Methods for the Determination of Mobam (4-Benzothienyl *N*-Methylcarbamate)

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Some of the analytical methods developed to determine Mobam (4-benzothienyl *N*-methylcarbamate, Mobil Chemical Co.), a new carbamate insecticide, in formulations and as a residue are described. The techniques applicable to the determination of macro quantities of the pesticide in technical product and in formulations are based on nonaqueous acidimetry, ultraviolet spectrophotometry, and an alkaline hydrolysis–methylamine evolution technique. The residue method is based on the measurement of the absorbance of the highly colored azo compound formed by the alkaline coupling of Mobam with *p*-nitrobenzenediazonium fluoborate. Procedures for isolating the carbamate from several fruits, forage crops, cottonseed products, vegetables, and milk are given. The milk procedure involves the direct extraction of the carbamate from only 25 ml. of whole milk with methylene chloride–hexane (3:1, v./v.). The limit of detection of the residue method is estimated to be 1 μg. The standard deviation of the method from 1 to 100 μg. of the carbamate is 0.4 μg.

Mobam (Mobil Chemical Co., previously designated as MC-A-600) is 4-benzothienyl *N*-methylcarbamate. The effectiveness of this compound in controlling a variety of insects was described by Kilsheimer *et al.* (1965). This paper presents some of the methods developed for the identification and determination of the pesticide in the technical product, in formulations, and as a trace residue in agricultural commodities. Determination of macro quantities of the carbamate in formulations is described as well as procedures for the determination of residues in a number of commodities.

The structure of Mobam suggested that analytical methods might be based on one or more of the following properties: nitrogen content, sulfur content, acid-base nature of the carbamate moiety, aromaticity of the benzothiophene ring, and the hydrolyzability of the carbamate. The direct determination of nitrogen and/or sulfur as a measure of the Mobam concentration lacks the selectivity required under most circumstances, and so methods based on heteroatom content were not investigated. Each of the other properties was studied and each led to a useful procedure for determining macro amounts of the pesticide. In addition, hydrolysis followed by the colorimetric determination of the resulting phenol (4-hydroxybenzothiophene) was utilized to determine microgram quantities of the carbamate in agricultural commodities.

MACRO METHODS

Nonaqueous Titration. Perusal of the literature suggested that the carbamate structure might exhibit a weakly acidic or basic character. Preliminary experiments with nonaqueous systems showed that the carbamate proton could be titrated in ethylenediamine in a manner similar to that described by Fritz (1952) for imides. In this laboratory, it was more convenient to use an established procedure involving dissolution of the pesticide in pyridine and titration with tetrabutylammonium hydroxide in benzene to the orange-red *o*-nitroaniline end point (Cundiff and Markunas, 1958). This titration system was used to obtain the data shown in Table I. The generally high results obtained for the lower concentration formulations are not due to a positive bias of the method, but rather to the generous addition of Mobam during the preparation of the formulations.

The principle expected impurity in Mobam, 4-hydroxybenzothiophene (4-HBT), is acidic under the conditions of the titration and is quantitatively titrated. Consequently, in materials containing only Mobam and 4-HBT, the concentration of each may be determined by titration and calculation via two simultaneous equations, one based on the sum of masses and the other on the sum of equivalents. Because of possible interferences (for example, cresols) found in some emulsifiable formulations, nonaqueous titrimetry is recommended only for rapid screening of the technical product.

Ultraviolet Spectrophotometry. The aromaticity of the benzothiophene ring would be expected to give intense, characteristic absorption bands in the ultraviolet region of the spectrum. The absorption spectra of Mobam and 4-HBT in methanol solution are shown in Figure 1. By taking advantage of the absorption band characteristic of Mobam at 289 mμ and of 4-HBT at 310 mμ, a method for determining both compounds simultaneously was devised. Typical results obtained when the method was applied to technical product and to a number of formulated products are shown in Table II. The standard deviation of the UV method calculated from the six duplicate Mobam determinations shown in the table is ±0.14% for the carbamate. The last entry represents a 57 to 43 mixture of Mobam and 4-HBT and is an example of the recoveries that can be expected. The ultraviolet method is rapid and is the method of choice for screening samples when both the Mobam and the 4-HBT contents must be determined.

