Literature Cited

(1) Beroza, M., J. AGR. FOOD CHEM. **4,** 49 (1956).
(2) Beroza, M., Berthel, W. F., *Ibid.*, **5,** 855 (1957).
(3) Bruson, H. A., "Organic Reactions," Vol. **V**, p. 110, Wiley, New York, 1949.
(4) Campbell, F. L., Barnhart, C. S., Hutzel, J. M., *Soap Sanit. Chemicals* **17,** 105, 109, 113, 115 (1941).
(5) Davidson, D., Bogert, M. T., *J. Am. Chem. Soc.* **57,** 905 (1935).
(6) Ek, A., Hartle, R. J., Gulf Research & Development Co., unpublished information.
(7) Ferguson, G. P., Alexander, C. C., J. AGR. FOOD CHEM. **1,** 888 (1953).
(8) Fukuto, T. R., Metcalf, R. L., Winton, M. Y., Roberts, P. A., *J. Econ. Entomol.* **55,** 341 (1962).
(9) Gersdorff, W. A., Piquett, P. G., Beroza, M., J. AGR. FOOD CHEM. **4,** 858 (1956).
(10) Gordon, H. T., Eldefrawi, M. E., *J. Econ. Entomol.* **53,** 1004 (1960).
(11) Gysin, H., *Chimia (Switz.)* **8,** 205 (1954).
(12) Hansens, E. J., Bartley, C. E., *J. Econ. Entomol.* **46,** 372 (1953).
(13) Hartle, R. J., Thayer, H. I. (to Gulf Research & Development Co.), U. S. Patent **2,951,850** (Sept. 6, 1960).
(14) *Ibid.*, **2,990,320** (June 27, 1961).
(15) *Ibid.*, **3,035,969** (May 22, 1962).
(16) Horning, E. C., ed., "Organic Syntheses," Col. Vol. **III**, p. 846, Wiley, New York, 1955.
(17) Hurd, V. N., Gwynn, B. H., *World Petrol. Congr., Proc. 5th., N. Y., 1959* **4,** 163 (pub. 1960).
(18) Kolbezen, M. J., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 864 (1954).
(19) Metcalf, R. L., *Agr. Chem.* **16,** No. 6, 20 (1961).
(20) Metcalf, R. L., "Organic Insecticides," pp. 317–30, Interscience, New York, 1955.
(21) Metcalf, R. L., Fukuto, T. R., Winton, M. Y., *J. Econ. Entomol.* **53,** 828 (1960).
(22) Miller, A. C., Mallis, A., Easterlin, W. C., *Ibid.*, **47,** 23 (1954).
(23) Moorefield, H. H., *Contribs. Boyce Thompson Inst.* **19,** 501 (1958).
(24) Prill, E. A. (to Boyce Thompson Institute for Plant Research), U. S. Patent **2,773,062** (Dec. 4, 1956).
(25) *Ibid.*, **2,856,411** (Oct. 14, 1958).
(26) Prill, E. A., Synerholm, M. E., *Contribs. Boyce Thompson Inst.* **14,** 221 (1946).
(27) Soap and Chem. Specialties Blue Book, New York, pp. 225–7, 1962.
(28) *Ibid.*, pp. 239, 240.
(29) Strain, F., Bissinger, W. E., Dial, W. R., Rudoff, H., DeWitt, B. J., Stevens, H. C., Lanston, J. H., *J. Am. Chem. Soc.* **72,** 1254 (1950).

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CARBAMATE INSECTICIDES

Gas Chromatographic Residue Determination of Sevin as Brominated 1-Naphthyl Acetate

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The insecticide, Sevin, has been determined in apples, broccoli, beans, corn, chicken, trout, bees, and soil at the 0.1-p.p.m. level by electron affinity gas chromatography. Following acetone extraction of samples and precipitation of interferences with ammonium chloride–phosphoric acid solution, Sevin is converted to a brominated 1-naphthyl acetate. This derivative is then chromatographed and determined by electron affinity detection. The recovery of Sevin from samples is good.

SEVIN (*N*-methyl-1-naphthyl carbamate) is one of the most widely used broad-spectrum carbamate insecticides. Methods for determining Sevin residues in crops, soil, poultry, fish, and other biological material are therefore essential.

Ralls and Cortes (*3*) brominated Sevin in a sealed tube with bromine in carbon tetrachloride to produce an electron-capturing derivative. The procedure was recommended as a screening method for detecting Sevin in green beans at the 1-p.p.m. level following a Florisil column cleanup, but not for quantitative analysis because of the production, in some instances, of mixed brominated derivatives. In the work reported, a procedure is described in which Sevin is hydrolyzed, brominated, and esterified in a single step to yield presumably brominated 1-naphthyl acetate. Following extraction of samples with acetone and precipitation of interferences with ammonium chloride in phosphoric acid solution (*2*), the brominated derivative is produced and chromatographed using electron affinity determination. The method is sensitive to about 0.05 p.p.m. and has been successfully applied to analysis of apples, broccoli, beans, corn, chicken, trout, bees, and soil.

