and about 3 grams of Celite. Filter through a folded filter into a 500-ml. Erlenmeyer flask and wash blender and filter thoroughly with acetone. Repeat the extraction and filtration using *n*-hexane. Concentrate the acetone extract to a volume of 50 ml. by distillation through a Snyder column. Transfer the condensed extract to a 500-ml. separatory funnel containing 200 ml. of 5% sodium sulfate, using 15 ml. of acetone to make the transfer. Extract three times with *n*-hexane, using the *n*-hexane from the extraction above for the first two extractions and 75 ml. of fresh *n*-hexane for the last extraction. Shake gently during the first extraction to avoid formation of an emulsion. Combine the hexane extracts in another separatory funnel and wash with a 50-ml. volume of 5% sodium sulfate solution. Discard the aqueous layer and drain the hexane solution through a 1-inch layer of anhydrous sodium sulfate into a 300-ml. Erlenmeyer flask. Evaporate the solvent to 5 or 10 ml. on a hot plate, then attach the flask to an aspirator, and remove the last traces at reduced pressure. Dissolve the residue in 5 ml. of *n*-hexane and proceed with the column cleanup and determination as described for fat.

Discussion

Recovery Experiments. The efficiency of the over-all procedure was tested by adding known amounts of ronnel to samples of the various tissues and milk before extraction. The recoveries from milk were 87% at the 0.01-p.p.m. level, from fat 77% at the 0.005-p.p.m. level, and from the other tissues 85 to 94% at the 0.005-p.p.m. level.

Sensitivity. With the range setting of 10^{-9} ampere, 0.1 nanogram of ronnel in 10 μl. of hexane injected into the column gives a response of 4% on the recorder. The control samples showed no peaks at the retention time for ronnel; however, there was some variation above the base line, which amounted to 2 to 3%. Under the conditions described, 0.1 nanogram of ronnel is readily detected, and with the sample sizes and dilutions used, 0.001 p.p.m. of ronnel can be detected in milk and 0.0005 p.p.m. in body tissues.

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INSECTICIDE RESIDUES

Determination of 2-Chloro-1-(2,4-dichlorophenyl)vinyl Diethyl Phosphate and 2,2′,4′-Trichloroacetophenone in Animal Tissues and Milk

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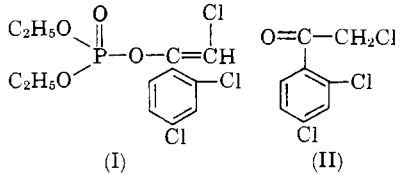
A gas chromatographic method is described for determining minute quantities of 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate (Shell Compound 4072) in body tissues and milk. A high degree of sensitivity is achieved by hydrolyzing the compound with dilute sulfuric acid to 2,2′,4′-trichloroacetophenone and examining the hydrolysis product by means of a gas-liquid chromatograph equipped with an electron-capture detector. Extraction and cleanup procedures are described for determining the presence of both Shell Compound 4072 and 2,2′,4′-trichloroacetophenone, a possible metabolite, in the same extract.

IN RESEARCH directed toward more effective control of livestock pests, the Entomology Research Division has a strong interest in finding new insecticides to substitute for those which are causing serious residue problems. Shell Compound 4072 [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] is very effective in controlling flies and ticks on livestock. Residue tests were required for approval of the use of this pesticide on livestock, but a sensitive method for its determination in milk and the body tissues was not available. P^{32}-labeled material was used by Roberts, Radeleff, and Claborn (*1*) to study its residues in milk, but this method was not adaptable to large-scale experiments. To fulfill the need for a method with a high degree of sensitivity we developed a gas chromatographic technique which makes possible determining as little as 5 parts per billion of Shell Compound 4072 in milk and body tissues. Shell Compound 4072 tends to decompose at high temperatures and therefore its presence cannot be determined directly by gas chromatographic methods. However, the compound is readily hydrolyzed in dilute sulfuric acid to produce 2,2′,4′-trichloroacetophenone, a volatile material that lends itself readily to gas chromatographic methods. The high percentage of chlorine in 2,2′,4′-trichloroacetophenone makes it possible to determine minimal quantities of the compound by using the electron-capture detector. 2,2′,4′-Trichloroacetophenone is also a likely metabolite of Shell Compound 4072, and any residue study of the latter should also include analyses for this metabolite. The structural formulas for Shell Compound 4072(I) and 2,2′,4′-trichloroacetophenone(II) are:



(I) (II)

Procedures are described for determining both of these compounds in the same extract. In developing these methods, it was necessary to use individual extraction and cleanup methods for milk, fat, and other body tissues.

