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Determination of Toxaphene in Milk, Butter and Meat

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Toxaphene (camphechlor) is currently one of the most abundantly used organochlorine pesticides with an annual world production in 1970 of 17 million kg (ANDRILENAS 1970). Its area of application covers small grains, rice, soyabeans, vegetables, control of cotton insects and the eradication of ticks on livestock. Our interest stems from the latter use which usually involves the dipping of livestock, a practice, which it is believed, leads to raised levels of toxaphene in the milk and meat of animals.

Toxaphene is produced by the chlorination of camphene to give a complex mixture consisting of some seventy closely related compounds which possess an average molecular formula of $C_{10}H_{10}Cl_8$. Like all organochlorine (OC) pesticides, toxaphene is toxic to mammals and maximum residue levels have, in most countries, been set at 0.5 parts per million (ppm), measured in terms of the weight of fat in the sample.

Notwithstanding, no recognised procedure for the reliable determination of toxaphene residues at this level, has to date been established. The reason for this may be attributed to its multicomponent and close inter-related structural make-up, as well as the close chromatographic character which toxaphene bears to other OC pesticides. These facts in consequence result in a substantial decrease in the sensitivity of its detection, as well as possible interference from coextractants such as DDT.

A number of findings have, however, emerged from recent studies which have been found helpful in a search for a reliable method for the routine analysis of toxaphene in fatty material. The first was contributed by SCHECHTER et al.(1945) and KAWANO (1969) who observed that while H_2SO_4 - fuming HNO_3 (0°C) exerted little effect on toxaphene, it completely destroyed most other pesticides. Apart from an evaluation study made by KLEIN and LINK (1970) this finding appears to have been neglected. A second development was the application of alkali hydrolysis for the simultaneous extraction and cleanup of OC in fatty substrates, a method first used by SCHAFER et al.(1963) and later, CROSBY and ARCHER (1966). Finally STALLING et al.(1972) and JOHNSON et al.(1976) developed gel permeation chromatography (GPC) as an efficient means for the

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isolation of OC pesticides from fat.

MATERIALS AND APPARATUS

The exact composition of toxaphene varies with producer, and standards must be selected accordingly. Stock solutions of 100 ppm were prepared weekly and were subjected to the exact treatment as were the extracts. Standard solutions of 0.05, 0.1, 0.5, 1.0 and 10 ppm were then prepared daily.

Florisil 60/100 mesh, supplied by Supelco Inc., was heated at 175°C for 24 h prior to use. Although petroleum ether-dichloromethane (4 + 1 $^{v}/_{v}$) is currently favoured for eluting OC from fat, it was found that toxaphene eluted very efficiently with hexane. 5% diethyl ether-hexane was therefore used. Unlike DDT, toxaphene showed no sign of adhering to the Florisil. Each 500 g batch of Florisil was tested with a toxaphene standard prior to use. The KOH reagent was prepared daily by diluting 10 g KOH with 6 mL H_2O and making up the volume to 35 mL with 95% ethanol.

The GPC material was Bio Beads S-X3 (3% divinyl benzene-polystyrene copolymer). GPC columns were prepared as described by JOHNSON et al. (1976) and eluted with ethyl acetate - toluene (3 + 1 $^{v}/_{v}$). The first 92 mL of the eluent was discarded, the next 83 mL fraction was collected and the column was purged with a final 50 mL of the eluant.

Analysis was by gas chromatograph equipped with an electron capture detector (^{63}Ni). Columns used were 5% OV-101 or 1.5% OV-17/1.95% OV-210 on Chromosorb W - HP, 100 - 120 mesh. The column lengths were 0.75 m and the bore was 3 mm. The carrier gas was nitrogen with a flow rate of 27 mL/min. Temperatures were: inlet 225°, column 205° or 180° and detector 250°C.

SAMPLE PREPARATION

(a) Milk. Samples were obtained in 100-mL glass bottles and stored at 1 - 2°C. After the bottles had been heated to 40°C and stirred to give a homogenous solution, 20 mL samples were taken for analysis. A separate sample was taken for the determination of per cent fat content.

(b) Butter. 50 g was heated to 50°C and the fat decanted. Two 1 g fat samples were weighed for analysis.

