Determination of Toxaphene by Basic Alcoholic Hydrolysis and Florisil Separation

Edward D. Gomes
Texas State Department of Health
Epidemiologic Studies Program
152 East Stenger
San Benito, Tex. 78586

Introduction

The detection and quantitation of sub-microgram quantities of toxaphene is difficult especially in the presence of other pesticides.

Because of its composition, toxaphene chromatographs as a series of hills and valleys with three main peaks (Fig. 1). Witt et al. (1962) reported using a short gas chromatographic column with microcoulometric detection to pronounce the toxaphene peaks. The use of a flash heater, filled with various reagents in the injection port of the gas chromatograph, produces definite changes in the chromatogram of the pesticides (Minyard & Jackson 1965). The idea of rearrangement or partially dehalogenating the structure of toxaphene by treatment with KOH in ethyl alcohol (Crosby & Archer 1966). In 1969 Miller and Wells described an alkaline pre-column for use in gas chromatographic pesticide residue analysis. Another method modified from the work of Crosby and Archer (Carlin 1970) involves a sulfuric acid-celite cleanup followed by dehydrohalogenation and EC-GLC. A micro scale alkali treatment for use in pesticide residue confirmation has also been described (Young & Burke 1972).

Modified GLC methods, such as those mentioned, are an improvement over the previously reported general methods. In this paper, a similar dehydrohalogenation method is described to determine toxaphene in the presence of other organochlorine pesticides with the aid of a small florisil column in order to isolate a specific peak.

Materials

The toxaphene used was obtained from the Hercules Powder Company. Pesticide residue grade petroleum ether and reagent grade potassium hydroxide (Matheson, Coleman, and Bell) and reagent grade granular sodium sulfate (Mallenckrodt Chemical Works) were used. The

pesticide residue grade florisil (Floridin Company) was 60/100 mesh, activated at 1200°F. The chromatographic column support materials and silicone oils were from Analabs, Inc. The gas chromatograph (Micro-Tek 220) was equipped with an electron capture detector. The chromatographic columns for the florisil, the West condenser, and the Kuderna-Danish evaporative concentrator are available from Kontes Glass Company.

The florisil column used in the procedure was prepared by first activating the florisil in a constant temperature oven at 160°C for 24 hours. A glass chromatograph column (600 mm long and 10 mm ID, 13 mm OD), with a 250 ml reservoir on top, was rinsed thoroughly with petroleum ether and secured with a clamp on either a ringstand or flex-frame apparatus. 5.6 g of previously activated florisil is placed in the glass column topped with 3/4" sodium sulfate. The florisil is tamped down by hitting the sides of the glass column gently with a small rubber mallet and prerinsed with 50 ml of petroleum ether. The petroleum ether is allowed to flow through, leaving enough to prevent drying. The column is then ready for use.

Analysis of the toxaphene and the reaction products were accomplished with a Micro-Tek 220 gas chromatograph equipped with electron capture detector (tritium source) and the following parameters:

Columns - 10% DC-200 on 80/100 Anakrom ABS
1.6% OV-210/6.4 OV-17 on 100/120 Gas Chrom
4% SE-30/6% QF-1 Chromosorb W 80/100
5% OV-210 Gas Chrom Q 100/120

Temperatures - Inlet - 225°
Detector - 205°

Column:

OV-17/QF-1	- 200°
DC 200	- 200°
OV-210/SE-30	- 200°
SE-30/QF-1	- 200°
OV-210	- 175°

Carrier Gas - Nitrogen

Flow Rate

DC-200	- 80 cc/min
OV-17/OV-210	- 65 cc/min
SE-30/QF-1	- 70 cc/min
OV-210	- 60 cc/min

Electrometer Attenuation - 10 X 16

Column Theoretical Plates:

