Determination of Insecticide, [*O*-(4-Bromo-2,5-dichlorophenyl) *O*-Methyl Phenylphosphonothioate] (Velsicol VCS-506), Its Oxygen Analog, and Its Phenolic Hydrolysis Product in Corn and Milk by Gas Chromatography

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A method was devised for determining residues of Velsicol VCS-506 [*O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothioate], its oxygen analog, and its phenolic hydrolysis product in corn and milk. The compounds are separated by liquid chromatography of the extract on two columns, buffer-deactivated silica gel and alumina, prior to gas chromatographic analysis using a flame-photometric detector for the parent compound and the oxygen analog and a Ni^{63} electron-capture detector for the phenol. Recoveries of the parent insecticide and its oxygen analog added at levels between 0.05 and 5.0 p.p.m. were 91 to 99% and 72 to 92%, respectively. Recoveries of the phenol were 55 to 69% from corn and 93 to 95% from milk. Minimum detectable levels for the three compounds were less than 0.01 p.p.m.

Velsicol VCS-506[*O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothioate], hereafter referred to as VCS-506, is an experimental insecticide produced by Velsicol Chemical Corp., Chicago, Ill. Since the compound is being considered for the control of insects attacking corn intended for use as forage, a method was needed to determine residues of the compound and its metabolites in corn and in the milk of livestock consuming treated feed. Residues may consist chiefly of the parent insecticide (I), its oxygen analog (II, hereafter *O*-analog), and the phenolic hydrolysis product (III). Formulas of these compounds are shown in Figure 1.

The method that was devised required the use of two liquid chromatographic columns to separate the three compounds; one was a pH 7 buffered silica gel column (buffered to prevent hydrolysis of the labile *O*-analog) and the other an alumina column. With the two columns in tandem, VCS-506 was eluted from both columns with benzene. The columns were then separated, and acetone was used to elute the *O*-analog from the silica gel, and 2% acetic acid in benzene to elute the phenol from the alumina; the acetic acid was removed with a sodium bicarbonate wash prior to the injection of the phenol fraction into an electron-capture gas chromatograph. VCS-506 and its *O*-analog were determined with a gas chromatograph equipped with the phosphorus-sensing flame-photometric detector of Brody and Chaney (1966). A hexane-acetonitrile partition was used to remove excessive fat from the VCS-506 fraction of the milk extract prior to its analysis.

Attempts made to recover other possible metabolites (IV to VIII in Figure 1) were generally unsuccessful, probably because of their instability.

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EXPERIMENTAL

Gas Chromatographs. Two instruments were used. One was a Hewlett-Packard (Avondale, Pa.) Model 5750 gas chromatograph equipped with the phosphorus-sensing flame-photometric detector (526-mμ filter) of Brody and Chaney (1966) (MicroTek Instruments, Inc., Baton Rouge, La.). The other was a MicroTek Model 2000R gas chromatograph equipped with a Ni^{63} electron-capture detector.

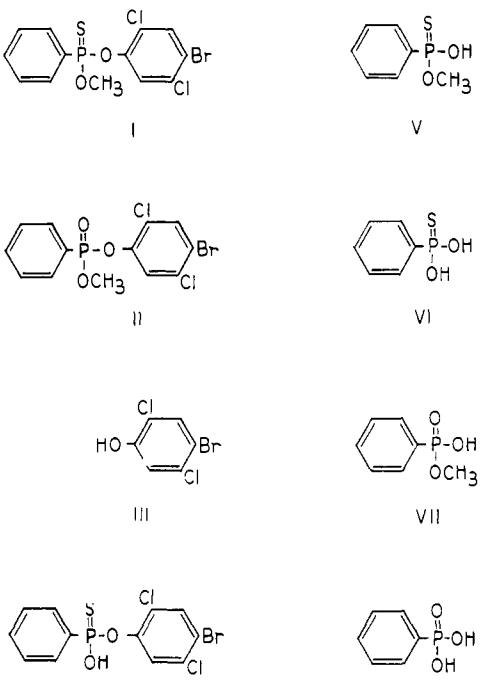

Figure 1. VCS-506 (I) and possible metabolites

Reagents and Solvents. The silica gel (J. T. Baker Chemical Co., No. 3405) used was found to contain 2.8% water based on loss in weight after overnight heating at 110° C. Its water content was adjusted to 10% by adding the appropriate amount of pH 7.00 buffer (W. H. Curtin & Co. No. 9730–16C) to a 500-gram batch of the adsorbent in a tightly sealed jar, and bringing the mixture to uniformity by rolling the jar overnight on a ball mill (no balls in jar). The alumina (Fisher Scientific Co., A-540 adsorption alumina, 80- to 200-mesh) was used as received; its loss in weight after overnight heating at 110° C. was 3.1%.

