**Table III. Characterization of Water Samples Used in Accuracy Experiments**

| source | iron (mg/L) | nitrate (mg/L) | nitrite (mg/L) | sodium chloride (M) | pH | hardness $CaCO_3$ (mg/L) |
|---|---|---|---|---|---|---|
| well | 1.9 | <4.4 | <0.03 | na[a] | 6.5 | 861 |
| municipal water | 0.2 | 17.6 | 0.13 | na | 7.5 | 120 |
| bay | 0.2 | 4.4 | <0.03 | 0.17 | 7.5 | 2054 |
| creek | na | 7.0 | na | na | 7.7 | na |

[a] na, information not available.

**Table IV. Accuracy of Carbendazim Measurement in Water by Immunoassay**

| amount of carbendazim added (ppb) | carbendazim recovered mean (ppb) | $n$ | SD (ppb) | % |
|---|---|---|---|---|
| 0.5 | 0.55 | 12 | 0.15 | 110 |
| 1.0 | 1.05 | 12 | 0.11 | 105 |
| 3.0 | 2.95 | 12 | 0.18 | 98 |
| 4.5 | 4.22 | 12 | 0.35 | 94 |
| av | | | | 102 |

recoveries. Added amounts of carbendazim were recovered accurately in all cases with an average assay recovery of 102%, indicating the assay is linear across the range of the assay.

**Correlation.** A study was conducted to compare results of the ELISA method with existing HPLC methods. Thirty-four water samples ranging from 10 to 100 ppb of carbendazim, plus sample blanks ("0" ppb), were prepared and analyzed by HPLC and the immunoassay. Due to the high levels of carbendazim required for the HPLC analysis, samples were diluted in the zero standard for analysis in the immunoassay. Correlation of the samples by the ELISA method ($y$) and the HPLC method ($x$) is illustrated in Figure 1. Correlation of the immunoassay with the 15 samples analyzed by lab A was significantly better than those analyzed at lab B: $r = 0.991$, slope of 1.04 at lab A, vs $r = 0.968$, slope of 1.57 at lab B. Immunoassay recoveries averaged 106% (SD = 5). The average recovery from lab A was 88% (SD = 6); the average recovery from lab B was 68% (SD = 8). The lower recoveries seen from lab B may have been due to their use of direct injection analysis (with no extraction) and reported problems with injection carryover and blank interference.

**Interferences.** Sample pH and substances that could potentially be found in water were evaluated for interferences in the immunoassay. Sample pH had no adverse effect on neat samples or spiked recoveries in the assay from pH 3 to 11. No interferences were seen up to 250 ppm with calcium, copper, magnesium, nitrate, sulfate, and sulfite, up to 100 ppm of nickel and iron, and up to 500 ppm of silicates. In addition, concentrations up to 1 M sodium chloride showed no effect.

These results suggest that the assay is reliable and free from interferences from commonly found groundwater components. To examine this, 426 water samples from around the United States were evaluated neat and spiked with 2.5 ppb of carbendazim to determine recovery and possible matrix interferences. Six samples (1.4%) contained flocculant material or precipitate that required filtration with a 1.2-μm syringe tip filter (Gelman Sciences, Ann Arbor, MI). One sample was positive (0.26 ppb) in the immunoassay and recovered outside the acceptable range of 100 ± 20%. This sample recovered accurately (100%) by linear dilution and was determined to be a true positive. A mean recovery of 103% (SD = 8) was observed for all 426 samples.

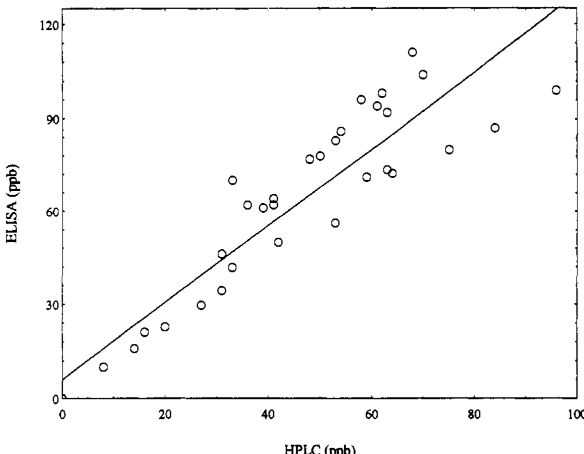


**Figure 1.** Correlation between carbendazim concentrations as determined by ELISA and HPLC methods. $n = 34$, $r = 0.920$, $y = 1.24x + 5.99$.

**Table V. Recovery (Percent) of Carbendazim from 100% Fruit Juice**

| sample | carbendazim spike level[a] (ppb) 100 | 200 | 400 | 1000 | mean | SD |
|---|---|---|---|---|---|---|
| orange juice 1 | 108 | 95 | 94 | 81 | 95 | 11 |
| orange juice 2 | 87 | 111 | 89 | 117 | 101 | 15 |
| apple juice | 116 | 113 | 105 | 97 | 108 | 9 |
| grapefruit juice | 111 | 120 | 109 | 112 | 113 | 5 |
| pineapple juice | 84 | 90 | 115 | 103 | 98 | 12 |

[a] $N = 2$ for each juice fortification level.

