Determination of Accothion, Its Oxygen Analog, and Its Cresol in Corn, Grass, and Milk by Gas Chromatography

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Residues of Accothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate), its oxygen analog, and its cresol hydrolysis product were determined in corn plants, coastal Bermuda grass, and milk. The compounds in the extract were separated by liquid chromatography on silica gel containing 20% water; a solvent partition was applied to one milk fraction and an alumina column cleanup to one fraction from the corn or grass. The Accothion and oxygen analog fractions were analyzed by gas chromatography with a flame-photometric detector sensitive to phosphorus and the cresol fractions by gas chromatography with a Ni^{63} electron-capture detector. Recoveries of Accothion were 94 to 100%; those of its oxygen analog were 90 to 96% from corn and grass and 80 to 82% from milk; those of the cresol were 90 to 97%. The sensitivity of the method was 0.001 to 0.002 p.p.m. for Accothion and its oxygen analog and about 0.01 to 0.02 p.p.m. for the cresol.

Accothion (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate, also known as Sumithion, Bay 41831, fenitrothion, methylnitrophos, Folithion) gives good control of a variety of insect pests attacking forage and other crops (Anderson *et al.*, 1963; Armbrust and Gyrisco, 1965; Cogburn, 1967; Cowan and Davis, 1968; Green and Tyler, 1966; Shapovalova and Bocharova, 1965; Strong and Sbur, 1968; Waites and Habeck, 1968). As part of an investigation to assure its safe usage on corn and grass intended for animal feed, a method was needed to determine residues of the insecticide in the treated crops and in the milk of cows consuming these crops. Residues may consist of the parent insecticide, the oxygen analog (hereafter *O*-analog), and the cresol hydrolysis product. Formulas of the three compounds are shown in Figure 1.

Despite the large number of residue methods for Accothion that have appeared, few can be used to determine other than the parent compound, and none could be found for determining quantitatively Accothion, its *O*-analog, and the cresol. Accothion has been determined by colorimetry or spectrophotometry (Franz and Kovac, 1965; Hais and Franz, 1965; Horler, 1966; Yuen, 1966), infrared spectrophotometry (Delves and Williams, 1966; Oi and Umeda, 1966), thin-layer chromatography (Barney, 1965; Ebing, 1967; Fischer, 1968; Guth, 1967; Kovac and Sohler, 1965; Oi *et al.*, 1966), and gas chromatography with an electron-capture detector (Dawson *et al.*, 1964; Kanazawa and Kawahara, 1966; Kawai and Shitaya, 1965), an argon β-ionization detector (Horiguchi *et al.*, 1964), and a Varian Aerograph phosphorus detector (Ruzicka *et al.*, 1967).

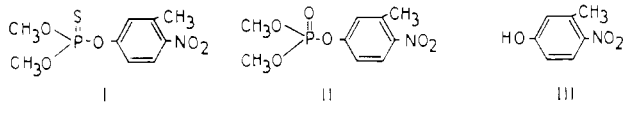

Figure 1. Formulas of Accothion (I), its *O*-analog (II), and cresol hydrolysis product (III)

This paper describes a highly sensitive method of determining residues of the three compounds. The extract of the crop or milk is separated by chromatography on silica gel into three fractions, each containing one of the compounds; after an additional cleanup that is required for some fractions, each fraction is analyzed by gas chromatography.

EXPERIMENTAL

Reagents and Solvents. Silica gel (J. T. Baker Chemical Co., No. 3405) was found to contain 2.8% water (determined by weight loss after overnight drying at 110° C.). For the liquid chromatography its water content was adjusted to 20% by weight by adding the required amount of distilled water to a 500-gram batch of the adsorbent in a reagent jar and rolling it overnight on a ball mill (with no balls in jar). The alumina (Fisher Scientific Co., A-540 adsorption alumina 80- to 200-mesh) was used as received; it lost 3.1% of its weight after drying overnight at 110° C.

