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A Gas Chromatographic Method for Determination of 2,4-D Residues in Urine after Occupational Exposure

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Derivatives of phenoxy herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D), are used extensively as selective herbicides against broad-leaf weeds in cereal crops and pasture and non-crop land in this country. The active principle of 2,4-D are formulated in local herbicide manufacturing plants. Although 2,4-D and its derivatives do not seem to pose a great risk for the general population, they might be the risk of mutagenicity, potential cancer hazard and fetotoxicity for herbicide formulators, agricultural spraymen and applicators who directly exposed to these chemicals (Ross et al. 1977 ; Seiler 1978 ; Elina 1979).

The level of the free acid form of 2,4-D in the urine can be used as an index of exposure to 2,4-D group herbicides (Khanna and Fang 1966). Thus, a rapid, sensitive and simple procedure is needed for the detection in human urine of the free acids in order to achieve the toxicological and environmental monitoring of these compounds. Not only does this study describe a gas chromatographic method for the determination of trace quantities of 2,4-D in human urine after methylation but also the application of the method for the measurement of exposure to 2,4-D group herbicides.

METARIALS AND METHODS

Analytical standards of 2,4- dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid(2,4,5-T) used as an internal standard (I.S) were obtained from the BDH chemicals. Standard stock solutions were prepared in methanol 100 mg/mL. Methylating reagent, dimethyl sulphate from the Riedel Chemical Co., Hannover, West Germany, and its 5% solution in methanol stored over anhydrous sodium sulphate. Silicagel (adsorbent), 0.05-0.2 mm, Merck, was dried for 30 h at 170°C and stored in a desiccator. On the day of use, 1 g of the silicagel was deactivated by adding 1.5% weight of water in a stoppered flask and allowed to equilibrate for 1-2 h with periodic shaking.Saturated solution of sodium chloride was prepared in distilled water. All solvents were distilled. Methylation procedure with dimethyl sulphate was carried out by the method of Scoggins and Fitzgerald (1969). 1 mL stock solution of free phenoxyacetic acid was methylated, methyl esters were extracted

with 1x2 mL benzene from the aqueous solution and benzene layer was evaporated. Methyl esters of 2,4-D (2,4-D Me) and 2,4,5-T (2,4,5-T Me) were recrystallized from hot benzene and characterized by the infrared (KBr) absorption and spectra and by melting points (Van Peteghem and Heyndrickx 1975).

Methyl esters of 2,4-D and 2,4,5-T were prepared as described above and 1-2 μ L aliquots of benzene extract was examined gas chromatographically. Packard-Becker gas chromatograph, model 419 equipped with a ^{63}Ni electroncapture (EC) detector and column 150 cm in length x 0.6cm o.d, packed with 4% SE-30 and 6% QF_1,on Chromosorb W, high performance, 80/100 mesh; operated under following conditions: nitrogen carrier gas with a flow rate of 30 mL/min; column temperature 190 ^{o}C; injection port 210 ^{o}C ; detector 220^{o}C.

Urine specimens were taken from healthy men. Aliquot of 100 mL portion was kept for control (blank), while further 100 mL portion was spiked with 20 μ L and of the stock solution of 2,4-D acid and 20 μ L of the stock solution of 2,4,5-T acid as an internal standard so that the fortified urine sample would contain 0.4 ppm 2,4-D and 0.5 ppm 2,4,5-T. Aliquot of 5 mL urine was acidified with 1mL concentrated hydrochloric acid (HCL) in a glass stoppered tube and extracted twice with 3 mL of benzene.Benzene extracts were collected and shaken in a tube containing 1 g of anhydrous sodium sulphate. The methylation reaction was carried out by adding 3 mL of the dimethyl sulphate solution to the benzene extract and by keeping the mixture at 55 ^{o}C in water bath for 10 min. After the tube under a water-stream, 3 mL of saturated sodium chloride solution and 1 mL of benzene added.The mixture was shaked and centrifuged at 2500 rpm for 5 min. Benzene extract containing methyl esters was evaporated to 0.5 mL.

The elution pattern of methylated 2,4-D and 2,4,5-T on silicagel column with using benzene-hexane mixtures was determined. Elution of 2,4-D Me and 2,4,5-T Me from urine extracts carried out by using chromatographic glass column (i.d 7 mmx200 mm length) lightly plugged with glass wool and containing 1 g of the partially deactivated silicagel. Urine extract, containing methyl esters of 2,4-D and 2,4,5-T, was added to the column prewashed with 10 mL of each 40 % benzene-hexane and 60% benzene-hexane. The eluates were collected and concentrated to 5 mL. 5 μ L of aliquots was used for the gas chromatographic analysis.

