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A RAPID GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SEVERAL PHENOXYALKANOIC ACID HERBICIDES IN SOIL SAMPLES*

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*An in-house analytical technique developed by DFCBS to determine levels of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in soil biodegradation plots, AFLC Test Range Complex, Utah, and Eglin AFB, Florida, in support of the disposition of Herbicide Orange.

TECHNICAL MEMORANDUM FJSRL(NC)TM-76-5. FRANK J. SEILER RESEARCH LABORATORY, U.S. AIR FORCE ACADEMY, COLORADO

INTRODUCTION

Problems associated with the ecologically safe disposal of contaminated or excess pesticides have received increased attention in recent years. Among those compounds requiring a safe method for disposal are the halogenated phenoxyalkanoic acid herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their esters. One of the means of disposal of these herbicides which has been suggested is soil biodegradation, whereby the herbicides are incorporated in soil and degraded by soil microorganisms (1). In order to evaluate the effectiveness of this technique, analytical procedures were necessary for the measurement over a wide range of concentrations of both the n-butyl esters and the sodium salts of 2,4-D and 2,4,5-T. Since evaluation of the biodegradation technique required the analysis of large numbers of soil samples taken from relatively large field plots, an analytical procedure which was not only accurate and precise, but also rapid and simple to perform was required. A number of different techniques have been devised for this type of analysis (2, 4), however, many of these procedures tended to be complex and time consuming since they were designed for samples where herbicide concentrations were low and many interfering substances were present. This paper describes a simple, rapid, and accurate procedure for the simultaneous determination of the n-butyl esters of 2,4-D and 2,4,5-T and their corresponding hydrolysis products in soil samples.

EXPERIMENTAL

APPARATUS: A Beckman Gas Chromatograph, Model GC-45 equipped with a hydrogen flame ionization detector was employed. Columns were 6 ft x 2 mm i.d. glass u-tubes packed with 80/100 mesh chromosorb W coated with 10% DC-200 silicone gum as the stationary phase. All columns used were supplied by the instrument manufacturer. Conditions for the chromatography were: temperatures; inlet, 245°C; column, 220°C; detector, 250°C; air flow: carrier (helium), 60 cc/min; detector make up (helium), 60 cc/min; hydrogen, 50cc/min; air, 250 cc/min. One microliter samples were injected directly on column.

REAGENTS: Methanol, chloroform, acetylchloride and anthracene were ACS reagent grade chemicals (J.T. Baker) and were used without additional purification. The sodium salts of 2,4 dichlorophenoxyacetic acid (2,4-D) and 2,4,5 trichlorophenoxyacetic acid (2,4,5-T) were practical grade (Dow Chemical Co.). The n-butyl esters of 2,4-D and 2,4,5-T were analyzed to be greater than 99% purity (Hercules, Inc.): 3% solution of anhydrous hydrogen chloride in methanol was prepared by reacting 5 ml of acetyl chloride with 100 ml of methanol. (5).

PROCEDURES: Standard solutions of the n-butyl esters and the sodium salts of 2,4-D and 2,4,5-T were prepared by dissolving weighed amounts of each compound in either chloroform (n-butyl esters) or methanol (sodium salts). After serial dilutions were made, the sodium salt solutions were reacted with a 3% (w/w) solution of anhydrous HCl in methanol (5 ml herbicide salt standard solution plus 1.5 ml 3% HCl: methanol) in order to form methyl esters suitable for chromatography. After heating for 15 minutes at 55°C, a sufficient volume of a chloroform solution of anthracene internal standard was added to obtain a

final internal standard concentration of 10 µg/ml, 50 µg/ml or 250 µg/ml depending upon the herbicide concentration of the standard solution. Sufficient internal standard solution was also added to the n-butyl ester solutions to obtain the same range of internal standard concentrations as used with the sodium salts. All standard solution volumes were adjusted to 10 ml with chloroform prior to chromatography.

Standard curves for each herbicide were prepared by the chromatography of standard solutions of varying herbicide concentration, calculating the ratios of the individual herbicide GC peak areas to the GC peak area of the internal standard and plotting these ratios against the actual herbicide concentrations in the standard solutions. Concentration curves were prepared for each of the four herbicides at three different concentration ranges, (0-10 µg/ml, 10-100 µg/ml, 100-1000 µg/ml).

Soil samples used for evaluating the procedures were prepared by dissolving weighed amounts of each of the four herbicides in anhydrous methanol and adding this solution to representative control soil samples contained in 1 liter round bottom flasks. Solvent was then removed from the samples using a rotary flash evaporator. Control soil samples had been previously analyzed and shown to be free from herbicide residues.

