Determination of DDT and Metabolites, including DDA, in Human Urine by Gas Chromatography

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INTRODUCTION

The need to investigate the residues and metabolism of chlorinated hydrocarbons has stimulated the development of many analytical methods.[1-8] The earlier procedures utilizing colorimetry and bioassay[2] have, for the most part, given way to analysis by selective detector gas chromatography.[3-6] Currently, different biological tissues, fluids and excrement require separate clean-up and determinative analysis. Furthermore, these methods often necessitate separate analysis of polar and non-polar components.

This paper reports the development of a technique suitable for the routine analysis of DDT, DDE, DDD and free DDA present in human urine. The procedure includes joint electron-capture gas chromatographic determination of DDT, DDE, DDD, and DDA-methyl ester, as well as other extractable chlorinated hydrocarbons such as lindane, dieldrin and dichloran. Detection of these compounds is possible at levels as low as 1.0 ppb by the combination of the Coulson conductivity and parallel plate electron-capture detectors. Identification is facilitated by comparison of the relative responses of different detection systems and relative retention times on columns with different retention characteristics.

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MATERIALS AND METHODS

Reagents: Hexane, petroleum ether, acetronitrile, toluene and acetone were either the Mallinkrodt* "Nanno-grade" or Burdick & Jackson "Distilled in Glass" products. Boron trifloride was obtained from the Matheson Co. in lecture bottles, and in nanograde Methanol reagent from Applied Science Laboratories. The coatings QF-1, DC-200, and SE-30 and the solid support Gas Chrom-Q were obtained from Applied Science Laboratories. Standard pesticides and pesticide metabolites were Food & Drug Administration Reference Standards.

Instruments: The gas chromatographs used were the Micro-Tek Model MT-220 fitted with an electron-capture detection system. One instrument was also equipped with the Coulson Conductivity detector, obtained from the Coulson Instrument Co. Measurement of osmolalities were made on an Osmette from Precision Systems.

Preparation of Glassware: When using electron-capture detection, thorough cleaning of glassware and the avoidance of plastics is mandatory in all steps of the procedure in order to avoid spurious peaks arising from sample contamination.

*Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Public Health Service.

Sample Collection and Preparation: Twenty-three individuals were selected to supply urine samples to test the method. They were divided into two approximately equal groups, the "general population" and "occupationally exposed".

Urine collections were made in sterile, screw-cap bottles to which 1 cc of toluene had been added as a preservative. Donors were requested to collect their specimens immediately after arising in the morning. The volume of urine routinely extracted varied for those individuals classified as normal (20-50 cc) and those with known or suspected exposure to unusual quantities of DDT or other chlorinated pesticides (5-10 cc).

The osmolality of each specimen was measured shortly after receipt using a Precision Systems Osmette.

Extraction Procedure: Each sample was mixed with equal volumes of 2% acetic acid in hexane by shaking the mixture in Teflon Stoppered separatory funnels for two minutes or by the use of an omni vortex mixer for samples in glass stoppered test tubes. When test tubes are used, centrifugation after mixing is desirable since any emulsion is eliminated. Test tubes were found to be more convenient for the extraction of 2- to 20-cc samples. Three equal volumes of 2% acetic acid in hexane extractant were used with each being equal to the volume of the urine sample.

In cases where persistant emulsions appeared, a few drops of acetonitrile were added to obviate the problem. The extracts were combined and evaporated to dryness in vacuo at 40-50°C, taking care that no residual traces of water remained (See Table I for recovery information).

Clean-up: Occasionally samples of urine with "general population" levels produced extracts containing sufficient amount of materials other than chlorinated hydrocarbons to warrant a "clean-up" step if electron capture determination was to be made. For this purpose the extract, after methylation and volume adjustment, was passed over a micro florisil column, according to the procedure of Enos and his co-workers.[9] This results in the removal of many interfering substances and the partition of the chlorinated pesticides present.