Calibration curve was prepared with methylated standard 2.4-D and I.S solutions. Results were calculated by comparison of peak height of 2,4-D Me / peak height of 2,4,5-T Me. Recovery studies were performed using control urine spiked with different levels of 2,4-D.

Urine samples were taken from :
15 males ranging in age from 23 to 47 who were full-time (6 h daily) employed in the manufacture of isooctyl esterification of 2,4-D acid and in the formulation of isobutyl ester, isooctyl es-

ter of 2,4-D and dimethyl amine salt of 2,4-D.

Urine samples were collected at the end of the week (Friday). 13 males ranging in age from 26 to 47 who were involved in the application of 2,4-D formulations. Urine samples were collected at the end of application period, during the period from March 1982 to May 1982.

Urine specimens collected in polyethylene bottles were kept at (+ 4° C) without adding any preservative until subsequently analyzed for 2,4-D. Aliquots of 5 mL urine were acidified with 1 mL concentrated HCl and hydrolyzed on boiling water bath for 1 h. Concentration adjustments were made to creatinine factor (Elkins et al. 1974).

RESULTS AND DISCUSSION

The method we developed for the determination of 2,4-D in urine was mainly a combination and modification of various methods (Scoggins and Fitzgerald 1969 ; Shafik et al. 1971). We preferred, the method of Scoggins and Fitzgerald (1969) for the methylation of phenoxyacetic acids with dimethyl sulphate. This was adapted to urine samples as the procedure was simple and rapid. Methylation with dimethyl sulphate gave reproducible and satisfactory results when compared with the criteria of pesticide analysis as described by Cochrane (1980).

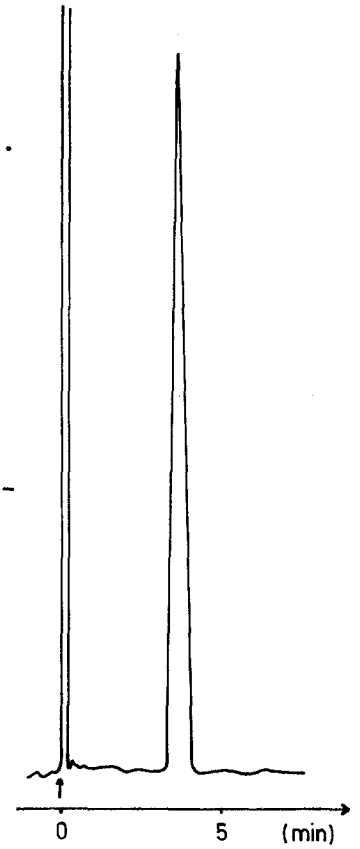

Fig 1. Gas Chromatogram of Methyl Ester of 2,4-D(0.1 µg 2,4-D)

Gas chromatograms of methyl ester of 2,4-D ; methylated urine extract containing 0.4 ppm 2,4-D and 0.5 ppm 2,4,5-T (I.S) and control human urine treated the whole procedure were shown in Figure 1 and Figure 2.As it was seen from Figure 2, control urine extract showed no interfering substances with the same retention time as 2,4-D Me. The sensivitiy of 2,4-D Me was 100 pg. The recoveries of 2,4-D from the 5 fortified urine specimens were excellent with a mean recovery and standard deviation 96.20 ± 1.90 %.

Urine samples from exposed persons were also subjected to hydrolysis with HCI (Shafik et al. 1971 ; Sauerhoff et al. 1977).

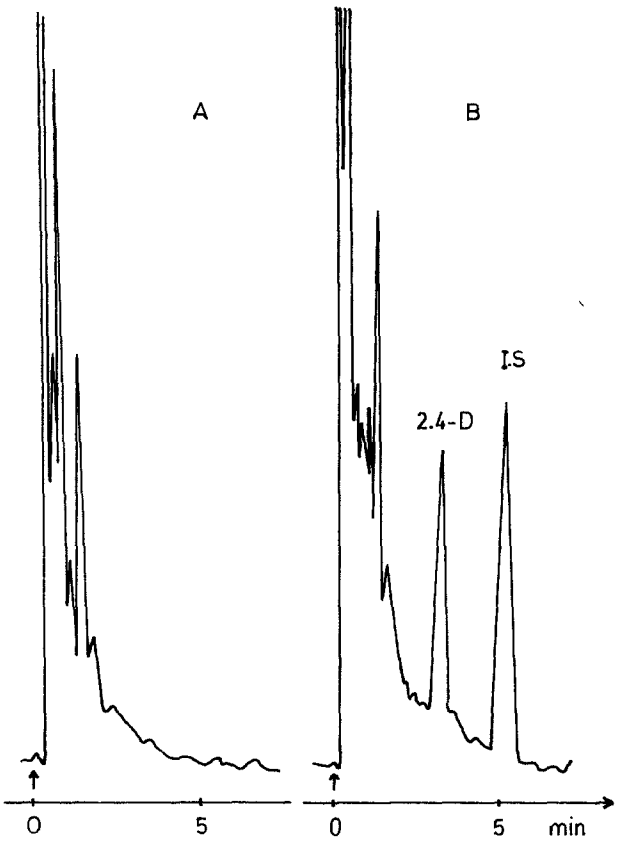

Fig 2. Gas Chromatograms Derived from 5 μL Injections of (A) Methylated Control Urine Extract ; (B) Methylated Extract Containing 0.4 ppm 2,4-D and 0.5 ppm 2,4,5-T (I.S).