Procedure

Blend 25 grams of a representative subsample with distilled acetone and filter to a total volume of 100 ml. according to the procedure described earlier (*1*). The remainder of the isolation procedure is an adaptation of that of Niessen and Frehse (*2*). Transfer the total aqueous acetone extract to a 250-ml. separatory funnel and partition the solution for 1 minute successively with 100, 50, and 50 ml. of distilled chloroform. With samples low in moisture content, such as bees or soil, add about 20 ml. of water to the acetone extract before partitioning. Drain the chloroform layers through a 60° funnel containing filter paper and about 20 grams of anhydrous sodium sulfate and combine the layers in a 400-ml. beaker. Evaporate the solution to dryness using air and a 60° C. water bath.

Dissolve the residue in 15 ml. of acetone. Add 50 ml. of the aqueous precipitating solution containing 1.25 grams of ammonium chloride and 2.5 ml. of orthophosphoric acid per liter and allow the solution to stand for 30 minutes. Filter the solution through a Büchner funnel containing a 3-mm. layer of Celite 521 filter aid. Rinse the filter with 50 ml. of the precipitating solution. To the filtrate, add 20 ml. of a buffer solution (pH 12) containing 6.4 grams of citric acid, 3.5 grams of boric acid, 13.7 grams of sodium hydroxide, and 2.1 ml. of orthophosphoric acid per liter. Transfer the solution to a 250-ml. separatory funnel and partition successively with 75, 25, and 25 ml. of distilled carbon tetrachloride. Filter the carbon tetrachloride layers through sodium sulfate and evaporate the combined solutions to about 3 ml. Transfer the solution to a 10-ml. volumetric flask using carbon tetrachloride and evaporate the contents to dryness with air.

Hydrolysis, Bromination, and Esterification. Add 1 ml. of glacial acetic acid and 10 drops of concentrated sulfuric acid to the flask. Mix the

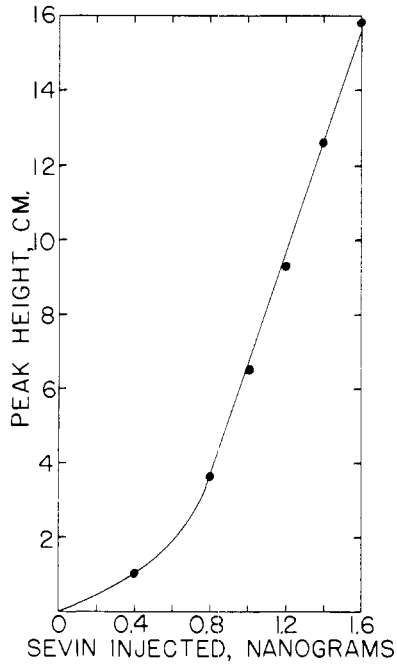

Figure 1. Sevin standard curve

Table I. Per Cent Recovery of Sevin from Various Samples

	Amount Added, P.P.M.		
Sample	0.1	0.2	0.5
Cortland apples	100	105	112
Green beans	96	105	108
Broccoli	120	112	110
Sweet corn	100	91	98
Canfield silt loam soil	85	85	82
Bees			92, 92
Whole chicken	97	85	100
Brook trout (whole fish)	77	75	

