Extraction and Cleanup Studies for Parathion Residues on Leafy Vegetables

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Crop blending in benzene for 5 minutes plus 30 minutes of tumbling was three times as effective in removing parathion residues from field-treated celery as tumbling only. The use of cosolvent blending (IPA-benzene) plus tumbling for the removal of parathion residues in frozen mustard greens proved superior to single solvent blending plus tumbling. Column chromatographic cleanup resulted in more consistent recoveries of both solvents and pesticide. Comparable parathion recoveries of field-treated cabbage and spinach resulted from 2 minutes of IPA-benzene blending only versus blending plus 30 minutes of additional tumbling. One-half hour of tumbling in addition to the preliminary blending proved to be unnecessary on the crops tested.

As was pointed out by Bann (*1*), most published residue research has emphasized the development and improvement of analytical methods for the detection of the pesticide per se. Unfortunately, only minor emphasis has been placed on techniques for the most practical and quantitative separation of a given toxicant from the plant or animal tissue and the subsequent cleanup of the extract obtained. Consequently, regardless of their precision and accuracy, analytical methods are often limited in effectiveness due to inadequate removal and incomplete cleanup of the pesticide residue being measured. Initially, the toxicant must be efficiently isolated by a suitable solvent from its previous environment on or in the plant or animal tissues. In addition, a cleanup procedure must be devised to quantitatively separate the originally applied toxicant from closely associated interfering materials co-extracted from the original biological environment. It is not until the extracted toxicant is free of most of its accompanying interfering extractants that precise and valid chemical analysis can be initiated.

Organic phosphorus pesticides such as parathion, unlike the chlorinated hydrocarbons, range in solubility from highly oil-soluble to highly water-soluble compounds. Parathion is almost insoluble in water but is quite oil-soluble. Jones and Riddick (*4*) when isolating Dilan from interfering biological systems resorted to partitioning between acetonitrile and petroleum ether to eliminate fats and waxes. They found that acetonitrile selectively extracted this insecticide and many other organic compounds from *n*-hexane. Moddes and Cook (*8*) extracted parathion from lettuce with acetonitrile, diluting it with water and transferring the parathion into pentane before cleanup on an alumina column. The experiments reported here are a sequence of studies to determine what extractive and cleanup techniques were more efficient in the removal of field-treated parathion residues from commercial leafy vegetables.

Methods and Results

The extraction and cleanup studies in the laboratory were superimposed on regular pesticide residue disappearance experiments conducted under field conditions. By following this procedure, not only was information obtained on the efficacy of extraction and cleanup, but simultaneously valuable information on the disappearance of the parathion on the crop was determined.

Experiment I. Although some information was available from previous investigations on other pesticides which indicated blending plus extraction was superior to extraction only, it was decided initially to test two extraction procedures for the removal of field-applied parathion residues from celery. In this experiment, frozen celery samples were cut finely in a Hobart food chopper prior to extraction. Two extraction procedures were compared, differing in that one was blended 5 minutes in benzene with a Lourdes multimixer prior to 30 minutes of tumbling (Extraction *A*) whereas Extraction *B* involved only the 30-minute tumbling in benzene. Table I shows the essential differences in efficiency of parathion removal on celery between Extractions *A* and *B*. Results in the Table I column (Extraction *A* divided by Extraction *B*) indicate that residues recovered from blending in the presence of the solvent increased extraction efficiency threefold over tumbling only. A similar study conducted on unwashed celery samples treated in the field with parathion in the wettable powder and emulsifiable concentrate forms, resulted in similar threefold recoveries due to the blending in the solvent.

Experiment II. The second extraction study attempted involved parathion-treated mustard greens that had been finely chopped and frozen prior to extraction. The three extraction studies with their respective cleanup procedures are outlined in Table II. Extraction *A* is essentially that described by Klein (*5–7*) and Ford and Ottes (*2*). Extraction *B* is generally the extractive procedure outlined by Bann (*1*). Extraction

Table I. Parathion Residues (P.P.M.) Extracted from Celery

Interval[a]	Lb. Active per Acre[b]	Extraction A	Extraction B	Extraction A/ Extraction B[c]
0	0.50	2.68	0.84	3.15
	1.00	7.98	2.20	3.63
2	0.50	2.30	0.62	3.71
	1.00	6.31	1.64	3.85
7	0.50	1.44	0.48	3.00
	1.00	3.11	1.00	3.11
14	0.50	0.89	0.37	2.41
	1.00	1.80	0.72	2.50

[a] Days since last application. [b] Wettable powder. [c] Extraction *A* = Blending + tumbling; Extraction *B* = Tumbling only.

