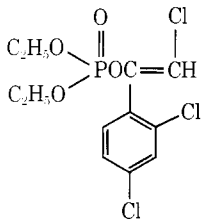
Gas–Liquid Chromatographic Determination of Chlorfenvinphos in Milk, Eggs, and Body Tissues of Cattle and Chickens

Marcellus C. Ivey,* Delbert D. Oehler,[1] and Houston V. Claborn[2]

A gas chromatograph equipped with a flame photometric detector provided a highly sensitive method of determining residues of chlorfenvinphos in milk, eggs, and body tissues of cattle and chickens. With extraction and cleanup, 0.002 ppm of the insecticide could be detected in milk and 0.001 ppm in body tissues and eggs. Recoveries of 83–100% were obtained from the fat, muscle, kidney, liver, and heart of cattle and from the fat, muscle, liver, skin, and manure of chickens. Recoveries of 93% were obtained from milk and 81% was obtained from eggs.

Chlorfenvinphos, a phosphate pesticide that effectively controls flies (Roberts *et al.*, 1961) and ticks (Drummond *et al.*, 1964) on livestock, has the following structure:



Claborn and Ivey (1965) determined chlorfenvinphos in animal tissues by acid hydrolysis to trichloroacetophenone, followed by gas–liquid chromatography (glc) with an electron capture detector. However, the glc methods (Beroza and Bowman, 1966; Beynon *et al.*, 1966) reported for the analysis of residues of chlorfenvinphos in crops and soils are not suitable without modification for analysis of residues in animal tissues. The present paper describes a reliable and sensitive glc method in which a flame photometric detector is used to determine micro amounts of chlorfenvinphos in the milk and body tissues of cattle and in the body tissues, eggs, and manure of chickens.

EXPERIMENTAL SECTION

Reagents and Equipment. All solvents were redistilled in glass. The silicic acid was Mallinckrodt's 100-mesh powder, analytical reagent grade (each lot of silicic acid must be calibrated to determine correct volume of eluting solvent). The Florisil was heated 16 hr at 140° and cooled, and 5% water was added and allowed to equilibrate. The chromatographic columns were Kontes technical glassware drawing No. 11416-B with 24/40 joints. The gas chromatograph was a Micro-Tek model 160 or equivalent equipped with a Melpar flame photometric detector.

Gas Chromatography. A borosilicate glass column 4 mm i.d. × 1.22 m filled with 5% DC-200 coated Gas Chrom Q, 80–100 mesh, was used. Carrier gas was prepurified nitrogen adjusted to a flow rate of 120 ml/min (exhaust). The column was heated isothermally to 205°, the injector to 265°, and the detector to 170°. Hydrogen and oxygen flowing to the detector were adjusted to 200 and 18 ml/min, respectively. At these conditions, the retention time for chlorfenvinphos was about 2 min.

A series of standard solutions containing 0.1 to 0.5 μg of chlorfenvinphos in 5-ml volumes of hexane was prepared. Ten microliters of each concentration was injected into the gas chromatograph, and a standard curve was prepared by plotting peak heights against nanograms of the insecticide. This curve was used to estimate the residues in test samples. A standard solution of about the same concentration as the test sample was then injected to determine the true value more accurately. Peak heights were proportional to the amounts of solute if they were injected in the same volume of solvent. An injection containing 0.2 ng of chlorfenvinphos in 10 μl of hexane gave a response of about 25% full-scale deflection (FSD).

Extraction and Cleanup of Milk. The method of Langlois *et al.* (1964), slightly modified, was used for the extraction and cleanup of milk.

Ten milliliters of well-mixed milk was added to 20 g of Florisil and mixed with a stainless steel spatula to a free-flowing mass. Then 20 g of Florisil was added to a chromatographic column, followed by the milk–Florisil mixture. 1:1 dichloromethane–hexane (50 ml) was used to complete the transfer. The column was washed with 150 ml of additional mixed solvent and 100 ml of dichloromethane. The receiver was changed, and the chlorfenvinphos was eluted with 100 ml of 1:1 acetone–hexane. A Snyder column was attached to the receiver, and the solvent was condensed to 5–10 ml. The remaining solvent was removed with a jet of clean dry air, and the residue was dissolved in 5 ml of hexane and stoppered tightly. The residue of chlorfenvinphos was determined by injecting a 10-μl aliquot into the gas chromatograph and comparing the peak height with a standard of about the same concentration.

