Determination of Chlorothalonil in Water and Agricultural Products by a Magnetic Particle-Based Enzyme Immunoassay

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A competitive enzyme immunoassay for the quantitation of chlorothalonil in water and agricultural products was developed. This immunoassay utililizes amine-terminated superparamagnetic particles as the solid phase to covalently attach polyclonal rabbit anti-chlorothalonil antisera. Specificity studies indicate that the antibody can distinguish chlorothalonil from its major metabolites and structurally similar pesticides. The immunoassay has a detection limit of 0.07 parts per billion (ppb, ng/mL) in water. The assay compares favorably with GC measurements when water samples are analyzed (r = 0.984). Studies indicate that the assay can potentially be used to analyze agricultural products after homogenization with acetone (detection limit of 20 ppb for celery) or to analyze dislodgable residues after a methanol surface wash (detection limit of 5 ppb for snow pea pods).

Keywords: *Chlorothalonil; immunoassay; water; produce; magnetic particle; ELISA*

INTRODUCTION

Increased worldwide demand for pesticide residue monitoring in food and water has led to a need for faster, easier methods that permit the screening of large numbers of samples. Interest in immunoassay technologies for the analysis of pesticide residues and environmental contaminants has dramatically increased due to the sensitivity, reliability, speed, and cost-effectiveness of these methods. Currrent testing methods involving gas (GC and GC/MS) and liquid (HPLC) chromatography are time-consuming and expensive and require specialized instrumentation. Immunological assays provide the residue chemist with a cost-effective, sensitive, rapid, and reliable method suitable for both laboratory and field analyses (Van Emon and Lopez-Avila, 1992).

Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile) is a broad spectrum, nonsystemic fungicide used on a wide variety of fruits and vegetables including tomatoes, celery, bananas, cucumbers, and soybeans. The active ingredient of Bravo fungicide (ISK Biotech, Mentor, OH), chlorothalonil is one of the most widely used fungicides in the United States with between 10 and 15 million pounds applied per year for crop protection (U.S. EPA, 1994). As a result of its usage, chlorothalonil residues may be present in food, wells, and streams due to runoff, spills, and spraying. Chlorothalonil residues have been detected in various agricultural commodities, usually below tolerance levels, although violative levels have been reported (Schattenberg and Hsu, 1992). Contamination in the aquatic environment is of particular concern because of its toxicity to fish (Davies and White, 1985); however, chlorothalonil toxicity under actual use conditions may be less than suggested by laboratory studies due to the rapid breakdown and binding to sediment in water. Chlorothalonil readily hydrolyzes to 2,3,5-trichloro-4-hydroxyisophthalonitrile, and this degradation is increased by the presence of algal material, aeration, and adsorption to suspended materials, with half-lives as short as 4 h (Davies, 1988).

Chlorothalonil is classified as a probable human carcinogen, Group B2, by the U.S. EPA. This category is for chemicals for which there is inadequate evidence of carcinogenicity from human studies and sufficient data from animal studies. The Drinking Water Equivalent Level (DWEL), the highest concentration that would not be expected to produce an adverse health effect if 2 L of water were consumed daily for a lifetime, is 500 ppb. The U.S. EPA has recommended that no Lifetime Health Advisory in drinking water be established for chlorothalonil (U.S. EPA, 1989b). The U.S. EPA tolerances for chlorothalonil residues in or on raw agricultural commodities range from 0.1 to 15 ppm (U.S. EPA, 1985). For example, the maximum residue limit (MRL) for chlorothalonil on celery is 15 ppm. The Codex Maximum Limit for chlorothalonil residues ranges from 0.1 to 25 ppm on various crops (Codex Alimentarius Commission, 1992). The use of chlorothalonil by growers on agricultural commodities for which it is not registered is of concern to both importers and exporters of produce. Chlorothalonil residues have been detected on imported snow peas and apples (Yeung and Newsome, 1995). Immunoassays offer the importer/exporter a reliable, rapid, and sensitive method to screen their product for illegal residues prior to shipment.

