Determination of *O,O*-Dimethyl *O*-2, 4, 5-Trichlorophenyl Phosphorothioate in Animal Tissues and Milk

H. V. CLABORN and M. C. IVEY
Entomology Research Division, U. S. Department of Agriculture, Kerrville, Tex.

A method for the determination of ronnel (O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate) in animal tissues and milk is described. A high degree of sensitivity is obtained with a gas chromatograph equipped with an electron-capture detector. Extraction and cleanup methods make possible detection of 0.001 p.p.m. of ronnel in milk and 0.0005 p.p.m. in body tissues. The over-all procedure gives recoveries of 87% from milk, 77% from fat, and 85 to 94% from other tissues.

RONNEL (*O,O*-dimethyl *O*-2,4,5,-trichlorophenyl phosphorothioate), also known as Dow Et-57, Trolene, and Korlan, has been shown to be a valuable insecticide for the control of flies (*5*), grubs (*4*), and screwworms (*2*) on livestock. To make the residue studies required for approval to use this pesticide on livestock, a sensitive method was needed for determination in milk and all the body tissues. Methods for determining ronnel described by Webley (*7*), Smith (*6*), and Claborn and Ivey (*1*) do not have the required sensitivity for an acceptable residue study. Consequently, a method was developed that met the rigid sensitivity requirements and was adaptable to the analysis of all the body tissues. By this method, which makes use of a gas chomatograph equipped with an electron-capture detector, residues of ronnel in the body tissues can be accurately determined in amounts as small as 0.5 part per billion.

Method

Reagents and Equipment. Acetone, *n*-hexane, acetonitrile, and dichloromethane, redistilled.

Florisil, 30- to 60-mesh, heated 16 hours at 190° C.

Florisil, partially deactivated by adding 5 or 10% water.

Celite, analytical filter aid.

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

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

Gas Chromatography. Use a glass column 1/4 inch in o.d. × 4 feet, filled with 80- to 100-mesh Chromosorb W treated with 5% SF-96 and, as carrier gas, prepurified grade nitrogen at 35 pounds pressure or a flow rate of 275 ml. per minute. Heat the column isothermally at 180° C., the detector at 200° C., and the injector at 190° C. Set the instrument range at 10^{-9} ampere. The retention time for ronnel is 2.75 minutes. Prepare a series of standard solutions of ronnel in hexane ranging in concentration from 0.02 to 0.20 μg. per ml. Inject into the gas chromatograph 10-μl. portions of each concentration and prepare a standard curve by plotting peak heights against nanograms of ronnel. Use this curve to estimate the residues in test samples. Then inject a standard solution of approximately the same concentration as the test sample to determine the true value more accurately. Peak heights are directly proportional to the amounts of solute if injected in the same volume of solvent. For this reason, it is necessary to have a series of standard solutions. One nanogram of ronnel in 10 μl. of hexane should give a response of 25 to 40% on the recorder, depending on the sensitivity of the detector.

Analysis of Milk. The method of Langlois, Stemp, and Liska (*3*), slightly modified, was used for the extraction and cleanup of milk.

Add 31 grams of Florisil deactivated with 5% water to a chromatographic column and wash it with 100 ml. of hexane containing 25% dichloromethane. Mix with a spatula 10 ml. of milk and 20 grams of dry Florisil in a 50-ml. beaker to a free-flowing powder, then add it to the column on top of the washed Florisil. Elute the ronnel with 200 ml. of 25% dichloromethane in *n*-hexane. Evaporate the solvent to 5 ml. on a hot plate. Transfer the residue to a 10-ml. Kolmer centrifuge tube, using *n*-hexane to make the transfer. Reduce to a volume of 1 ml. with a jet of clean, dry air. Inject a portion of the extract into the gas chromatograph, then dilute it as indicated. Run a control sample through the procedure first to make sure that there is no interference at the retention time for ronnel (2.75 minutes). For checking recovery, add a hexane solution of ronnel to 10 ml. of milk in a 100-ml. beaker. Evaporate the hexane with a jet of clean, dry air, then add the Florisil and proceed as described above.