**Table VI. Efficacy of Methanol and 0.5 N Sodium Hydroxide Solutions in Extracting Carbendazim from Plano Loam Soil[a]**

| extracting solution | carbendazim recovery[b] neat | 1000 ppb | % |
|---|---|---|---|
| methanol | <37.5[c] | 615 | 62 |
| 75% MeOH/25% 0.5 N NaOH | <37.5 | 1088 | 109 |
| 50% MeOH/50% 0.5 N NaOH | <37.5 | 975 | 98 |
| 25% MeOH/75% 0.5 N NaOH | <37.5 | 975 | 98 |
| 0.5N NaOH | <37.5 | 1035 | 104 |

[a] Extraction time of 30 min. [b] Analyzed by ELISA. [c] Detection limit of procedure.

**Fruit Juice Study.** Recoveries from five 100% juice samples fortified at four levels with carbendazim are shown in Table V. The estimated sensitivity of this procedure is 30 ppb based on the dilution factor and sensitivity estimates in water. All neat samples were determined to contain less than 30 ppb of carbendazim; recoveries of carbendazim spikes averaged 103% (SD = 12). U.S. tolerances range from 7 to 35 ppm of benomyl for these commodities (U.S. EPA, 1990).

**Soil Sample Analysis.** A Plano loam soil fortified with carbendazim was extracted and analyzed by immunoassay. In preliminary studies (data not shown), methanol was a better extractant than other commonly used solvents (e.g., acetone, acetonitrile, chloroform, and ethyl acetate). Tables VI and VII summarize soil spike recoveries using methanol solvent systems. Results shown are the average of duplicate immunoassay results of single extractions. Extraction efficiency at 30 min was greatly increased by the addition of sodium hydroxide (Table VI). However, on organic soils (e.g., muck and potting soils) aqueous solutions of sodium hydroxide can solubilize organic compounds from soil that can interfere with the immunoassay. A combination of 75% methanol/25% 0.5 N NaOH was therefore chosen and would be recommended

**Table VII. Recovery (Percent) of Carbendazim from Soil by ELISA**

| extracting solution | spike (ppb) | extraction time (h) | | |
|---|---|---|---|---|
| | | 0.5 | 3 | 16 |
| methanol | 100 | 72 | 80 | 74 |
| | 500 | 62 | 72 | 79 |
| | 1000 | 73 | 78 | 73 |
| 75% methanol/ 25% 0.5 N NaOH | 100 | 93 | 111 | 114 |
| | 500 | 86 | 89 | 91 |
| | 1000 | 87 | 95 | 95 |

to reduce the amount of organics solubilized by sodium hydroxide but still achieve maximum extraction efficiency.

Results also showed that increasing extraction time from 0.5 to 3 h with 75% methanol/25% 0.5 N NaOH did improve recoveries; however, no significant increase was seen by extending extraction time from 3 to 16 h (Table VII). As with any analytical method, the extraction efficiency may vary with soil type. Additional substances that may be extracted along with carbendazim and interfere in the immunoassay may also vary with soil type and should be evaluated.

Taking into account dilution factors and using the lowest assay calibrator as the LDD, the detection range of the assay for soil analysis was estimated to be 37.5–750 ppb of carbendazim. The 1:50 dilution of the soil extract was chosen to minimize matrix effects in the assay due to solvent interferences and other substances extracted along with the carbendazim in the sample; therefore, the same standards used to assay water were used.

**Conclusions.** The quick turnaround time and performance characteristics of the immunoassay described have shown it to be ideal for the screening of large numbers of environmental water samples. Recovery and precision results are good even at very low levels of detection. The assay compares favorably with traditional HPLC analysis and is free from interferences from pH and compounds commonly found in water. It has also been shown that the assay can easily be adapted to measurement of low levels of pesticide concentrations on soil and in fruit juice.

## LITERATURE CITED


Bardalaye, P. C.; Wheeler, W. B. Simplified Method for the Cleanup and Reversed-phase High Performance Liquid Chromatographic Determination of Benomyl in Mangoes. *J. Chromatogr.* **1985**, *330*, 403–407.

Ben-Aziz, A.; Aharonson, N. Dynamics of Uptake, Translocation, and Disappearance of Thiabendazole and Methyl-2-benzimidazole Carbamate in Pepper and Tomato Plants. *Pestic. Biochem. Physiol.* **1974**, *4*, 120–126.

Bookbinder, M. J.; Panosian, K. J. Correct and Incorrect Estimation of Within-Day and Between-Day Variation. *Clin. Chem.* **1986**, *32*, 1734–1737.

Calmon, J. P.; Sayag, D. R. Instability of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl) in Various Solvents. *J. Agric. Food Chem.* **1976**, *24*, 426–428.

Clemens, G. P.; Sisler, H. D. Formation of a Fungitoxic Derivative from Benlate. *Phytopathology* **1969**, *59*, 705–706.

