Comparison of Extraction/Hydrolysis Procedures for the Determination of Acidic Herbicides in Plants: Residues of Mecoprop in Barley following Postemergence Application

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Two extraction/hydrolysis procedures for the determination of acidic herbicides in plant tissue were compared for the analysis of mecoprop residues in barley tissue. It was found that organic solvent extraction followed by alkaline hydrolysis was less tedious and time-consuming and provided better recoveries than an extended alkaline extraction/hydrolysis. Following a postemergence application of mecoprop at 1.1 kg ha^{-1} to barley at the 5-leaf stage, initial residues were on the order of 100 mg kg^{-1}. Six weeks after application, whole plant (above ground) residues had decreased to 0.1–0.2 mg kg^{-1}. At maturity, residues in the straw were <0.1 mg kg^{-1}, and residues, at the limit of quantification of 0.05 mg kg^{-1}, were not detected in the seed.

INTRODUCTION

Acidic herbicides, such as mecoprop [(±)-2-(4-chloro-2-methylphenoxy)propanoic acid; Chow et al., 1971], 2,4-D [(2,4-dichlorophenoxy)acetic acid; Feung et al., 1973], dichlorprop [(±)-2-(2,4-dichlorophenoxy)propanoic acid; Løkke, 1975], diclofop [(±)-2-[4-(2,4-dichlorophenoxy)-phenoxy]propanoic acid; Jacobson and Shimabukuro, 1984], picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid; Eliasson and Hallmén, 1973), and benzoylprop [*N*-benzoyl-*N*-(3,4-dichlorophenyl)-DL-alanine; Beynon et al., 1974], are known to form conjugates with plant substituents. The site of conjugation is the carboxyl moiety and generally involves the formation of an amido linkage by reaction with amino acids/proteins and/or an ester linkage by reaction with sugars. Both linkages are susceptible to hydrolysis, and Chow et al. (1971) and Løkke (1975) have shown that acidic herbicides are most effectively released from plant tissues when the extraction includes a hydrolytic step.

In several residue studies, the analytical method has included a hydrolytic step to ensure more accurate quantitation of the parent herbicide. Acid, base, and enzymatic hydrolysis have been used; however, enzymatic hydrolysis has been used more extensively in herbicide metabolism studies. With acid and base hydrolysis, two strategies have been employed by analysts when incorporating the hydrolytic step. In one approach, the plant tissue is subjected to extended hydrolysis followed by partitioning of the "free" acidic herbicide into an organic phase (Bjerke et al., 1967; Cessna, 1980; Bristol et al., 1982; Frank et al., 1983; Galoux et al., 1983; Smith, 1984; Smith et al., 1986; Steinwandter, 1989). The other approach involves extracting the free acidic herbicide plus any conjugates directly into an organic phase and then effecting hydrolysis (Hamilton et al., 1971; Buckland et al., 1973; Feung et al., 1973; Beynon et al., 1974; Shimabukuro et al., 1979; Eronen et al., 1979; Scheel and Sandermann, 1981).

The objective of the present study was to compare the effectiveness and convenience of these two strategies by using both approaches to analyze green tissue and mature straw and seed of barley following postemergent field treatment with mecoprop at 1.1 kg ha^{-1}.

EXPERIMENTAL PROCEDURES

Sample Collection. Samples for residue analysis were collected on the Agriculture Canada Experimental Farm at Indian Head, SK. The check and herbicide treatments were replicated four times in a randomized complete block design. Mecoprop [formulated as an amine salt (mecoprop liquid herbicide; Ciba-Geigy Canada Ltd., Mississauga, ON) at 150 g of mecoprop acid equivalent (ae) L^{-1}], at 1.12 kg ha^{-1}, was applied to 0.9-m × 5.6-m plots of Bonanza barley on June 21, 1982, when the crop was in the 5-leaf stage. The application was made in 120 L of water ha^{-1} at 207 kPa with a compressed air operated bicycle-type small plot sprayer. Composite samples for residue analysis, consisting of 12 subsamples per plot, were collected at the following times after application: 30 min, 3 and 6 weeks, and maturity (8 weeks), that is, when the crop would normally be swathed. Subsamples were collected randomly from each plot and consisted of 15 (day of spraying, 3 weeks), 7.5 (6 weeks), or 30 cm (maturity) of a single row. Green tissue samples were collected directly into polyethylene freezer bags and immediately stored at −10 °C. These samples were then chopped in a food chopper and stored at −10 °C in polyethylene bags until extraction. The mature samples were collected directly into cotton bags and allowed to dry at room temperature prior to separation into straw/chaff and seed fractions using a laboratory thresher. These fractions were then milled through a 1-mm screen and stored until analysis as described above.