Analysis of Omental, Renal, and Subcutaneous Fat. Blend a 20-gram sample with 50 grams of anhydrous sodium sulfate and 150 ml. of *n*-hexane. Transfer the mixture to a 600-ml. beaker, stir in 2 grams of Celite, heat on a hot plate to nearly boiling, then decant the liquid onto a folded filter paper and filter into a tared 500-ml. glass-stoppered Erlenmeyer flask. Wash the blender, beaker, and filter with another 100 ml. of hot hexane. Concentrate the solvent by distillation to 30 ml., then attach the flask to an aspirator and remove the last trace of solvent at reduced pressure while heating in a boiling water bath. Cool the flask and weigh. Record the weight of extracted fat as weight of sample. Dissolve the fatty residue and transfer to a 500-ml. separatory funnel, using 200 ml. of *n*-hexane to make the transfer. Add 100 ml. of hexane to a second separatory funnel, then extract the dissolved fat four times with 50-ml. portions of acetonitrile, each time draining the acetonitrile extract into the second separatory funnel and shaking with the hexane. Combine the acetonitrile extracts in a 300-ml. Erlenmeyer flask and concentrate to a volume of 5 to 10 ml. by distillation through a Snyder column. Remove the last traces of acetonitrile by the addition and evaporation of three 25-ml. portions of *n*-hexane. Dissolve the residue in 5 ml. of hexane and transfer to a 20-mm. chromatographic column prepared by adding 1 inch of anhydrous sodium sulfate followed by 5 inches of well packed Florisil containing 10% water and then another 1-inch layer of sodium sulfate. Wet the column with hexane, then transfer the residue to the column with 20 ml. more of hexane. Wash the column with 155 ml. more of *n*-hexane. Change the receiver and elute the ronnel with 170 ml. of hexane. Evaporate the solvent to 5 ml., transfer the residue to a 10-ml. centrifuge tube as described above for milk analysis, and dilute as needed for injection. Calculate the residue as parts per million based on the weight of the extracted fat.

Analysis of Muscle, Liver, Kidney, Heart, Brain, and Spleen. Blend a 20-gram sample of any of these tissues 3 to 5 minutes with 150 ml. of acetone

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.

Literature Cited

(1) Claborn, H. V., Ivey, M. C., *J. Assoc. Offic. Agr. Chemists* **47,** 871 (1964).
(2) Graham, O. H., Moore, Bobby, Writch, M. J., Kunz, Sidney, Warren, Jack, Drummond, R. O., *J. Econ. Entomol.* **52** (6), 1217 (1959).
(3) Langlois, B. E., Stemp, A. R., Liska, B. J., J. AGR. FOOD CHEM. **12,** 243 (1964).
(4) McGregor, W. S., Bushland, R. C., *J. Econ. Entomol.* **50** (3), 246 (1957).
(5) Roberts, R. H., *Ibid.*, **52** (6), 1216 (1959).
(6) Smith, Grant N., Thiegs, B. J., J. AGR. FOOD CHEM. **10,** 467 (1962).
(7) Webley, D. J., *Analyst* **86,** 478 (1961).

Received for review February 4, 1965. Accepted May 18, 1965. Mention of proprietary products does not necessarily imply their endorsement by the U. S. Department of Agriculture.

INSECTICIDE RESIDUES

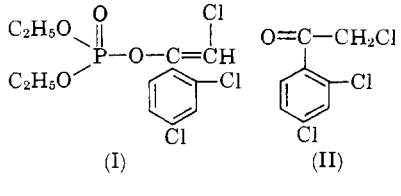
Determination of 2-Chloro-1-(2,4-dichlorophenyl)vinyl Diethyl Phosphate and 2,2′,4′-Trichloroacetophenone in Animal Tissues and Milk

H. V. CLABORN and M. C. IVEY

Entomology Research Division, U. S. Department of Agriculture, Kerrville, Tex.

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)