Engvall, B. Enzyme Immunoassay ELISA and EMIT. In *Methods in Enzymology*; VanVunakis, H., Langone, J. J., Eds.; Academic Press: New York, 1980; pp 419–439.

Harrison, R. O.; Braun, A. L.; Gee, S. J.; O'Brien, D. J.; Hammock, B. D. Evaluation of an Enzyme-linked Immunosorbent Assay (ELISA) for the Direct Analysis of Molinate (Odram®) in Rice Field Water. *Food Agric. Immunol.* **1989**, *1*, 37–51.

Howell, E. H.; Nasser, J.; Schray, K. J. Coated Tube Enzyme Immunoassays: Factors Affecting Sensitivity and Effects of Reversible Binding to Polystyrene. *J. Immunoassay* **1981**, *2*, 205–225.

Itak, J. A.; Selisker, M. Y.; Herzog, D. P. Development and Evaluation of a Magnetic Particle-based Enzyme Immunoassay for Aldicarb, Aldicarb Sulfone and Aldicarb Sulfoxide. *Chemosphere* **1992**, *24*, 11–21.

Kilgore, W. K.; White, E. R. Decomposition of the Systemic Fungicide 1991 (Benlate). *Environ. Contam. Toxicol. Bull.* **1970**, *5*, 67–69.

Kirkland, J. J.; Holt, R. F.; Pease, H. L. Determination of Benomyl Residues in Soils and Plant Tissues by High-Speed Cation Exchange Liquid Chromatography. *J. Agric. Food Chem.* **1973**, *21*, 368–371.

Kitada, Y.; Sasaki, M.; Tanigawa, K. Simultaneous Liquid Chromatographic Determination of Thiabendazole, o-Phenylphenol, and Diphenyl Residues in Citrus Fruits, Without Prior Cleanup. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 1302–1304.

Langone, J. J.; Van Vunakis, H. Radioimmunoassay for Dieldrin and Aldrin. *Res. Commun. Chem. Pathol. Pharmacol.* **1975**, *10*, 163–171.

Lawruk, T. S.; Lachman, C. E.; Jourdan, S. W.; Fleeker, J. R.; Herzog, D. P.; Rubio, F. M. Quantification of Cyanazine in Water and Soil by a Magnetic Particle-based ELISA. *J. Agric. Food Chem.* **1993**, *41*, 747–752.

Lehtonen, O. P.; Viljanen, M. K. Antigen Attachment in ELISA. *J. Immunol. Methods* **1980**, *34*, 61–70.

Newsome, W. H.; Collins, P. G. Enzyme-linked Immunosorbent Assay of Benomyl and Thiabendazole in Some Foods. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 1025–1027.

Newsome, W. H.; Shields, J. B. A Radioimmunoassay for Benomyl and Methyl 2-Benzimidazolecarbamate on Food Crops. *J. Agric. Food Chem.* **1981**, *29*, 220–222.

Peterson, C. A.; Edgington, L. V. Transport of Benomyl into Various Plant Organs. *Phytopathology* **1971**, *61*, 91–92.

Rubio, F. M.; Itak, J. A.; Scutellaro, A. M.; Selisker, M. Y.; Herzog, D. P. Performance Characteristics of a Novel Magnetic Particle Based ELISA for the Quantitative Analysis of Atrazine and Related Triazines in Water Samples. *Food Agric. Immunol.* **1991**, *3*, 113–125.

SAS Institute. *SAS/STAT User's Guide*, release 6.03 ed.; SAS Institute: Cary, NC, 1988; pp 549–640.

Singh, R. P.; Brindle, I. D.; Hall, C. D.; Chiba, M. Kinetic Study of the Decomposition of Methyl [1-(Butylcarbamoyl)-1*H*-benzimidazol-2-yl] carbamate (Benomyl) to Methyl 1*H*-Benzimidazol-2-ylcarbamate (MBC). *J. Agric. Food Chem.* **1990**, *38*, 1758–1762.

Tang, C. S.; Yanagihara, K.; Zhang, Y. 1-Butyl Isocyanate from Aqueous Benlate® Formulations. *Arch. Environ. Contam. Toxicol.* **1992**, *23*, 270–272.

Tijssen, P. 5.2.4 Immunization Procedures. In *Practice and Theory of Enzyme Immunoassays*; Burdon, R., vanKnippenburg, P., Eds.; Elsevier Science Publishers: New York, 1985.

U.S. EPA. Code of Federal Regulations, Title 40, Part 180, section 294; U.S. GPO: Washington, DC, 1990.

Van Emon, J. M.; Lopez-Avila, V. Immunological Methods for Environmental Analysis. *Anal. Chem.* **1992**, *64*, 79–88.

Watterson, A. *Pesticide Users' Health and Safety Handbook*; Van Nostrand Reinhold: New York, 1988.

Zweig, G.; Gao, R. Determination of Benomyl by Reversed-Phase Liquid Chromatography. *Anal. Chem.* **1983**, *55*, 1448–1451.


Received for review June 28, 1993. Accepted September 27, 1993.®

® Abstract published in *Advance ACS Abstracts*, November 15, 1993.