

A Method for the Determination of Toxaphene in the Presence of DDT

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The experienced residue analyst is quite familiar with the indecisive and continuum-type curve produced by the pesticide toxaphene (a mixture of compounds described as "chlorinated camphene") when it is analyzed by gas chromatography and the concomitant problems involved in the interpretation of the analytical results obtained from a sample of an agricultural commodity that may be suspected of containing excess quantities (above legal tolerance limits) of this pesticide. The problem is enhanced if other pesticides, such as DDT, are present in the same residue mixture and it becomes acute in regulatory laboratories where the time factor between the receipt of the market sample and the required report of the chemical analysis may be no greater than twenty-four hours.

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Several procedures have been proposed to clarify the situation that exists when the identity and the quantities of residues of mixtures of toxaphene and DDT found on an agricultural crop require confirmation. Faucheux (1) used the spray reagent diphenylamine-zinc chloride on aluminum oxide thin-layer plates to distinguish, by different color reactions, the pesticides Strobane, toxaphene, chlordane, and DDT. Strobane and toxaphene produced the same gray-green color, chlordane streaks were purple, and DDT spots were reddish orange; the method was useful for the qualitative confirmation of chlordane and DDT, but toxaphene confirmation was inconclusive. Archer and Crosby (2) applied the alkaline dehydrohalogenation technique to plant and animal extracts prior to gas chromatographic analysis. They produced a modified toxaphene curve with a shorter retention time than the modified DDT (DDE and related compounds) curve. Gaul (3) proposed the construction of a baseline between the extremities of the toxaphene curve on the gas chromatogram, or between the "last four peaks" (which appeared beyond the DDT peaks on the curve if a mixture was present), plus the construction of parallel lines at those points where the DDT compounds appeared above the toxaphene continuum. A comparison of the amount of toxaphene in the presence of DDT, using the area of the entire curve versus the area of the last four peaks of the toxaphene curve, versus the cut-and-weigh method (weight of paper area of the curve) showed that the measurements were quantitatively related. It should be noted that the three laboratories that were involved in the present study have observed

in past experiences that the "last four peak" phenomena is not always obtained on a toxaphene gas chromatograph curve. The inconsistency may be attributed to different weathering conditions of the sample, and/or the condition of the gas chromatograph column that is used for the analysis.

The stability of toxaphene in strong acids has been noted (4) and mixtures of sulfuric-fuming sulfuric acids have been used to separate this pesticide from fats and oils. This type of acid mixture has also been used to isolate DDT and other pesticides from fats (5, 6, 7), whereby the pesticide was subsequently measured by either a colorimetric or total chloride procedure. The amount of fuming sulfuric acid in the mixture was limited to a ratio of one part fuming sulfuric to three parts concentrated sulfuric acid to avoid sulfonation of some pesticides. Claborn and Beckman (8) noted that the pesticide methoxychlor [1, 1, 1-trichloro-2, 2-bis (p-methoxyphenyl) ethane] is destroyed by strong sulfuric acid. Schechter et al (9) applied intensive nitration to DDT residues from plant extracts and used a 1:1 (volume/volume) mixture of concentrated sulfuric acid and fuming nitric acid and heated the sample for one hour in a steam bath. The extended heating period was considered necessary to destroy extraneous plant extractives that would otherwise interfere with the subsequent colorimetric analysis procedure.

The following procedure is suggested for measuring the amounts of suspected residues of mixtures of toxaphene and DDT on agricultural commodities. Briefly stated, it involves analyzing separate portions of the crop extract for pesticides by gas chromatography, before and

after nitration of the crop extract residue, under milder conditions than originally suggested by Schechter et al (9). The chromatographic pattern of toxaphene is not affected by nitration, and the nitrated DDT does not chromatograph under the conditions specified below. The amount of toxaphene present in the sample can be determined by comparing the area of the curve of the acid-treated sample to the area of the acid-treated toxaphene standard curve. To determine the amount of DDT present in the sample, it is first necessary to obtain the area of the curve of the untreated sample. If the curve areas of a toxaphene standard and an acid-treated toxaphene standard (equal amounts of toxaphene used in both standards) are the same, then the amount of DDT in the sample is determined by subtracting the area of the acid-treated sample from the area of the untreated sample. If, however, 100 percent recovery of toxaphene is not obtained by acid treatment of the toxaphene standard, then the ratio value of the toxaphene standard : acid-treated toxaphene standard multiplied by the area of the acid-treated sample will give the amount of toxaphene present in the untreated sample and this value subtracted from the area of the untreated sample will give the amount of DDT in the original sample. The method should be useful not only in regulatory work but in various areas of pesticide research; for example, Klein and Link (10), in their field weathering study of toxaphene on kale, noted that large amounts of DDT interfered with the proper evaluation of toxaphene residues determined by gas chromatography.

Method

To test the proposed procedure, pesticide solutions were prepared

as listed in the tabulation below for subsequent analysis by gas chromatography using an electron capture detector.

