Development of an Enzyme-Linked Immunosorbent Assay for the Detection of Dicamba

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A competitive indirect enzyme-linked immunosorbent assay (CI-ELISA) was developed to quantitate the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) in water. The CI-ELISA has a detection limit of 2.3 μg L^{-1} and a linear working range of 10−10000 μg L^{-1} with an IC_{50} value of 195 μg L^{-1}. The dicamba polyclonal antisera did not cross-react with a number of other herbicides tested but did cross-react with a dicamba metabolite, 5-hydroxydicamba, and structurally related chlorobenzoic acids. The assay was used to estimate quantitatively dicamba concentrations in water samples. Water samples were analyzed directly, and no sample preparation was required. To improve detection limits, a C_{18} (reversed phase) column concentration step was devised prior to analysis, and the detection limits were increased by at least by 10-fold. After the sample preconcentration, the detection limit, IC_{50}, and linear working range were 0.23, 19.5, and 5−200 μg L^{-1}, respectively. The CI-ELISA estimations in water correlated well with those from gas chromatography−mass spectrometry (GC-MS) analysis ($r^2 = 0.9991$). This assay contributes to reducing laboratory costs associated with the conventional GC-MS residue analysis techniques for the quantitation of dicamba in water.

Keywords: *Dicamba; ELISA; 3,6 dichloro-2-methoxybenzoic acid; herbicide; residue; immunoassay; gas chromatography mass spectrometry; GC-MS; analysis*

INTRODUCTION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) (Figure 1) is the active ingredient in Banvel herbicide produced by BASF Canada Inc. and Novartis Crop Protection Inc. In Ontario, Canada, various formulations are scheduled for use that include products with the trade names Banvel 2 and Dycleer. Dicamba is a selective herbicide used for the control of annual, perennial, and biennial weeds and numerous brush species including conifers. Dicamba is used in field corn, spring and winter wheat, spring barley, spring rye, oats, and summer fallow (*1*). Over the past 15 years, the use of the herbicide dicamba in the Province of Ontario has increased. For example, in the years 1978, 1983, 1988, and 1993, dicamba usage has been 0.5, 0.8, 2.7, and 6%, respectively, of the total kilograms used for all herbicides (*2*). Dicamba is a benzoic acid herbicide and its mode of action is similar to that of other plant growth regulators, that is, the phenoxy herbicides 2,4-D and 2,4-DB. Dicamba is rapidly taken up by the leaves and roots of plants, and it is readily translocated to other plant parts, where it produces rapid abnormal cell growth in plants (*3*). In some plant species, dicamba accumulates in the tips of mature leaves, and as a result, desirable broad-leaf plants such as fruit trees and tomatoes may be harmed during these growth and development stages.

In water and soil, the main route of dicamba disappearance is microbial degradation and the main degradation product on soil is 3,6-dichlorosalicyclic acid (3,6-DCSA) (*4*).

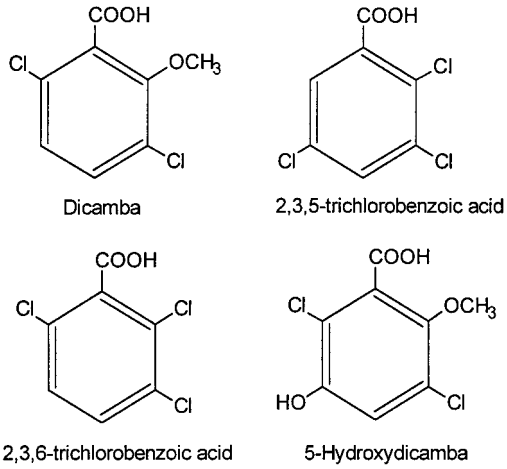

Figure 1. Structures of dicamba, 5-OH-dicamba, and selected chlorinated benzoic acids used in the synthesis of immunogens and cross-reactivity studies.

Dicamba is moderately persistent in soil with a variable half-life ranging from 10 days (*5*) to 30 days (*6*). In wetland and hardwood forest soils, dicamba residues were observed for up to 80 days (*7*). Dicamba degradation is influenced by many factors, including soil moisture content, pH, temperature, organic matter, and application rates (*8*, *9*). Dicamba is highly volatile, and special precautions must be followed when the herbicide is applied in the field. Dicamba is highly soluble in water, and as an acid with a pK_a of 1.95, it is highly mobile in the soil (*10*) and may contaminate water supplies (*11*, *12*). As a result dicamba has been detected in groundwater, farm ponds, and streams (*13*−*15*).