Table I. Tetrabutylammonium Hydroxide Titration of Mobam

Sample	Nominal Concn., %	Mobam Found, %
Attaclay	5	5.9
Corncobs	10	11.5, 11.1
Pike's Peak clay	10	12.5, 12.9
Attaclay	50	50.0, 50.9
Technical grade		97.4, 97.1
Analytical grade		99.9, 100.3

Mobil Chemical Co., Metuchen, N.J. 08840.

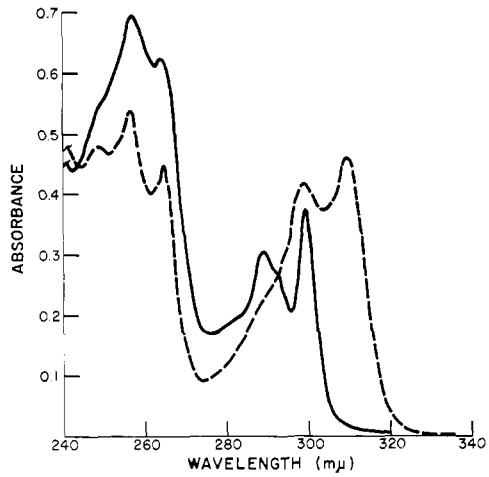
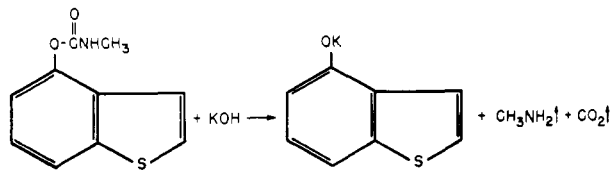


Figure 1. Ultraviolet spectra in methanol

—— Mobam
---- 4-HBT

Hydrolysis. The hydrolysis procedure is based on the following reactions:



(1)

$$CH_3NH_2 + H_3BO_3 \rightleftharpoons [CH_3NH_3]^+[H_2BO_3]^- \quad (2)$$

$$H_2BO_3^- + H^+ \rightleftharpoons H_3BO_3 \quad (3)$$

The first reaction is a high temperature alkaline hydrolysis carried out in ethylene glycol. The methylamine evolved is trapped in boric acid solution to form the borate as shown in Reaction 2. The borate ion is then titrated with standard acid as shown in Reaction 3.

A high temperature alkaline hydrolysis–methylamine evolution technique of this type was described by Zweig (1964) for the assay of another carbamate. Successful determinations by this procedure require high heat input and rapid purging of the alkaline digest with nitrogen. Some typical results obtained with formulated products and analytical grade Mobam are given in Table III. These results show that the precision of the method, expressed as the coefficient of variation, is 0.25%.

It is advantageous to have both the ultraviolet and methylamine methods for the determination of Mobam because certain formulations may not be amenable to examination by one or the other technique. A comparison of results obtained by each method is given in Table IV.

RESIDUE METHODS

Residue methods may be divided into essentially two parts, the isolation of the trace concentration of the pesticide and the detection or measurement of the isolated material. Considering the measurement step first, two spectrophotometric methods and a paper chromatographic procedure were developed. One of the spectrophotometric procedures is a slight modification of the above ultraviolet method, and the second involves the formation and measurement of a highly colored azo compound. A Cary spectrophotometer was used for both spectrophotometric procedures. The paper chromatographic method is used primarily for the identification of the carbamate and 4-HBT at the microgram level.

Table II. Ultraviolet Determination Employing Simultaneous Equations

	Found, %	
	Mobam	4-HBT
Wettable powder, 50%	53.0, 53.3	0.32, 0.16
Wettable powder, 10%	10.7, 10.9	0.21, 0.28
Pecan shells, 5%	5.5, 5.6	0.11, 0.12
Corncobs, 10%	10.1, 10.4	0.18, 0.26
Technical	96.9, 96.7	0.00, 0.21
Technical	98.8, 99.0	0.00, 0.00
Mixture of 2.26 mg. of Mobam (anal. grade) and 1.72 mg. of 4-HBT	2.27 mg.	1.73 mg.