The principles of enzyme-linked immunosorbent assay (ELISA) have previously been described (Hammock and Mumma, 1980). Magnetic particle-based ELISAs have previously been described and applied to the detection of pesticide residues in water (Lawruk et al., 1992; Gruessner et al., 1995), wine (Lawruk et al., 1994), fruit (Itak et al., 1994), fruit juice (Itak et al., 1993, 1994), meat products (Nam and King, 1994), soil (Lawruk et al., 1993a,b), and produce (Yeung and Newsome, 1994; Selisker et al., 1995). These immunoassays eliminate the imprecision associated with antibody-coated tubes and microtiter plates (Howell et al., 1981; Engvall, 1980; Lehtonen and Viljanen, 1980) through the precise addition of the antibody and covalent coupling of

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antibody to the magnetic solid phase. The magnetic particle-based immunoassay system has been shown to be more precise than microtiter plate systems (Aga and Thurman, 1993). The chlorothalonil magnetic particle-based immunoassay described in this paper combines a polyclonal antibody specific for chlorothalonil with an enzyme-labeled chlorothalonil analog. The assay takes less than 1 h to perform and requires no sample preparation for the analysis of water samples. Simple sample preparation procedures are described to adapt the immunoassay to the analysis of agricultural commodities.

MATERIALS AND METHODS

Immunochemicals. To prepare the chlorothalonil immunogen, *N*-(pentachlorophenoxyacetyl)glycine (343 mg, 0.9 mmol) and 140 μL of triethylamine were dissolved in 6 mL of dry dioxane. The solution was cooled, and 80 μL of isobutyl chloroformate was added. A precipitate of triethylammonium chloride formed. After 30 min, the solution of the mixed anhydride was added in one portion to an ice-cold solution of 1 g (0.015 mmol) of bovine serum albumin (BSA) (Miles, Inc., Kankakee, IL) in 26 mL of dioxane/water and 1.0 mL of 1.0 M NaOH. After 2 min, another 1.0 mL of 1.0 M NaOH was added. The solution was stirred in an ice bath for 2 h. The pH was adjusted to 4.5 with 1.0 M HCl and the resulting precipitate collected by centrifugation. The resulting pellet was resuspended in half the original volume of deionized water with a few drops of 1.0 M NaOH added to facilitate dissolution. The chlorothalonil–BSA immunogen was lyophilized, and treatment of the final product with picryl sulfonate indicated 40% of the amino groups were blocked. The chlorothalonil immunogen was dissolved in sterile saline solution to a concentration of 4 mg/mL. This solution was emulsified with an equal volume of Freund's complete adjuvant, and a total of 0.5 mL of the emulsion was injected in the hip muscle of three rabbits. After 20 and 45 days and at 30-day intervals thereafter, the rabbits were boosted with 0.5 mL of the emulsion using Freund's incomplete adjuvant. Whole blood (30–50 mL) was obtained 10 days after each boost, allowed to coagulate, and centrifuged to obtain the antiserum, which was stored at −70 °C.

The antiserum was covalently attached to amine-terminated superparamagnetic particles of approximately 1-μm diameter (Perseptive Diagnostics Inc., Cambridge, MA) by glutaraldehyde (Sigma Chemical Co., St. Louis, MO) activation of the solid phase as previously described by Rubio et al. (1991). Superparamagnetic particles of this size separate quickly in magnetic fields but have no magnetic memory, which allows for repeated magnetic separations and resuspension of the particles. The small particle size permits the particles to remain suspended in solution for over 1 h. Efficiency of the antiserum coupling to the magnetic particles exceeded 90%. The particle stocks were diluted 1:1000 in Tris-buffered saline (pH 7.4) containing 0.1% gelatin and 1 mM EDTA for use in the immunoassay.

The chlorothalonil hapten–horseradish peroxidase (HRP) was synthesized by active ester activation of the hapten followed by carbodiimide coupling of the enzyme (Langone and Van Vanukis, 1975). Peroxidase from horseradish was purchased from Sigma.

Additional Chemicals. Hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Kirkegaard and Perry (Gaithersburg, MD). Chlorothalonil and related compounds as well as nonrelated cross-reactants were purchased from ChemService Inc. (West Chester, PA). The chlorothalonil degradation products, 2,4,5,6-tetrachloro-3-cyanobenzamide, 2,5,6-trichloro-4-hydroxyisophthalonitrile, and 3-carbamyl-2,4,5-trichlorobenzoic acid, were obtained from ISK Biotech. All other chemicals were of reagent grade or chemically pure.

