Gas Chromatographic Measurement of Toxaphene in Milk, Fat, Blood, and Alfalfa Hay

by T. E. ARCHER and D. G. CROSBY

Agricultural Toxicology and Residue Research Laboratory
University of California, Davis, California

The need for a sensitive and reproducible method for determination of submicrogram quantities of toxaphene in food, forage crops, milk, and meat has become apparent with the widespread use of this pesticide. Graupner and Dunn (1) originally described a spectrophotometric procedure that could determine toxaphene in the 20 to 100 μg range. Extensive cleanup procedures were employed before analysis.

Kovacs (2) and Faucheux (3) used thin-layer chromatographic techniques for the rapid detection of micro quantities of several chlorinated pesticide residues. Cleanup of the samples was of critical importance, and toxaphene streaked on the TLC plates with the solvent systems employed. Burke and Guiffrida (4) investigated electron capture gas chromatography for the analysis of multiple chlorinated pesticide residues in vegetables; vigorous cleanup procedures were required, and, under their conditions, ten peaks were obtained for toxaphene. Bevenue and Beckman (5) gas chromatographed toxaphene in the nanogram range and concluded that in the presence of other chlorinated hydrocarbon pesticides, irrespective of the type of column or column packing used, no characteristic fingerprint for toxaphene could be obtained with one possible exception.

Contrary to the comments of these latter authors, the method described below for the analysis of toxaphene utilized a simple alkali treatment for cleanup and partial dehydrohalogenation of the substance, and gas chromatography with an electron capture detector permitted quantitative analysis. Nanogram quantities of toxaphene were detected reproducibly.

Bulletin of Experimental Contamination & Toxicology,
Vol. 1, No. 2, 1966, published by Springer-Verlag New York Inc.

Experimental

Chemicals and Equipment. Toxaphene was supplied by the manufacturer, all other chemicals were reagent grade, and the solvents were redistilled before use. The gas chromatograph was an Aerograph Model 200 (Wilkens Instrument Co.) equipped with an electron capture detector and a Texas Instruments Inc. Servo/Riter II 1 mv. potentiometric recorder. The chromatographic column was 9' x 1/8" stainless steel packed with 60/80 mesh Chromosorb W (HMDS treated) coated with 5% Dow 710 silicone oil and 5% SE-30 gum rubber. The 12" section of the column at the injection port was packed with 20/30 mesh calcium carbide for removal of traces of water and alcohol entrained during cleanup. Nitrogen carrier gas (40 p.s.i., 40-60 ml./min.), a column temperature of 200°C, a detector temperature of 190°C, an injector temperature of 250°C, and an instrument attentuation and sensitivity of 1X was used. The recorder chart speed was 15"/hour, and the peak areas were measured with a polar planimeter for quantitation.

The infrared spectra were obtained on a Perkin-Elmer Model 221 spectrophotometer, samples being mixed into potassium bromide and analyzed as micro pellets. Microgram amounts of toxaphene were dehydrohalogenated, extracted into benzene, and concentrated on the potassium bromide prior to pellet preparation. Background interference, found to be insignificant, was determined on a benzene blank treated in a manner similar to the toxaphene solution.

The dehydrohalogenation reagent was prepared fresh for each sample by dissolving 5 g. of C.P. potassium hydroxide in 3 ml. of distilled water followed by the addition of 17 ml. of ethanol.

Analysis of alfalfa hay, milk, fat, and blood. The preparation and analysis of the samples for toxaphene were performed as described by Crosby and Archer (6) with the exception that recoveries of toxaphene were higher if benzene rather than pentane was used for extraction.

Results and Discussion

Five different batches of toxaphene (Hercules Powder Co., Wilmington, Delaware) were analyzed with reproducible results. Fig. 1A represents the gas chromatogram from 30 ng. of untreated toxaphene; the total retention time was approximately 17.5 min. in several poorly resolved peaks. Fig. 1B represents the gas chromatogram of 30 ng. of toxaphene after dehydrohalogenation; the total retention time was approximately 11.0 min. with the major peak at 3.50 min. DDE and related compounds exhibited a longer relative retention time (6.25 min.) than that of the major toxaphene peak. For analytical data, either the measurement of the area of the entire trace from the dehydrohalogenated sample or measurement of the area of the major peak at 3.50 min. was easily accomplished with a planimeter.