Accothion, its *O*-analog, and cresol were analytical grade reagents kindly supplied by the American Cyanamid Co., Princeton, N.J.

Hexane was refluxed over potassium hydroxide and distilled before use. Acetonitrile, benzene, chloroform, and methylene chloride were C.P. grade solvents redistilled. C.P. absolute methanol, acetone, and acetic acid were used as received. Sodium sulfate was the anhydrous reagent grade chemical.

Equipment. A Hewlett-Packard (Avondale, Pa.) F & M Model 5750 gas chromatograph was equipped with the flame-photometric detector of Brody and Chaney (1966) (MicroTek Instruments, Inc., Baton Rouge, La.) and a 526-mμ interference filter for detecting phosphorus-containing compounds (Accothion

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and its *O*-analog). A MicroTek Model 2000 R gas chromatograph equipped with a Ni^{63} electron-capture detector was used for determining the cresol.

Extraction of Milk. The milk is shaken to disperse the cream uniformly, and a 100-gram sample is added to a Waring Blendor with 300 ml. of acetone. The mixture is blended for 3 minutes and filtered through Whatman No. 1 paper on a Büchner funnel. After the blender and filter funnel are washed with an additional 25 ml. of acetone, the combined filtrate and washings are extracted with a 200- and a 100-ml. portion of methylene chloride, and the extracts are percolated successively through a plug of sodium sulfate 4 cm. in diameter and 5 cm. thick. The percolate is evaporated almost to dryness under a Snyder column on a steam bath and then just to dryness at room temperature under water pump vacuum. The residue is taken up in 10 ml. of benzene for liquid chromatography.

Sample Preparation and Extraction of Corn and Grass. Twenty grams of the finely chopped and well mixed plant material is transferred to 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 the solvent exchanges. The sample is extracted under nitrogen for 4 hours at the rate of about six solvent exchanges per hour with 150 ml. of chloroform-methanol (9 to 1 by volume). The cooled extract is filtered through a plug of sodium sulfate about 2.5 cm. in diameter and 3.0 cm. thick, and the container and plug are washed with 10 ml. of chloroform. The combined extract and washings are evaporated to dryness with water pump vacuum (ca. 35 mm. of Hg) and a 50° C. water bath. (The presence of excessive solvent in the residue can impair the ensuing liquid chromatography.) The residue is taken up in 10 ml. of benzene for the liquid chromatography.

Liquid Chromatographic Separation of Compounds in Milk. The column is prepared by adding successively to a 2-cm. i.d. Shell-type column 15 grams of sodium sulfate, 10 grams of silica gel containing 20% water, and 10 grams of sodium sulfate. The column is prewashed with 30 ml. of benzene and the eluate is discarded; then the 10-ml. benzene extract of milk is added, and the collection of eluate is begun. The container is rinsed, and the extract is washed into the column with small portions of benzene to a total of 10 ml. Another 40 ml. of benzene is added and allowed to percolate through the column. The eluate (60 ml.) contains the Accothion.

The receivers are changed, and 120 ml. more of benzene is allowed to percolate through the column. This eluate contains the cresol.

Again the receivers are changed, and 50 ml. of acetone is added to the column. The eluate contains the *O*-analog.

Preparation of Liquid Chromatographic Fractions of Milk for Gas Chromatography. The Accothion fraction is evaporated to dryness on a 50° C. water bath under water pump vacuum, and the fatty residue is transferred to a glass-stoppered test tube with 5 ml. each of pre-equilibrated hexane and acetonitrile. The contents are shaken for one minute, the layers are allowed to separate, and the upper hexane layer is removed with a syringe and cannula and discarded. Five microliters of the remaining acetonitrile layer (equivalent to 100 mg. of milk), or a dilution thereof, is injected into the gas chromatograph for analysis. Because 3.5% of the Accothion is lost in the partitioning step, the analytical results are corrected by dividing them by 0.965.

