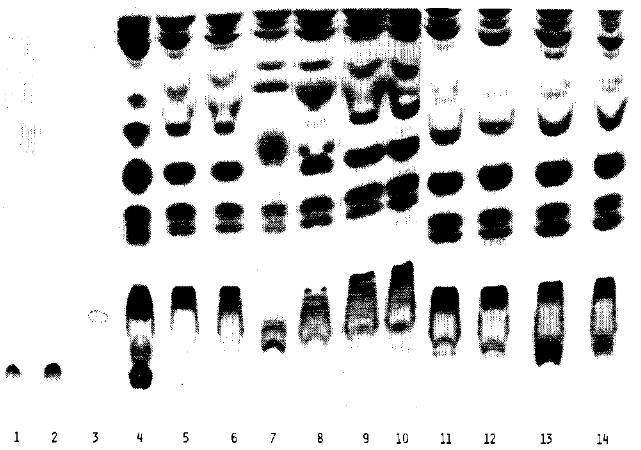

Figure 1. Lysinoalanine fractionation on cellulose TLC sheets (Eastman No. 6064 without fluorescent indicator) in protein hydrolysates from canned baby foods. (1) Lysinoalanine, 0.1 μg; (2) lysinoalanine, 0.2 μg; (3) D-(+)-galactosamine, 0.5 μg; (4) alkali-treated albumin; (5) baby food no. 4, unheated; (6) baby food no. 1, heated; (7) baby food no. 4, unheated; (8) baby food no. 4, heated; (9) baby food no. 5, unheated; (10) baby food no. 5, heated; (11) baby food no. 3, unheated; (12) baby food no. 3, heated; (13) baby food no. 2, unheated; and (14) baby food no. 2, heated. Spots 11–14 are from a separate developing.

observations presented by Rakowska (1972), who reported losses of 15–20% of the available lysine and NPU values in some canned baby foods.

Figure 1 shows the results of a lysinoalanine assay run on some of the baby food samples mentioned above. As can be seen from this figure, only traces of lysinoalanine were found in heat-treated samples. Alkali-treated albumin, however, gives a strong spot compared to the baby food samples. We figured out that lysinoalanine content is much less than 0.1 mg/g of protein, and thus much lower than toxic levels presented by Sternberg et al. (1975a).

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Gas–Liquid Chromatographic Analysis of Carbaryl as Its *N*-Thiomethyl Derivative

N-Thiomethyl- and *N*-thio-*p*-tolylcarbaryl were prepared, and a residue analytical procedure was developed for carbaryl using methylsulfenyl chloride as the derivatizing agent and using gas chromatography with a flame photometric detector operating in the sulfur-selective mode. The method was applied to beans, carrots, lettuce, and tomatoes fortified at the 5 and 10 ppm levels.

The thermal instability of the *N*-methylcarbamate insecticides has hampered the application of gas chromatographic techniques to the direct determination of their residues. One approach to the problem has been to replace the N-H proton with an organic moiety which would confer thermal stability to the carbamate. This derivatization step also offers an opportunity of adding a moiety which enhances the detectability of the carbamate to specific detectors such as the electron-capture or microcoulometric detectors. N derivatives that have been investigated for residue analytical purposes are *N*-trimethylsilyl (Fishbein and Zielinski, 1965), *N*-acetyl (Epstein et al., 1967; Sullivan et al., 1967; Magallona and Gunther, 1974), *N*-trifluoroacetyl (Lau and Marxmiller, 1970), *N*-pentafluoropropionyl and *N*-heptafluorobutyryl (Seiber, 1972), and the *N*-mono-, di-, and trichloroacetyl derivatives (Magallona and Gunther, 1977).

Westlake et al. (1972) used a flame photometric detector (FPD) in the sulfur mode to analyze for RE-11775 (*m*-*sec*-butylphenyl *N*-methyl-*N*-thiophenylcarbamate). Since sulfur allows the use of an element-specific detector and therefore some degree of selectivity over background interferences, the possibility of utilizing methylsulfenyl and *p*-toluenesulfenyl chloride to prepare *N*-thiomethyl and *N*-thio-*p*-tolyl derivatives of *N*-methylcarbamates for residue analytical purposes was investigated. Carbaryl (1-naphthyl *N*-methylcarbamate) was used as a model compound.