Acetone, acetonitrile, benzene, chloroform, hexane, and methylene chloride were redistilled C.P. grade solvents. Absolute methanol and acetic acid were used as received. Sodium sulfate was the anhydrous reagent grade chemical.

Analytical samples of VCS-506 (I), its *O*-analog (II), and its phenolic hydrolysis product (III) were furnished by Velsicol Chemical Corp.

Sample Preparation and Extraction of Whole Corn Plants and Ears (Husks and Silks Removed). Place 20 grams of the finely chopped and well mixed sample in a Soxhlet extraction apparatus (Fisher Scientific Co., No. 9–556 B) containing a plug of glass wool to prevent insoluble plant material from siphoning over during solvent exchanges, and extract under nitrogen for 16 hours (overnight) with 150 ml. of chloroform-methanol, 9-to-1 by volume, at the rate of about six solvent exchanges per hour. Let the extract cool, and percolate it through a plug of sodium sulfate about 2.5 cm. in diam. and 3 cm. thick; then wash the container and plug with 10 ml. of chloroform. Evaporate the extract and washings to complete dryness on a 50° C. water bath under water pump vacuum, and take up the residue in 10 ml. of benzene for the liquid chromatography.

Milk Extraction. After shaking the milk to disperse the cream uniformly, add 100 grams of the sample and 300 ml. of acetone to a Waring Blendor, and blend for 3 minutes. Filter the mixture through Whatman No. 1 paper on a Buchner funnel, and wash the blender, paper, and funnel with an additional 25 ml. of acetone. Extract the combined filtrate and washings with 200 ml. and then 100 ml. of methylene chloride, and pass the methylene chloride extracts successively through a plug of sodium sulfate about 4 cm. in diam. and 5 cm. thick. Evaporate the dried extracts almost to dryness on a steam bath under a Snyder column and then just to dryness under water pump vacuum. Take up the fatty residue in 10 ml. of benzene for the liquid chromatography step.

Liquid Chromatographic Separation of VCS-506, Its *O*-analog, and Its Phenol. The compounds are separated most conveniently on two columns as shown schematically in Figure 2. Prepare one column by adding successively to a 2-cm. i.d. Shell-type column (Scientific Glass Apparatus Co., No. JD 4010) 10 grams of sodium sulfate, 10 grams of the buffer-deactivated silica gel, and 10 grams of sodium sulfate. Prepare the other column by adding successively to a 12-mm. i.d. glass column (Kontes No. K-42000) a plug of glass wool, 2 grams of sodium sulfate, 5 grams of alumina, and 2 grams of sodium sulfate. Place the alumina column below the silica gel column, and add 50 ml. of benzene to the silica gel column to prewet both columns. Discard the filtrate. Add the 10 ml. of benzene extract of corn or milk to the silica gel column, and use the benzene rinsings of the container (10 ml.) to wash the extract into the adsorbent; then add 50 ml. more benzene. The eluate from the two columns in tandem (70 ml.) contains the VCS-506.

Separate the columns, and add 50 ml. of acetone to the silica gel column. The eluate contains the *O*-analog.

Add 50 ml. of benzene containing 1 ml. of glacial acetic acid to the alumina column. The eluate contains the phenol.

Preparation of Liquid Chromatography Fractions for Gas Chromatography. CORN PLANTS AND EARS. Concentrate the VCS-506 fraction on a 50° C. water bath under water pump vacuum, and dilute with benzene to an appropriate volume for gas chromatographic analysis.

Evaporate the *O*-analog fraction to near dryness on a 50° C. water bath under water pump vacuum, and adjust its volume with acetone as appropriate for gas chromatographic analysis.