Apparatus. The spectrophotometric results were determined using the RPA-I Analyzer (Ohmicron), the detailed functions of which have been previously described by Rubio et al. (1991). A two-piece magnetic separation rack consisting of a test tube holder which fits over a magnetic base containing permanently positioned rare earth magnets is required. This two-piece design allows for a 60-tube immunoassay batch to be set up, incubated, and magnetically separated without removing the tubes from the holders (Itak et al., 1992). Gilson P-200 (Rainin, Woburn, MA) and Eppendorf repeating pipets (Eppendorf, Hamburg, Germany) were used to dispense liquids.

Immunoassay Procedure. All standards, water samples, diluted food extracts and diluted surface washes were assayed by adding 200 μL of sample, 250 μL of chlorothalonil–HRP conjugate, and 500 μL of anti-chlorothalonil coupled magnetic particles to a disposable polystyrene test tube in the magnetic rack tube holder and incubating for 30 min at room temperature. The magnetic rack was used to magnetically separate the reaction mixture. After separation, the magnetic particles were washed twice with 1.0 mL of deionized water to remove unbound conjugate and eliminate any potential interfering substances. The colored product was developed for 20 min at room temperature by the addition of 500 μL of a 1:1 mixture of peroxide/TMB solution. Formation of the colored product was stopped and stabilized by the addition of 500 μL of 2 M sulfuric acid. The final concentrations of chlorothalonil for each sample were determined using the RPA-I Analyzer by measuring the absorbance at 450 nm. The RPA-I Analyzer was preprogrammed to compare the observed sample absorbances to a linear regression line using a logarithm of the concentration versus linear B/B_0 standard curve (where B/B_0 is the absorbance at 450 nm observed for a sample or standard divided by the absorbance at the zero standard). The calibrators were prepared in the zero standard (acetate-buffered saline preserved solution) and contained chlorothalonil at 0, 0.1, 1.0, and 5.0 ppb. Samples greater than 5.0 ppb were diluted in the zero standard for analysis, and sample concentrations were calculated by multiplying results by the appropriate dilution factor.

GC Analysis for Method Comparison. Water samples for method comparison were drinking and surface waters from Pennsylvania, New Jersey, Delaware, and North Carolina and were analytically spiked with chlorothalonil from 0.5 to 4.8 ppm. Samples were analyzed by the protocol of Newsome and Collins (1989) utilizing a gas chromatograph (GC) with a flame ionization detector. The GC results were not corrected for procedural recoveries.

Celery Sample Analysis. Celery samples (50 g) were coarsely chopped in a food processor and transferred to a polypropylene bottle containing 100 mL of acetone. The samples were shaken by hand for 15–20 s and then allowed to settle for approximately 5 min. The acetone extract (4 mL) was transferred to a test tube and 2 g of NaCl added (Steinwandter, 1992). The samples were mixed thoroughly and the acetone and aqueous layers allowed to separate (approximately 5 min). Fifty microliters (50 μL) of the acetone phase was added to 5 mL of the zero standard. The diluted extract was assayed as described above, and the chlorothalonil concentrations were calculated in each celery sample by multiplying the assay result by the appropriate factor, in this case, 200.

Snow Pea Pod Sample Analysis. Whole snow pea pods (approximately 20 g) were weighed into a 100 mL bottle and a volume of methanol (in milliliters) equal to twice the weight (in grams) of the snow pea pod sample (approximately 40 mL) was added. The snow pea pod/methanol mixture was shaken for 30 s–1 min, and particulates were allowed to settle for 3 min. After the methanol extract was prepared, removal of the extract from contact with the snow pea pods proceeded without delay. The methanol extract was diluted 1:25 into the zero standard (100 μL of extract into 2.4 mL of zero standard). The diluted snow pea pod extract was assayed as described above, and the results were multiplied by the appropriate dilution factor to determine the snow pea pod chlorothalonil concentration (i.e., multiply by 50 for a 1:25 dilution to correct for the initial 1:2 dilution of snow pea pods with methanol).