Soil samples were analyzed in the following manner. Five grams of treated soil were placed in 15 ml glass screw top culture tubes, and the herbicides extracted with 10 ml anhydrous methanol by heating the sealed tubes at 55°C in a water bath. Samples were shaken periodically during the heating period. After cooling, a 5 ml aliquot of the methanol extract was transferred to a clean culture tube and mixed with 1.5 ml of 3% HCl:methanol.

Reaction was allowed to proceed at 55°C for 15 minutes. The samples were allowed to cool to room temperature and 3.5 ml of a chloroform solution of internal standard added. The final concentration of internal standard obtained was either 10 µg/ml, 50 µg/ml or 250 µg/ml, depending upon the expected range of herbicide present. One microliter samples were analyzed by gas chromatography. Soil concentrations were determined by multiplying the concentrations in mg/ml obtained from the standard curves by a factor of four to obtain the concentrations in micrograms of each herbicide per gram of soil. Soil samples obtained from biodegradation test plots were analyzed in the same manner.

RESULTS AND DISCUSSION

Employing the chromatographic conditions outlined above, the four herbicides of interest could be separated from each other in less than ten minutes as shown in Figure I. This figure also illustrates the freedom of the samples from interfering substances which might be present in the soil. In the chromatogram, the herbicides which were present in the soil as either salts or free acids appear as methyl esters. The esterification reaction appeared to produce a reproducible yield of methyl esters regardless of the equilibrium form of the herbicide which was present.

Five replicate soil samples for each of five different herbicide concentrations were analyzed. All samples were analyzed on the same day as they were prepared in order to minimize hydrolysis of n-butyl esters due to the prevailing alkalinity (ph 8.4) of the soil which was used. The results of these analysis are given in Table I. Three additional replicates at two concentration levels were stored at 4°C for 24 hrs then analyzed for the purpose of evaluating the degree of hydrolysis. These data are presented