Method

Materials and Apparatus. Acetone, ethanol, ether, *n*-hexane, acetonitrile, and dichloromethane, redistilled.

Silicic acid, Malinckrodt's 100-mesh powder, analytical reagent grade.

Florex XXS grade, extracted with acetone, air-dried, and heated at 130° C. for 7 hours.

Alumina, Woelm, acid-washed, with 2.5% water added.

Chromatographic column, Shell design, Scientific Glass Apparatus Co. Catalog No. JD-4030.

Gas chromatograph, Jarrell-Ash Model 700 or equivalent with electron-capture detector.

Standard Curve. Prepare a standard solution of Shell Compound 4072 in *n*-hexane containing 0.1 μg. per ml. To five 125-ml. flasks add 1, 2, 3, 4, and 5 ml. of the standard solution and evaporate the solvent with a jet of clean dry air. Add 0.5 ml. of methanol to dissolve the residue, and follow with 10 ml. of 12*N* sulfuric acid. Connect the flask to a reflux condenser and boil for 30 minutes. Remove from heat and wash down the condenser with 10 ml. of 5% sodium sulfate and 5 ml. of water. Cool the flask to about 20° C. and add, with a pipet, 5 ml. of *n*-hexane. Stopper the flask and shake for 1 minute, then transfer the mixture to a 60-ml. separatory funnel and drain off the water layer completely. Transfer the *n*-hexane solution to a 10-ml. glass-stoppered flask containing a small amount of anhydrous sodium sulfate (the last two steps need not be quantitative). Inject 10 μl. of each solution into the gas chromatograph and prepare a standard curve, plotting nanograms of Shell Compound 4072 against peak heights. Keep the standards tightly stoppered and hold for future use. One nanogram in 10 μl. should give a recorder response of 45 to 60%, depending upon the sensitivity of the detector, and the curve will represent concentrations of 0.2 to 1.0 nanogram of Shell Compound 4072 and 0.124 to 0.62 nanogram of 2,2′,4′-trichloroacetophenone. If less sensitivity is desired, extract the trichloroacetophenone after hydrolysis with 10 ml. of *n*-hexane.

For gas chromatography use a glass column 6 mm. in o.d. and 4 feet long, filled with 80- to 100-mesh Chromosorb W treated with 5% SF 96, a column temperature of 200° C., injector temperature of 200° C., and detector temperature of 205° C., prepurified grade nitrogen as carrier gas at 15 p.s.i., gas flow of 67 ml. per minute, instrument range setting at 10^{-9} ampere, and a recorder chart speed of 1 inch per minute. The retention time for 2,2′,4′-trichloroacetophenone under these conditions is 50 seconds.

Analysis of Milk

Extraction. Dilute a 125-ml. sample of milk with an equal volume of ethanol and extract it four times with 100-ml. portions of a 3 to 1 mixture of ether and *n*-hexane. Combine the extracts and wash with 100 ml. of 5% sodium sulfate solution. Dry the extract with anhydrous sodium sulfate, filter, and remove the solvent. Transfer the fatty residue to a 500-ml. separatory funnel with 100 ml. of *n*-hexane. Place 100 ml. of *n*-hexane in another separatory funnel. Extract the fat solution four times with 40-ml. portions of acetonitrile. Each time drain the acetonitrile into the second separatory funnel and wash with *n*-hexane. Combine the acetonitrile extracts and reduce the volume to 10 ml. by distillation through a Snyder column. Transfer the extract to a 60-ml. separatory funnel and dilute with 30 ml. of 5% sodium sulfate solution. Extract with two 10-ml. portions of *n*-hexane. Combine the extracts in a 25-ml. volumetric flask and make to volume. Take 10 ml. of this solution for the determination of Shell Compound 4072 and 10 ml. for 2,2′,4′-trichloroacetophenone.

Cleanup of Extracts. For the cleanup of Shell Compound 4072 prepare a chromatographic column by adding, in order, 0.5 inch of sodium sulfate, a well packed 2-inch layer of silicic acid, and another 0.5 inch of sodium sulfate. Wet the column with the mixed solvent, 3 to 1 dichloromethane–*n*-hexane. Evaporate 10 ml. of milk extract to dryness, dissolve in 10 ml. of solvent mixture, and transfer to the column using 25 ml. of the mixed solvent, wash with 90 ml. more, then change the receiver and elute the Shell Compound 4072 with 225 ml. of the same solvent. Condense the eluate to 10 ml. by distillation, transfer to a 125-ml. Erlenmeyer flask with *n*-hexane, then evaporate the solvent completely with a jet of dry air. Hydrolyze, extract, and inject into the gas chromatograph as described for the standard curve.