(c) Meat. Normally 50 g samples were quick-frozen sealed in glass containers until required.

It was shown by BRADLEY and SCHULTZ (1970) that little change occurred to toxaphene either during the processing of milk to butter or cheese or during lengthy periods under refrigeration. In contrast to the persistent nature of most OC pesticides, the

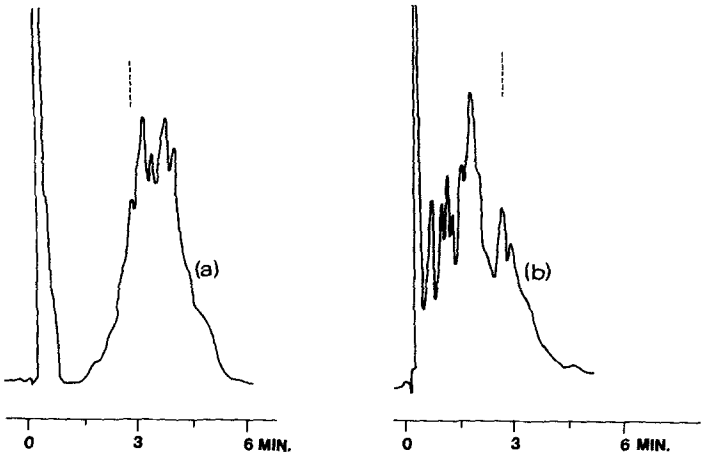

Figure 1. Chromatograms of Toxaphene(a) and the dehydrohalogenated product (b) on 5% OV101 at column temperature 205°C. Broken lines indicate the relative position of DDE.

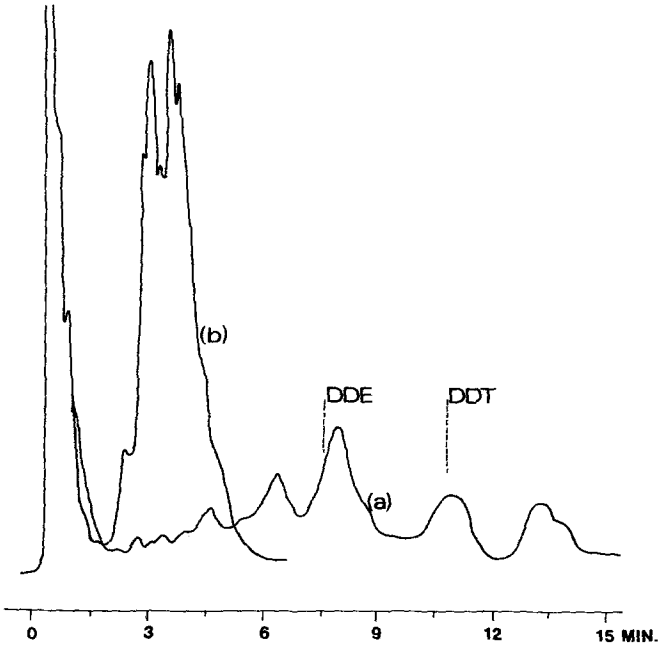

Figure 2. Chromatograms of Toxaphene on 5% OV101. Column temperatures (a) 180°C (b) 205°C.

depletion of toxaphene from livestock tissue is known to be relatively rapid BATEMAN et al. (1953) and ZWEIG et al. (1963).

ANALYTICAL PROCEDURE

A number of possible procedures for the analysis of toxaphene residues were investigated:

Study I. The method evaluated in this study was based on that described by SCHAFER et al. (1963) and CROSBY and ARCHER (1966). 20 mL whole milk was added to 35 mL of the alcoholic KOH reagent and heated on a waterbath. The dehydrohalogenated toxaphene was extracted with hexane and analysed (Fig. 1(b)).

Study II. The milk fat, containing the toxaphene was extracted from whole milk with diethyl ether-hexane. The toxaphene was isolated from the fat by GPC, after which the GPC solvent was removed. Cold H_2SO_4 - fumming HNO_3 was added to the dry extract, thereby oxidising interfering pesticides e.g. DDT. After the addition of cold water the toxaphene was extracted with hexane, cleaned up on Florisil and analysed (Fig 1(a)).