Currently, dicamba is registered under the Pesticide Control Products Act in Canada as a pre-emergent or

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postemergent treatment in the control of annual, biennial, and perennial broad-leaf weeds in various crops including field corn, wheat, barley, rye, and oats. Dicamba is also used in noncrop areas such as roadsides, utility rights-of-way, railways, and turfgrass (*11*).

The Canadian Food and Drugs Act and Regulations administered by Health Canada indicate that there are no tolerances established for dicamba. As a result, the maximum residue limit (MRL) permitted on crops is considered to be the negligible level of 0.1 ppm (*16*). The Canadian Drinking Water standard has been established at 120 μg L^{-1} (ppb), whereas the maximum contaminant level (MCL) for safe drinking water in the United States is 13.0 μg L^{-1} (ppb).

Early work on the analysis of samples of chlorophenoxy and benzoic acid herbicides involved solvent extraction with a subsequent derivatization or esterification (*17*). Perhaps the two most common methods of analyzing samples for dicamba and phenoxy herbicides are gas−liquid chromatography (GLC) and high-pressure liquid chromatography (HPLC).

The established method of dicamba analysis involves extraction of the herbicide residues from the matrix using solvents, sample concentration, derivatization, and sample cleanup, all followed by analysis by GLC and HPLC. Although these methods involve similar extraction procedures, the final determination of residues by gas chromatography (GC) is facilitated using a variety of different detectors such as electron capture, electrolytic conductivity, and mass selective detectors (*18*). Both GLC and HPLC methods remain very tedious and are considered time-consuming and difficult with poor and inconsistent recoveries. The continuing challenge for the analytical chemist involves ensuring the accuracy of the analytical method with an emphasis on keeping the method as uncomplicated as possible. As an alternative to determination by GLC, a number of methods have been published using HPLC. The HPLC methods have made use of amino-bonded solid-phase extraction columns (SPE) and are useful for the determination of dicamba in water and soil samples (*19*−*21*). GC methods have been developed that are very sensitive (*22*) and provide the analytical chemist with multiresidue capabilities (*23*).

The enzyme-linked immunosorbent assay (ELISA) is recognized as a valuable tool in residue analysis and complements conventional analytical methods (*24*, *25*).

ELISA provides rapid sample testing and accurate results and is more cost-effective than conventional chromatographic analysis (*26*). The sensitivity and specificity of the technique have made it useful in a variety of projects such as fate and persistence studies (*27*), environmental residue analysis (*24*), and worker exposure studies (*28*). ELISAs have been used successfully for the quantitative analysis of numerous pesticides in water matrices with little or no matrix interference (*29*−*32*). Two enzyme immunoassays were developed to screen for 2,4-D in water (*33*), and more recently a magnetic particle based ELISA has been developed for the quantitation of 2,4-D and related chlorophenoxy herbicides (*30*).

Currently, there is no immunochemical analytical method for the detection and quantitation of dicamba. In contrast to expensive and time-consuming HPLC and GLC methods, an ELISA would present a sensitive, cost-effective, and efficient method for analyzing environmental samples containing dicamba. In this paper, we report the quantitative performance of an indirect ELISA for dicamba detection and quantification in water.

MATERIALS AND METHODS

Chemicals and Instrumentation. The analytical standard of dicamba (Figure 1) was obtained from Chem. Service Inc. (West Chester, PA). Freund's incomplete adjuvant, 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid diammonium (ABTS) substrate tablets, *N*,*N*′-dicyclohexylcarbodiimide (DCC), and urea hydrogen peroxide were obtained from Sigma Chemical Co. (St. Louis, MO). Goat anti-rabbit−horseradish peroxidase (GARHRP) was purchased from Pierce Immunochemicals (Rockford, IL). Immulon 4 flat-bottom microtiter plates were purchased from Fisher Scientific (Don Mills, ON, Canada). ELISA plates were analyzed using a model 3550-UV microplate reader (Bio-Rad Laboratories, Richmond, CA). The C_{18} Chromosep cartridges were purchased from Chromatographic Specialties (Brockville, ON, Canada). The GC-MS system consisted of a Varian Saturn 4D GC-MS/MS mass spectrometer with a Varian 8300 autosampler. The chromatography column was a DB-1 capillary column (30 m length, 0.25 mm i.d., 0.25 μm thickness). All other chemicals were of reagent grade and obtained commercially.