For the cleanup of 2,2′,4′-trichloroacetophenone, prepare a chromatographic column by adding, in order, 0.5 inch of sodium sulfate, 5 grams of alumina, 6 grams of silicic acid, and 0.5 inch of sodium sulfate. Prepare a mixed solvent of 3% ether in *n*-hexane and wash the column with 100 ml. Transfer 10 ml. of milk extract to the column using 15 ml. of *n*-hexane for the transfer. Wash the column with 40 ml. of the mixed solvent. Change the receiver and elute the 2,2′,4′-trichloroacetophenone with 90 ml. of the same solvent. Condense the eluate to 5 ml. by distillation through a three-ball Snyder column. Transfer the extract to a 15-ml. centrifuge tube with *n*-hexane and evaporate the solvent to a 5-ml. volume with a jet of dry, clean air. Inject an aliquot into the gas chromatograph and from the peak height and standard curve make an approximate estimation of the concentration. Then inject a standard of the nearest concentration, so that a more accurate estimation of the 2,2′,4′-trichloroacetophenone can be made.

Fat

Extraction. Blend a 25-gram sample with about 50 grams of anhydrous sodium sulfate and 150 ml. of *n*-hexane. Transfer the mixture to a 400-ml. beaker, stir in 2 grams of Celite, and heat to nearly boiling. Decant the liquid onto a folded filter and filter into a 500-ml. Erlenmeyer flask. Wash the blender and filter with 150 ml. more of hot *n*-hexane. Concentrate the solvent to 100 ml. by distillation through a Snyder column. Remove the fat by partitioning into acetonitrile as described for milk. Divide the extract for the determination of Shell Compound 4072 and 2,2′,4′-trichloroacetophenone. If it is desired to base the analysis on extracted fat, transfer the hexane solutions remaining after the acetonitrile extraction into a tared flask, evaporate the solvent, weigh the fatty residue, and record it as the weight of sample.

Cleanup of Extract. Use the same cleanup columns and complete the analyses as described above for milk.

Analysis of Muscle, Liver, Heart, Kidney, Spleen, and Brain

Extraction. Extract 25-gram samples of these tissues by blending with 150 ml. of acetone. Stir in 2 grams of Celite and filter. Wash the blender and filter with acetone. Repeat the extraction and filtration with *n*-hexane. Concentrate the acetone extracts to a volume of 50 ml. by distillation through a Snyder column, then transfer the extract to a 500-ml. separatory funnel and dilute with 200 ml. of 5% sodium sulfate. Extract three times with *n*-hexane, using the *n*-hexane from the extraction above for the first two extractions and 75 ml. of fresh *n*-hexane for the last extraction. Combine the extracts and filter through a 1-inch layer of anhydrous sodium sulfate. Concentrate the extract to 150 ml. by distillation through a Snyder column and transfer to a 500-ml. separatory funnel with 50 ml. of *n*-hexane. Partition into acetonitrile, and back into hexane as described for milk. Make the hexane extract to 25 ml., and use 10 ml. each for the determination of 2,2′,4′-trichloroacetophenone and Shell Compound 4072.

Cleanup of Extracts. For the determination of 2,2′,4′-trichloroacetophenone, clean up the extract using the column and procedure described for milk.

For the cleanup in preparation for the determination of Shell Compound 4072, prepare a chromatographic column for milk, but use a 2-inch layer of Florex in place of the silicic acid. Wet the column with dichloromethane containing 1.5% acetone. Transfer 10 ml. of extract to the column and use a total of 100 ml. of the same solvent. Change the receiver and elute the Shell Compound 4072 with 200 ml. of dichloromethane containing 3% acetone. Concentrate the solvent to 10 ml. and transfer to a 125-ml. Erlenmeyer flask with *n*-hexane. Evaporate to dryness, hydrolyze, and complete the analysis as described for the standard curve.