The cresol and *O*-analog fractions are evaporated under a Snyder column on a steam bath to near dryness, and the residues are made up to appropriate volumes (with benzene for the cresol fraction and acetone for the *O*-analog fraction). A 5-μl. portion is injected into the gas chromatograph for analysis. (If the concentrates are adjusted to 5 ml., the 5-μl. injection is equivalent to 100 mg. of milk.)

Liquid Chromatographic Separation of Accothion, Its *O*-Analog, and Its Cresol in Corn and Grass. The silica gel chromatography of the corn and grass extracts is carried out exactly as described for the milk until the first 60 ml. of eluate (Accothion fraction) is collected.

The column is then placed directly over another (in tandem) previously prepared 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; then 200 ml. of benzene is percolated through both columns, and the eluate is discarded. (Because the plant material delayed the elution of the cresol, 200 ml. of benzene instead of the 120-ml. volume used for milk was required for complete transfer of the cresol to the alumina column.) The *O*-analog, now on the silica gel, and the cresol, on the alumina, are eluted from the separate columns. Fifty milliliters of an equivolume mixture of benzene and acetone is percolated through the alumina column, and the eluate, which contains most of the plant interferences, is discarded. Fifty milliliters of benzene containing 1 ml. of acetic acid is now passed through the alumina column; the eluate contains the cresol.

Fifty milliliters of acetone is added to the silica gel column. The eluate contains the *O*-analog.

Preparation of Liquid Chromatographic Fractions of Corn and Grass for Gas Chromatography. The Accothion fraction and that of its *O*-analog are concentrated to appropriate volumes, and 5-μl. portions are injected into the gas chromatograph for analysis.

The cresol fraction is added to a separatory funnel containing 50 ml. of 5% aqueous sodium bicarbonate. The mixture is shaken vigorously for 2 minutes (allowing the CO_2 to escape), and the benzene layer is percolated through a plug of sodium sulfate 2.5 cm. in diameter and 3 cm. thick. The extraction of the aqueous layer is repeated twice with 25-ml. portions of benzene, and the benzene is passed through the plug of sodium sulfate. The filtrate is concentrated to near dryness on a 50° C. water bath under water pump vacuum, its volume is adjusted as appropriate, and a 5-μl. portion is injected into the gas chromatograph.

If the concentrates of the fractions are adjusted to 5 ml., the 5-μl. injection is equivalent to 20 mg. of crop.

Gas Chromatographic Analysis. Fractions containing Accothion and its *O*-analog are analyzed with the

gas chromatograph having the flame-photometric detector. A glass column, 4-mm. i.d. (6-mm. o.d.) × 100 cm. long, containing 10% DC-200 (w./w.) on 80- to 100-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.) is maintained at 180° C. Temperatures of the injection port, transfer line (column to detector), and detector are 200°, 190°, and 200° C., respectively. The flow rates of the nitrogen (carrier), oxygen, and hydrogen gases are 160, 40, and 200 ml. per minute, respectively. The column is conditioned overnight at 240° C. prior to use. Conditioning of the column to the *O*-analog and the extract is necessary to achieve reproducibility, linearity, and high sensitivity. The *O*-analog in extract is injected repeatedly until trials with 5 ng. of the compound in the appropriate extract build up to a constant response. [Such conditioning has been found necessary or desirable in the analysis of organophosphorus and other types of insecticides (Beroza and Bowman, 1968; Shuman and Collie, 1963).] Once the column is conditioned, no further conditioning is required; however, the oven and the gases should be turned off overnight and during weekends. Response, which is based on peak height, is linear with concentration to at least the 250-ng. level. The amount of compound in an extract is determined by comparison of its response with that of a 5-ng. standard. These standards are injected frequently and help keep the column conditioned. The retention time of Accothion was 2.90 minutes and that of the *O*-analog 2.30 minutes.

