Table II. Recovery of 2-Imidazoline from Lettuce in the Presence of 7 ppm Zineb, 0.1 ppm Ethylenediamine, or 0.1 ppm DIDT[a]

2-Imidazoline hydrochloride added, ppm	2-Imidazoline hydrochloride found, ppm		
	Zineb	Ethylene-diamine	DIDT
0	0.005	0.009	0.009
0.110	0.089	0.095	0.101
0.550	0.485	0.562	0.483

[a] DIDT = 5,6-dihydro-3*H*-imidazo[2,1-*c*]-1,2,4-dithiazole-3-thione.

was made to remove them. A minimum detectable limit of at least 0.02 ppm is suggested from the peak heights of fortified samples compared to the background of controls.

As shown by the data in Table I, the recoveries of 2-imidazoline obtained from four commodities were constant over a tenfold range of concentration and varied from a mean of 87.3% in apples to 101% in grapes. The values are calculated relative to a standard of 2-imidazoline hydrochloride (1.1 μg) added to a blank extraction solvent and carried through the analytical procedure. The absolute recovery of the standard was 55% of the theoretical yield. A 80% yield of derivative was formed when the standard was reacted and determined without prior column chromatography, indicating a 25% loss on the columns. Attempts to improve the recovery by increasing the concentration of reagent or strength of eluting solvents resulted in unacceptably high background.

The effects of zineb and two of its degradation products which could be adsorbed on the ion-exchange resin and possibly interfere with the determination of imidazoline were examined. The results of this experiment are given in Table II and show that, at the levels studied, there is no reduction or enhancement of the recovery of imidazoline.

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Rapid, Simple Procedures for the Simultaneous Gas Chromatographic Analysis of Four Chlorophenoxy Herbicides in Water and Soil Samples

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Water and soil samples are acidified and extracted into organic solvent for the determination of 2,4-D, 2,4-DP (dichlorprop), 2,4,5-T, and 2,4,5-TP (silvex). A similar but not generally used compound, 2,3,4-trichlorophenoxyacetic acid (2,3,4-T), is added to the samples prior to extraction for use as an internal standard. Water samples require no further cleanup whereas soil samples are cleaned up by back-extraction into alkali, a single chloroform wash, acidification, and final ether extraction. Samples are derivatized with BF_3–methanol and analyzed by electron-capture gas chromatography. The simultaneous extraction of fortified and blank control samples provides a means for recovery correction for quantitation and reduces the risk of false positives from glassware or reagent contamination. Minimum quantitation level for all four herbicides in both media is 0.001 ppm.

Although numerous gas chromatographic methods for the simultaneous analysis of chlorophenoxy herbicides in soil or water have appeared in the literature, they have, in general, been more time consuming, less sensitive, and/or less easily quantitated (Devine and Zweig, 1969; Glas, 1976; Goerlitz and Lamar, 1967; Purkayastha, 1974) than the procedures described herein. Methods reported for the analysis of a single chlorophenoxy herbicide in soil or water, while useful for their intended applications, have had some of the same relative drawbacks (Gutenmann and Lisk, 1964; McKone and Hance, 1972; Schultz and Harman, 1974; Schultz and Whitney, 1974; Woodham et al., 1971). Considerable additional work has been reported on the analysis of these compounds in a variety of environmental media, including plant and animal tissue.

Several of the chlorinated phenoxyalkanoic acid herbicides have been used routinely in Los Angeles County weed abatement programs for many years. They have proved extremely useful in brush control for firebreaks and control of broadleaf weeds in recreational turf areas. They are also used for weed control by private operators in the county.

Because of their frequent application, a monitoring program was set up in 1973 to test environmental samples from various treated and untreated sites throughout Los Angeles County for these herbicide residues. It was necessary to devise sensitive, rapid, and consistently accurate methods of analysis for the many specimens collected under this program. Resultant procedures for the analysis of water and soil samples for 2,4-D, 2,4-DP (di-

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chlorprop), 2,4,5-TP (silvex), and 2,4,5-T with minimum quantitation levels of 0.001 ppm are reported herein.