Transfer the phenol fraction (from the alumina column) to a separatory funnel containing 50 ml. of 5% aqueous sodium bicarbonate, and shake well (periodically allowing the CO_2 to escape) for about 1 minute. Percolate the benzene layer through a plug of sodium sulfate 2.5 cm. in diam. and 3 cm. thick. Extract the aqueous layer twice more with 25-ml. portions of benzene, and percolate them through the plug. Concentrate the combined benzene extract under water pump vacuum at 50° C. to near dryness, and adjust to an appropriate volume with benzene for gas chromatographic analysis.

MILK. Evaporate the VCS-506 fraction to dryness under water pump vacuum at 50° C., and transfer the residue to a small separatory funnel with 5 ml. each of hexane and acetonitrile pre-equilibrated with each other. Shake the contents for 1 minute, and remove and reserve the acetonitrile layer. Extract the hexane layer with another 5 ml. of preequilibrated acetonitrile. Discard the hexane layer, and adjust the combined acetonitrile extracts to 10 ml. (if necessary) for gas chromatography. Correct the results for a 12% loss of compound in the partitioning cleanup.

Work up the *O*-analog and phenol fractions of milk as described for the corn.

Gas Chromatographic Analysis. Solutions containing the parent VCS-506 and the *O*-analog were analyzed with the gas chromatograph equipped with the phosphorus flame-photometric detector, and solutions containing the phenol were analyzed with the instrument having the electron-capture detector; 5-μl. aliquots were injected. Injections of fractions from corn plants or ears made up to 2 and 5 ml. were equivalent to 50 and 20 mg. of plant, respectively, and injections of fractions from milk adjusted to 5 and 10 ml. were equivalent to 100 and 50 mg. of milk, respectively. All quantitative determinations were based on peak height.

Use the following conditions for analyses with the flame-photometric detector: column, 50-cm. × 4-mm. i.d. glass; packing, 5% w./w. OV-17 on 80- to 100-mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) preconditioned overnight at 250° C.; gases: nitrogen (carrier), 160 ml. per minute; oxygen, 40 ml. per minute; hydrogen, 200 ml. per minute; temperatures: column, 225° C.; injection port, 230° C.; transfer line from column to detector and detector, 230° C. The column is conditioned to the compounds by repeated injections of 250-ng. amounts of the compounds in the corn or milk extract until the response to trial injections containing 5 ng. of each compound with the same amount of extract does not increase in several successive injections. Once the column is conditioned in this manner, no further conditioning is required for that day. With these conditions, the respective retention times of VCS-506 and its *O*-analog were 2.50 and 2.00 minutes, and the peak height was proportional to concentration to the upper limit of the electrometer settings (5×10^{-6} amp. full scale).

Use the following conditions for analyses with the electron-capture instrument: column, 180-cm. × 4-mm. i.d. glass; packing, 20% w./w. OV-101 on 80- to 100-mesh Gas Chrom

Q preconditioned overnight at 280° C.; carrier gas, nitrogen at 190 ml. per minute; temperatures: column, 240° C.; injection port, 260° C.; detector, 280° C., electrometer setting 4×10^{-9} amp. full scale with 1-mv. recorder. Conditioning of the column to the phenol and substrates was required for reproducible, linear, and sensitive responses. With these conditions, the retention time of the phenol was 1.20 minutes. (With the same conditions, retention times of the *O*-analog and VCS-506, which can also be analyzed by electron-capture gas chromatography, were 13.3 and 17.2 minutes, respectively.) Response (peak height) of the phenol was linear with concentration to at least the 4-ng. level.

Recovery Experiments. Appropriate amounts of the three compounds in chloroform were added to control corn plants and ears, and similar additions of the compounds in acetone were made to milk. The fortified samples were then carried through the entire analytical procedure. The dry-matter contents of the control corn plants and ears were 13.5 and 39.1%, respectively.