Synthesis of Immunogens and Coating Conjugates (CC). The Fleeker method (*33*) was used to synthesize the immunogens and CC. Briefly, the dicamba bovine serum albumin (BSA) immunogen was prepared by dissolving the acid form of dicamba (0.019 mM) and 22 mg of *N*-hydroxysuccinimide (0.019 mM) in 2.0 mL of dioxane. To this was added 39 mg (0.019 mM) of DCC in 0.50 mL of dioxane, and the resultant solution was allowed to react overnight. At that time, 200 mg of BSA was dissolved in 3.0 mL of 0.15 M sodium borate (pH 9), added to the solution containing the herbicide, and allowed to react for 2 h at room temperature. The solution containing the DIC−BSA conjugate was dialyzed (Spectrapor 1; 6000−8000 MW cutoff) for 24 h at 4 °C against four 1-L changes of distilled water. The solution containing the conjugate was frozen in 500 μL aliquots. Two CCs of dicamba (DIC−PSA and DIC−OVA) were synthesized for the study by conjugating dicamba to porcine serum albumin (PSA) and ovalbumin (OVA), respectively. The synthetic route was similar to that used for the BSA immunogen described previously.

Polyclonal Antisera Production. For the immunogen, dicamba−BSA, a pair of New Zealand white rabbits were injected, intramuscularly, with 500 μg of immunogen in 1.0 mL of a 10 mM phosphate-buffered 15 mM NaC1 (PBS) pH 7.5/Freund's incomplete adjuvant (1:1 v/v) emulsion. The primary immunizations were repeated at weekly intervals for 3 weeks, and following a 4-week rest period, secondary immunizations (boosts) of immunogen (200 μg) in 1.0 mL of PBS/Freund's incomplete adjuvant (1:1 v/v) were injected intramuscularly every 3 weeks. The rabbits were bled monthly, and the serum was tested for anti-dicamba antibody titer 1 week after each secondary boost.

Antisera Titer Determination. Dicamba specific antisera titers were monitored as described by Campbell (*34*) and Gee et al. (*35*). Checkerboard binding studies between dicamba antiserum and dicamba CC were used to determine the optimal dilution of each. The optimal dilutions chosen were those that provided an absorbance of 1.0−1.2 at 405 nm, following an incubation of 30 min at room temperature (22 °C).

Standard Curve and Sample Analysis. The ELISA tests were performed as follows. Immulon 4 flat-bottom plates were coated with CC diluted 1/1000 in PBS (0.2 mg mL^{-1}; 100 μL $well^{-1}$) and allowed to incubate overnight at 4 °C. The plates were washed three times with PBS containing 0.05% (v/v) Tween 20 (PBS-Tween) and patted dry on paper towels. Sites not containing CC were blocked by adding 200 μL $well^{-1}$ of 0.01% gelatin in water (w/v). After 20 min, the plates were washed and dried as previously described. Anti-dicamba antibody was diluted 1/100 with PBS (optimal predilution).

Following this dilution, dicamba standards or samples containing dicamba were prepared in PBS. The antisera and sample or standard solutions were mixed 1:1 (v/v) and allowed to incubate in test tubes for 60 min. The final antisera dilution (optimal dilution) was 1/200. The preincubated mixtures were then transferred to the plates (100 μL well^{-1}). The plates were incubated in the dark for another 60 min at 22 °C before being washed with PBS-Tween and dried. Secondary antibody GARHRP was diluted 1/5000 in PBS, and 100 μL was added to each well and allowed to incubate at 22 °C for 60 min. The wells were washed as described earlier prior to the addition of 100 μL of substrate (1 mg mL^{-1} ABTS) and 1.0 mg mL^{-1} urea hydrogen peroxide in 10 mM citric acid/10 mM sodium phosphate (citrate buffer, pH 9.0). The reaction proceeded for 30 min and was stopped with 0.5 M citric acid (100 μL well^{-1}).

Standard Curve. The absorbance (A) was measured at 405 nm and was inversely proportional to the concentration of dicamba in the standards and samples. Relative absorbance was calculated using the formula A/A_0, where A_0 is the absorbance of the well in which the antibody was not challenged with dicamba. The standard curves were constructed by plotting A/A_0 values against the log values of the dicamba concentration. A commercial computerized graphing program (SigmaPlot) was used for data analysis and presentation. Dicamba concentrations in the water samples were interpolated from the standard curve.