EXPERIMENTAL SECTION

Reagents. All solvents are pesticide grade. Internal standard solution consists of 0.1 ng/μL of 2,3,4-trichlorophenoxyacetic acid (2,3,4-T) in water. Herbicide spiking solution contains 0.1 ng/μL each of 2,4-DP; 2,4-D; 2,4,5-TP and 2,4,5-T in water. Herbicide stock solutions (1 mg/mL) are prepared in acetonitrile.

Standards of 2,4-D, 2,4,5-TP, and 2,4,5-T were obtained from the EPA, Environmental Toxicology Division, Research Triangle Park, N.C. Standards of 2,4-DP and 2,3,4-T were supplied by Amchem, Inc., Ambler, Pa.

Controls. For every specimen or group of specimens worked up, a blank (internal standard only) and a spiked (internal standard plus spiking solution) control sample are also prepared. The controls utilize previously analyzed negative water or soil and are extracted along with and analyzed identically with the actual samples. For the blanks, 1 mL of internal standard solution is added to the substrate at the beginning of the procedure and, for the spiked controls, 1 mL of internal standard solution plus 1 mL of spiking solution is added.

Water Extraction. Add 0.5 mL of 50% H_2SO_4, 50 mL of benzene, and 1 mL of internal standard solution to a 100-mL water sample in a 250-mL separatory funnel. Shake vigorously by hand for 1 min. After layers separate, discard lower aqueous phase. Transfer benzene to a round-bottom flask, being careful to exclude any water and evaporate to dryness at 90 °C using rotary evaporator.

Methylation. Reconstitute with 5–10 mL of methanol and transfer to a 15-mL conical centrifuge tube. Add 1 mL of BF_3–methanol (14%) and evaporate to 0.5 mL in 80–90 °C water bath under a gentle stream of nitrogen. Add approximately 0.5 mL of hexane and vortex-mix.

Soil Extraction and Cleanup. Screen soil through a 40-mesh screen and weigh 100 g into a 500-mL Erlenmeyer flask. Add 1 mL of internal standard solution and 75 mL of 1 N H_2SO_4 to make slurry. Add 150 mL of ethyl ether, stopper flask tightly, and shake upright for 15 min on platform shaker at 150 cpm. Vacuum-filter extract through Buchner funnel fitted with Whatman No. 1 filter paper into a 500-mL filtering flask, then transfer filtrate to a 250-mL separatory funnel. Discard aqueous layer, add 50 mL of 1 N NaOH, and shake vigorously by hand for 1 min. Aspirate top organic layer, then wash aqueous phase with 50 mL of $CHCl_3$. Discard $CHCl_3$, acidify aqueous phase by adding 2 mL of concentrated H_2SO_4, extract into 50 mL of ethyl ether by shaking for 1 min, then discard aqueous layer. Pour ether phase into a round-bottom flask and evaporate to dryness at 50 °C on rotary evaporator. Methylate as with water specimens.

GC Analysis. Gas chromatographic analyses were performed isothermally using a Tracor 222 model gas chromatograph equipped with a ^{63}Ni electron-capture detector. General operating conditions were as follows: detector mode, DC; carrier gas, nitrogen; nitrogen purge, off; detector temperature, 300 °C; injector temperature, 250 °C; chart speed, 1 cm/min; output attenuation, ×1024 (×2 at GC; ×512 at electronic integrator); input attenuation, ×1; bucking range, 4.

Several different columns have been used successfully. Three typical columns are as follows: (1) 3% OV-17 on Gas-Chrom Q (80/100 mesh): column temperature 195 °C; carrier flow, 55 mL/min; glass column, 6 ft × 4 mm (i.d.); (2) 3% Versamide-900 (V-900) on Supelcoport (80/100 mesh): column temperature 200 °C; carrier flow, 60 mL/min; glass column, 6 ft × 4 mm (i.d.); (3) 15% QF-1/10% DC-200 on Chromosorb W (80/100 mesh): column temperature, 195 °C; carrier flow, 45 mL/min; glass column, 5 ft × 4 mm (i.d.).

Inject an aliquot (1–3 μL) from the hexane layer of the methylated extract onto the gas chromatograph.