Table III. Methylamine Evolution Determination

Sample	Mobam Found, %	Std. Dev.
Attaclay, 5%	5.32, 5.27	...
Wettable powder, 50%	48.4, 48.7, 48.8 48.7, 48.8, 48.8	0.12
Mobam (anal. grade)	100.2, 99.9, 99.6, 99.8	0.25

Table IV. Comparison of Mobam Determinations by Ultraviolet and Methylamine Procedures

	Mobam, %	
	Ultraviolet	Methylamine
Attaclay, 5%	6.5	6.4
Attaclay, 10%	10.7	11.2
Corncobs, 10%	10.4	10.8
Wettable powder, 50%	53.3	53.5
Technical	99.0	99.2

Spectrophotometric Measurement. A linear relationship between the concentration of Mobam in methanol and the absorbance at 299 mμ was established for the concentration range 10 to 100 μg. per 5 ml. of methanol in 1.00-cm. cells. Apparently, 5 μg. is the least detectable amount by the ultraviolet procedure in laboratory solvents. As mentioned earlier, the ultraviolet approach has the advantage of distinguishing between Mobam and 4-HBT.

The methods of Miskus and Gordon (1959) and Johnson (1963) for the colorimetric determination of 1-napthyl *N*-methylcarbamate (Sevin) by coupling with *p*-nitrobenzenediazonium fluoborate were investigated for application to the Mobam determination. Miskus and Gordon employed an alkaline coupling procedure whereas Johnson employed coupling with the diazonium compound in acetic acid solution. The alkaline coupling reaction was chosen for the Mobam determination because it yields a stable colored product which follows the Beer-Bouguer absorption law. The colored product has a constant absorbance at 530 mμ for at least 2 hours. The absorbance of the product formed with 25 μg. of Mobam in

Table V. Effect of Time on Absorbance of Alkaline Color for 25 μg. of Mobam at 530 mμ

Minutes	Absorbance *vs.* Reagent Blank 22° C.	30° C.
0	0.455	0.419
15	0.459	0.431
30	0.459	0.438
45	0.460	0.439
60	0.453	0.438
90	0.449	0.432
120	0.453	...

10 ml. of methanol at 530 mμ (1.00-cm. cell) as a function of time is shown in Table V at two ambient temperatures. Table V shows that the absorbance does not change by more than 5% during the entire 2-hour period and only by 1.5% after the first 30 minutes. The two temperatures selected for the color stability study are expected to represent the extremes in ambient temperature that might be encountered in a residue laboratory. Of course, the color of calibration standards and of samples must be developed and measured at the same temperature.

The absorption spectrum of the colored reaction product formed by the alkaline coupling of Mobam (actually 4-HBT) is shown in Figure 2 (solid line); the spectrum of the coupling product with 1-naphthol is also shown. The difference in the positions of the absorption maxima of the coupling products makes it possible to differentiate between the two phenolic substances. The colored azo compound obtained with 4-HBT is most likely the sodium salt of 4-hydroxy-7-(4′-nitrophenylazo)benzo[*b*]thiophene.

Processing and Cleanup. After establishing the reliability of the ultraviolet and colorimetric measurement steps for determining the carbamate at trace levels, several methods for isolating the carbamate were evaluated. The procedure finally adopted is a modification of procedures published by Johnson (1963) and Miskus and Gordon (1959) for carbaryl. Reagent grade chemicals are required throughout the residue determination approach outlined, unless otherwise noted.

The exact procedure differs somewhat with the commodity examined, but generally it involves stripping the residue from 100 grams of crop with methylene chloride, evaporating the total solvent or an aliquot under vacuum at 40° C., and dissolving the recovered material in acetone. Oils and waxes are precipitated from the acetone solution with an aqueous coagulating solution (Johnson, 1963) and are removed by filtration. The carbamate is extracted from the water-acetone phase with methylene chloride which is then removed by evaporation. At this point, the residue obtained from some crops (apples, peaches, plums, lettuce, potatoes, and green tobacco) is dissolved in methanol and is ready for the color development step. Further cleanup is necessary for the extracts of range grass, flue-cured tobacco, alfalfa, corn silage, corn kernels, and grapes and involves transferring the methylene chloride extract of the water-acetone phase to a 3-inch × 24 mm.-I.D. chromatographic column of 11% water-loaded Florisil [prepared as described by Miskus (1959), except dried at 130° C.]. The pesticide is then eluted from the column with water-saturated methylene chloride as described by Johnson and Stansbury (1965) for carbaryl. The solvent is removed by vacuum evaporation, and the sample is ready for color development.