<u>Sample</u>	<u>Toxaphene (micrograms)</u>	<u>Technical grade DDT (micrograms)</u>	<u>Treatment of Sample</u>
A	60	30	None
B	60	None	None
C	None	30	None
D	60	60	sulfuric- fuming nitric acid, 1:1, v/v
E	60	None	ditto
F	None	30	ditto

Sample solutions of greater concentration (400 micrograms toxaphene and 100 micrograms DDT) were similarly prepared for gas chromatographic analysis on the Dohrmann microcoulometer equipped with the halide detector. The sensitivity of the Dohrmann instrument was set at 100 ohms for the determination of toxaphene-DDT in the range of 400 and 100 nanograms, respectively.

In the suggested procedure, the pesticide solutions and the solvent extracts of the plant samples were evaporated to dryness in suitable glass containers and five ml. of a 1:1 (volume/volume) chilled mixture of sulfuric acid-fuming nitric acid was added to each residue. Each mixture was allowed to stand for 15 minutes at room temperature, then quantitatively transferred to a separatory funnel (60 ml. or 125 ml. capacity) containing 25 ml. ice-cold distilled water, mixed thoroughly, and allowed to stand until mixture attained room temperature. The aqueous mixture was extracted twice with re-distilled benzene (11), using 10 ml. of benzene for each extraction.

The benzene extracts were combined and washed twice with 10 ml. portions of distilled water, filtered through a plug of anhydrous sodium sulfate and made to a suitable volume for gas chromatography. Gas chromatograms were obtained with two Aerograph Pestilyzers, an Aerograph Model 204-1B (equipped with electron capture detectors), and two Dohrmann microcoulometer instruments equipped with halide detectors. The gas chromatograph columns used were 2% SE-30 on Chromosorb G. AW, DMCS, 80/90 mesh; 3% SE-30 on Chromosorb W, DMCS, 80/100 mesh; 5% SE-30 on Chromosorb W, DMCS, 60/80 mesh; and a 50-50 mixture of 5% Dow-11 and 5% QF-1 on Chromosorb P, DMCS, 60/80 mesh. The columns for the Aerograph instruments were 1/8" x 5 ft. glass, and for the Dohrmann instruments 1/4" x 5 ft. glass. Column temperatures were 195° C. for the Aerographs and 220° C. for the Dohrmanns. Nitrogen was the carrier gas. The variety of gas chromatograph columns was occasioned by the fact that three differently located laboratories collaborated in this study and the designated columns were available for immediate use in the instruments mentioned above.

Discussion

The chromatographic data showed that the DDT component of the residue mixtures was quantitatively removed by the acid treatment, and that high and consistent levels of toxaphene were obtained. The efficiency of the removal of the DDT component from the mixture was consistent and comparable in all three laboratories involved in this study. No differences in the results were noted by extending the nitration period to one, two, four, or 18 hours. Experiments with

sample residues (red leaf lettuce) had undergone a Florisil cleanup step prior to the nitration step. Further study may show that nitration at room temperature may be sufficient for the elimination of any extraneous materials that might interfere with DDT-toxaphene mixtures. In studies made thus far, toxaphene was not affected by the more severe nitration treatment prescribed by Schechter et al (9) for the removal of interfering plant extractives.

Nanogram quantities of the pesticides can, of course, be determined with the electron capture detector, and somewhat larger amounts with the Dohrmann halide detector, and the limits of detectability will be governed by the attenuation setting of the electrometer, the sensitivity of the detector, and the efficiency of the gas chromatograph column. Burke and Holswade (12) have indicated an average toxaphene-p-p'-DDT ratio of 8:1 for comparative responses on the gas chromatograph with the electron capture detector and a ratio of about 6:1 with the microcoulmeter detector (13). In the work reported herein, toxaphene and p,p'-DDT were distinctive in ratios of 4:1 and 2:1 (toxaphene:DDT).

In addition to DDT and its related products, nitration removed aldrin, heptachlor, Kelthane, Perthane, Tedion, Telodrin, and Trithion from the chromatographic picture. Lindane and heptachlor epoxide were not removed by nitration, and preliminary studies indicated that chlordane was not affected by the nitration treatment. Strobane (a mixture of chlorinated terpenes) produced a gas chromatographic pattern very similar to toxaphene, and the Strobane pattern, also, was not affected by the nitration treatment. Without doubt, toxaphene-DDT

formulation mixtures are more commonly used than a Strobane mixture, and this is evident when one compares the lengthy list of uses for toxaphene with the brief list for Strobane in the USDA Summary of Registered Agricultural Pesticide Chemical Uses. To date, pesticides other than the ones mentioned above have not been studied with the nitration procedure. If toxaphene-DDT formulations are used on a crop, usually in ratios of 3:1, 2:1, and 1:1, other residues will not ordinarily be present, at least not in the over-tolerance range.

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