Fractions containing the cresol are analyzed with the instrument having the electron-capture detector. A glass column, 4-mm. i.d. (6-mm. o.d.) × 180 cm. long, containing 20% (w./w.) OV-101 (Applied Science Laboratories) on 80- to 100-mesh Gas Chrom Q is maintained at 240° C. Temperatures of the injection port and detector are 255° and 270° C., respectively. The nitrogen carrier gas flow rate is 200 ml. per minute. The electrometer setting was 4×10^{-9} ampere full scale with a 1-mv. recorder. The column is conditioned overnight at 280° C. Conditioning of the column by repeated injections of the cresol in the appropriate extract is also required to obtain reproducible, linear, and sensitive responses to the cresol. After the column is conditioned, response of the cresol in terms of peak height is linear with concentration to at least the 10-ng. level. The retention time of the cresol was 1.10 minutes. (Accothion and its *O*-analog can also be analyzed with the electron-capture instrument; with the conditions given, the retention times of Accothion and its *O*-analog were 3.20 and 2.55 minutes, respectively).

RESULTS AND DISCUSSION

A summary of the separation and cleanup operations is given in Figure 2. The silica gel chromatography separates the extracts into fractions, each of which contains one of the compounds. The 15 grams of sodium sulfate used in this column is needed to retain the water that acetone elutes from the silica gel (*O*-analog fraction). The fat in the Accothion fraction from the milk is removed by the hexane-acetonitrile partition to avoid depositing it on the gas chromatographic column. A

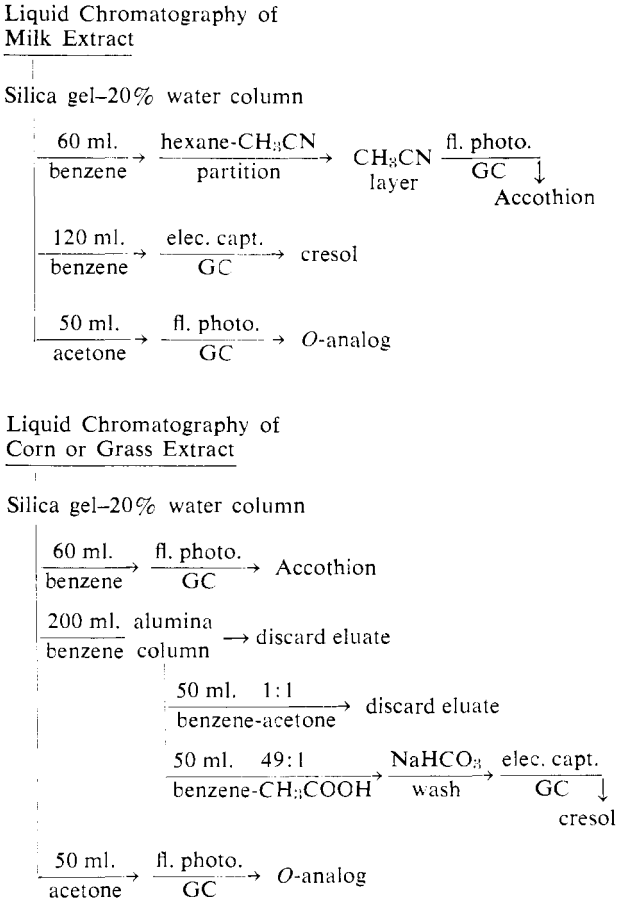

Figure 2. Summary of separation and cleanup operations preceding gas chromatography (GC)

correction is made for the 3.5% of Accothion discarded with the fat-containing hexane layer. The cresol fraction from the silica gel chromatography of the plant extracts requires an additional cleanup on alumina to remove interferences encountered in the electron-capture gas chromatography of this fraction. A similar cleanup of the cresol fraction from milk was not necessary; the fat, which would contaminate the electron-capture detector, was removed with the Accothion fraction, and the one peak that did appear in the gas chromatography of the cresol fraction did not interfere.