Extraction of Fat and Skin. A 20-g sample was blended in a Waring blender with 50 g of anhydrous sodium sulfate and 150 ml of hexane. The mixture was transferred to a 600-ml beaker and stirred with 2 g of Celite. After the mass was heated to near boiling, it was transferred onto a folded filter paper and filtered into a 500-ml Erlenmeyer flask. The blender, beaker, and filter were washed with another 150 ml of hot hexane, the solvent was concentrated to 100 ml by distillation through a Snyder column and cooled to room temperature, and the extract was transferred to a 500-ml separatory funnel by using 100 ml of hexane to make the transfer. After 100 ml of hexane was added to a second separatory funnel, the fat solution was extracted four times with 40-ml portions of acetonitrile; each time the acetonitrile was drained into the second separatory funnel and shaken with the hexane. The acetonitrile extracts were combined in a 300-ml Erlenmeyer flask and concentrated to a volume of 10 ml by distillation through a Snyder column. Then it was transferred to a 60-ml separatory funnel and diluted with 30 ml of 5% sodium sulfate solution. The solution was extracted with two 10-ml aliquots of hexane, and the extracts were combined and evaporated just to dryness. The residue was dissolved in 10 ml of 3:1 dichloromethane–hexane and

U. S. Livestock Insects Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Kerrville, Texas 78028.

[1] Present address: Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, College Station, Texas 77840.

[2] Retired.

Table I. Recovery of Chlorfenvinphos (0.025 ppm) from Body Tissues, Eggs, and Manure of Chickens[a]

Material	Chlorfenvinphos		
	Added, ng	Found, ng	% recovered
Fat	500	500	100
Breast muscle	500	490	98
Thigh muscle	500	465	93
Liver	500	475	95
Skin	500	415	83
Eggs	500	405	81
Manure	500	435	87

[a] Control values = <0.001 ppm of chlorfenvinphos.

Table II. Recovery of Chlorfenvinphos (0.01 ppm) from Body Tissues and Milk of Cattle[a]

Material	Chlorfenvinphos		
	Added, ng	Found, ng	% recovered
Milk	100	93	93
Fat	200	184	92
Muscle	200	200	100
Kidney	200	200	100
Liver	200	166	83
Heart	200	194	97

[a] Control values = <0.001 ppm of chlorfenvinphos in tissues and 0.002 ppm in milk.

reserved for the chromatographic cleanup column. For fat samples, the hexane solutions remaining after the acetonitrile extraction were transferred into a tared flask, the solvent was evaporated, and the fatty residue was weighed and recorded as the weight of sample so we could base the analysis on extracted fat.

Extraction of Muscle, Liver, Heart, Kidney, Eggs, and Manure. A 20-g sample of any of the above tissues was blended with 150 ml of acetone. The mixture was transferred to a 600-ml beaker, stirred with 2 g of Celite, and filtered into a 300-ml Erlenmeyer flask. The blender, beaker, and filter were washed thoroughly with acetone. The filter and mass were returned to the blender, and the extraction and filtration steps were repeated with hexane. The acetone extract was concentrated to a volume of about 50 ml by distillation through a Snyder column, cooled to room temperature, and transferred to a 500-ml separatory funnel containing 200 ml of 5% sodium sulfate solution. The solution was extracted three times with hexane by using the hexane from the extraction and filtration for the first two extractions and 75 ml of fresh hexane for the last extraction. The hexane extracts were combined in another separatory funnel and washed with 50 ml of 5% sodium sulfate solution; the water phase was discarded. The extract was filtered through a 2.5-cm plug of anhydrous sodium sulfate into a 300-ml Erlenmeyer flask. After the solvent was condensed to 150 ml by distillation through a Snyder column, it was cooled and transferred to a 500-ml separatory funnel with 50 ml of hexane. One-hundred milliliters of hexane was added to a second separatory funnel. The extract was partitioned with acetonitrile and back into hexane as described for fat and skin.