EXPERIMENTAL SECTION

Synthesis. *Methylsulfenyl Chloride.* A round-bottom flask containing 50 mmol of dimethyl disulfide was equipped with a magnetic stirrer bar, condenser, and dropping funnel containing 50 mmol of sulfuryl chloride. With the flask cooled to about −10 °C, one-half the SO_2Cl_2 was added dropwise to the stirred dimethyl disulfide. The temperature was allowed to rise slowly to room temperature and the remainder of the SO_2Cl_2 was added. The

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mixture was stirred for 4 h at room temperature. The fraction collected at 65–70 °C upon distillation at atmospheric pressure was stored in sealed ampules at 0 °C until used. Kühle (1970) reported bp 27–28 °C at 53–63 mmHg.

p-Tolylsulfenyl Chloride. The procedure differed from above in that 25 mmol of each reactant was used, the *p*-tolyl disulfide was dissolved in 75 mL of CCl_4, and the second half of the SO_2Cl_2 was added after the temperature had been allowed to rise to about 0 °C. The fraction collected at 70–75 °C (0.5 mmHg) was stored in sealed ampules at 0 °C until used.

N-Thiomethylcarbaryl. To a stirred solution of 10 mmol of carbaryl in 10 mL of dry pyridine was added 20 mmol of CH_3SCl; stirring was continued for an additional 4 h. After adding 100 mL of water, the reaction mixture was extracted with 75 mL of $CHCl_3$. The organic phase was washed thrice with 1 N HCl and then thrice with water. The $CHCl_3$ first dried with Na_2SO_4 was then removed to yield 1.8 g of reddish-brown oil. The oil was placed on a chromatographic column of 15 g of Florisil and eluted with 500 mL of hexane, discarding the first 100 mL of eluate. The hexane was removed to yield 1.3 g of reddish-brown oil. The ^1H NMR spectrum taken in $CDCl_3$ contained signals at δ 3.5 (singlet, NCH_3), 2.7 (singlet, SCH_3), and 7.2–8.2 (multiplet, aromatic).

N-Thio-p-tolylcarbaryl. The procedure differed from above in that 10 mmol of carbaryl in 5 mL of dry pyridine was reacted with 11 mmol of *p*-tolylsulfenyl chloride and the mixture was stirred for 24 h. The residue obtained upon removing the $CHCl_3$ was crystallized from $CHCl_3$–hexane to yield a product of mp 98–99 °C. The ^1H NMR spectrum taken in $CDCl_3$ contained signals at δ 3.5 (singlet, NCH_3), 2.4 (singlet, CH_3), and 7–8 (multiplet, aromatic).

Residue Procedure. The carbaryl was recovered from fortified 100-g crop samples using the "Extraction of Pesticide" procedure of Holden (1973). To this final crop extract was added 0.2 mL of dry pyridine and 3 drops of methylsulfenyl chloride, and the mixture was heated at 60–65 °C for 2 h. The mixture was cooled and transferred to a separatory funnel using 50 mL of CH_2Cl_2. The CH_2Cl_2 phase was shaken for 30 s with 50 mL of 1 N HCl. The organic phase was transferred to a second separatory funnel and the aqueous phase was extracted twice using 10 mL of CH_2Cl_2 each time and adding each CH_2Cl_2 extract to the second separatory funnel. The combined CH_2Cl_2 extract was shaken for 30 s with 50 mL of 1 N HCl. The CH_2Cl_2 was passed into a flask through a funnel containing Na_2SO_4; the latter was rinsed with two 10-mL portions of CH_2Cl_2. After removing the solvent, 30 mL of a $HgCl_2/CaCO_3$ solution was added to the residue and the mixture stirred for 4 h at room temperature. The $HgCl_2/CaCO_3$ solution was prepared by adding 8.7 g of $HgCl_2$ and 3.7 g of $CaCO_3$ to 156 mL of an acetonitrile–water (4:1) mixture, shaking the mixture, and decanting the clear supernatant solution for use (Hankock et al., 1977). The reaction mixture was transferred to a separatory funnel using 75 mL of ether. The ether solution was washed thrice with 10-mL portions of 5% NaOAc and thrice with 15-mL portions of 10% NaCl. The ether phase was passed into a flask through a funnel containing Na_2SO_4; the latter was rinsed with some ether. The ether was removed and the residue was dissolved in acetone for gas chromatographic quantitation.