Discussion

Shell Compound 4072 is quantitatively hydrolyzed by boiling with 12*N* sulfuric acid for 30 minutes However, the heating time is not critical and a heating period of 1 hour neither increased or decreased the amount of 2,2′,4′-trichloroacetophenone produced. The concentration of sulfuric acid is not too critical, either, since 9*N* sulfuric gave complete hydrolysis in 1 hour.

In the description of the chromatographic columns used for cleanup, specific volumes of solvents are given. However, because of the variation in adsorbents these volumes may vary. All chromatographic adsorbents used should be calibrated.

The column temperature of 200° C. is higher than necessary for determination of 2,2′,4′-trichloroacetophenone; however, this temperature is required to remove DDT and other less volatile unknown compounds in a reasonable length of time. Under some circumstances a lower column temperature might be better for separation of interfering materials.

Trichloroacetophenone is very volatile and care must be taken to prevent its loss. Solutions should be concentrated by distillation through a Snyder column, or a *n*-hexane solution may be concentrated by evaporation at room temperature with a jet of air. Care should be taken to remove dichloromethane completely before the gas chromatographic analysis is performed.

When Shell Compound 4072 was added to the body tissues at the 0.05-p.p.m. level and to milk at the 0.1-p.p.m. level, and carried through the complete procedure described above, 71% was obtained from fat, 95% from milk, and 82 to 92% from the other tissues.

Recoveries of 2,2′,4′-trichloroacetophenone added to milk and the various body tissues amounted to 68%. By using the method as described above, maximum sensitivity would not be achieved, since the analysis is run on aliquots of a 5-ml. solution. With a more efficient cleanup procedure, this volume could be decreased to 1 ml. with a corresponding increase of sensitivity. However, this amount of solution is not recommended for analysis with the cleanup methods described. With the range setting at 10^{-9} ampere, 0.1 nanogram of Shell Compound 4072 or 0.062 nanogram of 2,2′,4′-trichloroacetophenone will give a response of 4% on the recorder. Control samples give no peaks at the retention time of 2,2′,4′-trichloroacetophenone, so that these amounts are readily detected. The amounts detectable represent residues of 0.005 p.p.m. of Shell Compound 4072 and 0.003 p.p.m. of trichloroacetophenone in the tissues, and 0.001 and 0.0006 p.p.m., respectively, in milk.

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METABOLISM OF INSECTICIDE RESIDUES

Fate of Inhaled C^{14}-TDE in Rabbits

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New Zealand Red rabbits, selected for their tolerance to mainstream tobacco smoke, were exposed to smoke from cigarettes containing 12 and 48 μg. of C^{14}-TDE per cigarette in Holland smoking boxes. The animals received smoke from 20 cigarettes per day for periods ranging from 2 weeks to 6 months, after which times they were sacrificed and 20 tissues examined for total and organosoluble radioactivity. The deposition via inhalation appears to follow that of oral ingestion with accumulation of TDE in the fat, followed by slow metabolism and elimination. There was no evidence of accumulation in the inhalation system or other vital organs. Human noninhaling smokers appear to exhale all TDE components of main-stream smoke, whereas inhaling smokers appear to retain (for subsequent storage and metabolism) about 3% of the TDE contained in a cigarette.

The fate of TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] on tobacco from application through the various phases of production and utilization has been reported (*2*). Commercial cigarettes (70 mm. long) contained approximately 12 p.p.m. of TDE, and the mainstream smoke from these cigarettes contained 1.6 μg. of TDE and 1.4 μg. of the major heat-induced degradation product TDEE (1-chloro-2,2-bis - *p* - chlorophenylethylene) per "smoked" cigarette (one 35-ml. puff per minute, 2-second duration for seven puffs). The presence of TDE in commercial cigarette smoke was confirmed subsequently (*11*). Although the toxicity and fate of ingested and dermal doses of TDE in mammalian systems have been investigated (*3, 8, 9*), similar investigations have not been made with inhaled TDE. A report has been made of the vapor toxicity of chlordan (*6*) and hexachlorocyclopentadiene, an intermediate in the manufacture of aldrin (*12*). Studies on the toxicity of lindane, aldrin, and DDT dispensed from vaporizers have been reported (*1*).

Studies with arsenic-74-supplemented mainstream tobacco smoke showed that rabbits retained 0.01% of the volatilized arsenic, and the distribution of the arsenic in the respiratory tract 2 hours after smoking was discussed (*5*). Following withdrawal from tobacco smoke containing As^{74}, the radioactivity decreased rapidly during the first 2 days and then tapered off slowly. The general distribution and elimination of

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