Color Development. The walls of the flask containing the carbamate residue are rinsed with 5.0 ml. of methanol followed by the addition of 4.0 ml. of methanolic alkali (0.1*F* NaOH) and then 1.0 ml. of freshly prepared chromogenic agent, *p*-nitrobenzenediazonium fluoborate (25 mg. per 25 ml. of methanol). The resulting 10 ml. of solution are allowed to develop for 30 minutes, after which the colored solution is transferred to a 1.00-cm. cell to obtain its absorption spectrum *vs.* a similarly treated reagent blank. The absorbance at 530 mμ, obtained from a chart scan over the range from 550 to 510 mμ, is then corrected by subtracting the absorbance obtained with a crop check or control. The concentration of Mobam is read from the corrected absorbance by means of a calibration curve constructed with the typical data shown in Table VI. These data were obtained by carrying methanol solutions (standards) of analytical grade Mobam through the entire residue method. Calibration based on processed standards was chosen because if there were any losses of Mobam due to method treatment and manipulations, a

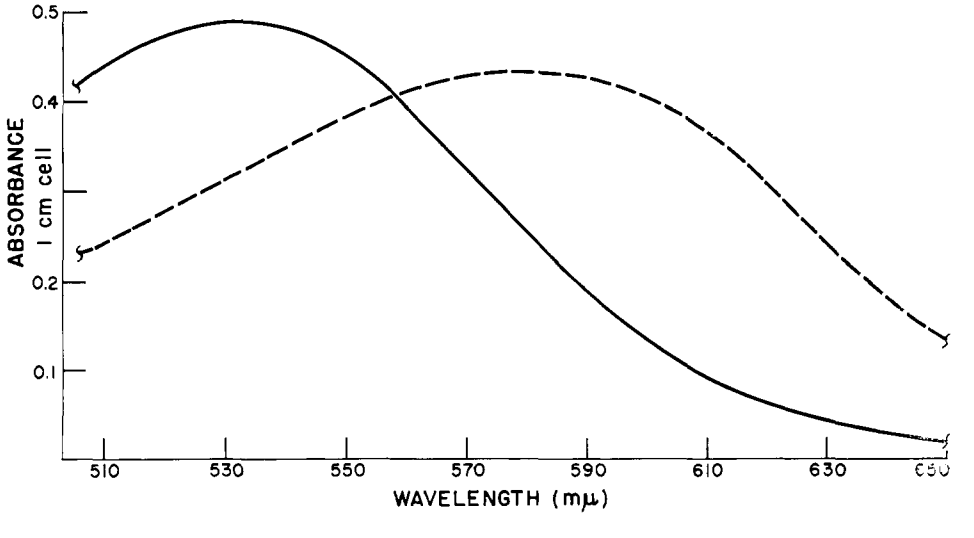


Figure 2. Alkaline *p*-nitrobenzenediazonium fluoborate color

—— Mobam, solid
---- Carbaryl, 25 μg./10 ml.

Table VI. Processed Calibration Curve Data

(10 ml. solution volume)

Mobam, μg.	$A^{a\,1\text{ cm}}_{530\text{ m}\mu}$
10	0.169 (0.182)[b]
25	0.466 (0.470)
50	0.918 (0.940)
100	1.835 (1.860)

$A = 0.0184\,C - 0.0053$, with A representing absorbance and C, concentration in μg.

[a] Average of duplicate determinations.

[b] Typical unprocessed Mobam absorbance.

more accurate (higher) value for a crop residue would be obtained from the processed curve than from standards that had not been processed. Actually, the absorbance data from processed standards regularly averaged greater than 95% of the absorbance observed for unprocessed standards. Johnson (1963) has also recommended the use of a processed calibration curve in his method for carbaryl.

This type of calibration curve was used for determining the recoveries of Mobam added to crop samples or stripping extracts in the 0.5- to 4.0-p.p.m. concentration range processed according to the colorimetric residue method. These data are given in Table VII as well as the apparent level of Mobam in crop checks as determined by carrying untreated crops through the entire procedure. These recoveries establish the lack of interference of the crops examined with the Mobam determination.

Cottonseed Products. Different approaches for stripping and cleanup were developed for examining cottonseed products and milk. Fortified cottonseed oil samples are processed by diluting the oil with petroleum ether (permanganate purified grade, b.p. 30° to 60° C.) and extracting with acetonitrile. The acetonitrile extractables are then recovered, treated with a coagulating solution, and passed through Florisil columns as indicated above. The Mobam recoveries in the 1- to 4-p.p.m. range averaged 79% for cottonseed oil. Cottonseed meal is extracted with methylene chloride in the normal manner and then processed further as described for cottonseed oil.