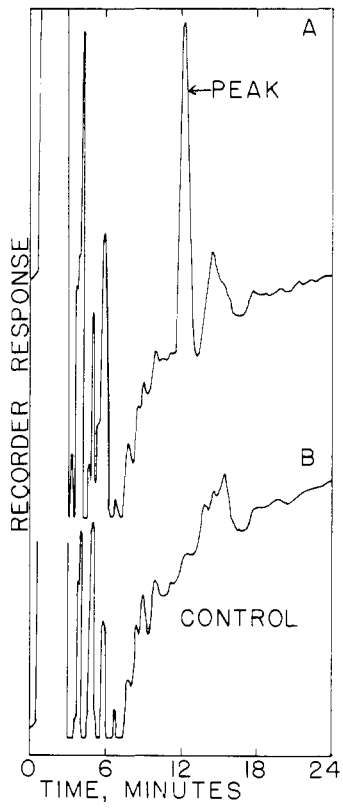

Figure 2. Chromatograms of chickens

A. Whole chicken containing 0.1 p.p.m. of added Sevin
B. Control chicken

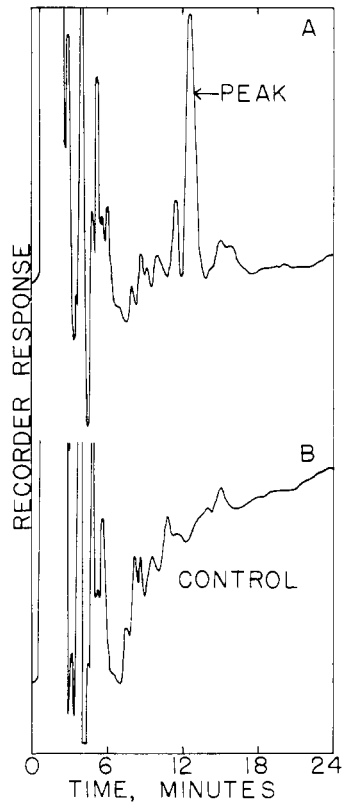

Figure 3. Chromatograms of bees

A. Bees containing 0.5 p.p.m. of added Sevin
B. Control bees

contents and allow the flask to stand at room temperature for 5 minutes. Add 0.2 ml. of a solution of acetic acid saturated with iodine crystals and containing 5% (by volume) of liquid bromine. Mix the contents and place a 10/30 standard-taper male ground joint (with the full 14.5-cm. length of glass tubing attached) in the top (as an air condenser). Immerse the flask to a depth of 1 cm. in a 130° C. constant temperature oil bath for 10 minutes.

Remove the flask and rinse down the condenser with distilled water (about 5 ml.). Add 2 ml. of benzene to the flask, make to volume with water, and shake the contents vigorously for 1 minute. Transfer 1 ml. of the upper benzene layer to a 10-ml. test tube and evaporate the contents to dryness. If all of the excess bromine and iodine are not visibly absent, add small amounts of benzene to the tube and evaporate again. Rotating the tube by hand during evaporation is helpful. After removal of the halogens in this manner, dissolve the residue in the tube in 1 ml. of benzene and inject up to 4 μl. of the solution into the gas chromatograph. The column, instrument, and operating conditions were identical to those used earlier (*7*). The nitrogen flow rate was 60 cc. per minute. The retention time for brominated 1-naphthyl acetate was about 12 minutes.

The standard curve for Sevin is developed as follows. Pipet 0, 0.2, 0.4, 0.5, 0.6, 0.7, and 0.8 ml. of Sevin (1 μg. per ml.) in acetone into a series of 10-ml. volumetric flasks. Evaporate the solutions with air and proceed as in the analysis of samples, beginning with the addition of 1 ml. of acetic acid. Inject 4 μl. of each standard into the column.

Results and Discussion

Figure 1 shows a typical standard curve for Sevin. Figure 2 shows chromatograms of whole chicken to which 0.1 p.p.m. of Sevin was added and chicken control. Figure 3 shows chromatograms of bees to which 0.5 p.p.m. of Sevin was added and control bees. The chromatograms of the other samples (Table I) were very similar to those for chicken and bees, with the majority of large peaks appearing within 6 minutes after sample injection. The recorder pen dropped below scale between peaks from about 3 to 6 minutes with all samples, probably because of the large space charge imposed on the detector by the sample injection. It returned on scale of its own accord, however, in advance of the brominated 1-naphthyl acetate peak.

The recoveries of Sevin from samples are listed in Table I. The insecticide in acetone was added to the samples in the Waring Blendor. The chicken and brook trout represented the whole carcass minus internal organs. The largest sample of bees which could be extracted and analyzed was 5 grams. The method is sensitive to about 0.05 p.p.m. for each of the samples and about 0.25 p.p.m. for bees. The check value was consistently less than 0.02 p.p.m. A separate standard curve was developed with each set of samples. The precipitation cleanup (*2*) was very efficient. Only with bees was it necessary to precipitate the interferences twice.

The peak appearing at 12 minutes was also obtained when 1-naphthol dissolved in acetic acid was brominated and esterified. No attempts were made to apply the method to determinations of 1-naphthol in samples. This hydrolysis product of Sevin was found previously to volatilize rapidly from various samples during solvent evaporations. In several years' work by Union Carbide Corp., 1-naphthol has never been found as a significant residue in Sevin-treated samples. Apparently its reactivity in biological systems (glucuronide formation,

etc.) results in its rapid metabolism.

The method was applied to the analysis of field-treated samples. McIntosh apple trees from an orchard in Lagrangeville, N. Y., were treated seven times at regular intervals between June 6 and August 16, 1963, with 1 pound of 50% wettable Sevin per 100 gallons of water. Replicate apple samples harvested on September 17 showed residues of 1.5, 1.2, 1.5, and 1.4 p.p.m. of Sevin. Untreated apples showed less than 0.02 p.p.m. of Sevin and recovery of Sevin at the 0.1-p.p.m. level was 94%.