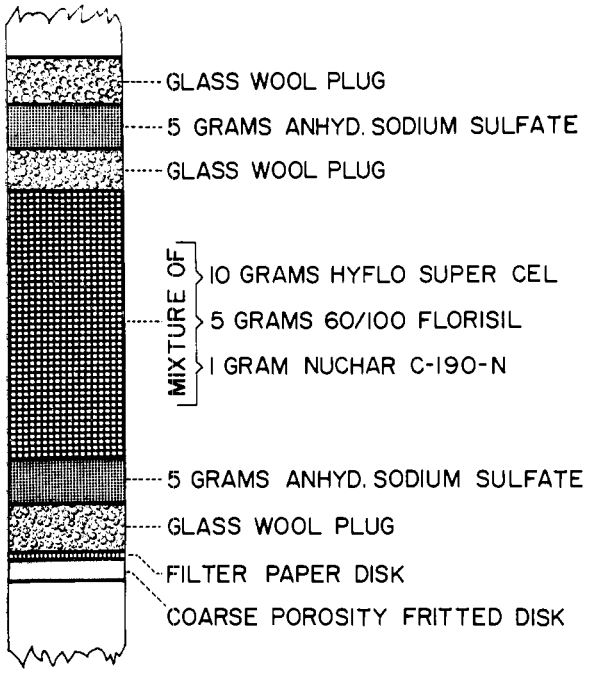

Figure 1. Chromatographic column of cleanup of IPA-benzene extracts of frozen leafy vegetables containing parathion residues

Table II. Parathion Residue Extraction from Mustard Greens

	Extraction A	*Extraction B*	*Extraction C*
Finely-chopped crop (grams)	200	200	200
Solvent in blendor (ml.)	200 (IPA)	200 (IPA)	400 (benzene)
Anhyd. sodium sulfate (grams)	None	None	50
1st blending period (min.)	5	5	5
2nd solvent (ml.)	400 (benzene)	400 (benzene)	None
2nd blending period (min.)	5	5	None
End-over-end (min.)	None	60	30
Subsequent cleanup	10 grams A.O.A.C. decolorizer per 100 ml. of extract in Erlenmeyer flask on wrist action shaker for 10 min.	Washed through chromatographic column packed with mixture of 5 grams of Florisil and 7 grams of Hyflo Super Cel	Same as for Extraction *B*

C employs the one solvent technique plus the addition of anhydrous sodium sulfate to tie-up the excess water in the extract. The chromatographic column cleanup in Extractions *B* and *C* were identical.

Results of this study are summarized in Table III. The parathion recoveries that resulted from the three extractive procedures were not significantly different except in isolated instances. Observations drawn from this study were that isopropanol and water washing is considerably more time-consuming than merely blending one solvent in the presence of anhydrous sodium sulfate. Despite the added time, however, the cosolvent technique resulted in more consistent and reproducible extracts. Conclusions similar to this had been made earlier by Gunther and Blinn (*3*) regarding tomato extractions. The chromatographic column cleanup was found to be superior to the cleanup of shaking the raw extract in the presence of a decolorizing mixture. Volume of cleaned up solvent was more closely controlled when run through the columns whereas considerable losses in volume were noted to occur when the solvent was filtered from the decolorizing mixture. This study stressed the fact that although promising, the chromatographic column adsorbent mixture could be further improved. For cosolvent extracted leafy crops particularly, the florisil–Hyflo Super Cel column was not always adequate for complete cleanup of chlorophyll and other color-producing extraneous agents in the sample extract.

Experiment III. Further research to improve the over-all extraction and accompanying cleanup was undertaken on parathion-treated spinach. Previous experiments showed that the cosolvent technique was necessary for extraction of parathion from thawed-out leafy vegetables. Two successive 5-minute blendings in the previous experiment resulted in over-heating of the macerate, and therefore, the two blending periods were shortened to 2 minutes. At high speeds, this shorter blending time was found to be adequate. Since excessive time-consumption was one of the primary disadvantages of this procedure, it was decided to verify the necessity of further extraction by tumbling the previously blended samples. If the tumbling procedure could be eliminated, several hours could be saved in extracting one dozen samples. In addition to the blending only (*IZ*) and the blending plus tumbling (*IZT*), a new chromatographic column for better cleanup of the resultant extracts was tried. This column, as illustrated in Figure 1, proved to be quite satisfactory. Not only did the adsorbent combinations effectively remove all coloring constituents in the extracts (even from large aliquots), but also the pesticide was allowed to elute more or less quantitatively through the column and be recovered.

Results of this study are shown in Table IV and indicate rather conclusively that there appear to be no significant differences in parathion residue removal from spinach between the blending only procedure compared with the blending plus additional tumbling.

Table III. Comparison of Three Extraction Techniques for Removal of Parathion[a] Residues[b] from Mustard Greens

Day Interval	*Extraction A*	*Extraction B*	*Extraction C*
1	13.40	13.02	11.97
7	1.55	1.92	2.01
14	0.73	0.70	0.65

[a] 0.50 pound active parathion emulsifiable application per acre.
[b] Expressed in p.p.m. parathion. Average of two replications.