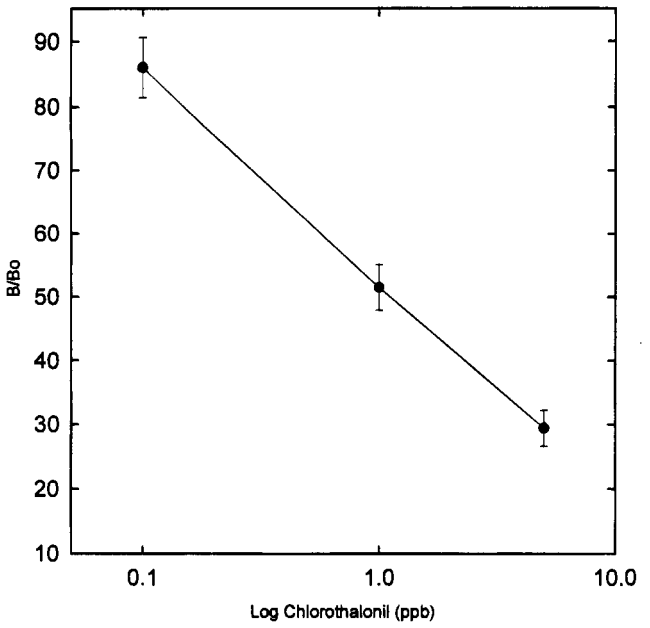


Figure 1. Chlorothalonil dose response curve. Each point represents the mean of 51 determinations. Vertical bars indicate ±2 SD about the mean.

RESULTS AND DISCUSSION

Dose Response Curve and Sensitivity. Figure 1 illustrates the mean standard curve for the chlorothalonil calibrators, linearly transformed using a log/linear curve fit, collected over 51 assays with error bars representing 2 standard deviations. To ensure accurate results, a calibration curve was included with every assay to correct for variability. The assay sensitivity, the lowest concentration that can be distinguished from zero, based on 90% B/B_0 is 70 ppt chlorothalonil in water (Midgley et al., 1969). This sensitivity approaches the estimated detection limit reported for U.S. EPA Method 508 of 25 ppt using gas chromatography and an electron capture detector (U.S. EPA, 1989a). The least detectable dose of the immunoassay exceeds the HPLC method detection limit of 5 ppb reported by Jongen et al. (1991). Quantitation with the immunoassay should be limited to within the range of the standard curve, from 0.1 to 5.0 ppb in water. To analyze samples with higher chlorothalonil concentration, the water samples are diluted in the zero standard for analysis and sample concentrations calculated by multiplying results by the appropriate dilution factor.

Precision. A precision study in which three surface water and one municipal water samples were fortified with chlorothalonil at 0.25, 0.5, 1.5, and 3.0 ppb and each assayed five times in singlet on five different days is shown in Table 1. The within- and between-day variation was determined by analysis of variance (ANOVA) (Bookbinder and Panosian, 1986). Coefficients of variation (%CV) within and between day were less than 11% and 9%, respectively. The total %CV (n = 25) was less than 12% at all concentrations tested.

Method Comparison. Method correlation of 35 spiked water samples obtained by the immunoassay (y) and an established (Newsome and Collins, 1989) GC method (x) is illustrated in Figure 2. The regression analysis yields a correlation (r) of 0.984 and a slope of 1.14 between methods. The apparent higher chlorothalonil concentrations by the immunoassay could be due to the loss of analyte during the sample concentration and extraction steps of the GC method. This observation is supported by the lower recovery of the spiked water samples by the GC method (mean recovery = 91%), possibly due to the C_{18} solid phase extraction, whereas the water samples were analyzed directly, without sample extraction, by the immunoassay (mean recovery = 108%).

Table 1. Precision of Chlorothalonil Measurement by Immunoassay[a]

	sample			
	1	2	3	4
replicates	5	5	5	5
days	5	5	5	5
N	25	25	25	25
mean (ppb)	0.19	0.50	1.66	3.11
%CV (within assay)	10.6	3.9	4.9	4.1
%CV (between assay)	5.7	6.4	4.6	8.2
%CV (total assay)	11.8	7.1	6.5	8.6

[a] Water samples were fortified with 0.25, 0.50, 1.5, and 3.0 ppb chlorothalonil and assayed in five singlets each over 5 days. Sample 1, surface water fortified with 0.25 ppb; sample 2, surface water fortified with 0.5 ppb; sample 3, surface water fortified with 1.5 ppb; sample 4, municipal water fortified with 3.0 ppb.