Administration of 2,4-D derivatives to men and animals showed that excretion occur mainly as free acidic form 2,4-D from 75% to 96% and conjugated form excreted in smaller amounts from 4% to 25 % (Erne 1966; Sauerhoff et al. 1977). Many investigators concluded that determination of free acids in urine after hydrolysis could be an index of low level exposure of 2,4-D derivatives and phenolic metabolites can only be used as an index of the high exposure (Shafik et al. 1971; Taskar et al.1982).

This procedure using hydrolysis, extraction, methylation with dimethyl sulphate,cleaning on silicagel column and GLC appears satisfactory for the analysis of 0.03 mg per L 2,4-D in urine.

Gas chromatograms and the results of the 2,4-D levels in the urine of occupationally exposed people were analyzed and found to contain 0.10 to 9.51 ppm. 2,4-D levels determined in urine samples

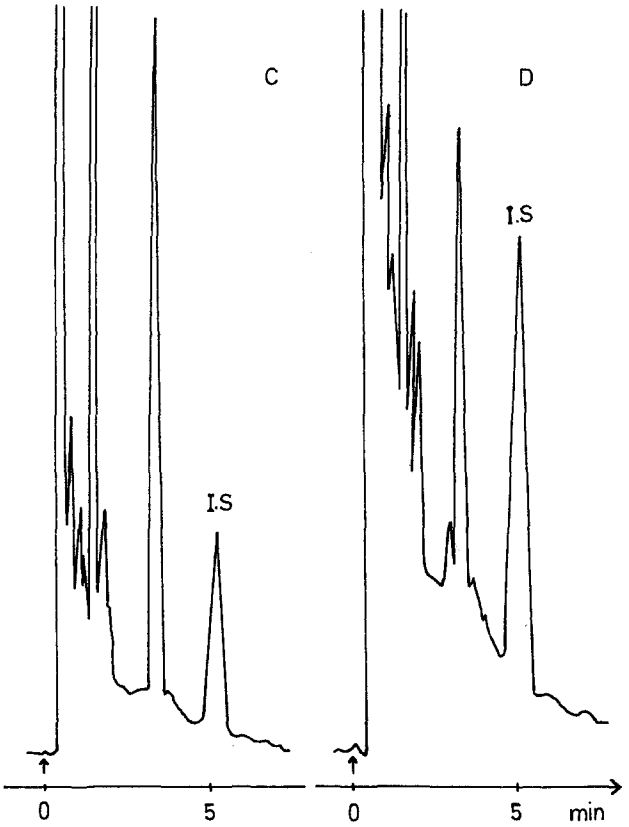

Fig 3. Gas Chromatograms of Occupationally Exposed Urine Samples (C): Sample No.4 (D) : Sample No.2.

from people involved in the application of herbicide formulations are shown in Table 1.The average results as shown in Table 1, indicate a higher degree of exposure for pesticide formulators (1.37 ppm) than for those employed in pesticide application (0.71 ppm).

Our results showed higher levels as compared with the results of Shafik et al (1971). It can be generally concluded that the method we developed for the determination of 2,4-D in urine is satisfactory to indicate low exposure to 2,4-D herbicides.

Table 1. Results of Analysis of Urine from People Involved in the Formulation and Application of 2,4-D Derivatives.

Crew and Duty	2,4-D Residue (ppm)	Ave. $\bar{X}$	Crew and Duty	2,4-D Residue (ppm)	Ave. $\bar{X}$
Pesticide Formulator			Helicopter Pilot		
1	2.40		1	-	
2	0.65		2	0.40	
3	0.75		3	-	
4	3.44		4	1.09	
5	9.51		Flagman		
6	0.49		5	1.01	
7	0.30		6	1.92	
8	0.35		Mixer		
9	0.06		7	0.58	
10	0.10		Supervisor		
11	0.59		8	0.75	
12	0.17		9	0.54	
13	1.55		10	1.16	
14	0.08		11	0.85	
Pesticide Project Engineer			12	-	
15	0.15		13	0.99	
		1.37			0.71

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