Study III. The technique of single-ion monitoring in gas chromatography - mass spectrometry (GC - MS) was studied. Using this approach it was hoped to eliminate the lengthy acid oxidation treatment used in study II. The $^{m}/_{e}$ 159 ion species were found to be present in 70% of the toxaphene isomers, but absent from the spectra of other OC pesticides.

The method outlined in study I involving alkali hydrolysis, proved to be extremely rapid and as many as 30 milk samples could be analyzed per day. The average recovery of toxaphene by this method was found to be good. Quantitation, however, presented numerous difficulties, especially where low levels of toxaphene were to be measured by a GC integrator.

The method described in study II gave a high average recovery of toxaphene. Results proved to be reproducible as well as reliable. The method was however, demanding and a maximum of only 6 - 8 samples per day could be handled by a trained person.

Study III was attractive from the point of view of rapid extraction and cleanup (study I), followed by the removal of interfering substances by single ion monitoring GC - MS. It was found, however, that the approach of study III failed to provide sufficient sensitivity for the analysis of toxaphene at the residue level. A Varian CH 7 (1967) instrument, fitted with a Bieman gas separator and a Pye 104 gas chromatograph was used for this purpose.

The findings resulting from above studies are equally valid for the case of butter and meat.

In deciding on a method, our strategy was determined by the fact that of all the samples analysed in the laboratory, less than 5% revealed measurable levels of toxaphene. It was, therefore, decided to utilise the alkali hydrolysis method outlined in study I as a screening method, on a yes or no basis, while reserving the longer method outlined in study II for the precise analysis of the ca 6% of samples in which toxaphene was either confirmed or suspected of being present.

I. Screening Method for Toxaphene

(a) Milk. A 20 mL sub-sample of milk was pipeted into a 80 - 100-mL test tube (with a B19 ground joint) containing 35 mL freshly prepared KOH reagent. The solution was shaken, a 15-cm condenser tube with a B19 ground joint attached and the tube heated on a water bath at 80°C for 25 min with intermittent swirling. The reacted solution was cooled under tap water and 10 mL hexane was added by washing the ground joint surface by pipet. The condenser tube was removed, the tube stoppered and the solution shaken on a vortex mixer for 4 - 5 min. The organic and aqueous layers were then separated by centrifuging the tubes for 5 min at ca 1500 rpm. 5 µL of the extract was examined by EC - GC.

(b) Butter. 35 mL KOH reagent was added to a 80-mL test tube containing 1 g extracted fat. The analysis was then conducted according to the procedure for milk.

(c) Meat. A 10 g sub-sample of meat was blended with 400 mL ethyl acetate, the homogenate dried on Na_2SO_4 and filtered through glass paper using a Buchner funnel. The solvent was evaporated and the resultant extract treated with the KOH reagent according to the procedure for milk.

Blank and fortified samples were included in the screening procedure. The analysis was abandoned if 0.05 ppm (0.05 ng/µL) or less toxaphene was present, i.e. could not be identified from the chromatogram. In instances where the presence of toxaphene could be identified, was suspected or where uncertainty arose, a second sub-sample was taken and analysed according to method II.

II. Analysis of Toxaphene

(a) Milk. 20 g milk was pipeted into a 75 - 100-mL centrifuge tube (fitted with Teflon stopper) containing 20 mL ethanol. After swirling, 0.4 g sodium oxalate was added and the tube shaken. 10 mL Di-ethyl ether, 15 mL hexane and an internal standard were added and the solution thoroughly shaken on a vortex mixer. The tubes were centrifuged at ca 1500 rpm for 10 min and the ether layer then removed by Pasteur pipet. A second extraction was performed with a further 25 mL of the

solvent. The extract was concentrated to less than 0.5 mL with a two ball micro-Snyder column and made up to 10 mL with the GPC elution solvent, ethyl acetate-toluene (3 + 1 $^{v}/_{v}$). A separate 20 mL milk sample was taken for per cent fat determination.

(b) Butter. 1 g fat was diluted in 10 mL GPC solvent.