DC-200	-
OV-210/OV-17	2800
SE-30/QF-1	3900
OV-210	3000

Analytical Procedures

It is well known that basic alcoholic solutions will partially dehalogenate chlorinated pesticides (Fig. 1). With this idea in mind, a toxaphene standard was prepared to a concentration of 10 ng/µl, using currently approved pesticide residue analytical techniques. Ten ml of this standard solution was evaporated down to 1 ml and placed in a 250 ml Erlenmeyer flask with a ground glass joint and allowed to dry. To this, 25 ml of 50% methanolic potassium hydroxide (w/v) was added and refluxed for one hour. After this reflux, the solution was cooled immediately and the reflux condenser washed. The contents were poured into a 1 liter separatory funnel containing 500 ml of water. Petroleum ether (100 ml) was added to the methanolic KOH-water mixture and was shaken for 1 minute. After the layers separated, the water layer was discarded. A second portion of 500 ml of water was added and two layers shaken vigorously for 1 minute. After the layers separated, the water layer was again discarded. This step was repeated a third time so that the petroleum ether was washed with three successive portions of water in order to extract the methanol and potassium hydroxide from the solution. (If an interfacial cuff developed, it was easily broken with sodium sulfate.) At this stage the refluxed toxaphene product chromatographed with shrap peaks instead of hills and valleys (Fig. 2).

The petroleum ether layer was then transferred to a Kuderna-Danish evaporative concentrator and evaporated to 1 ml. The 1 ml solution of reaction extract was

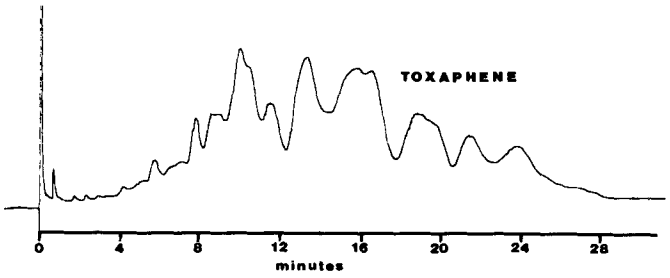

Figure 1. Chromatogram of Toxaphene on 10%DC-200 column

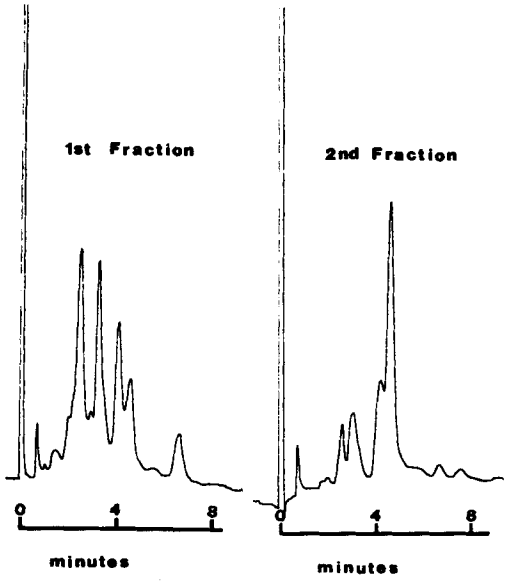

Figure 3. Chromatograms of 1st and 2nd fractions of Toxaphene refluxed product on 10%DC-200 column.

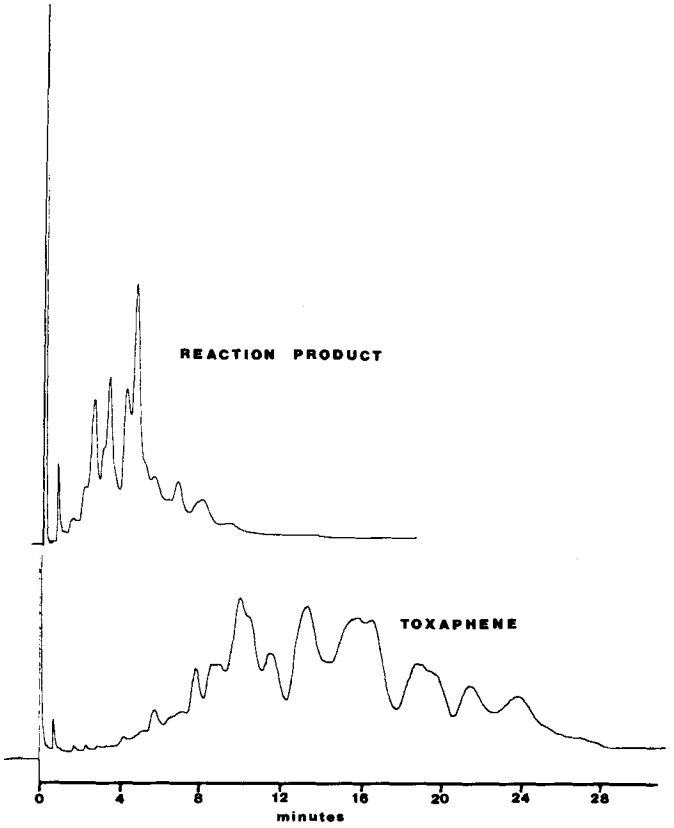

Figure 2. Comparison of chromatograms of Toxaphene and the reaction product, both on 10%DC-200 column.