Table VII. Recovery of Mobam Added to Crop Samples and Crop Check Values

Crop	Recovered, %[a]	Crop Check: Apparent p.p.m.	Crop Check: No. of samples[b]	Crop Check: Std. dev., p.p.m.
Apples	100	0.2	6(3)	0.06
Peaches	96	0.01	3(1)	0.01
Grapes	91	0.03	3(1)	0.00
Potatoes	98	0.3	3(1)	0.13
Lettuce	95	0.2	3(1)	0.02
Tobacco, green	99	1.5	3(1)	0.09
Tobacco, flue-cured	90	1.4	3(1)	0.12
Range grass	92	1.8	8(1)	0.8
Alfalfa	84	0.3	14(4)	0.09
Soil	94	0.04	6(1)	0.01

[a] Represents an average of recoveries observed at four levels of Mobam, accomplished in duplicate.

[b] Value in parentheses represents number of geographical locations represented in the number of crop checks examined.

The recoveries in the 1- to 2.5-p.p.m. range for fortified cottonseed meal samples averaged 100%.

Milk Residue Method. A rapid, sensitive method for determining Mobam residues in milk involved selection of a stripping solvent that was effective, convenient, and safe from a flammability standpoint. Methylene chloride–hexane (3:1, v./v.) was chosen and used to extract the carbamate from whole milk, resulting in an average recovery of over 90%. Although extraction of only the separated cream portion has been used in other pesticide residue methods (Whitehurst, 1963), the method reported here is based on the direct extraction of whole milk. Experiments in this laboratory showed that the amount of carbamate found in the separated cream varied from 20 to 30% of the amount of carbamate added to the whole milk.

The direct extraction procedure involves successive extraction of 25 ml. of mixed whole milk with two 50-ml. portions of methylene chloride–hexane (3:1, v./v.). The stripping extract is filtered through dry cotton and evaporated to dryness. The isolated butterfat and pesticide are then dissolved in petroleum ether (permanganate purified grade, b.p. 30° to 60° C.) and transferred to a separatory funnel. The Mobam is separated from the petroleum ether by successive extractions with chilled acetonitrile and is subsequently taken to dryness. The residue is used to develop the color in methanol as described earlier. A single determination requires about 2 hours, but only about 4 hours are required for six determinations.

A calibration curve for the whole milk approach was obtained by processing standards in the 1- to 10-μg range. The least-squares equation of the line and typical absorbance data for the low level calibration curve are given in Table VIII. Recovery of from 1 to 10 μg. of Mobam from 25 ml. of fortified milk (40 to 400 p.p.b. residue) ranged from 92 to 118% with an average of 108%.

Precision and Detection Limit. Comparison of the equation of the calibration curve for the 10- to 100-μg. concentration range (Table VI) with the equation for the curve covering the 1- to 10-μg. range (Table VIII) indicated the data for both curves could be combined. The combined data gave a linear, over-all calibration curve represented by the following least-squares equation: $A = 0.01834\,C + 0.0005$, where A is the absorbance at 530 mμ in a 1.00-cm. cell, and C is the concentration of Mobam in μg. per 10 ml. A more convenient form of the equation, ignoring the insignificant intercept term, is $C = 54.5\,A$. The over-all equation is applicable to any sample containing from 1 to 100 μg. of Mobam. The standard deviation of the method, calculated from the deviation of each ex-

Table VIII. Low Level Processed Calibration Curve Data

(10 ml. solution volume)

Mobam, μg.	$A^{a\,1\text{ cm}^a}_{530\text{ m}\mu}$
1.0	0.020
2.0	0.044
3.0	0.048
5.0	0.099
7.5	0.137
10.0	0.174

$A = 0.0172\,C + 0.0052$

[a] Average of duplicate determinations.

perimental point from the over-all least-squares line, is ±0.007 absorbance unit which is equivalent to ±0.4 μg. of Mobam. Assuming that two standard deviations from the calibration curve represent the 95% confidence limit and this is selected as the detection limit of the method, then the minimum detectable amount of Mobam is 0.8 μg.

The standard deviation of six replicate determinations of Mobam for corn silage fortified at the 0.5-p.p.m. level was 0.12 p.p.m.. The standard deviation of five replicate determinations of the same stripping extract from a fortified silage sample was 0.04 p.p.m. of Mobam. Sample size for these samples was 20 grams.

Identification of Residues. In general, determining the residue concentration by ultraviolet is not reliable because of the presence of ultraviolet interferences in many crops and/or solvents. Ultraviolet examination has proved useful for identification purposes; however, paper and thin-layer chromatography are more reliable.