Esterification of Extracts: Each dry extract was treated with 2-3 cc of fresh methylation reagent, 10% boron triflouride in Nanograde Methanol. The reagent-sample mixture was heated at 50°C for 30 minutes in open tubes. To quench the reaction, 5 cc of distilled water was added to each tube. The resulting mixture was extracted three times with 5 cc volumes of hexane. The combined hexane extracts, after being reduced in vacuo and adjusted to the appropriate volume (usually 2.0cc), were then ready for analysis by electron-capture gas chromatography.

TABLE I

RECOVERIES FROM URINE SPIKED AT THREE LEVELS UPON EXTRACTION WITH 2% ACETIC ACID IN HEXANE FOLLOWED BY METHYLATION

Pesticide	I	II	III
Aldrin	91-95 93	91-95 94	92-96 95
Dieldrin	92-96 94	93-97 94	94-98 96
p,p'-DDA	91-95 93	90-94 93	93-96 95
p,p'-DDE	93-97 94	92-96 94	92-99 96
p,p'-DDD	92-96 94	94-97 95	95-100 97
p,p'-DDT	93-97 95	93-100 96	94-99 96
o,p'-DDT	93-97 94	94-98 95	93-101 97
o,p'-DDE	89-95 91	88 95 91	90-97 93
Endrin	92-96 93	92-97 93	95-102 97
Lindane	92-96 94	92-96 94	95-100 97

I. 10ppb, II. 25ppb, III. 100ppb

Results are reported as the Range and Average obtained upon analysis of four identical samples.

TABLE II

LEVELS OF DDT-DERIVED MATERIALS IN HUMAN URINE IN P. P. B.

Pesticide	General Population		Occupationally Exposed	
	Range	Mean	Range	Mean
p,p'-DDA	8.1-19.0	14.2	33.0-148	75.3
p,p'-DDT	0.48-0.67	0.36	0.80-7.2	2.90
p,p'-DDE	0.41-0.55	0.42	1.10-1.3	1.20
p,p'-DDD	0.25-0.43	0.34	0.89-1.4	1.10

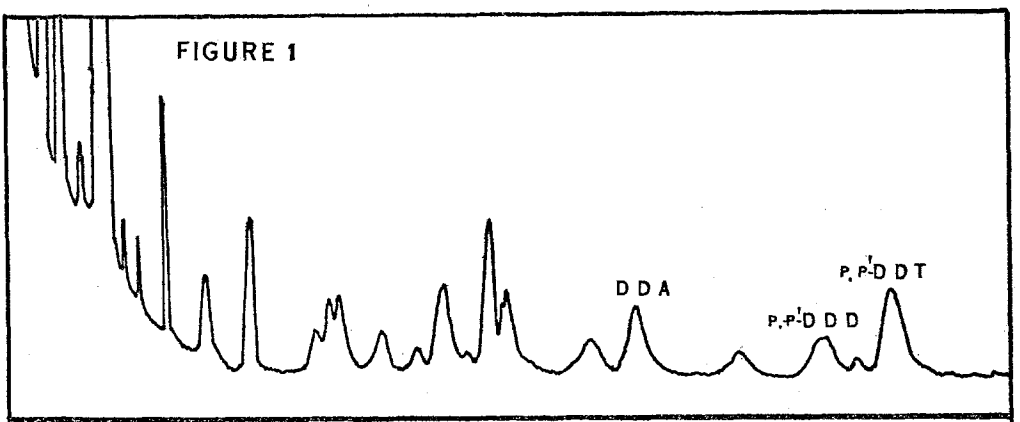
Analysis by Gas-Liquid Chromatography: Micro-Tek Model MT-220 gas chromatographs, equipped with electron capture or Coulson Conductivity systems, were used throughout the study. The Dohrmann microcoulometer detection system can be used in place of the Coulson conductivity system. These systems are roughly equal in their sensitivity to chlorinated hydrocarbons.

The most useful column type proved to be a 6'x1/4" O.D. Pyrex glass U-tube packed with 5% QF-1 on 80/100 mesh Gas Chrom-Q. Conditions for chromatography using 5% QF-1 columns were an oven temperature of 170-175°C and a nitrogen carrier flow of 80-100 cc/min. Other successful column packings were 5% QF-1 + 7.5% DC-200 and 3% SE-30 on 80/100 mesh Gas Chrom-Q.