Fig. 2 presents the infrared spectra of dehydrohalogenated toxaphene (I) and untreated toxaphene (II). As expected, comparison revealed a decrease in the number of the carbon-chlorine bands in the 12-15 μ range and an increase in the carbon-hydrogen absorption at 3.5 μ. A new carbonyl absorption was evident at 5.8 μ.

Numerous samples of alfalfa hay, milk, bovine fat, and blood have been analyzed by this method in our Laboratory. Background for injections representing as much as 50 mg. of prepared sample was negligible, while recoveries ranged from approximately 74% to 95% when the samples were fortified at the 0.1 ppm. and 0.5 ppm. level. Table I lists recoveries for a few of these samples.

The advantage of this procedure for toxaphene analysis is the very rapid and effective cleanup of the feedstuff and animal products by alkali treatment as well as the chemical conversion of the pesticide into derivatives that are more readily gas chromatographed than the parent substance. The reproducible single peak at 3.50 min. serves for quantitative and qualitative identification, and this peak cannot be confused with those of the DDT group also commonly present in most samples. An additional advantage is that the dehydrohalogenation procedure in-

creased the sensitivity of the electron capture detector to toxaphene by approximately two fold.

We are indebted to Nels Larsen and Eugene Whitehead for technical assistance and to Paul Allen and Joseph Thomas for assistance with the infrared data.

TABLE I

Recovery of Toxaphene in Animal Products and Feedstuffs

Product	Fortification p.p.m.	Recovery %
Alfalfa hay	0.5	95.2
Cow milk	0.1	74.1
Cow milk	0.5	92.7
Cow body fat	0.5	82.2
Cow blood	0.5	88.8
Rat blood	0.5	91.2

References

1. A. J. GRAUPNER and C. L. DUNN, J. Agr. Food Chem. 4, 286 (1960)
2. M. F. KOVACS, JR., J. Assoc. Offic. Agr. Chem. 48, 1018 (1965)
3. L. J. FAUCHEUX, JR., J. Assoc. Offic. Agr. Chem. 48, 955 (1965)
4. J. BURKE and L. GUIFFRIDA, J. Assoc. Offic. Agr. Chem. 47, 326 (1964)
5. A. BEVENUE and H. BECKMAN, Bull. Environ. Contam. and Tox. 1, 1 (1966)
6. D. G. CROSBY and T. E. ARCHER, Bull. Environ. Contam. and Tox. 1, 16 (1966)