On May 31, 1963, in Cambridge, N. Y., an airplane application of 1.25 pounds of Sevin per acre (formulation 80 W) was made on a 590-acre woodlot and pasture in which bee hives were located. Dead bees taken 14 and 24 hours after treatment showed, respectively, 0.3 and 0.5 p.p.m. of Sevin. Separate bee samples taken from two different hives after 48 hours showed 0.4 and 0.6 p.p.m. Control bees showed less than 0.02 p.p.m. and recovery of 0.5 p.p.m. of Sevin was 92%.

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Literature Cited

(1) Gutenmann, W. H., Lisk, D. J., J. AGR. FOOD CHEM. **12,** 46 (1964).
(2) Niessen, H., Frehse, H., *Pflanzenschutz-Nachrichten "Bayer"* **16,** 205 (1963).
(3) Ralls, J. W., Cortes, A., *J. Gas Chromatog.* **1,** 132–3 (April 1964).

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PESTICIDE RESIDUE METHODOLOGY

Gas Chromatographic Determination of Flash Heater–Modified Pesticides

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The possibility of direct chemical modification of a number of the more common chlorinated pesticides in the flash heater of a gas chromatograph with electron capture detection is explored. The action of sodium carbonate, cupric oxide, cadmium chloride, aluminum chloride, and potassium dichromate at 240° C. on BHC isomers, DDD, DDE, DDT, methoxychlor, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, chlordan, toxaphene, and methyl parathion is described in terms of aldrin retention ratios and specific aldrin area ratios of the maxima produced. Mild and severe modifications are noted. A system utilizing multiple-injection modifiers ahead of a single column and electron capture detector is suggested to provide more positive identification of specific pesticides in a residue extract and to eliminate interferences normally occurring on silicone columns.

SINCE the introduction by Goodwin, Goulden, Richardson, and Reynolds (*8*) of electron capture gas chromatography (EC/GC) for the determination of pesticide residues, the method has been applied widely by a number of investigators (*2, 3, 7, 11, 12, 16, 17, 19, 22, 24, 28, 29*). The high sensitivity of the electron-capture detector for molecules containing electronegative substituents such as halogens and aromatic nitro groups has uniquely fitted it to the problem of detecting trace quantities of many pesticides which contain such groups.

Nevertheless, extracts of plant and animal origin can contain electron-capturing materials other than the sought-after chlorinated or thiophosphoryl pesticides. Incorrect identifications of chromatographic maxima based solely on retention times or ratios can occur for this reason. Interferences also occur between certain of the commonly occurring pesticides chromatographed on the usual variants of silicone greases that have been employed. Examples of this latter are the nearly identical aldrin retention ratios as defined by Burke and Johnson (*6*) and the present authors (*24*) for aldrin and a chlordan component, aldrin and methyl parathion, *p,p'*-DDD and *o,p*-DDT, *p,p'*-DDE and dieldrin, endrin and *p,p'*-DDD, and a methoxychlor degradation product, endrin, and *p,p'*-DDT. The positive identification of a particular pesticide residue in a biological product is considered to be a major problem by these and other investigators (*9, 27*), particularly if the extract is not well cleaned up before chromatography.

Several methods have been employed to minimize these difficulties. Extensive cleanup of the extracts prior to gas chromatography is helpful in preventing the occurrence of many natural electron-capturing contaminants in the final mixture, and is recommended by most groups utilizing the gas chromatographic technique.

Chemical modification of the residues followed by EC/GC is a generally useful technique for confirmatory identification. Klein, Watts, and Daminco (*17*) confirmed the presence of DDT and DDD in butter by chromatographing extracts before and after treatment with NaOH to convert the pesticides to the dehydrochlorinated ethylenic derivatives. Gutenmann and Lisk have shown that pesticides insensitive to electron capture may be converted to appropriately sensitive forms by bromination (*13, 15*) or the Zeisel alkoxy method (*14*), where the alkyl iodides formed are detected. A similar approach was employed by Beckman and Berkenkotter (*1*), who utilized gas chromatography with flame ionization detection to determine residues before and after reduction with sodium in anhydrous ammonia. All of this modification work was carried out on a macro or semimicro scale in separate reaction vessels prior to sampling and chromatography.

Use of well developed paper chromatographic methods (*20, 21, 23*) as well as recently advanced thin layer chromatographic techniques (*18, 31*) as a supplemental identification is an obvious technique.

The possibility of finding other stationary phases suitable for gas chromatog-

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