Chemicals. All solvents were distilled-in-glass grade (BDH Inc.). Florisil (150–250 μm) was heated at 600 °C for 48 h, cooled, and then deactivated by the addition of 5% water (w/w). Sodium sulfate was heated at 600 °C for 24 h. The extraction solvent was prepared as an ethanol/water (80/20 v/v) solution. The mecoprop analytical standard was obtained from Ciba-Geigy Canada.

Preparation of Mecoprop Methyl Ester. Mecoprop (15 g, 0.07 mol) and boron trifluoride/methanol reagent (50 mL, 14% by weight) were heated at 80 °C for 1 h in a 100-mL round-bottom flask equipped with a reflux condenser and drying tube. After cooling by immersing the round-bottom flask in an ice–water bath, the reaction mixture was poured into a 250-mL separatory funnel containing 100 mL of saturated NaCl solution and the resulting mixture was extracted twice with 100-mL portions of hexane. The hexane extracts, combined in a 250-mL separatory funnel, were washed twice with 50 mL of water and then concentrated using a rotary evaporator. The resulting residue was vacuum distilled to yield mecoprop methyl ester (bp 91.8 °C at 3.0 mmHg).

Organic Solvent Extraction with Subsequent Hydrolysis. (*a*) *Green Samples.* Chopped barley tissue (25 g) was blended at high speed in a 250-mL stainless steel blender jar with 100 mL of extraction solvent (ethanol/water, 80/20 v/v) for 5 min. The mixture was filtered under reduced pressure through a Büchner funnel equipped with a glass fiber filter paper and the filter cake washed with 100 mL of extraction solvent. The

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combined filtrates were then taken to volume (200 mL) with extraction solvent.

The plant extract (40 mL; equivalent to 5 g of plant tissue) was transferred to a 250-mL round-bottom flask containing 1 mL of 1 N NaOH solution and a few glass beads and concentrated to an aqueous residue (approximately 10 mL) using a rotary evaporator (water bath 40 °C). The aqueous residue was transferred to a 125-mL Erlenmeyer flask followed by two 20-mL 0.1 N NaOH solution rinses of the 250-mL round-bottom flask. The basic extract was then heated at 80 °C (water bath) for 30 min. After the extract was cooled by immersing the flask in an ice–water bath, saturated NaCl solution (25 mL) was added and the pH was lowered to approximately 1 using 6 N H_2SO_4. The acidified mixture was transferred to a 125-mL separatory funnel and extracted twice with 50-mL portions of diethyl ether. The ether extracts, combined in a 250-mL separatory funnel, were partitioned twice with 25-mL portions of 4% $NaHCO_3$ solution. The combined $NaHCO_3$ extracts were acidified to pH 1 by the careful addition of 6 N H_2SO_4 solution and then extracted twice with 25-mL portions of chloroform. Each chloroform extract was passed through 30 mL of anhydrous sodium sulfate (contained in a 9 cm diameter long-stemmed funnel on top of a glass wook plug) into a 100-mL round-bottom flask containing 10 mL of acetone, and these were followed by a 25-mL chloroform wash of the sodium sulfate. The combined chloroform extracts were then taken just to dryness using a rotary evaporator (water bath 40 °C).

(*b*) *Seed and Straw.* Milled seed or straw (10 g) and 100 mL of extraction solvent were blended and filtered as described for the green tissue. The total filtrate was transferred to a 1-L round-bottom flask containing 5 mL of 1 N NaOH solution and the ethanol removed using a rotary evaporator (water bath 40 °C). The aqueous residue (approximately 45 mL), along with two 15-mL 0.1 N NaOH solution rinses of the 1-L round-bottom flask, was transferred to a 250-mL Erlenmeyer flask. The sample workup was then continued as described for the green tissue commencing with heating the basic extract, except that 50 mL of saturated NaCl solution was used prior to acidification and diethyl ether extraction.