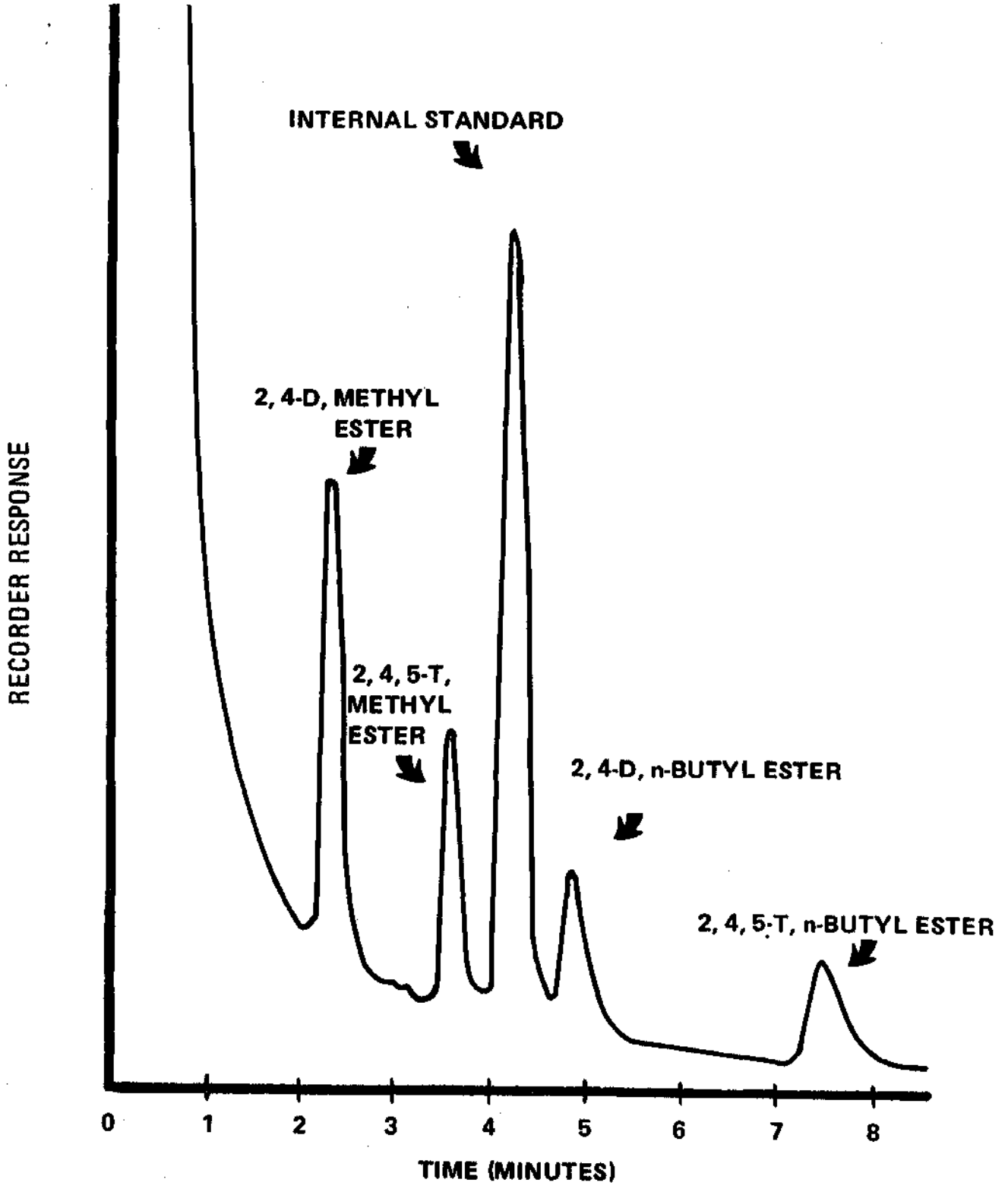

Figure 1: Gas chromatogram of the extract of a prepared soil sample containing 1 mg/g each 2,4-D and 2,4,5-T sodium salts and 1 mg/g each 2,4-D and 2,4,5-T n-butyl esters. Salts were converted to methyl esters prior to chromatography. Chromatographic conditions as outlined in experimental section, attenuation = 1000x.

in Table II. Average recovery of total added herbicide ranged from 99.7% at soil concentration of 8 mg/g to 87.5% at soil concentrations of 0.04 mg/g. The mean relative standard deviation for all four compounds of interest was $\pm$ 4.9%. It appears from the data contained in Table I that approximately 8% of the n-butyl esters of 2,4,5-T and 12% of the n-butyl esters of 2,4-D contained in any sample are converted to methyl esters by the derivative forming reaction. Since this rate of conversion is relatively constant at all herbicide concentrations, a conversion factor may be added in order to improve the accuracy of the analysis. However, this conversion factor was not used in any of the calculations shown in the tables. The data presented in Table II indicates a rapid hydrolysis of the n-butyl esters of 2,4-D and 2,4,5-T after application to alkaline (pH 8.1) soils. In the 24 hour period after sample preparation, a 21% reduction in concentration of the n-butyl esters of 2,4-D and a 16% reduction in the concentration of the n-butyl esters of 2,4,5-T were experienced. If a conversion factor is utilized as mentioned previously, it can be calculated that 9% of the n-butyl esters of 2,4-D and 8% of the n-butyl esters of 2,4,5-T were hydrolyzed to the acid forms by the alkaline soil even though the samples were maintained at a temperature of 4°C for the entire 24 hour period. These data illustrate the need for rapid sample analysis when attempting to accurately determine degradation rates and breakdown patterns in field situations.

At the conclusion of the analysis of the prepared soil samples, a number of samples were obtained from USAF soil biodegradation test plots and analyzed for herbicide residues. A herbicide formulation of the n-butyl esters of 2,4-D and 2,4,5-T had been incorporated at several different

application rates into the soil of these plots. Table III contains data from five replicate analyses from three different samples taken from the test plots. As can be seen from the data in the table, the concentrations of the different herbicide residues in these plots varied greatly; however, the mean relative standard deviation ($\pm$ 6.1%) for all samples varied only slightly from that determined when prepared samples were used. This indicates that the analytical technique is applicable for the determination of herbicide residues over the large concentration ranges found in actual field samples.

While the procedure described does not offer the absolute sensitivity of methods employing electron capture detection (6), it's major advantage lies in the large number of samples which can simultaneously be analyzed accurately for four different components in a very short time period. The time required for one individual to analyze 12 soil samples was 3-4 hours or about 30 minutes per sample. Sensitivity of the procedure allows accurate measurement of any of the four compounds at levels greater than 4 microgram/gram soil. By the addition of a final concentration step (solvent evaporation) this sensitivity could be increased to the ppb level without compromising precision or accuracy. While the techniques prescribed could be utilized for the measurement of phenoxyherbicide residues after large applications, their primary usefulness is in the monitoring of herbicide disposal sites and research directed toward the development of ecologically safe methods of herbicide waste disposal. In these applications, extreme sensitivity is often a secondary consideration when compared to the rapidity of analysis and the range of concentration which can be accurately measured.