Quantitation. An Autolab System IV electronic integrator was used for resolution of peak areas. All specimens are quantitated against the spiked control sample to correct for recovery, using the following general formula:

$$\frac{X_s}{\text{I.S.}_s} \times \frac{\text{I.S.}_c}{X_c} \times 0.001 = \text{H}$$

where X_s = area of herbicide peak in specimen, I.S._s = area of internal standard peak in specimen, I.S._c = area of internal standard peak in spiked control, X_c = area of herbicide peak in spiked control, 0.001 is the concentration (ppm) of the internal standard in the specimen, and H = herbicide concentration (ppm).

RESULTS AND DISCUSSION

The procedures described provide very reliable, accurate, and sensitive methods for the simultaneous analysis of four chlorophenoxy herbicides in soil and water specimens. The methods are simple and rapid enough to be suited for routine monitoring projects.

The use of 2,3,4-T as an internal standard included at the beginning of the procedure provides a monitoring mechanism for the extraction efficiency of each individual specimen and, in conjunction with the fortified control sample, allows consistently accurate quantitation of positive specimens based on herbicide recovery. Storrs and Burchfield described the use of internal standards for analyzing chlorine-containing herbicides in 1962. When all quantitations are made relative to an internal standard, much time is saved, e.g., aliquots for GC injection and the amount of hexane used to extract the methylated sample prior to GC injection need not be exact or consistent between samples or injections. In addition, the fortified control provides a regular qualitative and quantitative check on the efficiency of the procedures.

The blank control sample acts as a solvent and glassware contamination check. Contamination from glassware is a common problem, particularly when samples are analyzed which contain a high level of any of the herbicides. Best results are obtained when all glassware and pipets are rinsed with acetone immediately after use; washed with detergent and rinsed; soaked in concentrated acid–dichromate solution and rinsed with distilled water; then rinsed with pesticide grade acetone and/or hexane immediately prior to use.

Excellent separation of the five compounds is obtained on the three columns listed as well as several others (5% QF-1, 3% SE-30, 3% OV-101/3% OV-1, 2% OV-210). Figure 1 shows spiked control tracings for soil and water on 3% V-900 and 3% OV-17 and Figure 2 shows blank control tracings on 3% OV-17. It can be seen that the peaks are quite symmetrical. No significant background peaks have been encountered in the water samples and those few present in the soil tracings have not interfered with the herbicide peaks. An occasional soil sample will present greater interference and may necessitate additional chloroform washes of the alkaline phase; however, this is rare. Any sample which appears positive on one column is confirmed on a second column of differing polarity. Quantitation on both columns must be similar for a sample to be reported out as positive. Occasionally it may be necessary to use a third column for absolute confirmation if interferences are encountered on one of the first two