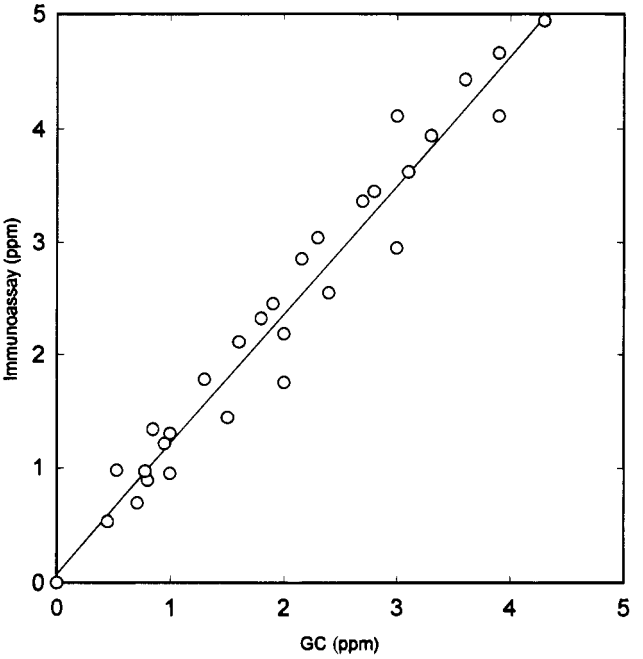


Figure 2. Correlation between chlorothalonil concentrations in spiked water samples as determined by the magnetic particle-based immunoassay and GC/FID method. n = 35, r = 0.984, $y = 1.14x + 0.07$ ppb.

Accuracy in Water. The accuracy of the immunoassay was assessed by evaluating four water samples each fortified with chlorothalonil at 0.25, 0.5, 1.5, and 3.0 ppb. The water samples included a small pond, a small stream, the Delaware River, and a municipal drinking water. Each sample was evaluated three times in duplicate to verify reproducibility. Table 2 summarizes the accuracy of the chlorothalonil immunoassay in environmental water samples. Added amounts of chlorothalonil were recovered quantitatively in all cases, with an average assay recovery of 98%. The accurate recovery of the spiked water samples suggests that no sample matrix problems or interferences were present in the samples tested, and the immunoassay is accurate across the range of the method.

Sample Dilution. Water and celery extracts that contain detectable concentrations of chlorothalonil by the immunoassay can be diluted in the zero standard

Table 2. Accuracy of the Chlorothalonil Immunoassay in Water[a]

chlorothalonil added (ppb)	mean chlorothalonil recovered (ppb)	n	SD (ppb)	% recovery
0.25	0.23	12	0.03	92
0.50	0.48	12	0.04	96
1.50	1.58	12	0.09	105
3.00	2.97	12	0.22	99
av				98

[a] Three surface water samples and one municipal water sample were each fortified at the described concentrations and assayed in duplicate in three separate immunoassays.

Table 3. Linearity upon Sample Dilution (Water and Celery)[a]

sample	undiluted	1:2	1:4	1:8
water sample 1				
obtained (ppb)	2.92	1.55	0.75	0.33
expected[b] (ppb)	2.92	1.46	0.73	0.37
recovery (%)		106	103	89
water sample 2				
obtained (ppb)	3.10	1.54	0.71	0.36
expected (ppb)	3.10	1.55	0.78	0.39
recovery (%)		99	91	92
celery sample 1[c]				
obtained (ppb)	19,400	11,300	5,560	2,900
expected (ppb)	19,400	9,730	4,860	2,430
recovery (%)		116	114	119
celery sample 2[c]				
obtained (ppb)	15,100	8,100	3,900	NA[d]
expected (ppb)	15,100	7,550	3,780	NA
recovery (%)		107	103	NA

[a] Samples were diluted with the zero standard. [b] Expected concentrations are derived from the chlorothalonil concentration obtained from the undiluted sample. [c] Initial 1:5000 dilution of celery homogenate extract. [d] NA, analysis not available.