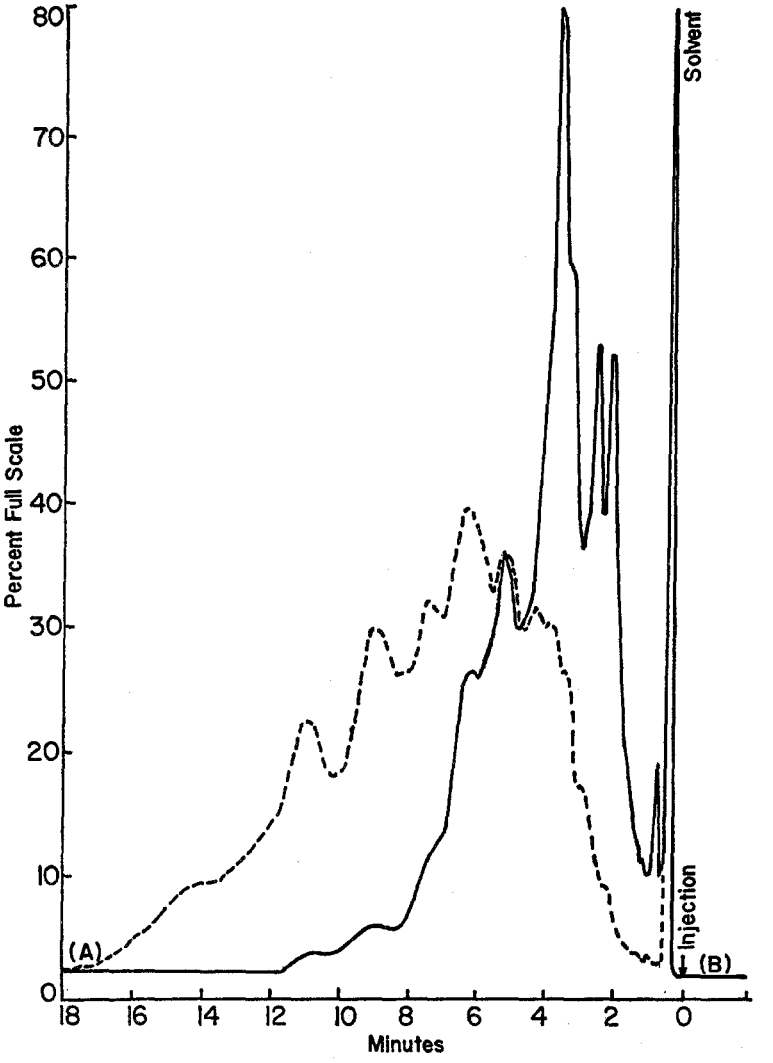

Fig. 1. Gas chromatogram from 30 ng. of toxaphene (A) before alkali treatment and after alkali treatment (B).

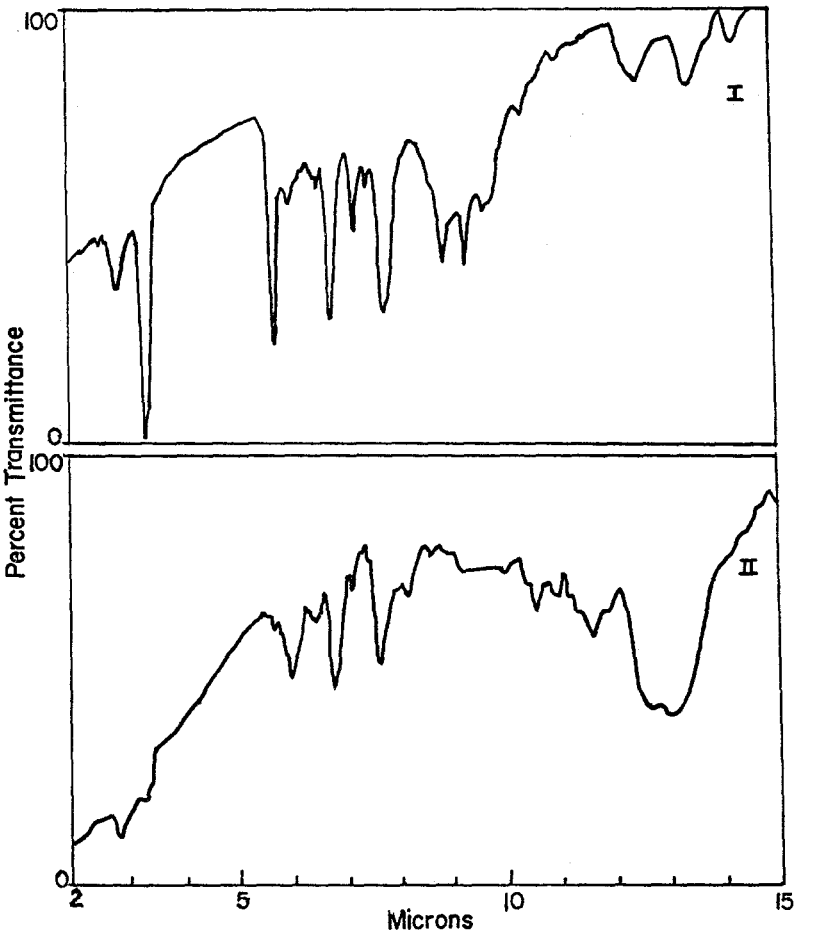

Fig. 2. Infrared spectra of toxaphene after alkali treatment (I) and before alkali treatment (II).