Cleanup of Tissue Extracts. For the cleanup of chlorfenvinphos, a chromatographic column was prepared by adding, in order, 1.5 cm of sodium sulfate, a well-packed 5-cm layer of silicic acid, and another 1.5 cm of sodium sulfate. After the column was wetted with the mixed solvent, 3:1 dichloromethane–hexane, the extract was transferred to the column by using 25 ml of the mixed solvent and the column was washed with 90 ml more. The receiver was then changed, and the chlorfenvinphos was eluted with 225 ml of the same solvent. The eluate was condensed to 10 ml by distillation through a Snyder column, cooled, and transferred to a 125-ml Erlenmeyer flask with hexane. The solvent was evaporated with a jet of clean dry air, and the residue was dissolved in 5 ml of hexane. Gas chromatographic determination was then conducted as described for milk.

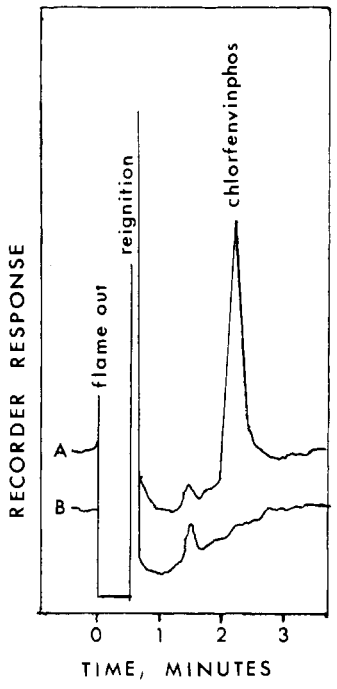


Figure 1. Chromatograms of extracts from beef fat: A, control sample containing 0.2 ng of chlorfenvinphos; B, untreated sample.

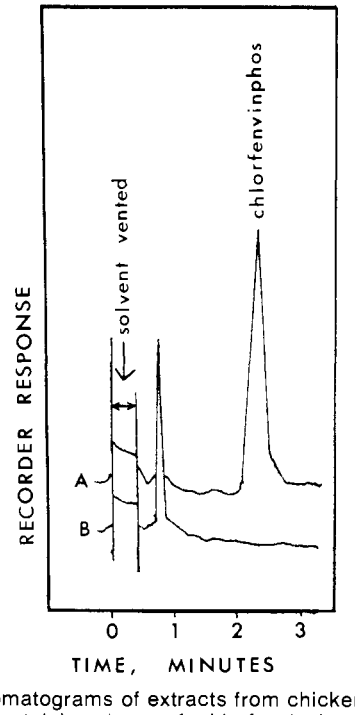


Figure 2. Chromatograms of extracts from chicken liver: A, control sample containing 1 ng of chlorfenvinphos; B, untreated sample.

RESULTS AND DISCUSSION

Recovery Experiments. The efficiency of the overall procedure was tested by adding known amounts of chlorfenvinphos to control samples of the various tissues before blending. The recovery of chlorfenvinphos from fortified

chicken tissues, eggs, and manure is reported in Table I, and that from milk and cattle tissues is reported in Table II. Figure 1 shows a recovery from 20 g of beef fat fortified with 0.1 μg of chlorfenvinphos. The final volume was 5 ml and 10 μl was injected into the chromatograph. Figure 2 shows a recovery from 20 g of chicken liver fortified with 0.5 μg of chlorfenvinphos. Final volume and injection were the same as for beef fat. As Figure 1 shows, this chromatograph did not have a switching valve to vent the solvent, and the detector was slightly more sensitive but also less stable than the detector in Figure 2.