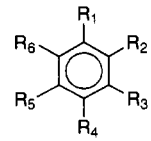
and reassayed to determine "parallel" dilution. If the positive chlorothalonil result was due to specific or nonspecific interferences, the concentrations of the diluted samples would not assay as expected; i.e., the standard curve should be parallel to a curve obtained by diluting a sample (Jung et al., 1989). Values obtained from spiked water samples and spiked celery samples diluted in the zero standard showed agreement between measured and expected values (Table 3). The expected values are derived from the chlorothalonil concentration of the undiluted sample.

Specificity. Table 4 summarizes the specificity of the chlorothalonil immunoassay with various degradation products and structurally related compounds. The least detectable dose (LDD) was determined as the amount of each compound necessary to achieve 90% B/B_0. At the 90% B/B_0 concentration, each compound would yield an apparent chlorothalonil concentration greater than the LDD of chlorothalonil. The 50% inhibition concentration (I_{50}) was determined by estimating the amount of each compound necessary for 50% displacement of the chlorothalonil–HRP conjugate. Reactivity to the three major metabolites of chlorothalonil, 2,4,5,6-tetrachloro-3-cyanobenzamide, 2,5,6-trichloro-4-hydroxyisophthalonitrile, and 3-carbamyl-2,4,5-trichlorobenzoic acid, was dependent upon the position of the substituent group substitution. In the case of 2,4,5,6-tetrachloro-3-cyanobenzamide, substitution of an amide to the R_1 position provides 10.7% of the reactivity as compared to chlorothalonil. Substitution at the R_4 position, the most distal from the immunogen side chain, as exhibited by the major hydrolysis product 2,5,6-trichloro-4-hydroxyisophthalonitrile, provides the most substantial decrease in immunoreactivity (<0.1%). 3-Carbamyl-2,4,5-trichlorobenzoic acid, which is substituted at the R_1, R_3, and R_4 positions, also yields low reactivity (<0.1%). The influence of position R_4 on immunoreactivity is also evidenced in the substantial reactivity of pentachloronitrobenzene and hexachlorobenzene (>50%), both of which retain the chlorine at R_4. However, position R_1 also influences the specificity as exhibited by the dramatic decrease in reactivity when a hydroxyl group is added to R_1, as with pentachlorophenol (<0.1%).

Interferences. The following compounds were added to blank and fortified chlorothalonil water samples at 250 parts per million (ppm, μg/mL) and evaluated for possible interference in the immunoassay: copper (chloride), magnesium (chloride), manganese (chloride), mercury (chloride), nickel (sulfate), zinc (chloride), nitrate (sodium), phosphate (sodium), and thiosulfate (sodium). In addition, calcium (chloride) to 500 ppm, sulfate (sodium) to 10 000 ppm, sulfite (sodium) to 100 ppm, silicates (sodium meta-) to 1000 ppm, peroxide (hydrogen) to 5000 ppm, iron (chloride) to 100 ppm, and NaCl to 1.0 M exhibited no interference in the assay (Table 5). The concentrations of the compounds evaluated most likely exceed levels found in environmental water samples (American Public Health Association, 1989).

Accuracy in Agricultural Products. Two sample preparation techniques were evaluated for chlorotha-

Table 4. Specificity (Cross-Reactivity) of Degradation Products and Unrelated Agrochemicals in the Chlorothalonil Immunoassay



compd	R_1	R_2	R_3	R_4	R_5	R_6	LDD[a] (ppb)	I_{50}[b] (ppb)
chlorothalonil	CN	Cl	CN	Cl	Cl	Cl	0.07	1.12
2,4,5,6-tetrachloro-3-cyanobenzamide	$CONH_2$	Cl	CN	Cl	Cl	Cl	0.29	10.5
2,5,6-trichloro-4-hydroxyisophthalonitrile	CN	Cl	CN	OH	Cl	Cl	18.7	1450
3-carbamyl-2,4,5-trichlorobenzoic acid	$CONH_2$	Cl	COOH	H	Cl	Cl	48.1	1210
pentachloronitrobenzene	NO_2	Cl	Cl	Cl	Cl	Cl	0.14	1.90
hexachlorobenzene	Cl	Cl	Cl	Cl	Cl	Cl	0.16	2.00
pentachlorophenol	OH	Cl	Cl	Cl	Cl	Cl	29.2	1700
2,4,5,6-tetrachlorophenol	OH	Cl	H	Cl	Cl	Cl	157	4990