Cross-Reactivity. A variety of agrochemicals were tested for cross-reactivity to the dicamba antisera. The 5-hydroxy (OH) metabolite of dicamba and a number of other structurally related chlorinated benzoic acid molecules were also tested for their cross-reactivity. A 1000 μg mL^{-1} (ppm) standard of each chemical was prepared using distilled water and tested against the dicamba antibodies.

Gas Chromatography—Mass Spectrometry (GC-MS). Dicamba and phenoxy herbicides are separated and detected by GLC using a capillary column. After this GC separation, the residues of dicamba and the internal standard (2,4,5-TP) were determined by reconstruction of selected ions 203 and 196, respectively. The analytical results for dicamba in fortified samples (distilled water) were standardized on the basis of a single value of the internal standard (2,4,5-TP) prior to quantitation. Water samples were filtered through 0.45 μm nylon filters and stored refrigerated until analyzed. A volume of 1.0 μL of standard and/or sample extract was injected into the GLC, which was equipped with a DB-1 capillary column (30 m length, 0.25 mm i.d., 0.25 μm thickness) with a helium carrier gas pressure of 12 psi and a flow rate of 1.0 mL min^{-1}. The inlet temperature was 90 °C, and the detector was 280 °C. A temperature gradient was used starting at 90 °C for 1.0 min, increased to 150 °C at 20 °C min^{-1}, and then increased to 280 °C at a rate of 5 °C min^{-1}. The final temperature was held for 5 min. Peak heights of reconstructed ion chromatograms of the standards were plotted against the concentration of dicamba, and the resulting standard curve was used to interpolate dicamba concentrations in the water samples.

C_{18} Column Concentration. A simple concentration step was devised using a Chromosep C_{18} chromatography column. The column consisted of 200 mg of C_{18} packing in a disposable 3.0 mL cartridge. The column was prewashed with 2 × 5 mL of methanol followed by washes (2 × 5 mL) with acidified water at pH 2. A 10.0 mL aliquot of each sample and/or fortified (distilled) water was put on the top of each of column and was allowed to filter through the column by gravity, ensuring that the column never went dry during elution. Once the elution was complete, each of the columns was put under vacuum and dried for 15 min. A volume of 1.0 mL of methanol was used to elute the samples. This represented a 10× concentration factor for each sample (10.0 mL sample in a 1.0 mL final volume). Due to the sensitivity of the GC method, the sample extracts were diluted prior to analysis.

RESULTS AND DISCUSSION

Generation of Antibodies. Dicamba is considered to be a small molecule (MW= 221.04 amu) and as such is not immunogenic. To render small molecules such as this immunogenic, it is normal practice to conjugate them to larger protein carriers such as BSA. The classical conjugation or linkages to proteins are via free primary amines or carboxylic acid functionalities. Examination of the structure of dicamba indicated a free carboxylic acid; hence, the conjugation of dicamba to the protein was directed through the carboxylic functional group.

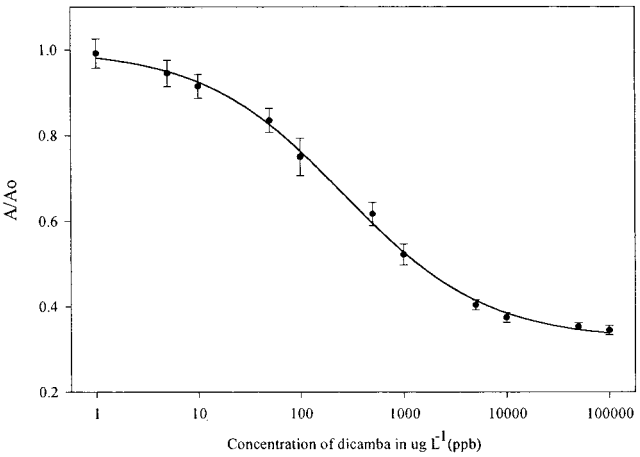

Figure 2. Plot of dicamba concentration in micrograms per liter (ppb) versus the normalized absorbance. The line presented is fit to a logistic four-parameter regression and has an $r^2 = 0.9983$.