RESULTS AND DISCUSSION

The results of analyzing the samples of corn plants and ears with and without the compounds are given in Table I. The three compounds were added singly and together. In no instance was there any evidence of the formation of one compound from the others. Recoveries at levels between 0.05 and 5.0 p.p.m. were 92 to 99% for VCS-506, 72 to 92% for the *O*-analog, and 55 to 69% for the phenol. The minimum detectable level (twice noise) was 0.004 p.p.m. for the parent compound and its *O*-analog and 0.009 p.p.m. (twice the interference of the unfortified sample) for the phenol. Recoveries of VCS-506 and its *O*-analog from corn were satisfactory while recoveries of the phenol were low. Recoveries of the phenol at higher levels, namely 2, 10, and 25 p.p.m., were about the same (65, 73, and 69%, respectively). In general, recoveries of the phenols of other insecticides tended to be similarly low (Bowman and Beroza, 1967a, 1967b, 1968), probably because phenols form conjugates with ingredients in the corn or other crop. When untreated corn plants were spiked with 1 p.p.m. of the phenol and acidified to at least pH 2 (citric, phosphoric, and sulfuric acids were tried), the recovery of the phenol was not improved over that obtained with no added acid, and no phenol could be recovered from the silicic acid column. (Recovery of VCS-506 and its *O*-analog is not affected by the acidification.) Although the determination of the phenol is normally not required in residue analyses because it is not considered a toxicant, the determination does provide an estimate of the amount present, which is of interest in following the degradation of the insecticide.

As shown in Table II, recoveries of VCS-506, the *O*-analog, and the phenol added to milk at levels between 0.05 and 0.50 p.p.m. were 91 to 95%, 83 to 88%, and 93 to 95%, respectively, and minimum detectable levels were, respectively, 0.004, 0.002 (based on twice the noise level), and 0.001 p.p.m. (based on twice the interference of the unfortified sample).

Typical chromatograms of VCS-506 and its *O*-analog are shown in Figure 3; those of the standards are shown on the right and those of the two compounds (2.5 ng. each) in the presence of the extract of corn plants in the center of the figure; no appreciable interference appeared in the flame-photometric analyses. The peak at 1.30 minutes in the center chromatogram of the *O*-analog fraction was traced to the solvents used; it does not interfere. Chromatograms of the compounds in the extracts of milk and corn ears are not given because they did not differ appreciably from those shown in Figure 3.

Table I. Gas Chromatographic Analysis of VCS-506, Its Oxygen Analog, and Its Phenol in Corn Plants and Ears[a]

Trial	Compound	Added μg.[c]	Added p.p.m.	Mg. Equivalent of Crop/ Analysis	Recovered[b] μg.[c]	Recovered[b] p.p.m.	Recovered[b] %
1	VCS-506	0	0	50	<0.08	<0.004	—
	O-Analog	0	0	50	<0.08	<0.004	—
	Phenol	0	0	20	<0.18	<0.009	—
2	VCS-506	10	0.50	20	9.7	0.485	97
	O-Analog	10	0.50	20	8.0	0.40	80
	Phenol	10	0.50	5	5.9	0.295	59
3	VCS-506	100	5.0	20	99	4.95	99
	O-Analog	0	0	50	<0.08	<0.004	—
	Phenol	0	0	20	<0.18	<0.009	—
4	VCS-506	0	0	20	<0.08	<0.004	—
	O-Analog	10	0.50	20	8.2	0.41	82
	Phenol	0	0	20	<0.18	<0.009	—
5	VCS-506	0	0	50	<0.08	<0.004	—
	O-Analog	0	0	50	<0.08	<0.004	—
	Phenol	10	0.50	5	5.5	0.275	55
6	VCS-506	100	5.00	20	98	4.9	98
	O-Analog	2	0.10	50	1.58	0.079	79
	Phenol	2	0.10	20	1.15	0.058	58
7	VCS-506	1	0.050	50	0.94	0.047	94
	O-Analog	1	0.050	50	0.75	0.038	75
	Phenol	1	0.050	20	0.62	0.031	62
8	VCS-506	0	0	50	<0.08	<0.004	—
	O-Analog	0	0	50	<0.08	<0.004	—
	Phenol	0	0	20	<0.18	<0.009	—
9	VCS-506	10	0.50	20	9.6	0.480	96
	O-Analog	10	0.50	20	7.8	0.389	78
	Phenol	10	0.50	5	6.9	0.345	69
10	VCS-506	1	0.050	50	0.92	0.046	92
	O-Analog	1	0.050	50	0.72	0.036	72
	Phenol	1	0.050	20	0.67	0.034	67

[a] Trials 1 to 7 on corn plants; trials 8 to 10 on ears.
[b] Mean of duplicate analyses.
[c] Per 20 grams of plant material.