[a] Least detectable dose (90% B/B_0). [b] Fifty percent inhibition concentration (50% B/B_0). The following compounds had no reactivity in the chlorothalonil immunoassay up to 10 000 ppb: alachlor, aldicarb, aldicarb sulfone, aldicarb sulfoxide, atrazine, benomyl, butylate, captan, captafol, carbaryl, carbofuran, 2,4-D, dicamba, dinoseb, MCPA, MCPP, metolachlor, metribuzin, simazine, terbufos, thiophanat-methyl, and thiabendazol.

Table 5. Effect of Possible Interfering Substances

compd[a]	max concn of compd tested (ppm)	0 ppb chlorothalonil sample (ppb)	2 ppb chlorothalonil sample (ppb)
calcium (chloride)	500	ND[b]	1.99
copper (chloride)	250	ND	2.27
iron (chloride)	100	ND	1.78
magnesium (chloride)	250	ND	1.91
manganese (chloride)	250	ND	2.16
mercury (chloride)	250	ND	2.20
nickel (sulfate)	250	ND	1.91
zinc (chloride)	250	ND	1.95
nitrate (sodium)	250	ND	1.66
peroxide (hydrogen)	5000	ND	1.67
phosphate (sodium)	250	ND	1.80
sulfate (sodium)	10000	ND	1.92
sulfite (sodium)	100	ND	1.72
silicates (sodium meta-)	1000	ND	2.00
thiosulfate (sodium)	250	ND	1.89
NaCl	1.0 M	ND	1.82

[a] Concentrations were corrected for the compound of interest. [b] ND, none detected (<0.07 ppb).

Table 6. Accuracy of the Chlorothalonil Immunoassay in Celery Homogenates[a]

chlorothalonil fortification (ppb)	sample	assayed (ppb)	% recovery
100	1	101	101
	2	113	113
	3	86	86
	av	100	100
500	1	520	104
	2	499	100
	3	491	98
	av	503	101

[a] Fifty grams of three chlorothalonil-spiked celery samples were macerated and then shaken with 100 mL of acetone for 15–20 s. After phase separation with NaCl, the extracts were diluted in zero standard and analyzed in duplicate in the immunoassay. All unspiked celery extract assayed as less than the detection limit of 20 ppb.

lonil residue detection on agricultural commodities with the magnetic particle-based immunoassay. The recovery of chlorothalonil fortified into celery samples was evaluated by extracting macerated celery with acetone and diluting the extract in the zero standard. The average recovery of chlorothalonil fortified at 100 and 500 ppb in the celery samples without filtering the extract was 100% (Table 6). The unspiked celery samples assayed as less than the detection limit of 20 ppb. At least a 1:100 dilution of the celery extract into the zero standard is necessary to be below the 1% maximum acetone tolerance for this immunoassay. Using this dilution provides a detection range of 20 ppb–1 ppm of chlorothalonil in celery. Samples containing greater than 1 ppm must be diluted further. A similar extraction procedure for produce by Yeung and Newsome (1995) utilizing 70% aqueous methanol and analysis by the magnetic particle immunoassay has been reported. This protocol required filtering of the produce extracts before analysis by immunoassay to remove matrix interferences extracted by the methanol. Extraction of macerated produce with acetone eliminated the necessity of extract filtration to remove matrix interferences before immunoassay analysis.