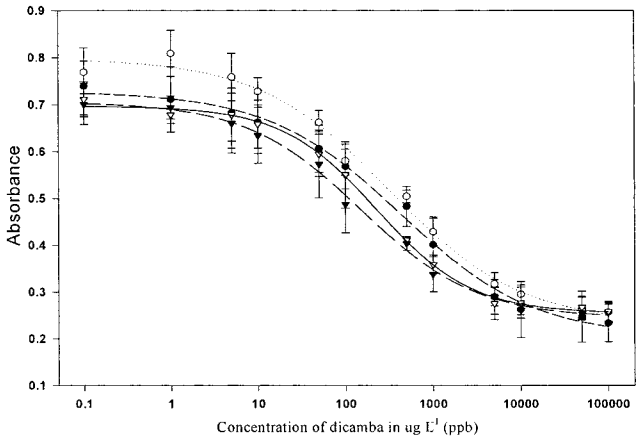

Figure 3. Effect of CC dilutions on the sensitivity of inhibition of the dicamba ELISA. Corresponding concentrations and r^2 are (●, short dash) CC 1/1000, $r^2 = 0.9954$; (○, dotted) CC 1/2000, $r^2 = 0.9938$; (▼, long dash) CC 1/5000, $r^2 = 0.9968$; (▽, solid) CC 1/10000, $r^2 = 0.9983$ for each curve. Overall $r^2 = 0.9982$.

ELISA Optimization. Dicamba specific antisera titers were monitored as described by Campbell (*34*) and Gee et al. (*35*). The checkerboard binding studies between dicamba antiserum and dicamba CCs were used to determine the optimal dilution of each. The optimal dilutions chosen were those that provided an absorbance of 1.0−1.2 at 405 nm, following a 30 min incubation at room temperature (22 °C). A representative inhibition curve for dicamba is shown in Figure 2. Initially, the checkerboards indicated that dilutions of 1/200 and 1/1000 were suitable for sera and CCs, respectively. The effects of CC concentrations can be seen in Figure 3. It is evident from Figure 3 that other CC concentrations may have been examined, but the 1/1000 CC was chosen for this study. As observed in a number of immunoassays for other pesticides (*36*−*38*)

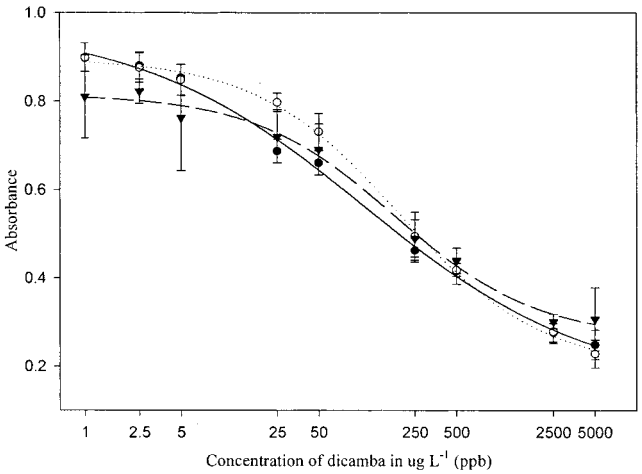

Figure 4. Effect of incubation temperature of the CC step in the assay with polyclonal dicamba antibodies. Temperatures were 37 °C (●, solid), 22 °C (room temperature; ○, dotted), and 4 °C (refrigerated, ▼, dash). The r^2 values are 0.9984, 0.9969, and 0.9855, respectively.

the dicamba assay was sensitive to matrix effects such as pH and solvent concentration of the final sample solutions. The optimum pH for the assay was determined to be 7.5, and the assay was found to tolerate solvent (methanol) concentration as high as 5%. During the assay, the plates are routinely coated with the CC and incubated overnight at 4 °C. The length of time required to complete the immunoassay would be reduced if the coating step could be performed just prior to blocking of the plates with gelatin. The incubations times and temperatures of this step were varied, and the effects of a 4 h incubation at 22 and 37 °C compared to the overnight incubation at 4 °C are presented in Figure 4. Because no dramatic improvement was noted, the overnight incubation at 4 °C was used for the assay.

This assay is the first polyclonal-based immunoassay reported for a benzoic acid herbicide and is sensitive in the 10−10000 μg L^{-1} range. A similar sensitivity range (5−5000 μg L^{-1}) has been reported for another polyclonal-based assay for the acidic herbicide picloram (*39*). The dicamba hapten conjugate was linked to a carboxylic acid substituent, whereas the picloram hapten conjugate was linked to a pyridinecarboxylic acid. Both carboxylic acid groups are adjacent to the ring structure and demonstrate that the sensitivity of an assay is dependent upon the structure of the hapten conjugate and that it is important to preserve structural similarities between the major functional groups which characterize the pesticide chemical family.