pipetted on the previously prepared florisil column. The column was eluted with 350 ml of petroleum ether at the rate of 5 ml/min. The first 150 ml fraction was discarded. The second 200 ml fraction of petroleum ether was eluted into a Kuderna-Danish concentrator apparatus and evaporated to the original volume of standard which was 10 ml. This extract was equivalent to the original standard which was 10 ng/ul. Figure 3 shows chromatograms of the two fractions of refluxed toxaphene separated by the florisil column.

Discussion

Treatment of toxaphene with 50% methanolic potassium hydroxide for a one hour period produced the configuration in Figure 2. Three prominent, well-defined peaks are evident. Eluting this mixture of reaction product through a 5.6 g column of florisil isolates the major spiked peak of detection (Fig. 3). The relative retention time (relative to aldrin) of this peak is very close to that of aldrin, but on close examination the relative retention time is calculated to be 1.01 using the 10% DC-200 column. On the OV17/OV210 column, the relative retention time is 1.32. The two chromatograms in Figure 2 illustrate the same concentration for the isolated peak as for the standard unreacted toxaphene.

The peak height of the spiked peak on the gas chromatograph was linear with concentration. To prove this, various amounts of toxaphene were reacted and then injected into the gas chromatograph and the height of the spiked peak was measured. The concentrations used were between 1 ug and 32 ug. This step also revealed the lower limit of detection, which was one ng (10^{-9} grams).

The last phase involved was the determination of interfering pesticides with this procedure. Chlordane, Strobane, and PCB's interfere with the determination of toxaphene, whether the method is colorimetric or gas chromatographic employing microcoulometric or electron capture detectors. Chlordane, Strobane, and PCB were reacted together with toxaphene by this alkaline hydrolysis procedure. The major reaction products of the three compounds eluted through the florisil column in the first 150 ml fraction. The second 200 ml fraction shows the

presence of toxaphene by the spiked peak (Fig. 4).

Various formulations of pesticides were prepared in order to confirm the value of the analytical procedure developed. Each formulation was subjected to the procedure developed and the results indicated the spiked peak to be specific for toxaphene with little or no interference from other pesticides in the sample.

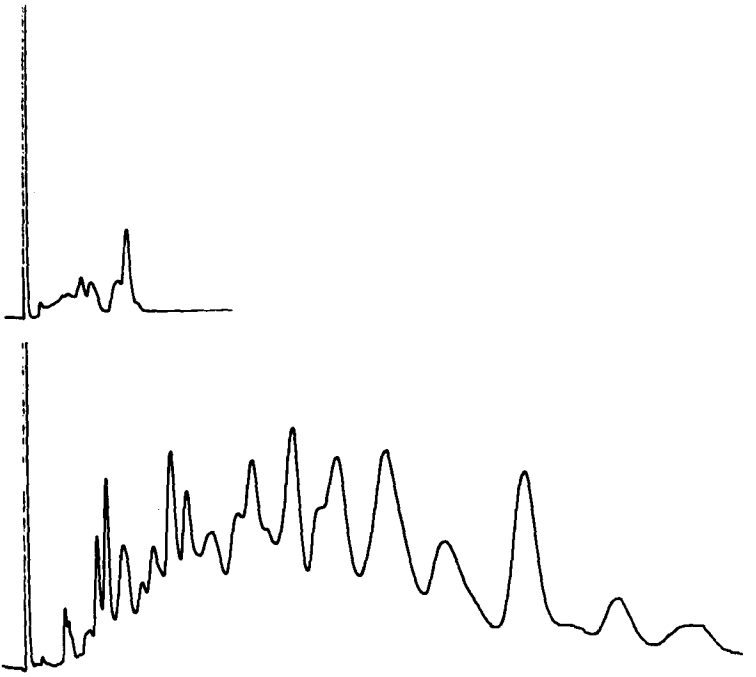

Figure 4. Chromatograms of before and after procedure of mixture having Toxaphene, Strobane, Chlordane, PCB 1260, BHC, Aldrin,Heptachlor Epoxide, p-p'DDE,Dieldrin,p-p'DDD, o-p'DDT and p-p'DDT.

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