Chlorothalonil is a nonsystemic fungicide with no evidence of translocation in plants (FAO Surveillance Index, 1981). The second sample preparation technique utilized a methanol surface wash of snow pea pods to quantitate dislodgable chlorothalonil residues. Methanol was used as the extraction solvent in this case because the immunoassay can tolerate up to 5% methanol. This procedure allowed for smaller extract dilutions, thereby increasing sensitivity to dislodgable residues. Snow pea pods were fortified with chlorothalonil at 5, 50, 100, and 200 ppb and extracted with 100% methanol by shaking for at least 30 s. After settling, the extract was diluted in the zero standard. The average recovery of chlorothalonil using this surface wash was 103% (Table 7). The unspiked snow pea pod samples assayed as less than the detection limit of 5 ppb. Using this dilution scheme provides a detection range of 5–250 ppb chlorothalonil on snow pea pods, making it ideal to screen for illegal chlorothalonil residues on this commodity. Samples containing greater than 250 ppb must be diluted further. Yeung and Newsome (1995) have previously reported a 70% methanol surface wash of various agricultural commodities using the magnetic particle immunoassay and obtained elevated recoveries. However, a methanol surface wash of snow peas was not attempted, indicating that matrix interferences may be extracted from different produce but not snow pea pods.

Table 7. Accuracy of the Chlorothalonil Immunoassay on Snow Pea Pods (Dislodgable Residues)[a]

chlorothalonil added (ppb)	mean chlorothalonil recovered (ppb)	n	SD (ppb)	% recovery
5	5.8	8	1.2	116
50	45.4	8	11.2	90
100	98.3	12	10.6	98
200	217.8	24	34.7	109
av				103

[a] Twenty grams of chlorothalonil-spiked whole pea pods were shaken for 30 s with 40 mL of methanol and allowed to settle for 3 min. The methanol extracts were diluted in the zero standard and analyzed in duplicate in the immunoassay. All unspiked snow pea extracts assayed as less than the detection limit of 5 ppb.

The stability of chlorothalonil in snow pea pod and celery methanol extracts was investigated (Table 8). The chlorothalonil appeared to degrade rapidly in the snow pea pod extracts (<1.5 h) but not the celery extracts (>3.5 h). Rinsing the snow pea pods with water to remove possible interfering substances before extraction had no effect on the chlorothalonil stability in the methanol extract. Due to this instability, all methanol extract dilutions were made immediately following the 3-min settling time. Yeung and Newsome (1995) have reported a similar chlorothalonil instability of snow pea pod homogenates in 70% methanol after 3 days at room temperature. With both sample preparation techniques,

Table 8. Chlorothalonil Stability in Methanol Extracts by Immunoassay[a]

	obsd chlorothalonil concn (ppb)					
	100 ppb added			200 ppb added		
product	initial	1.5 h	3.5 h	initial	1.5 h	3.5 h
snow pea pod A	85	ND[b]	ND	220	11	9
snow pea pod B	97	ND	ND	219	10	12
snow pea pod rinsed	102	ND	ND	249	10	12
celery[c]	112	109	125	224	204	201

[a] The snow pea pod (20 g)/methanol (40 mL) mixture was shaken for 30 s−1 min, and particulates were allowed to settle for 3 min. Initial dilution into the zero standard occurred immediately after the 3 min settling time. The dilutions for the 1.5 and 3.5 h testing occurred after the snow pea pods sat in the methanol extract for that time period. [b] ND, none detected (<5 ppb). [c] The celery samples were analyzed for dislodgable residues using the procedure previously described.

diluting the celery or snow pea pod extract in the zero standard eliminates the need for solvent evaporation and solvent interferences in the assayed sample while providing parts per billion sensitivity to chlorothalonil residues.

Conclusions. This method demonstrates the advantages of the magnetic particle-based enzyme immunoassay for the quantitation of chlorothalonil in water and agricultural products. The assay described allows for results to be obtained in less than 1 h without the problems of variability encountered with coated tubes and microtiter plates (e.g., coating variabilty and antibody desorption). The immunoassay compares favorably to GC results for water samples ($r = 0.984$) and exhibits within- and between-assay precision of less than 11%. Agricultural products can be prepared for immunoassay analysis by extraction of homogenates with acetone or a surface wash with methanol to determine dislodgable residues. Studies have shown the magnetic particle-based immunoassay to be accurate in water with no sample preparation (average recovery of 98%), fortified celery (average recovery of 101%), and fortified snow pea pods (average recovery of 103%) employing minimum sample preparation. The specificity of the antibody used allows for the detection of chlorothalonil in the presence of other pesticides, metabolites, and commonly found ground water components. The method's sensitivity in water (0.07 ppb) and dislodgable residues on food (5 ppb) makes it ideal as a screening tool.

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