The overall immunoassay performance can also be altered by factors that include the addition of immunogen spacer groups and type of CC and assay format (*40*, *41*). More sensitive assays based upon polyclonal antibodies (low parts per billion range) have been reported for triazine herbicides, atrazine (*42*), and atrazine metabolites (*43*), and the observed increase in sensitivity may be the result of the introduction of a spacer between the parent herbicide moiety and the protein carrier. Sensitive assays based upon polyclonal antibodies have also been reported for the "chloroacetanilide" herbicides, alachlor (*44*), at a sensitivity of 0.2−8.0 μg L^{-1}, and metolachlor, amidochlor, and butachlor (*45*), at sensitivities of <10 μg L^{-1} (ppb). The chloroacetanilde immunoassays also have spacer arms in the hapten conjugates, and further studies are warranted with dicamba immunogens to determine if the sensitivity of the assay is affected by the addition of spacer arms in the hapten conjugate.

The introduction of phage display technology along with the development of recombinant antibody technology has resulted in assays being developed for environmental contaminants such as diuron (*46*), atrazine (*47*), parathion (*48*), *s*-triazines (*49*), cyclohexanediones (*50*), and dioxin (*51*). The difficulties involved in the development of an assay that is extremely sensitive to dicamba may be overcome by use of these technologies. Recently, these "third-generation" antibodies have been shown to be useful for the development of assays for the herbicide picloram; this assay was demonstrated to be as sensitive as assays derived from original monoclonal antibodies (*52*). Charlton et al. (*53*) estimate that sensitivities of detection of the immunochemical assays may be increased by 10−100-fold if recombinant antibodies are used.

Cross-Reactivity. In the determination of the selectivity of the assay, it is important to examine the potential for inhibition of dicamba antibodies by structurally related compounds, metabolites, and/or other agrochemicals. Degradation studies of dicamba have shown that 5-OH-dicamba is a minor metabolite in soil (*6*) and plants (*8*). This metabolite was tested for cross-reactivity as were the other structurally related herbicides clopyralid, picloram, triclopyr, chloramben, chlorfenac, and dicloran (Figure 5). The antibodies produced were specific for dicamba, and there was cross-reactivity observed for the 5-OH-dicamba. The percent cross-reactivity was 9.3% with an IC_{50} value of 2096 μg L^{-1}. No cross-reactivity was found for structurally related herbicides and the other agricultural chemicals alachlor, metolachlor, metribuzin, cyanazine, tebuthiuron, simazine, prometryn, bromacil, fusilade, 2,4-D, 2,4-DP, 2,4-DB, PCP, MCPP, MCPB, MCPA, 2,4,5-TP, 2,3,6-TBA, monuron, chloroxuron, nitrofen, clopyralid, picloram, triclopyr, chloramben, chlorfenac, and dicloran. A variety of chlorinated benzoic acids (mono-, di-, tri-, tetra-, and pentachlorinated acids) were also tested for cross-reactivity. The 2,3,5- and 2,3,6-trichlorobenzoic acids were determined to be cross-reactive at 8.4 and 12.8%, respectively, with corresponding IC_{50} values of 2321 and 1525 μg L^{-1}. The 5-OH-dicamba is considered to be a minor metabolite of dicamba with no phytotoxicity and therefore does not pose an agronomic problem to rotational crops. The two chlorobenzoic acids are not metabolites of dicamba and, therefore, are not likely to be found in environmental samples.

Fortified Water Samples. The linear working range of the ELISA was 10−10000 μg L^{-1} (Figure 2). The utility of the dicamba ELISA assay was tested by a comparison of analytical results obtained using a GC method. This method used a Saturn 4D GC-MS system, and prior to GC analysis each water sample was concentrated 10 times (10.0 mL sample in 1.0 mL final volume), evaporated to incipient dryness, and methylated with diazomethane. The methylated extracts were then analyzed by GC-MS, and the recovery of dicamba from fortified water samples is presented in Table 1 along with ELISA results. The GC-MS method was linear from 0.1 to 10 μg L^{-1} and had a correlation coefficient of $r^2 = 0.983$. The highest fortification level of 100 μg L^{-1} was above the linear working range of the mass spectrometer and was considered to be too high for accurate quantitation. Any sample found to contain