Little or no difference between the two procedures was noticed regardless of the time interval the residue had remained on or in the spinach following the last application in the field.

Experiment IV. To further verify or dispute these results, an additional extraction study was conducted comparing the *IZ* and *IZT* procedures in removing field-applied parathion residues from cabbage. The same chromatographic column was used for extract cleanup as was used in the previous spinach experiment. Results of this study, also shown in Table IV, tend to verify the previous conclusion that there is no significant difference in residue removal efficiency between the *IZ* and *IZT* methods of extraction.

Discussion

Pesticides applied to leafy vegetables present the residue chemist with a perplexing set of analytical problems.

Table IV. Comparison of Two Extraction Procedures for Removal of Parathion Residues[a]

Lb. Active per Acre	IZ Extraction			IZT Extraction		
	Days 0	Cut-off 7	Interval 11	Days 0	Cut-off 7	Interval 11
			Spinach			
0.25	17.69	3.10	1.14	18.93	3.16	1.07
0.50	28.39	6.09	2.33	29.95	4.73	1.91
			Cabbage			
0.50	5.89	0.94	0.35	6.13	0.87	0.30

[a] Expressed in p.p.m. parathion. Average of three replications.

When dealing with an organic phosphate pesticide, such as parathion, some quick and efficient method of storing large quantities of samples must be devised. Placing field-cut samples immediately into a Hobart food cutter for 5 minutes and then quick-freezing answered the storage and possible subsequent breakdown-prior-to-analysis problems. However, when the frozen leafy vegetable sample is thawed immediately prior to extraction, the analyst is faced with considerable quantities of water which must be quantitatively removed without excessive loss of pesticide.

To quantitatively remove the pesticide from the crop and the accompanying water, a rigorous cosolvent extraction blending technique should be employed. This procedure results, not only in efficient residue removal from its crop environment, but also in extracts containing unwanted and interfering extractants. In other words, rigorous cosolvent extraction ensures more or less quantitative residue removal but can accentuate cleanup problems.

The first experiment verified the need for crop blending in the presence of a suitable solvent over merely tumbling the crop end-over-end in the solvent. The advantage of using a cosolvent extraction, such as isopropanol and benzene, to remove pesticides from thawed leafy vegetables was apparent from the results obtained in the second experiment. From the experience gained in this experiment, it was also apparent that column chromatography had definite cleanup advantages when compared with the shaking of the extract in the presence of a mixture of adsorbents for decolorization, etc. Once it was decided to go to the cosolvent extraction technique, additional research was necessary to determine whether or not tumbling in addition to blending extraction was definitely superior. Simultaneously, experimentation with more efficient chromatographic columns for cleanup was undertaken in the third experiment. Results of this study indicated that with the improved chromatographic column for extraction cleanup, there appeared to be very little difference in efficiency of parathion residue removal from frozen spinach, regardless of whether cosolvent blending extraction alone was used or when this treatment was augmented by an additional 30 minutes of end-over-end tumbling. A fourth and last extraction experiment utilizing parathion-treated cabbage tended to verify the results found previously on parathion-treated spinach.

It was concluded from the four experiments evaluated in this study that when extracting a pesticide, such as parathion, from frozen field-treated leafy vegetable samples, the highest and most consistent recoveries were made with cosolvent blending with or without subsequent tumbling. Also, column chromatography employing the proper combinations of adsorbents for the particular pesticide in question was found to be an excellent and reproducible technique for cleanup of highly contaminated extracts.

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INSECTICIDE RESIDUES

Residue Analysis of Phorate by Cholinesterase Inhibition after Oxidation

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THE SYSTEMIC organophosphorus insecticide *O,O*-diethyl *S*- (ethylthiomethyl) phosphorodithioate (phorate) is used in the seed treatment and side dressing of sugar beets, potatoes, and cottonseed for protection from insect attack. Since one may expect to find extremely small residual amounts of the pesticide and its metabolites in the harvested plant tissue, the method of analysis must be highly sensitive.

The recently reported residue method for phorate (*6*) involves the direct enzymatic determination of phorate phosphorothiolate sulfone without prior oxidation, assuming that complete conversion of phorate had already occurred in the plant by a metabolic reaction. This procedure is based on the observation of Bowman and Casida (*2*) and Metcalf *et al.* (*11*) that phorate is metabolized to the corresponding phosphorothiolate sulfone by many plant species. If, however, the oxidation has not gone to completion at the time the analyses are performed, the residue data may be expected to be low, since phorate phosphorothiolate sulfone is the most potent cholinesterase inhibitor of the known phorate metabolites (*1*).

The colorimetric method (*9*) for the determination of phorate residues did not prove successful in the authors'