Chromatograms of 1 ng. of the phenol alone and in the phenol-containing fractions from milk, corn ears, and corn plants are shown in Figure 4. The interference that was present was minor.

Because a recent study on the extraction of phosphorus-containing insecticides from several field-weathered crops (including corn) indicated that an exhaustive extraction was needed for maximum recovery of insecticides and their metabolites and that 10% methanol in chloroform was the best of the solvents tested (Bowman *et al.*, 1968), an exhaustive Soxhlet extraction with this solvent system was used with the corn samples. Sixteen hours was required to remove more than 99% of the residues of the three compounds extractable by this procedure from field-weathered corn plants. Inasmuch as extractions were started in the late afternoon and terminated the following morning, the prolonged extraction period caused no undue holdup. (The time required to complete the extraction of the compounds should be checked for each crop.)

The *O*-analog was found to hydrolyze when it was chromatographed directly on the silica gel. The addition of water to silica gel, which is known to reduce its adsorptive properties, failed to eliminate the hydrolysis; however, the use of pH 7 buffer rather than water alone corrected this difficulty.

The amount of fat in a 100-gram sample of milk is appreciable; it passes the columns operated in tandem and accumulates in the fraction containing the VCS-506. Although

Table II. Gas Chromatographic Analysis of Velsicol VCS-506, Its Oxygen Analog, and Its Phenol in Milk

Trial	Compound	Added μg.[b]	Added P.p.m.	Mg. Equivalent of Milk/ Analysis	Recovered[a] μg.[b]	Recovered[a] P.p.m.	Recovered[a] %
1	VCS-506	0	0	50	<0.40	<0.004	—
	O-Analog	0	0	100	<0.20	<0.002	—
	Phenol	0	0	100	<0.14	<0.001	—
2	VCS-506	50	0.500	50	47.5[c]	0.475[c]	95
	O-Analog	10	0.100	100	8.8	0.088	88
	Phenol	10	0.100	5	9.5	0.095	95
3	VCS-506	5	0.050	50	4.55[c]	0.046[c]	91
	O-Analog	5	0.050	100	4.15	0.042	83
	Phenol	5	0.050	10	4.65	0.046	93

[a] Mean of duplicate analyses.
[b] Per 100 grams of milk.
[c] Corrected for 12% loss in the partitioning cleanup.

the fat does not contaminate the flame-photometric detector or interfere with its response, over a period of time it does contaminate the column and alter its characteristics. The fat is removed to avoid this contamination by partitioning the fraction between hexane and acetonitrile, and reextracting the hexane phase with acetonitrile. The *p*-value (fraction partitioning into the nonpolar phase of an equivolume two-phase system) of VCS-506 in this system was determined to be 0.35. In accordance with Equation 4 of Beroza and Bowman (1966) for such a partition, the amount extracted into the acetonitrile (polar) phase, the one analyzed, is $1 - (0.35)^2$ or 88%; thus, 12% of the compound is discarded with the hexane layer, and a correction for this appreciable loss is applied as noted. Because *p*-values may be used to confirm identities of pesticides at the nanogram level (Beroza and Bowman, 1965; Bowman and Beroza, 1965), the *p*-values of the *O*-analog and the phenol in the hexane-acetonitrile system were also determined; they are both 0.10.

The low residues of the *O*-analog in field crops (0.2 p.p.m. or less) required the use of highly concentrated extracts. Repeated injections of these extracts caused a build-up of the involatiles at the head of the column and a consequent diminution of the gas chromatographic response (probably because the extract does not pass). This difficulty was overcome by using a glass wool plug at the head of the column and replacing it as soon as standards (injected alternately with unknowns) exhibited a diminished response (usually every 10 analyses).