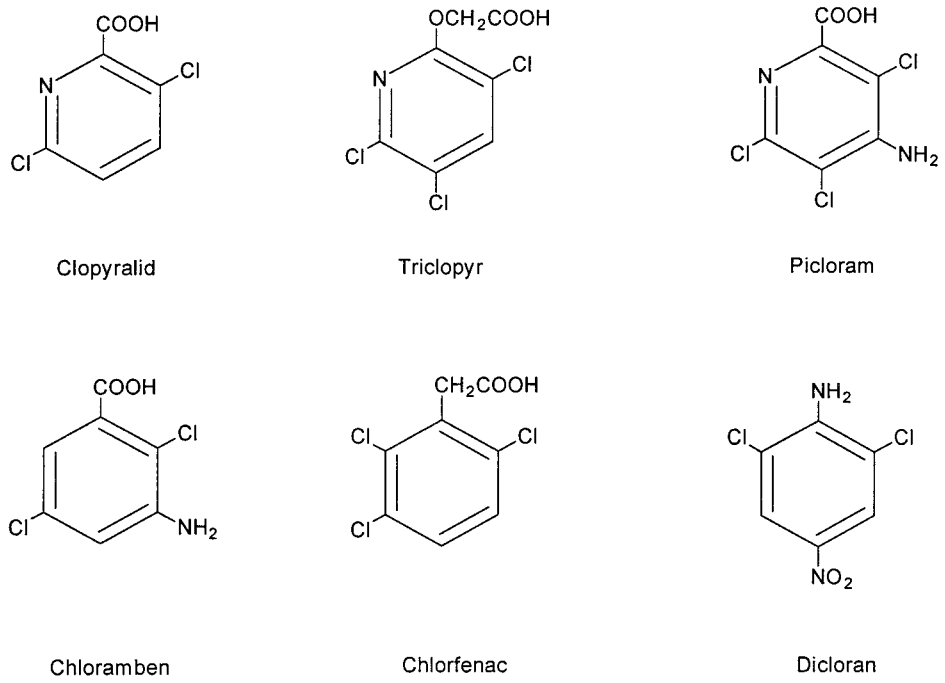

Figure 5. Series of selected agrochemicals and chlorinated herbicides structurally related to dicamba tested for cross-reactivity to polyclonal dicamba sera.

Table 1. Recovery of Dicamba from Fortified Water Samples Determined by Enzyme Immunoassay Using Polyclonal-Based ELISA and GC-MS

dicamba added (μg L^{-1})	mean[a] (μg L^{-1})	SD (μg L^{-1})	coeffiicient of variation (%) (replicates)
	ELISA		
0.1	84.1 (1.0)	2.2	2.6 (4)
1.0	21.7 (10.0)	0.33	1.5 (4)
5.0	51.2 (50)	0.89	1.8 (4)
10.0	126 (100)	1.6	1.3 (4)
25	181 (250)	2.2	1.2 (4)
50	406 (500)	3.9	0.96 (4)
100	966 (1000)	14.8	1.5 (4)
200	1751 (2000)	24.5	1.4 (4)
	GC-MS		
0.1	0.158	0.012	7.6 (3)
0.5	0.520	0.051	9.8 (3)
1.0	1.13	0.089	7.9 (3)
5.0	4.77	0.196	4.1 (3)
10.0	7.4	0.185	2.5 (3)
100	28.8	0.979	3.4 (3)
	105[b]	5.504	5.2 (3)

[a] Levels in parentheses are expected amounts after concentration step. [b] Diluted and reanalyzed.

dicamba at these levels would routinely be diluted and reanalyzed. As such these samples were diluted and reanalyzed, and the results are included in Table 1. Because the linear working range of the dicamba ELISA assay was 10−10000 μg L^{-1}, a concentration step was required to achieve detection limits similar to that of the GC-MS method. A 10.0 mL sample of water was concentrated using C_{18} columns. The final sample volume was 1.0 mL, and this represented a 10× concentration factor for each sample (i.e., 10.0 mL sample in a 1.0 mL final volume). The use of the concentration step allowed dicamba residues to be determined at or below quantities specified (120 μg L^{-1}) by Health and Welfare Canada (*54*) and the 13.0 μg L^{-1} (ppb) safe drinking water level in United States. The results obtained for waters fortified at levels of 0.1−200 μg L^{-1} (ppb) are presented in Table 1. A plot of the