RESULTS

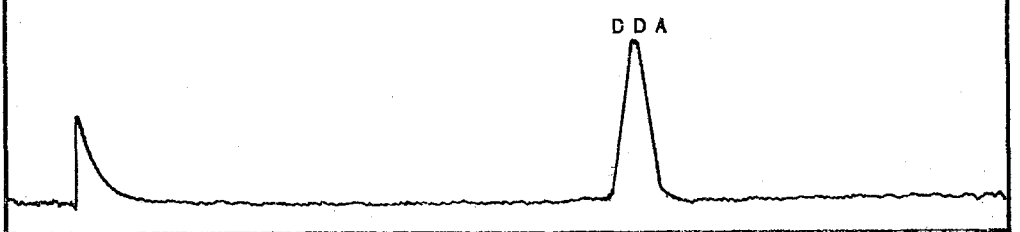
The differences in range of the two exposure groups are not as great as one might expect, however table II shows that mean values, particularly for DDA, are different. All values obtained from urinalysis were corrected to an osmolality of 1000 milliosmols by multiplying the observed value times a correction factor, K = 1000/observed osmolality. Satisfactory results are usually obtained, especially for the occupationally exposed, via the selective extraction method described and the use of an extra "clean-up" step is not always necessary. However, especially when examining lower level general population samples, materials which

FIGURE 1



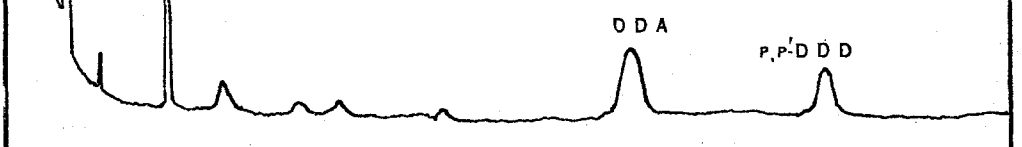
EXAMPLE OF A POOR EC CHROMATOGRAM OF A GENERAL POPULATION URINE EXTRACT

FIGURE 2



EXAMPLE OF COULSON CONDUCTIVITY CHROMATOGRAM OBTAINED BY CONCENTRATING EXTRACT SHOWN IN FIGURE 1

FIGURE 3



EXAMPLE OF AN EC CHROMATOGRAM OF FRACTION 2 FROM THE MICRO FLORISIL CLEAN UP OF THE EXTRACT SHOWN IN FIGURE 1

may not be chlorinated pesticides are extracted from the urine and interfere with analysis by resulting in electron-capture chromatograms too complicated for easy peak assignment (Figure 1). In these cases the simplest, most straight-forward method of analysis was found to be concentration of the extract to an appropriate volume (often about 0.20cc) with subsequent conductivity or microcoulometric detection (Figure 2). However, if electron-capture detection is to be used, clean-up by the micro florisil column is necessary (Figure 3).

DISCUSSION

The procedure described combines several desirable features including simplicity, speed of analysis, reproducibility and produces chromatograms with a minimum of interference. The lower limit level of detectability of the various chlorinated pesticides and metabolites can be adjusted by increasing the volume of urine originally extracted, concentrating the extracts to smaller volumes, injecting larger aliquots into gas chromatographs equipped with conductivity or microcoulometric detection systems, or by the inclusion of the micro-florisil clean-up step. One of the problems encountered in this procedure is the inherent lack of sensitivity of the methyl ester of DDA to electron-capture detection. In no case was it possible to detect the ester in a concentration of less than 2 ng.

total injected, whereas p,p'-DDT could be detected at the level of 50 pg. or less. In certain cases use of the conductivity or microcoulometric detectors was particularly valuable since sensitivity to chlorinated pesticides varies only with chlorine content. These detectors therefore will "see" the methyl ester of DDA while ignoring DDT and its other metabolites which are usually present in much lower concentrations.

It must be emphasized that the levels of pesticides reported for the two groups referred to as "general population" and "occupationally exposed" should not necessarily be considered representive of like groups throughout the country. The results obtained here are reported to demonstrate a method and not as a study of populational levels. However, it can be seen easily that the occupationally exposed group has urine DDA levels approximately ten times that of the general population group.

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