Analysis. A Tracor MT-220 gas chromatograph equipped with a flame photometric detector incorporating a 394-nm filter for selective response to sulfur-containing compounds was used. *N*-Thiomethylcarbaryl was analyzed using a 0.9 m × mm i.d. glass column packed with 5% OV-210 on 60–80 mesh Gas-Chrom Q. Inlet, column, and detector temperature were 225, 200, and 220 °C, respectively, and the nitrogen carrier gas flow rate was 80 mL/min. *N*-Thio-*p*-tolylcarbaryl was analyzed using a 0.9 m × 4 mm i.d. glass column packed with 3% SE-30 on 60–80 mesh Gas-Chrom Q. Inlet, column, and detector temperatures were 225, 220, and 220 °C, respectively, and the nitrogen carrier gas flow rate was 80 mL/min.

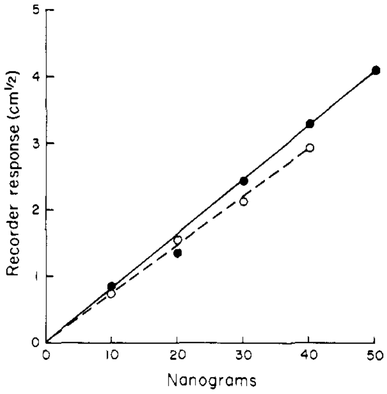

Figure 1. Flame photometric detector response to *N*-thiomethyl (○) and *N*-thio-*p*-tolylcarbaryl (●).

RESULTS AND DISCUSSION.

Moye (1975) developed a residue procedure for carbaryl and carbofuran which involved hydrolysis of the carbamate pesticides to their corresponding phenols and derivatization of the phenols with 2,5-dichlorobenzenesulfonyl chloride. Although electron-capture detection was used, Moye (1975) noted that 10 ng of 1-naphthyl 2,5-dichlorobenzenesulfonate responded well to a flame photometric detector operating in the sulfur mode. As an extension of this work, direct derivatization of the intact carbamate was attempted. However, no product could be isolated after treating carbaryl in pyridine with either methane or trifluoromethanesulfonyl chlorides under a variety of reaction conditions.

Black et al. (1973) synthesized several *N*-alkyl- and *N*-arylsulfenyl derivatives of several *N*-methylcarbamate pesticides in order to prepare more selective insecticides. A simple alkyl (methyl) and an aryl (*p*-tolyl) sulfenyl chloride were selected for evaluation for use as a derivatizing agent for carbaryl residues.

Figure 1 shows the flame photometric detector response to *N*-thiomethyl and *N*-thio-*p*-tolylcarbaryl. The nonlinear detector response to sulfur compounds makes data interpretation more time-consuming. However, commercial modules are available which electronically linearize the FPD signal by transmitting to the recorder the square root of the detector response (Tracor Instruments, 1975). Another means of simplifying data interpretation would be to use the method of Zehner and Simonaitis (1976) who obtained a relatively linear response and an eightfold increase in minimum detectability to aldicarb and malathion at low levels by addition of SO_2 to the hydrogen flame of the FPD. The extraction and cleanup procedure of Holden (1973) was used, so attention needs to be focused in this paper only on the derivative formation step. Following extraction and cleanup, the carbaryl in the extract was derivatized with sulfenyl chloride; Holden (1973) hydrolyzed the carbaryl to 1-naphthol which was then reacted with 2,4-dinitro-1-fluorobenzene. Four of the crops used by Holden (1973), for which a >95% recovery of carbaryl added at the 0.05 ppm level was reported, were used here.