TABLE I

RECOVERIES OF HERBICIDES ADDED TO SOIL SAMPLES*

Compound	Amount added mg/g soil	Average Recovered**(mg/g)	Percentage Recovery
2,4-D, Na	2.00	2.19	110
2,4,-D, n-butyl	2.00	1.85	93
2,4,5-T, Na	2.00	2.08	104
2,4,5-T, n-butyl	2.00	1.84	92
2.4-D, Na	1.00	1.12	112
2,4-D, n-butyl	1.00	0.86	86
2,4,5-T, Na	1.00	0.98	98
2,4,5-T, n-butyl	1.00	0.88	88
2,4-D, Na	0.250	0.264	106
2,4-D, n-butyl	0.250	0.212	85
2,4,5-T, Na	0.250	0.251	100
2,4,5-T, n-butyl	0.250	0.201	81
2,4-D, Na	0.050	0.061	112
2,4-D, n-butyl	0.050	0.048	96
2,4,5-T, Na	0.050	0.052	104
2,4,5-T, n-butyl	0.050	0.041	82
2,4-D, Na	0.010	0.012	120
2,4-D, n-butyl	0.010	0.007	70
2,4,5-T, Na	0.010	0.009	90
2,4,5-T, n-butyl	0.010	0.007	70
2,4-D, Na	0.004	0.005	120
2,4-D, n-butyl	0.004	0.004	100
2,4,5-T, Na	0.004	0.004	100
2,4,5-T, n-butyl	0.004	0.003	75

*Samples analyzed immediately following preparation.
**Mean value of 6 analyses.

TABLE II

HYDROLYSIS OF HERBICIDE ESTERS ADD TO ALKALINE SOIL SAMPLES

Compound	Amount added mg/g soil	Time of exposure hrs	Average amount recovered	Percent increase/ decrease of 24 hr exposure
2,4-D, Na	2.00	0	2.19	
2,4-D, Na	2.00	24	2.71	
				+26.0
2,4-D, n-butyl	2.00	0	1.85	
2,4-D, n-butyl	2.00	24	1.38	
				-24.0
2,4,5-T, Na	2.00	0	2.08	
2,4,5-T, Na	2.00	24	2.40	
				+16.0
2,4,5-T, n-butyl	2.00	0	1.84	
2,4,5-T, n-butyl	2.00	24	1.58	
				-13.0
2,4-D, Na	1.00	0	1.12	
2,4-D, Na	1.00	24	1.32	
				+20.0
2,4-D, n-butyl	1.00	0	0.86	
2,4-D, n-butyl	1.00	24	0.72	
				-14.0
2,4,5-T, Na	1.00	0	0.98	
2,4,5-T, Na	1.00	24	1.14	
				+16.0
2,4,5-T, n-butyl	1.00	0	0.88	
2,4,5-T, n-butyl	1.00	24	0.71	
				-19.0

TABLE III

DETERMINATION OF HERBICIDE CONCENTRATIONS IN SOIL BIODEGRADATION TEST PLOTS

Sample #	Soil Depth	2,4-D Na mg/g	2,4-D n-butyl mg/g	2,4,5-T Na mg/g	2,4,5-T n-butyl mg/g
1-1	0-6"	0.740	0.376	0.700	0.326
1-2	"	0.698	0.354	0.650	0.303
1-3	"	0.731	0.368	0.684	0.340
1-4	"	0.740	0.385	0.706	0.327
1-5	"	0.726	0.361	0.671	0.316
Mean ± σ		0.727 .018	0.369 .012	0.682 .023	0.322 .014
2-1	0-6"	0.835	1.05	0.410	1.62
2-2	"	0.910	1.15	0.426	1.70
2-3	"	0.856	1.08	0.410	1.66
2-4	"	0.795	1.06	0.392	1.57
2-5	"	0.852	1.10	0.408	1.60
Mean ± σ		0.850 .042	1.09 .040	0.409 .010	1.63 .043
3-1	6-12"	0.125	0.015	0.098	0.032
3-2	"	0.130	0.015	0.100	0.042
3-3	"	0.117	0.013	0.110	0.035
3-4	"	0.115	0.011	0.095	0.040
3-5	"	0.132	0.014	0.112	0.038
Mean ± σ		0.124 .015	0.013 .002	0.103 .008	0.037 .004

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