Extended Hydrolysis/Extraction. (*a*) *Green Tissue.* Chopped barley tissue (25 g) in 125 mL of aqueous 0.1 N NaOH was blended at high speed in a 250-mL stainless steel blender jar for 5 min. The contents of the blender jar were transferred to a 500-mL Erlenmeyer flask, followed by a 15-mL 0.1 N NaOH solution rinse of the blender jar, and then heated at 80 °C (water bath) with mechanical stirring for 30 min. After cooling by immersing the flask in an ice–water bath (with stirring), the mixture was centrifuged at 2000*g* for 5 min and the alkaline extract decanted into a 250-mL volumetric flask. Sodium hydroxide solution (0.1 N, 85 mL) was added to the plant tissue residue and the mixture shaken vigorously prior to centrifuging and decanting as before. The combined alkaline extracts were taken to volume (250 mL) with water.

The alkaline extract (50 mL, equivalent to 5 g of plant tissue) was added to 25 mL of saturated NaCl solution in a 100-mL beaker and the pH lowered to approximately 5 by the addition of 6 N H_2SO_4. After standing for about 15 min, the mixture was centrifuged at 2000*g* for 5 min and the pH of the decantate lowered to approximately 1 by the addition of 6 N H_2SO_4. The acidified mixture was transferred to a 125-mL separatory funnel and the sample workup continued as described above.

(*b*) *Straw.* Milled straw (10 g) was similarly blended in 150 mL of 0.1 N NaOH solution and the sample workup continued as described above for the green tissue. The alkaline extract (50 mL) was equivalent to 2 g of plant tissue.

(*c*) *Seed.* Milled seed (10 g) was similarly blended and heated in 225 mL of 0.1 N NaOH solution. The cooled mixture was transferred to a 250-mL volumetric flask and taken to volume with 0.1 N NaOH solution. The pH of the alkaline mixture (50 mL, equivalent to 2 g of plant tissue) was similarly adjusted to approximately 5, and then the mixture, divided among three 25-mL centrifuge tubes, was centrifuged at 35000*g* for 10 min and decanted. Water (8 mL) was added to each tube, the plant material was resuspended, and the mixtures were centrifuged and decanted as before. The pH of the combined decantates was lowered to approximately 1 by the addition of 6 N H_2SO_4 and the sample workup continued as described above.

Boron Trifluoride–Methanol Methylation. The extract residue resulting from either extraction procedure was transferred to a 20-mm i.d. × 150-mm test tube (19/26 joint) with two 1.5-mL methanol rinses of the 100-mL round-bottom flask, and boron trifluoride–methanol reagent (3 mL; 14% by weight) was added. The test tube was tightly stoppered and placed for 30 min in an aluminum block dry bath at 70 °C. After cooling the reaction mixture by immersing the test tube in an ice–water bath, hexane (10.0 mL), followed by 10 mL of saturated NaCl solution, was added. The test tube was tightly stoppered and shaken vigorously for 1 min. After separation of the organic and aqueous layers, the hexane layer was decanted into a second test tube containing 1–2 mL of anhydrous sodium sulfate and the test tube stoppered.

Florisil Column Cleanup. Florisil (4 mL) was added to a 10-mm i.d. × 200-mm column containing 10 mL of hexane and then topped with 1 cm of anhydrous sodium sulfate. A 5.0-mL aliquot of the hexane extract (equivalent to 2.5 g of green tissue or 5.0 g of seed or straw) was transferred to the Florisil column and the column eluted with 40 mL of 0.5% acetone in hexane, the last 30 mL of which were collected and concentrated using a rotary evaporator (water bath 40 °C) prior to being taken to an appropriate volume with hexane for gas chromatographic analysis.

Gas Chromatography. A Tracor Model 560 gas chromatograph, equipped with a Model 700A Hall electrolytic conductivity detector operated in the halogen mode, was used with a Varian Vista 400 data station. A 1.8-m × 4-mm i.d. coiled glass column, packed with 5% Dexsil 300 on 150–180-μm Chromosorb W, HP, was used under the following conditions: helium (carrier gas), 35 mL min^{-1}; injector, 220 °C; column, 210 °C; furnace base, 250 °C; furnace, 910 °C; reaction gas (hydrogen), 70 mL min^{-1}; conductivity solvent (1-propanol), 0.6 mL min^{-1}; vent time, 0.75 min. Under these conditions, mecoprop methyl ester had a retention time of 3.3 min. The methyl ester was quantitated from a calibration curve based on area count response. The electrolytic conductivity detector provided a linear response over the range 0.4–40 ng of mecoprop methyl ester using an injection size of 4 μL.