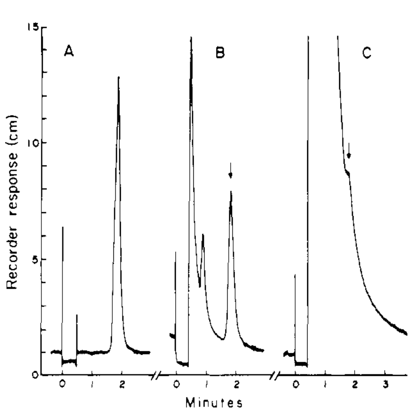

Figure 2. Flame photometric detector response to 20 ng of *N*-thiomethylcarbaryl standard (A) and to string beans fortified with 10 ppm carbaryl with (B) and without (C) treatment with $HgCl_2$–$CaCO_3$ solution after derivatization.

Table I. Recovery of Carbaryl Added to Various Crops and Quantitated as Its *N*-Thiomethyl Derivative

		Recovery, %			
		Replicate			
Crop	Fortification, ppm	A	B	C	Mean
Beans	10	118	96	68	94
	5	56	105	100	87
Carrots	10	118	105	90	104
	5	115	96	92	101
Tomatoes	10	118	78	78	91
	5	63	63	57	61
Lettuce	10	90	83	86	86

Attempts to analyze for the *N*-thio-*p*-tolyl derivative by gas chromatography were unsuccessful due to interfering peaks which coeluted with the derivative peak. Florisil column chromatography and treatment of the final solution with $HgCl_2$–$CaCO_3$ to remove interfering sulfur compounds were unsuccessful. Use of methylsulfenyl chloride as the derivatizing reagent also produced many interfering peaks, but they could be reduced by treatment with $HgCl_2$–$CaCO_3$ to the point where crop samples fortified at 5 to 10 ppm could be analyzed. Figure 2 shows a sample chromatogram for string beans fortified at 10 ppm carbaryl and analyzed by the *N*-thiomethyl derivatization technique. The figure demonstrates that much interference was produced that needed to be eliminated before quantitation could be accomplished. The chemical and physical properties of the interferences produced from the *p*-tolylsulfenyl chloride were probably too similar to that of *N*-thio-*p*-tolylcarbaryl to be readily separated.

Table I gives the recovery data for carbaryl added to beans, carrots, tomatoes, and lettuce prior to extraction and cleanup. Recoveries for a 10 ppm fortification were above 85%. Thus, direct derivatization of carbaryl residues with a sulfur-containing moiety to utilize the selective sulfur response of a flame photometric detector has been demonstrated. Unpleasant odors are produced by the reagent or its by-products, but can be largely contained by good ventilation. Due to interferences formed, the procedure cannot be applied to crops containing less than 5 ppm carbaryl without further refinement; however, most agricultural crops have a food tolerance of 10 ppm. The overall length of the procedure, though excessive for routine samples, is approximately equal to the 2,4-dinitrophenyl ether formation procedure.

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A Rapid Technique for Total Nonstructural Carbohydrate Determination of Plant Tissue

A simple and inexpensive technique was developed based on the digestion of total nonstructural carbohydrates by a MYLASE enzyme system followed by colorimetry at 250 nm using the Teles' reagent. The technique is very precise with a standard deviation of less than 0.3%. An average of 96 samples were analyzed easily in 24-h elapsed time by one technician.

The energy readily available to a plant is determined by total nonstructural carbohydrate (TNC) analyses. This total is sometimes termed total available carbohydrate (TAC) by plant scientistis, rather than TNC (Smith, 1969). There are various techniques published for TNC determination (Burris et al., 1967; Greub and Wedin, 1969; Heinze and Murneek, 1940; Smith et al., 1964; Smith, 1969; Weinmann, 1947; and Yemm and Willis, 1954). All of these require extensive time, reagents, and glassware, in addition to considerable skill and practice by the analyst.

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