Although the halogens present in VCS-506 and its *O*-analog would allow their analysis to be conducted by electron-capture gas chromatography, the use of the phosphorus flame-photometric detector was much preferred. The flame-photometric detector is not easily contaminated by unclean extracts as is the electron-capture detector, its response is more specific and is linear with concentration over a wide range, the baseline is more stable, and there is virtually no interference from crops so the need for a time-consuming cleanup is avoided. The analysis of the halogen-containing phenol with the electron-capture detector is, of course, necessary because the phenol contains no phosphorus. Should the analysis of the phenol not be needed, the alumina column and the electron-capture analysis of its eluate can be omitted. However, the dual chromatography does purify the phenol

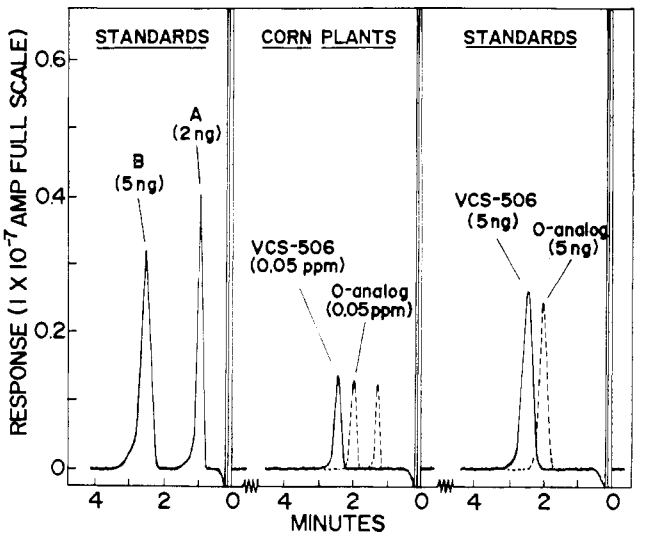

Figure 3. Chromatograms of VCS-506 (I), its *O*-analog (II), and two derivatives obtained with the flame-photometric detector

Right, VCS-506 and *O*-analog standards. Center, extract equivalent to 50 mg. of crop fortified with 0.05 p.p.m. of VCS-506 or *O*-analog. Left, dimethyl phenylphosphonate (*A*) from 2 ng. of compound VIII and *O,O*-dimethyl phenylphosphonothioate (*B*) from 5 ng. of compound V

fraction to the extent that electron-capturing and other interferences from the milk, corn plants, and ears are inconsequential. The chromatography also removes VCS-506 and the *O*-analog, which would otherwise interfere in the analysis of the phenol.

In the flame-photometric analyses, the widely used DC-200 and QF-1 liquid phases separated VCS-506 and its *O*-analog well, but they failed to resolve the aforementioned solvent peak (Figure 2, center) from that of the *O*-analog when retention times of these compounds were kept reasonably short. The OV-17, a 50% methyl-50% phenyl silicone liquid phase, was used because the two compounds and the solvent peak were easily and rapidly resolved. It may sometimes be possible to analyze for VCS-506 and its *O*-analog in nonfatty crops without the liquid chromatographic separation of the compounds; however, the retention times are only 0.5 minute apart, and a large amount of one compound could mask the presence of

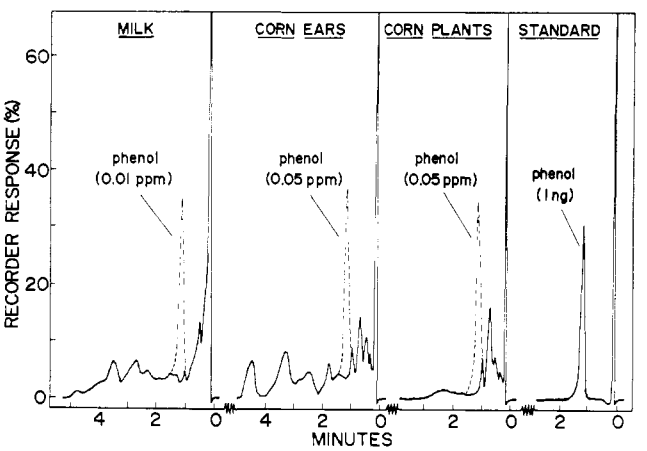

Figure 4. Chromatograms of phenol (III) obtained with the electron-capture detector

Right, the phenol standard. Right center, extract equivalent to 20 mg. of corn plant fortified with 0.05 p.p.m. of phenol. Left center, extract equivalent to 20 mg. of corn ears fortified with 0.05 p.p.m. of phenol. Left, extract equivalent to 100 mg. of milk fortified with 0.01 p.p.m. of phenol

the other or make quantification of the lesser ingredient uncertain.