(c) Meat. 10 g meat was blended with 400 mL ethyl acetate and the homogenate dried on Na_2SO_4. After filtering with a Buchner funnel, using glass filter paper, the solvent was evaporated to near dryness with a Kuderna-Danish evaporator. 1 g of the extract was diluted in a 10 mL ethyl acetate-toluene (3 + 1 $^{v}/_{v}$) solution and shaken on a vortex mixer.

GPC Isolation of Toxaphene. Since the loading capacity of the S-X3 gel column is a limiting factor, extracts were split into two 5 mL fractions. Working at a solvent flow rate of 4.5 mL/min, the first 92 mL containing the fat was discarded, the fraction 92 to 175 mL was collected, after which the column was purged with a further 50 mL of the solvent. The two 82 mL fractions containing the toxaphene were combined and concentrated with a Kuderna-Danish evaporator to 10 mL, then to near dryness with a two ball micro Snyder evaporator, in a 50 - 75 mL Erlenmeyer flask fitted with a ground joint.

Acid Oxidation. 5 mL H_2SO_4 - fumming HNO_3 (1 + 1 $^{v}/_{v}$) at $0^{o}C$ was added to the Erlenmeyer flask containing the dry extract, gently swirled for 30 sec and left at room temperature for 25 min. 25 mL distilled iced water was carefully added (in a fume cupboard), the solution was swirled for 30 sec and allowed to cool. The solution was transferred to a 100-mL separatory funnel with the aid of 20 mL hexane and after thorough shaking and allowing the layers to separate, the aqueous layer was drained to a second separatory funnel where it was re-extracted with 20 mL hexane. The extracts were combined in the first separotory funnel to which 20 mL of a 5% $NaHCO_3$ solution (to give pH 7) was added. After shaking, the aqueous layer was discarded and the organic extract collected in a 50 mL-Erlenmeyer flask. The extract was again concentrated to 5 mL and passed through a Na_2SO_4 - Florisil column.

Florisil Cleanup. 12 g Florisil was placed in a 25 x 500 mm column and 4 g Na_2SO_4 added to the top of the leveled Florisil. The column was pre-washed with 40 mL of the eluting solvent; 5% diethyl ether in hexane. After applying the extract and allowing it to enter the Florisil, the eluting solvent was added. Working at a flow rate of 3.5 mL/min, toxaphene eluted with 100 mL of the eluting solvent. The cleaned-up extract was concentrated down and 5 μL of the extract was examined by EC - GC.

RESULTS AND DISCUSSION

No difficulty was experienced with the extraction and cleanup procedures of either the screening or the analytical method. Recovery of toxaphene (from milk) was high, 86% for the screening and 83% for the analytical method. The latter case is tabulated below:

Toxaphene added (ppm)	Recoveries (%)		
	milk	butter	meat
0.5	82	79	76
0.5	78	-	-
1.0	83	85	78
1.0	86	-	-
2.0	88	84	79
2.0	84	-	-
5.0	84	86	78
average recovery	83%	84%	78%

The standard deviation for the analytical method (II) for milk was 7.8.

Two approaches for the evaluation of the chromatogram were investigated. In the one the average value of individual peak areas were considered while in the other the total area of the unresolved peak capsule was measured (Fig. 2). The practice of measuring the unresolved peak area provided greater sensitivity and reproducibility. This latter approach is made possible only because interfering substances are removed by the acid oxidation treatment. Interfering substances known to survive the acid treatment are chlordane, which we have to date not encountered, lindane and heptachlor epoxide. The latter two, particularly lindane, elute ahead of toxaphene on non-polar phases. As a precaution, however, all samples found to contain a significant level of toxaphene were re-chromatographed at 180°C (Fig. 2), in which case significant levels of chlordane etc. would be noticable. In this rare event the last two major peaks of the chromatogram would be used for the quantification of toxaphene. Organophosphorus pesticides are not encountered in milk or meat except in cases of poisoning.

To date no toxaphene has been found in either butter or meat, although the number of samples has been limited. Of 49 milk samples analysed (September to December 1977), 4 showed positive results: 0.2, 0.4, 0.4 and 1.0 ppm, and of 59 samples (February to May 1978), three were positive: 0.3, 0.4 and 2.9 ppm.

The procedure reported here has met requirements for the routine but accurate analysis of toxaphene residues in milk, butter and meat.

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