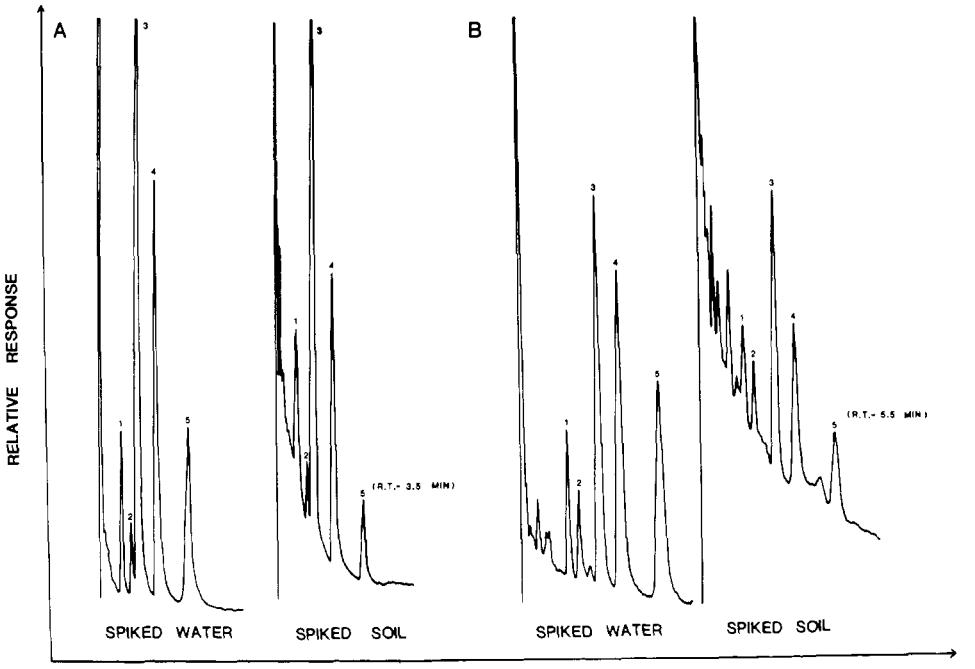

Figure 1. Chromatograms of control samples spiked with the four herbicides and internal standard at 0.001 ppm on (A) 3% Versamide-900 and (B) 3% OV-17. Peaks include (1) 2,4-DP, (2) 2,4-D, (3) 2,4,5-TP, (4) 2,4,5-T, and (5) 2,3,4-T (internal standard).

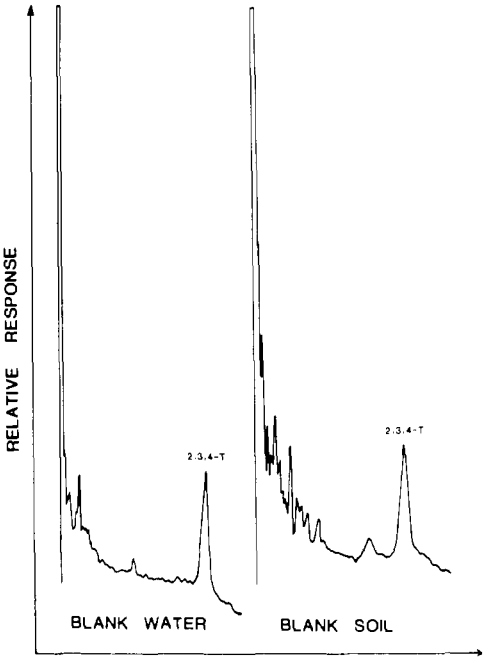

Figure 2. Chromatograms of blank control samples spiked with internal standard only (0.001 ppm) on 3% OV-17 column.

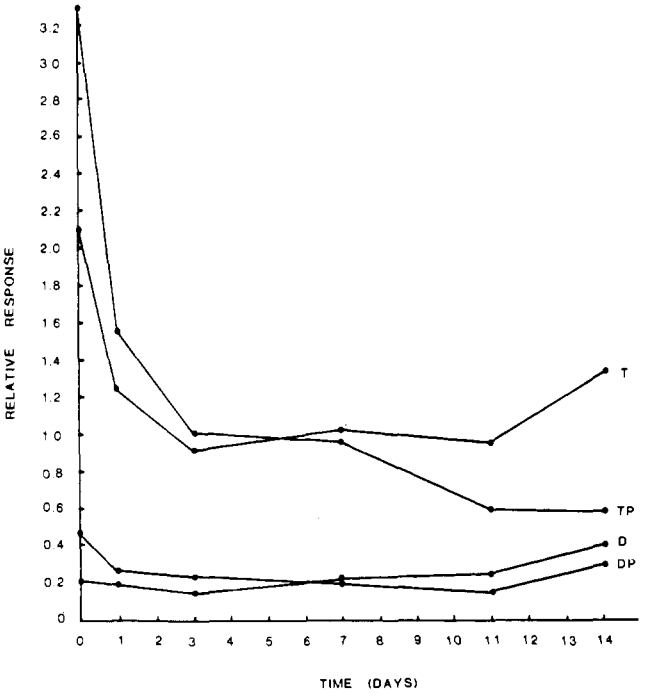

Figure 3. Degradation of methylated herbicide extracts relative to internal standard. A 0.1-μg sample each of herbicide and internal standard extracted from 100 mL of water and analyzed over a 2-week period.

columns. The spiked control is injected at the beginning of a group of GC analyses on a particular column as well as intermittently between samples and at the end. Because of the slight shifting of response characteristics inherent with the electron capture detector, best results are obtained by using sample injections and spiked control injections in close proximity for quantitation.