Attempted Analysis of Other Metabolites. Five other hydrolysis products and *O*-analogs (IV to VIII) that may be postulated as metabolites of VCS-506 are shown in Figure 1. Compounds IV and V are unstable, but the Velsicol Chemical Corp. supplied them to us as potassium salts. Compound VI is reported to be unstable by Kosolapoff (1950). Compound VII was not available. Compound VIII was obtained from Aldrich Chemical Co., Milwaukee, Wis. The phosphonic acids (IV to VIII) could not be analyzed directly by gas chromatography, but the possibility of analyzing them as their methyl esters, formed by reaction with diazomethane, seemed worth exploring. (Note that compounds V and VI form the same dimethyl phenylphosphonothioate, and compounds VII and VIII form the same dimethyl phenylphosphonate.) The dimethyl esters were readily formed by an adaptation of the procedure described by Kirkland (1961) from compounds V (potassium salt was acidified) and VIII; as shown in Figure 3 (left), they (*A* and *B*) could be determined by gas chromatography with the flame-photometric detector. The gas chromatographic conditions used were the same as those used for VCS-506 and the *O*-analog except that the temperature of the column was 140° C. and the temperature of the injection port, transfer line, and detector was 150° C; the solvent was ethyl ether. With these conditions, the retention time of the dimethyl ester of compound VIII was 1.00 minute, and that of the ester of compound V was 2.55 minutes.

Specific directions used in methylating the two compounds follow: A weighed amount of compound V (K salt used, but weight was expressed as the acid) dissolved in water (standard solution) was acidified with sulfuric acid to Congo Red and extracted four times with equal volumes of ethyl ether. The ether was percolated through sodium sulfate and made to volume. A standard solution of compound VIII in ethyl ether was prepared by direct weighing and making to volume. The desired amount of compound in 3 ml. of ether was placed in a 10-ml. glass-stoppered tube and 2 ml. of diazomethane solution in ether, prepared from Diazald (Aldrich Chemical Co.), was added. After the mixture was shaken for 1 minute, 5 μl. of the solution was injected into the gas chromatograph set as described. The esterification was reproducible.

Essentially quantitative recoveries of the two compounds (as their esters) could be obtained when the compounds were added to raw extracts of corn plants, corn ears, and milk (fat removed by partitioning with hexane-acetonitrile) in ether and the solution was reacted with 2 ml. of the diazomethane reagent. (Recoveries were lower if less than 2 ml. of reagent was used.) However, attempts to recover the added compounds (as much as 10 p.p.m. in corn or milk) taken through the extraction procedure were completely negative. Only when sulfuric acid (3 ml. of 1*N* per 25 grams) or citric acid (3 ml. of 5*N* per 25 grams) was added to corn (spiked at the 1-p.p.m. level) was about 35% of V recovered; no VIII was recovered, nor could V or VIII be recovered from milk similarly spiked and acidified to pH 2. No V or VIII could be found in acidified and methylated field-treated corn known to contain 18 p.p.m. of VCS-506. For esterification, the corn extracts were evaporated to dryness and reconstituted in ether, and the diazomethane was added. The milk extracts were taken to dryness and partitioned in the hexane-acetonitrile system; aqueous salt solution was added to the acetonitrile layer, and the mixture was extracted with ether, which was dried and concentrated to 3 ml.; then 2 ml. of the diazomethane solution was added.

Compound IV (potassium salt) treated as described for compound V gave very little of the parent insecticide (5% or less). Virtually no product was obtained when IV was refluxed with 1*N* sulfuric acid prior to methylation.

Failure to achieve adequate recoveries of these postulated metabolites and the probability that they would not persist in appreciable quantity owing to their instability caused us to abandon attempts to analyze for them.

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