Samples of corn, coastal Bermuda grass, and milk were fortified at several levels with the three compounds and carried through the entire analytical procedure. (The dry-matter content of corn plants and coastal Bermuda grass used in this work was 13.5 and 30.8%, respectively.) Inasmuch as the minimum time required to remove by Soxhlet extraction 99% of the residues of the three compounds (extractable by this method) in field-treated corn or grass was determined to be 4 hours, extraction of the fortified samples was continued for this time. The results of the analysis of the corn and grass samples are shown in Table I and those from milk in Table II. The milk was fortified with an acetone solution of the compounds before blending. Recoveries

Table I. Gas Chromatographic Analysis of Accothion, Its Oxygen Analog, and Its Cresol in Corn Plants and Coastal Bermuda Grass

Trial	Compound	Added		Mg. Equiv. Crop/ Analysis	Recovered[a]					
					Corn Plants			Coastal Bermuda Grass		
		μg.[b]	P.p.m.		μg.[b]	P.p.m.	%	μg.[b]	P.p.m.	%
1	Accothion	0	0	50	<0.04	<0.002	—	<0.04	<0.002	—
	O-analog	0	0	50	<0.04	<0.002	—	<0.04	<0.002	—
	Cresol	0	0	20	<0.12	<0.006	—	<0.48	<0.024	—
2	Accothion	10	0.500	20	9.8	0.49	98	9.9	0.49	99
	O-analog	10	0.500	20	9.6	0.48	96	9.4	0.47	94
	Cresol	10	0.500	10	9.4	0.47	94	9.2	0.46	92
3	Accothion	100	5.00	20	98	4.9	98	99	4.93	99
	O-analog	0	0	50	<0.04	<0.002	—	<0.04	<0.002	—
	Cresol	0	0	20	<0.12	<0.006	—	<0.48	<0.024	—
4	Accothion	0	0	50	<0.04	<0.002	—	<0.04	<0.002	—
	O-analog	10	0.500	20	9.4	0.47	94	9.2	0.46	92
	Cresol	0	0	20	<0.12	<0.006	—	<0.48	<0.024	—
5	Accothion	0	0	50	<0.04	<0.002	—	<0.04	<0.002	—
	O-analog	0	0	50	<0.004	<0.002	—	<0.04	<0.002	—
	Cresol	10	0.500	10	9.2	0.46	92	9.4	0.47	94
6	Accothion	100	5.00	20	99	4.95	99	100	5.00	100
	O-analog	2	0.100	50	1.84	0.092	92	1.87	0.094	94
	Cresol	2	0.100	20	1.80	0.090	90	1.84	0.092	92
7	Accothion	1	0.050	50	0.98	0.049	98	0.097	0.048	97
	O-analog	1	0.050	50	0.92	0.046	92	0.90	0.045	90
	Cresol	1	0.050	20	0.90	0.045	90	0.90	0.045	90

[a] Mean of duplicate analyses.
[b] Per 20 grams of sample.

Table II. Gas Chromatographic Analysis of Accothion, Its Oxygen Analog, and Its Cresol in Milk

Trial	Compound	Added		Mg. Equiv. Milk/Analysis	Recovered[a]		
		μg.[b]	P.p.m.		μg.[b]	P.p.m.	%
1	Accothion	0	0.0	100	<0.10	<0.001	—
	O-analog	0	0.00	100	<0.10	<0.001	—
	Cresol	0	0.00	100	<0.082	<0.008	—
2	Accothion	50	0.500	50	48[c]	0.48	96
	O-analog	10	0.100	50	8.2	0.082	82
	Cresol	10	0.100	50	9.7	0.097	97
3	Accothion	5	0.050	50	4.7[c]	0.047	94
	O-analog	5	0.050	50	4.0	0.040	80
	Cresol	5	0.050	100	4.6	0.046	92

[a] Mean of duplicate analyses.
[b] Per 100 grams of milk.
[c] Corrected for 3.5% loss in hexane-acetonitrile partitioning.

of the compounds from solutions carried through the analytical procedure in the absence of crop or milk were essentially quantitative.