In specimens where there appears to be herbicide(s) present in concentrations considerably higher than the minimum detectable level (i.e., it is not possible to have an on-scale herbicide peak and still have a measurable peak for the internal standard) the specimen is reextracted with a concentration of internal standard estimated to approximate the concentration of the herbicide(s) present. In such a case, the spiked control sample would be prepared with both the internal standard and the herbicides included at the higher level. This procedure precludes errors resulting from differential extraction efficiencies of disparate herbicide concentrations.

The methylation procedure as described is efficient, simple, and rapid. Length of time in the water bath has not proved to be critical, but 10–15 min is the average time required to evaporate to 0.5 mL. Evaporating to as low as 0.1 mL does not appear to have a significant effect on the efficiency of this derivatization. The use of a Na_2SO_4 solution (Clark, 1969; Goerlitz and Lamar, 1967; Gutenmann and Lisk, 1964) in the methylation procedure was

Table I. Results of Intralaboratory Double Blind Quality Control Program for 2-Month Period[a]

Medium	Herbicide	Amount added, ppm	Amount detected, ppm
Water	2,4-D	0.001	0.001
Water	2,4,5-TP	0.001	0.001
Water	2,4-D	0.001	0.001
Water	2,4-DP	0.001	0.001
	2,4-D	0.001	0.001
	2,4,5-T	0.001	0.001
Water	2,4-D	0.008	0.008
	2,4,5-TP	0.005	0.005
	2,4,5-T	0.005	0.006
Soil	2,4-D	0.026	0.029
Soil	2,4-D	0.008	0.014
Soil	2,4-D	0.001	0.001
Soil	2,4-D	0.012	0.010
Soil	2,4-D	0.003	0.004

[a] Specimens fortified by individual not connected with analyses and submitted for analysis as routine samples.

Table II. Percent Recovery of Chlorophenoxy Herbicides and Internal Standard from Fortified Water and Soil Samples

	2,4-DP	2,4-D	2,4,5-TP	2,4,5-T	2,3,4-T
Water 1[a]	99	87	98	100	107
Water 2[a]	95	76	94	93	101
Water 3[a]	96	90	90	90	84
Av recov., water	97	84	94	94	97
Soil 1[b]	96	86	87	97	86
Soil 2[b]	98	91	91	75	84
Soil 3[b]	84	72	83	86	73
Av recov., soil	92	83	87	92	81

[a] 0.1 μg of each herbicide added to 100 mL of water (0.001 ppm). [b] 0.1 μg of each herbicide added to 100 g of soil (0.001 ppm).

not found to be necessary for our purposes. Although methylated extracts of the herbicides begin to break down within a day of their derivatization, this fact does not normally affect the accuracy of quantitations, since all calculations are based upon a comparison of the internal standard/herbicide relationship in the specimen to that in the control. Figure 3 shows that methylated extracts of silvex and 2,4,5-T appear to break down more rapidly, initially, than methylated 2,4-D and dichlorprop. The limiting factor in setting time deadlines for analyzing the methylated extracts would appear to be the sensitivity of the equipment and presence of interference in the specimens; however, it was generally not necessary to extend the analyses over more than 7 days. It is often possible to increase the peak sizes after several days by briefly reheating the samples at 80–90 °C and revortexing.

An in-house double-blind quality control program utilizing water and soil specimens spiked with one or more of the four herbicides at concentrations of 0.001 ppm and higher serves as a constant check on accuracy. Results for a 2-month period are shown in Table I. It should be noted that these results represent work performed by several different analysts using the procedures described.

Actual recoveries for the herbicides by both procedures were calculated against methylated herbicide standards and are illustrated in Table II. A relatively lower recovery of 2,4-D was also experienced by Devine and Zweig (1969) in their water extractions using benzene. Efforts were made to correct for differential extraction efficiencies due to sample differences, e.g., varying organic content of soils, by matching the substrate used in the fortified sample as much as possible to the actual specimens. There is some inherent error in this practice, of course, but less so than when standard recovery corrections for various sample types are used. Moreover, a regular and simultaneous check on recovery can serve as a more realistic and immediate correction for day-to-day variations in reagents, technician performance, etc.

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