Sensitivity. With the input attenuator at 10^3, the output attenuator at 16, and the bucking range at 10^{-8}, 0.2 ng of chlorfenvinphos in 10 μl of hexane gave a recorder response of about 25% FSD. The control samples showed no peaks at the retention time for chlorfenvinphos. At the conditions described, 0.04 ng was readily detected and gave a response of about 5% FSD. With the sample sizes and dilutions used, 0.001 ppm of chlorfenvinphos can be detected in the body tissues and manure and 0.002 ppm can be detected in milk. No effort was made to determine whether other phosphorous-containing compounds would interfere.

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Photochemical Decomposition of Heptachlor Epoxide

Robert E. Graham,* K. Ray Burson,[1] Charles F. Hammer,[2] Lydell B. Hansen, and Charles T. Kenner[3]

The progressive decomposition of solid heptachlor epoxide (I) in KBr disks is followed by a study of the changes which occur in the infrared spectrum with time of exposure to ultraviolet radiation and sunlight. The products obtained by photodecomposition in KBr disks are identical to those obtained by exposure of solid I to sunlight and ultraviolet radiation and include a semicage ketone and an intermediate which may be converted to an enantiomeric semicage ketone. The mechanism proposed accounts for the intermediate and final products and is based upon structure determinations by infrared, nuclear magnetic resonance, mass spectrometry, and ultraviolet absorption. A study of the factors which affect the rate and extent of decomposition in the KBr solid state technique shows that the time of exposure can be controlled to produce a maximum yield of intermediate or final products. The amount of decomposition in any given sample is calculated by the absorbance ratio technique.

Heptachlor epoxide (I) is a metabolic as well as environmental oxidation product of heptachlor (Davidow and Radomski, 1953; Davidow *et al.*, 1953; Lichtenstein and Schulz, 1965; Lichtenstein *et al.*, 1970; Weatherholz *et al.*, 1967).

The metabolic and environmental decomposition products of all pesticides are important, since the toxicity of certain derivatives of polycyclic pesticides has been found to be greater than that of the original compound (Rosen and Sutherland, 1967; Rosen *et al.*, 1969). The IUPAC Commission on terminal pesticide residues makes an annual report (Egan, 1967, 1968, 1969; Hill, 1970, 1971) on this subject.

The decomposition of I has not been studied extensively. Mitchell (1961) decomposed microgram amounts of I with ultraviolet light and detected two decomposition products by paper chromatography. Banks and Bills (1968) used gas chromatography to show that I in hexane is degraded by ultraviolet light to give two compounds. Glotfelty (1972) irradiated solid I with ultraviolet light and used the resulting glc pattern for identification. None of these investigators identified the products nor elucidated their structure. Fischler and Korte (1969) suggested a semicage structure for an isomeric photochemical derivative of I made by irradiation of I in acetone by ultraviolet light of 254 nm wavelength and Benson *et al.* (1971), in a report on chlordane photoalteration products, also suggested two possible structures. A recent article by Ivie *et al.* (1972) proposed a structure for a I intermediate photoisomer which is identical to the one (III) reported in this paper. Cochrane, in 1969, reported that basic reagents caused I to rearrange to an allylic alcohol isomer, the structure of which was elucidated by determination of the products formed by chlorination and oxidation.

This paper reports an investigation of the techniques of irradiation of solids in KBr disks and the decomposition of I by ultraviolet irradiation and by sunlight using this technique. The main derivatives are isomeric and consist of a semicage ketone and an intermediate ketone which, upon further ultraviolet irradiation, may be converted to a different semicage ketone. The photochemical degradation of I in a KBr disk is followed by a study of changes that occur in a series of infrared spectra related to time of irradiation with ultraviolet or sunlight. The residues obtained from sun-induced degradation and those obtained from the use of ultraviolet light are shown to be identical.

The structures of the derivatives shown in Figure 1 were determined by interpretation of spectra obtained from ultraviolet, infrared, mass spectrometry, and nuclear magnetic resonance instrumentation.

Dallas District Food and Drug Administration, Dallas, Texas 75204.
[1] Texas Instruments, Inc., Dallas, Texas 75222.
[2] Department of Chemistry, Georgetown University, Washington, D. C. 20007.
[3] Department of Chemistry, Southern Methodist University, Dallas, Texas 75275.