In each table, the sensitivity or the minimum detectable level of the residues is the value given for the zero level of fortification with the compounds. Sensitivity was based on twice the noise level in the flame-photometric analyses and on twice the interference of the unfortified sample in the electron-capture analyses. The sensitivity in the determinations of Accothion and its *O*-analog was 0.002 p.p.m. in corn and grass and 0.001 p.p.m. in milk. Sensitivity in the cresol determinations was 0.006 p.p.m. in corn, 0.024 p.p.m. in grass, and 0.008 p.p.m. in milk.

The compounds were added to the crops both individually and together. There was no indication of any interconversion of the compounds. The recoveries of Accothion, its *O*-analog, and cresol from corn and grass fortified at levels between 0.05 and 5 p.p.m. were between 90 and 100%. Because high levels of the cresol were found in field-treated samples, recoveries of higher levels of cresol in corn and grass were also determined;

at levels of 10 and 25 p.p.m., recoveries were between 95 and 100%. With 100-gram samples of milk fortified at levels between 0.05 and 0.5 p.p.m., recoveries were 94 to 96% for Accothion, 80 to 82% for its *O*-analog, and 92 to 97% for the cresol hydrolysis product.

Typical chromatograms of Accothion and its *O*-analog as standards and in the presence of the crop and milk extracts are shown in Figure 3. A peak with a retention time of a little more than one minute that appeared in the crop and milk chromatograms caused no difficulty; it is believed to arise from a solvent impurity. Figure 4 shows chromatograms of the cresol alone and in the presence of corn, grass, and milk.

The separation of 100-μg. amounts of the three compounds on the 10-gram silica gel column containing 20% water is illustrated in Figure 5, which is a plot of the amounts of the compounds in 5-ml. fractions of the eluate from the column. The 20% water was used because hydrolytic cleavage of the *O*-analog occurred with less water, and incomplete separation of the compounds occurred with greater amounts. Hydrolysis, when it occurred, was detected by the characteristic yellow color of the nitrocresol in alkaline solution or by electron-capture chromatography. (Alumina also caused hydrolysis of the *O*-analog.)

The *p*-values (fraction of total in the nonpolar phase after an equivolume two-phase distribution) of Accothion, its *O*-analog, and the cresol in the hexane-acetonitrile system at 25° C. are 0.035, 0.007, and 0.012, respectively. *p*-Values are useful for determining extraction procedures and for confirming identities of pesticides at nanogram levels such as are encountered

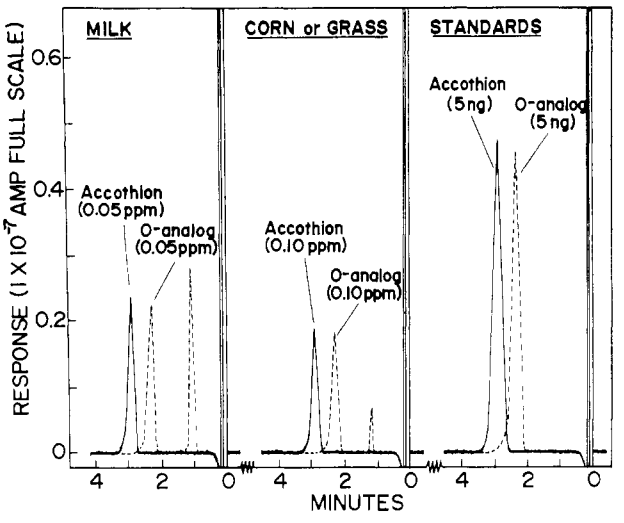

Figure 3. Chromatograms of Accothion (I) and its *O*-analog (II) with the flame-photometric detector

Right. 5 ng. in 5 μl. of benzene and acetone, respectively
Center. Extract equivalent to 20 mg. of corn or grass fortified with 2 ng. of Accothion or *O*-analog injected in 5 μl. of benzene or acetone, respectively
Left. Extract equivalent to 50 mg. of milk fortified with 2.5 ng. of Accothion or *O*-analog injected in 5 μl. of acetonitrile or acetone, respectively
Peaks with retention time slightly greater than 1 minute arise from impurities