Fortification Experiments. Recoveries of mecoprop were determined by the extraction of green tissue and seed fortified at 0.1 and 0.05 mg kg^{-1}. Mecoprop in 2.5 (2.5 and 1.25 μg) and 1.0 mL (1.0 and 0.5 μg) of methanol was added to 25 g of chopped untreated green tissue and 10 g of milled untreated seed, respectively, in 100-mm i.d. × 80-mm glass storage dishes. The storage dishes were placed in a fumehood until the methanol had evaporated and, with the lids on, maintained in the dark at −10 °C until extraction. Extractions were carried out at 0.5, 24, 48, and 96 h after fortification. Four replicates of each substrate at both fortification levels were analyzed using each of the extraction procedures.

RESULTS AND DISCUSSION

The necessity of a hydrolytic step in residue analysis methods for the determination of acidic herbicides, such as the chlorophenoxy acids, in plants has been demonstrated by Chow et al. (1971) and Løkke (1975). However, how the hydrolytic step is incorporated into an analytical method may affect the convenience and time requirements of the method, as well as its effectiveness and/or reliability in terms of herbicide residue determination. In the present study, a comparison of two extraction/hydrolysis procedures utilizing alkaline hydrolysis was made.

One extraction/hydrolysis procedure involved organic solvent extraction followed by hydrolysis. Briefly, the plant tissue was extracted with aqueous ethanol, the mixture filtered, and, after concentration to an aqueous residue, the extract hydrolyzed and then acidified prior to partitioning into diethyl ether. The other procedure involved an extended extraction/hydrolysis. In this case, the plant tissue was hydrolyzed, the mixture centrifuged, and the alkaline decantate acidified, centrifuged again,

Table I. Comparison of Percent Recoveries of Mecoprop from Fortified Barley Tissues Using Extended Hydrolysis/Extraction and Organic Solvent Extraction/Hydrolysis Procedures

tissue fortified	fortification level, mg kg^{-1}	% recoveries	mean ± SD
	Extended Hydrolysis/Extraction		
green	0.10	85, 69, 82, 70	77 ± 8
	0.05	78, 116, 77, 102	93 ± 19
seed	0.10	68, 52, 49, 52	58 ± 9
	0.05	53, 39, 43, 36	43 ± 7
	Organic Solvent Extraction/Hydrolysis		
green	0.10	86, 95, 89, 86	89 ± 4
	0.05	73, 80, 95, 95	86 ± 11
seed	0.10	102, 117, 94, 74	97 ± 18
	0.05	92, 110, 69, 89	90 ± 17

and then partitioned into ether. Subsequent partitioning, derivatization, and gas chromatographic quantitation steps were the same for both procedures.

Several differences in convenience between the two extraction/hydrolysis procedures were apparent. The filtration following aqueous ethanol extraction was less tedious and time-consuming than the centrifugation required after extended hydrolysis. This was especially apparent with the seed. The mixture resulting after extended hydrolysis was a viscous suspension that could not be effectively centrifuged until after acidification and then only at a high g value. Severe emulsions were also encountered during the diethyl ether partitioning of the resulting decantate. [Similar problems occurred with triticale (Cessna, 1990) and wheat (unpublished data) seed.] Such emulsions generally required centrifugation to effect partitioning.

Acidification of the alkaline extract resulting from extended alkaline hydrolysis resulted in the formation of a precipitate. Previous studies (unpublished data) using radiolabeled 2,4-D had indicated that the phenoxy herbicide was sorbed to this precipitate at a pH of 1 and was not completely removed during the diethyl ether extraction. Thus, to minimize such losses in the present study, the pH of the alkaline extract was initially lowered to pH 5 so that mecoprop remained in solution while the precipitate formed (pK_a = 3.1 for mecoprop; Cessna and Grover, 1978). The precipitate was then removed by centrifugation. Very little additional precipitate formed when the pH was subsequently lowered to 1. In contrast, although the aqueous ethanol extract had to be concentrated prior to hydrolysis, no such precipitate formed when the hydrolyzed aqueous ethanol extract was acidified to a pH of 1.

A major difference between the two extraction/hydrolysis procedures was lower mean recoveries of mecoprop from the seed with the extended extraction/hydrolysis procedure. Mean recoveries were 57.8 ± 8.8 and 42.7 ± 7.3% at the 0.1 and 0.05 mg kg^{-1} fortification levels, respectively, whereas analogous recoveries for the organic solvent extraction/hydrolysis procedure were on the order of 90% or greater (Table I). The low recoveries were most likely associated with the centrifugation and emulsion problems encountered with the former procedure.