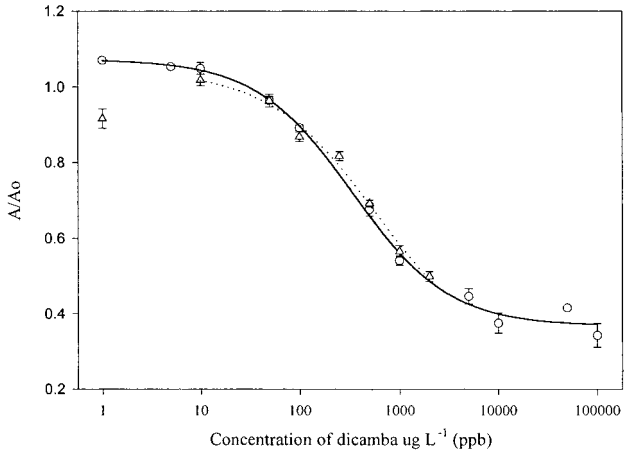

Figure 6. Plots of recovered dicamba after Sep-Pak concentration (△, dotted) compared to standard concentrations of dicamba in buffer (○, solid).

recovered dicamba is shown with a standard curve for dicamba in buffer in Figure 6. As indicated by the data in Table 1, a high recovery was observed for the fortification level of 0.1 μg L^{-1} (ppb), which is quantitated from the standard curve as 10 μg L^{-1} (ppb). Therefore, the linear range for quantitation following a 10-fold concentration step is 5−200 μg L^{-1} (ppb). Correlation of GC-MS and ELISA estimates was r^2 = 0.9991, and these results indicate a good agreement between the two analytical methods over the range of 1−100 μg L^{-1} (ppb). The slope of the line (1.33) was not equal to 1, indicating that the dicamba values determined by ELISA were greater than those obtained by the GC-MS method. The most likely explanation for this may be a matrix effect introduced by concentration of the water. The limit of detection (LOD) of the ELISA was determined to be 2.3 μg L^{-1} and was calculated according to methods used by the American Chemical Society (*55*). The LOD was calculated by taking the absorbance of the positive control and subtracting 3 times the standard deviation (SD) of the positive control.

The limit of quantitation (LOQ) was calculated in a similar fashion using 10 times the SD and was determined to be 11.6 μg L^{-1}. A reliable detection limit (RDL) was determined to be 4.6 μg L^{-1} using 6 times the SD. The IC_{50} (the concentration of dicamba required for 50% inhibition of the absorbance of the positive control) was 195 μg L^{-1}. If standards are made up in sample matrix rather than PBS buffer and run along with normal samples, the matrix effect can be eliminated or at least compensated. A variety of techniques are available that would be useful in improving the detection level and/or the linear working range of the immunoassay. Immunoaffinity chromatography is a technique that has been used favorably in some applications to increase sensitivity and has been shown to be useful for improving the sensitivity of polyclonal-based assays (*56*). It is quite likely that this technique may be useful here and would maximize the sensitivity of the assay. The ELISA assay was more rapid than the GC-MS method in that many samples could be analyzed on a single plate. As many as 40 samples (in duplicate) can be analyzed simultaneously by ELISA, whereas each single GC-MS analysis requires a minimum of 30 min per sample with a total analysis time of >20 h.

Conclusions. The dicamba CI-ELISA is suitable for the accurate determination of dicamba concentrations in the range of 10−10000 μg L^{-1} (ppb) without sample concentration. A simple concentration step using C_{18} cartridges improved the assay and allowed the concentration range to be decreased to 5−200 μg L^{-1} (ppb). The assay was sensitive to pH and organic solvents. The assay was found to be cross-reactive to several compounds: 5-OH-dicamba and 2,3,4- and 2,3,6-trichlorobenzoic acids. All other agrochemicals, related herbicides, and other chlorinated benzoic acids tested in the assay showed no cross-reactivity. The immunoassay showed a good correlation with conventional GC-MS with a correlation coefficient, r^2, equal to 0.9991. The assay provides a more rapid sample analysis, thereby increasing sample throughput. Less time is required for sample preparation, extraction, concentration, and analysis. The necessity for high-end expensive analytical equipment and highly trained personnel decreases and, as a result, overall laboratory costs as well as costs per sample are reduced.

LITERATURE CITED

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