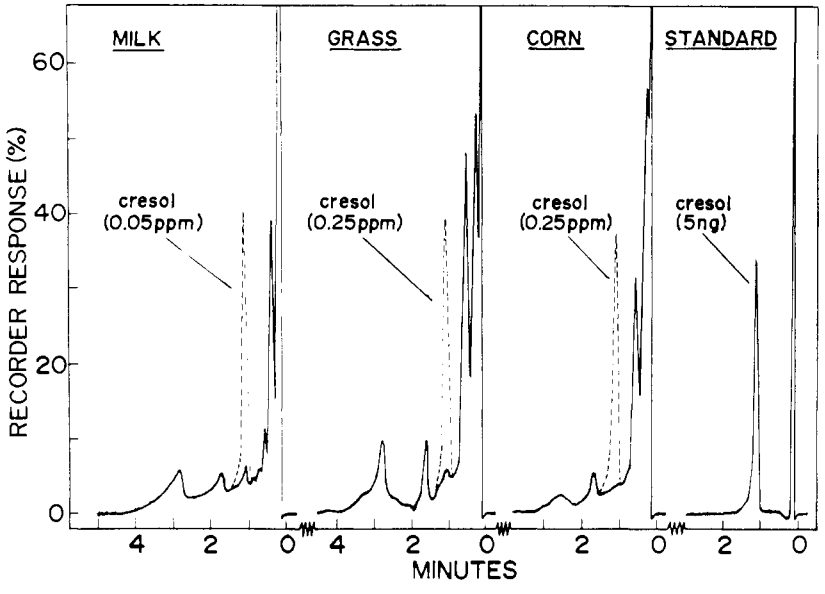

Figure 4. Chromatograms of cresol (III) with electron-capture detector

All injections in 5 μl. of benzene
Right. 5-ng. standard
Right center. Extract containing equivalent of 20 mg. of corn fortified with 5 ng. of cresol
Left center. Extract containing equivalent of 20 mg. of grass fortified with 5 ng. of cresol
Left. Extract containing equivalent of 100 mg. of milk fortified with 5 ng. of cresol

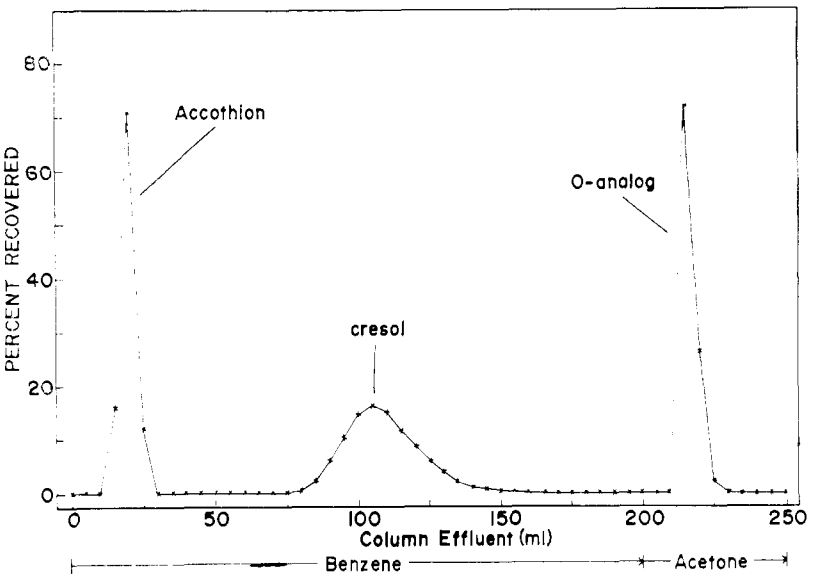

Figure 5. Separation of Accothion, its *O*-analog, and cresol by liquid chromatography on 10 grams of silica gel containing 20% water

in residue analyses (Beroza and Bowman, 1965; Bowman and Beroza, 1965).

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