

Methodology and Analysis for Residues of MCP and 2,4,5-T in Wheat*

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The chemical MCP [2-methyl-4-chlorophenoxyacetic acid] has proven to be an effective herbicide for controlling certain weeds in wheat. Therefore, it was necessary to ascertain what residue levels would be encountered in grain from the use of this chemical. At the time this study was initiated it was thought that 2,4,5-T might also be used for specialized weed problems in wheat so that it was included in the study. However, since then such a use of 2,4,5-T has been prohibited. In any event, we thought it would be informative to show what residue levels would accrue from an application of 2,4,5-T from wheat.

In surveying the literature it was noted that most analytical methods are designed to analyze "free" or unbound forms of the phenoxy herbicides. An organic solvent is used to extract the herbicide following acidification of the plant tissue [3,4,6]. However, there is ample evidence that the phenoxy herbicides undergo extensive conjugation with plant constituents [1,2,5]. Since most published methods do not include a hydrolytic step to liberate the free herbicide it was suspected that such adducts would go undetected. The study reported here was carried out to determine if there were conjugates of MCP and 2,4,5-T in wheat plants and to develop a method which would measure total herbicide residues.

Experiments and Results

The first part of the study was concerned with determining whether the herbicides were conjugated in wheat plants. Wheat was grown in small greenhouse pots until it attained a height of about 6 inches. The foliage was then dipped in a solution of the triethanolamine salt of ^{14}C carboxyl labeled MCP or 2,4,5-T. After two weeks the surface residues were removed by rinsing with 1% sodium bicarbonate solution and the plants were macerated and extracted with 80% ethanol. The extracts were subjected to thin layer chromatography on silica

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gel plates using petroleum ether:diethyl ether:formic acid [50:50:2]. A portion of the alcohol extract was evaporated to dryness, hydrolyzed with 2M sulfuric acid on a steam bath for 2 hours and extracted with ether. The ether extract was analyzed by the same TLC procedure.

Radioactive scanning of the plates showed both compounds formed conjugates in wheat. The conjugates were present as bands near the origin while the free herbicides had Rf values greater than 0.7. Upon hydrolysis, the conjugate bands disappeared and the free herbicides were released. The rate of conjugation of MCP was greater than for 2,4,5-T so that at the end of two weeks nearly all of the MCP was present as conjugate, while 40% of the 2,4,5-T was in a bound form.

The second part of the study was to develop a method which would extract and hydrolyze all of the herbicide in the tissue. In developing such a method it was felt that much time could be saved if the extraction and hydrolytic steps could be combined. An alkaline condition was selected since it would keep the compounds in a soluble form and reduce the tendency of the free acid to adsorb on plant material.

Plant material for checking the efficacy of an alkaline extraction method was obtained by dipping in non-labeled MCP solution. After a two week exposure period, the surface residues were removed with bicarbonate solution. The plants were cut into small pieces and mixed to obtain a homogenous sample. The tissue was analyzed by the alkaline extraction method described below and by the method of Yip and Ney [6].

In the alkaline extraction method, a 25 g sample of tissue was macerated in a food blender with 125 ml of water. The macerate was transferred to a 400 ml beaker and 25 ml of 0.6M sodium hydroxide was added. The mixture was heated on a steam bath for 2 hours, filtered, and the filter cake washed with 100 ml of water. The combined filtrates were acidified to at least pH 1 with dilute sulfuric acid and extracted with 3 successive 100 ml portions of ether.

In order to make certain the hydrolysis was complete, sufficient sulfuric acid was added to the extracted aqueous phase to give a 2M concentration of acid. The acidic mixture was heated for 2 hours on a steam bath and reextracted with ether.

For comparative purposes a sample of the wheat tissue was extracted by the method of Yip and Ney [6]. This involves blending of the tissue with a mixture of 10% sulfuric acid in ethanol, water, sodium chloride,

ether and petroleum ether. After blending and filtration, the aqueous phase was reextracted with ether:petroleum ether. The ethereal extracts contain the free herbicide.

In checking for presence of unhydrolyzed herbicide, which Yip and Ney indicate is present, the aqueous phase was made to a 2M concentration of sulfuric acid. After heating for 2 hours on a steam bath the mixture was extracted with 3 successive 100 ml portions of ether.

The ethereal extracts obtained from the above procedures were concentrated to about 25 ml by using a stream of nitrogen. The extracts were purified by passage through a 2.5 cm diameter column containing 30 g of Woelm basic alumina. The alumina was washed successively with 100 ml portions of chloroform and ether. After drawing air through the column to remove solvents, the herbicide was eluted with 125 ml of 1% sodium bicarbonate solution. The bicarbonate solution was acidified with dilute sulfuric acid and extracted with 3-50 ml portions of ether. The ether was concentrated to about 30 ml and methylated with diazomethane in ether. After boiling off about 15 ml of ether to dispel excess diazomethane, the sample was dried with sodium sulfate and concentrated to 10 ml. The sample was then analyzed on a Varian gas chromatograph having a Infotronics microcoulometric detector.

The results of this study are shown in Table I.

TABLE I. Recovery of MCP from Wheat
by Different Extraction Methods

	<u>Micrograms of MCP</u>
Yip and Ney Procedure	
Ether:petroleum ether extract	25.7
Acid hydrolysis of aqueous phase	86.0
Alkaline Extraction Procedure	
Alkaline extract	188.9
Acid digestion of alkaline extract	0.0

It is apparent from these data that a hydrolytic step is required to measure total herbicide residues. More than 75% of the residue would be missed by analyzing only the organic phase in the Yip and Ney method. The alkaline extraction hydrolyzes the conjugates without additional acid treatment since no detectable MCP is found following acid hydrolysis.

Comparison of the total amounts of MCP recovered also indicates the alkaline extraction method is more efficient at removing bound MCP from the tissue. Although some sampling variation is to be expected, the 70% higher concentration of MCP by one method is greater variation than would be expected since the plants were cut into small pieces and mixed before sampling.

The alkaline extraction method was employed to determine residue levels of MCP and 2,4,5-T in wheat treated in the tillering stage. The only difference between the method used and that previously described was that the final extracts were concentrated to 1 ml to obtain greater sensitivity. The percent recovery of MCP and 2,4,5-T added to grain at the 0.05 ppm level was 89 to 92%.

Table II gives the results of the analyses. The rates of application were 2.0 lbs/acre for MCP and 1.0 lb/acre for 2,4,5-T, applied as the amine salts.

TABLE II. Residues of MCP and 2,4,5-T in Wheat Treated with these Herbicides

<u>Days following treatment</u>	<u>PPM*</u>	
	<u>MCP</u>	<u>2,4,5-T</u>
7	13.89	9.21
14	1.39	4.98
28	0.38	1.22
56 straw	0.02	0.08
56 grain	0.00	0.00

* These results are an average of 2 samples which were a composite of 2 sub-samples.

The analyses show that there are appreciable concentrations of both chemicals in wheat shortly after application. However, weathering, growth dilution and metabolism reduce the amount of chemical to undetectable levels (> 0.01) in the grain.

Summary

The phenoxy herbicides MCP and 2,4,5-T undergo extensive conjugation with plant constituents. Therefore, in order to measure total herbicide residues, a method must be used which will extract and hydrolyze such conjugates. The studies reported here showed that

heating plant tissue under dilute alkaline conditions is adequate to measure total herbicide residues. Using the alkaline extraction method, it was shown that there were no detectable residues of MCP or 2,4,5-T in the grain of wheat plants treated with these herbicides.

References

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