Both extraction/hydrolysis strategies gave similar background interferences at the retention time for mecoprop methyl ester (Tables II and III) for all substrates. Green tissue check samples from the day of application showed elevated background interferences, and since there was only a 0.5-m separation between plots, these were most likely due to drift that occurred during spraying. Background interferences from the green tissue samples were on the order of 0.006 mg kg^{-1} and readily permitted quantitation at 0.05 mg kg^{-1}. Background interferences from the seed were higher (0.020 mg kg^{-1}), but reasonable standard deviation values were obtained at the same limit of quantification.

Table II. Residues of Mecoprop Determined in Barley following Postemergence Application Using the Extended Hydrolysis/Extraction Procedure

days after application	mg kg^{-1} rep 1	rep 2	rep 3	rep 4	mean ± SD
	Check Samples				
1[a]	0.02	0.01	0.01	0.01	0.01 ± 0.01
21	0.01	0.01	0.01	0.00	0.01 ± 0.01
42	0.00	0.00	0.00	0.02	0.01 ± 0.01
straw	0.02	0.01	0.01	0.01	0.01 ± 0.01
seed	0.02	0.02	0.01	0.03	0.02 ± 0.01
	Treated Samples				
1	59[b]	88	121	131	100 ± 33
21	0.78	1.40	0.86	0.62	0.92 ± 0.34
42	0.13	0.17	0.25	0.25	0.20 ± 0.06
straw	0.07	0.10	0.17	0.04	0.10 ± 0.06
seed	0.03	0.03	0.02	0.01	0.02 ± 0.01

[a] Day 1 = day of application. [b] Residue values for the treated samples have not had background interferences for the corresponding check samples subtracted and have not been corrected for recoveries.

Table III. Residues of Mecoprop Determined in Barley following Postemergence Application Using the Organic Solvent Extraction/Hydrolysis Procedure

days after application	mg kg^{-1} rep 1	rep 2	rep 3	rep 4	mean ± SD
	Check Samples				
1[a]	0.03	0.04	0.04	0.02	0.03 ± 0.01
21	0.01	0.01	0.01	0.00	0.01 ± 0.01
42	0.01	0.00	0.02	0.01	0.10 ± 0.01
straw	0.01	0.02	0.01	0.01	0.01 ± 0.01
seed	0.05	0.02	0.01	0.03	0.03 ± 0.02
	Treated Samples				
1	83[b]	118	115	136	113 ± 22
21	0.63	0.74	0.96	0.98	0.83 ± 0.17
42	0.11	0.10	0.26	0.17	0.16 ± 0.07
straw	0.03	0.07	0.12	0.03	0.06 ± 0.04
seed	0.02	0.01	0.01	0.01	0.01 ± 0.01

[a] Day 1 = day of application. [b] Residue values for the treated samples have not had background interferences for the corresponding check samples subtracted and have not been corrected for recoveries.

Residue values obtained for the treated plots by both extraction/hydrolysis procedures were also similar; however, standard deviation values for the extended extraction/hydrolyis procedure tended to be somewhat greater. Both extraction/hydrolysis procedures indicated that initial mean residues of mecoprop in the barley green tissues were on the order of 100 mg kg^{-1} (Tables II and III) and had decreased by 2 orders of magnitude 3 weeks after application. Growth dilution would have accounted for much of this decrease; however, rainfall washoff of mecoprop leaf deposits may have also occurred, primarily from a 22.4-mm rainfall on the third day (day 4) after application. (Lesser rainfalls of 0.4, 1.8, and 0.4 mm were also recorded on days 1, 2, and 3, respectively). Other processes may also have contributed to this rapid dissipation. For example, aqueous solutions of chlorophenoxy acids are readily photolyzed by sunlight (Cessna and Muir, 1991), and photodecomposition on leaf surfaces may have occurred. As well, biotransformations of chlorophenoxy acids in higher plants have been reported (Hatzios, 1991), and additional losses may have occurred due to metabolism following uptake.

By 6 weeks after application, there was a further 5-fold decrease in mecoprop residues and mean residue values ranged between 0.1 and 0.2 mg kg^{-1} (Tables II and III). At maturity, mecoprop residues still remained in the straw but were less than 0.1 mg kg^{-1}. Residues, at the limit of quantification of 0.05 mg kg^{-1}, were not detected in the seed. Detector response at the retention time for mecoprop methyl ester in the chromatograms of treated seed samples was not significantly different from that of the check samples. Rapid dissipation of residues leading to nondetectable residues in the seed as observed in the present study has also been reported for other chlorophenoxy acids in cereals, for example, 2,4-D in triticale (Cessna, 1990) and wheat (Cessna, 1980; Grover et al., 1985).

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