A paper chromatographic approach, adapted from one described by Miskus *et al.* (1961), was developed for identifying Mobam and three similar compounds: 4-HBT, 1,1-dioxo-4-benzo[*b*]thienyl-*N*-methylcarbamate (Mobam dioxide), and 4-hydroxybenzothiophene-1-dioxide (4-HBT dioxide). The R_f values observed in the systems are as follows: 4-HBT, 0.69; Mobam, 0.39; 4-HBT dioxide, 0.15; and Mobam dioxide, 0.03. The chromatographic system consists of No. 4 Whatman paper impregnated with a 15% solution of glutaronitrile in acetone (by volume) and a developing solution of isopropyl ether saturated with glutaronitrile. Development requires only 20 minutes in an ascending manner. Visualization of the separated materials is accomplished by spraying with alcoholic sodium hydroxide (1.5*F*) followed by a dilute methanol solution of *p*-nitrobenzenediazonium fluoborate. The approximate limits of detection for Mobam and 4-HBT are about 0.1 μg.

Subsequent to the development of the above paper chromatographic approach, Chiba and Morley (1964) and Finocchiaro and Benson (1965) described thin-layer chromatographic systems for determining carbaryl (Sevin) residues. Both of these procedures also proved suitable for separating Mobam and 4-HBT. Visualization of the spots on the thin-layer chromatograms is accomplished as described for the paper chromatographic system and results in lavender-colored spots for 4-HBT and Mobam and a brilliant blue spot for Sevin. Paper chromatography is preferred for characterizing Mobam crop residues because of the better resolution it has exhibited compared with the thin-layer approaches studied.

DISCUSSION

Gutenmann *et al.* (1964) reported the use of the whole milk extraction procedure for isolating Mobam. These investigators, however, used a bromination–electron capture gas chromatographic technique to measure the Mobam in milk samples from a cow fed 100 p.p.m. of Mobam, and reported that the pesticide could not be detected in the milk samples throughout the sampling period. They estimated the limit of detection of their method at about 0.1 p.p.m. of Mobam.

Since the original analytical method development effort, Epstein *et al.* (1967) of this laboratory have reported a gas chromatographic technique for the macro determination of Mobam and 4-HBT based on the preparation of thermally stable acetyl derivates. Efforts are presently underway to adopt the gas chromatographic approach to residue determinations. Boyd (1966) has reported a tentative microcoulometric gas chromatographic determination of sulfur as a means of determining Mobam at residue (microgram) levels. The microcoulometric approach appears to have a detection limit of about 0.2 μg. Mobam but is incapable of distinguishing between the carbamate and 4-HBT because of the immediate degradation of Mobam to 4-HBT in the injection port of the gas chromatograph.

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LITERATURE CITED

Boyd, G. R., Mobil Chemical Co., Industrial Chemical Division, Ashland, Va., private communication, April 1966.
Chiba, M., Morley, H. V., *J. Assoc. Offic. Agr. Chemists* **47,** 667 (1964).
Cundiff, R. H., Markunas, P. C., *Anal. Chem.* **30,** 1450 (1958).
Epstein, A. J., Gaskill, D. R., Lucchesi, C. A., *Anal. Chem.* **39,** 721 (1967).
Finocchiaro, J. M., Benson, W. R., *J. Assoc. Offic. Agr. Chemists* **48,** 736 (1965).
Fritz, J. S., *Anal. Chem.* **24,** 674 (1952).
Gutenmann, W. H., Wagner, D. G., Lisk, D. J., *J. Dairy Sci.* **47,** 821 (1964).
Johnson, D. P., *J. Assoc. Offic. Agr. Chemists* **46,** 234 (1963).
Johnson, D. P., Stansbury, H. A., *J. Assoc. Offic. Agr. Chemists* **48,** 771 (1965).
Kilsheimer, J. R., Kaufman, H. A., Roth, R. W., ACS Meeting, Atlantic City, September 1965.
Miskus, R., Eldefrawi, M. E., Menzel, D. B., Svoboda, W. A., J. AGR. FOOD CHEM. **9,** 190 (1961).
Miskus, R., Gordon, H. T., J. AGR. FOOD CHEM. **7,** 613 (1959).
Whitehurst, W. E., Bishop, E. T., Critchfield, F. E., J. AGR. FOOD CHEM. **11,** 167 (1963).
Zweig, G., "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," Vol. **II,** pp. 440–2, Academic Press, New York, 1964.

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