To the Graduate Council:

I am submitting herewith a thesis written by John S. Pretanik entitled "Degradation Rate of Carbaryl Following Thermal Processing as a Function of Liquid Matrix, Presence of Plant Material, and Container." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Ernest A. Childs, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

DEGRADATION RATE OF CARBARYL FOLLOWING THERMAL PROCESSING AS A FUNCTION OF LIQUID MATRIX, PRESENCE OF PLANT MATERIAL, AND CONTAINER

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

John S. Pretanik

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ABSTRACT

Experiments were undertaken to determine if carbaryl underwent further degradation in Phaseolus vulgaris var. Half Runner during storage following thermal processing. Experimental variables included: (1) enamel lined can versus glass container, (2) distilled water versus 2% brine solution, and (3) presence or absence of plant material. The nondegraded residue remaining after thermal processing was measured over a three month storage period.

The accuracy, precision, and sensitivity of the analytical method employed were defined as follows: (1) accuracy = 2.70 ± 1.722% error, (2) precision = 1.44 ± 1.136% error, and (3) analytical zero level = 0.0286 ± 0.0120 µg.

Although carbaryl was readily broken down during thermal processing, complete degradation of the compound did not occur. After three months storage, carbaryl was still detected in all of the experimental systems containing green beans.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

I. GENERAL HISTORY AND PROPERTIES OF CARBARYL

The general insecticide carbaryl was discovered in 1953 and introduced in 1956 by Union Carbide Corporation under the code number Experimental Insecticide 7744, and trademark Sevin (29,46). Chemically it is 1-naphthyl N-methylcarbamate. Carbaryl may be produced by the reaction of 1-naphthyl and methyl isocyanate, or by the reaction of 1-naphthyl, phosgene and methylamine (46). Its physical and chemical properties are presented in Table 1 (39,45,46).

Carbaryl is a contact insecticide with slight systemic properties (7,46). Its insecticidal action is attributed to its ability to inhibit the nerve enzyme cholinesterase (13). The enzyme is carbamylated by the N-methylcarbamyl group which is the toxophoric group of the insecticide compound (28,33,34).

Carbaryl has been suggested to be established as a broad spectrum pesticide of relatively low mammalian toxicity. It presently is registered for use on over 100 field, vegetable and fruit crops as well as for dermal treatment of most domestic and livestock animals (29,36). Due to its reported low mammalian toxicity, residue tolerances have been established at relatively high levels, in the range of 5 to 12 ppm for most fruits and vegetables (17,36).

TABLE 1

PHYSICAL AND CHEMICAL PROPERTIES
OF CARBARYL AND DDT

Properties	Carbaryl	DDT
Physical state	white crystalline solid	white crystalline solid
Empirical formula	$C_{12}H_{11}NO_2$	$C_{14}H_9Cl_5$
Molecular weight	201.2	354.5
Melting point	142°C	108.5-109.0°C
Water solubility	<0.1%	<1 ppm
Organic solvent solubility	high	high
Heat stability	up to 70°C	up to 110°C
Mammalian toxicity acute oral LD 50 (rats)	500-800 mg/Kg	150-420 mg/Kg

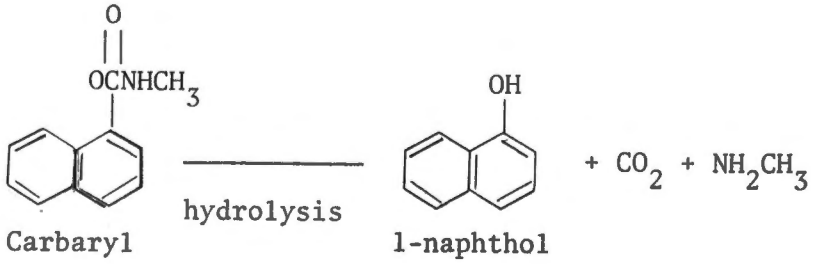
II. TOXICITY

The toxicological significance of most foreign compounds entering the animal body may be partially dependent on the manner in which they are metabolized. Some chemicals, such as strong bases, strong acids, and highly chlorinated hydrocarbons, are highly resistant to metabolism (13). These compounds tend to retain their toxic potential until excreted from the body.

Most chemicals entering the body usually undergo some type of metabolism. The metabolite may be more toxic, less toxic, or equal in toxicity to the original compound. All three of these possibilities are known to occur with certain carbamate insecticides in animal systems (13).

Metabolites

Prior to the mid 1960's, little was known concerning the metabolism of insecticidal carbamates. Hydrolysis of the ester linkage was assumed to be the predominant metabolic pathway.



The techniques employed at that time were of such a nature that only hydrolytic products could be detected. Consequently there was no

convincing evidence that nonhydrolytic mechanisms might be important in the metabolism of the carbamates.

With the development of radiotracer techniques and the refinement of thin-layer chromatography, intensive investigation of the metabolic fate of carbaryl began (1,9,11,12,32,33,34,35,38,40,41,42). In a review of the pathways of carbaryl metabolism in plants, Kuhr (36) reported that oxidative metabolism predominates. The primary metabolites were formed by hydroxylation of the aromatic ring, ring substituents and the carbamate N-methyl group. Few of these hydroxylated metabolites were recovered in their free form. They were converted to stable water-soluble glycosides by conjugation and tended to be stored by the plant.

The metabolites formed in plants are similar to those found in insects and mammals and may be represented by the proposed metabolic pathway for carbaryl in animals (Figure 1). Those differences which do exist, occur in their rate of formation, their conjugating moieties, and their ultimate fate within the organism. For example, Dorough (13) reported in a study using rats, that 25 to 30% of a carbaryl C^{14} dose was hydrolyzed, and 70 to 80% of the administered radioactivity was excreted in the urine within 24 hours. The hydrolytic and carbamate metabolites were excreted primarily as sulfate and/or glucuronide conjugates.

The toxicological importance of the hydrolytic and oxidative metabolites of carbaryl is indicated by the data presented in Table 2. Hydrolysis is a detoxication reaction while oxidation may result in metabolites which are still biologically toxic. The 5-hydroxy

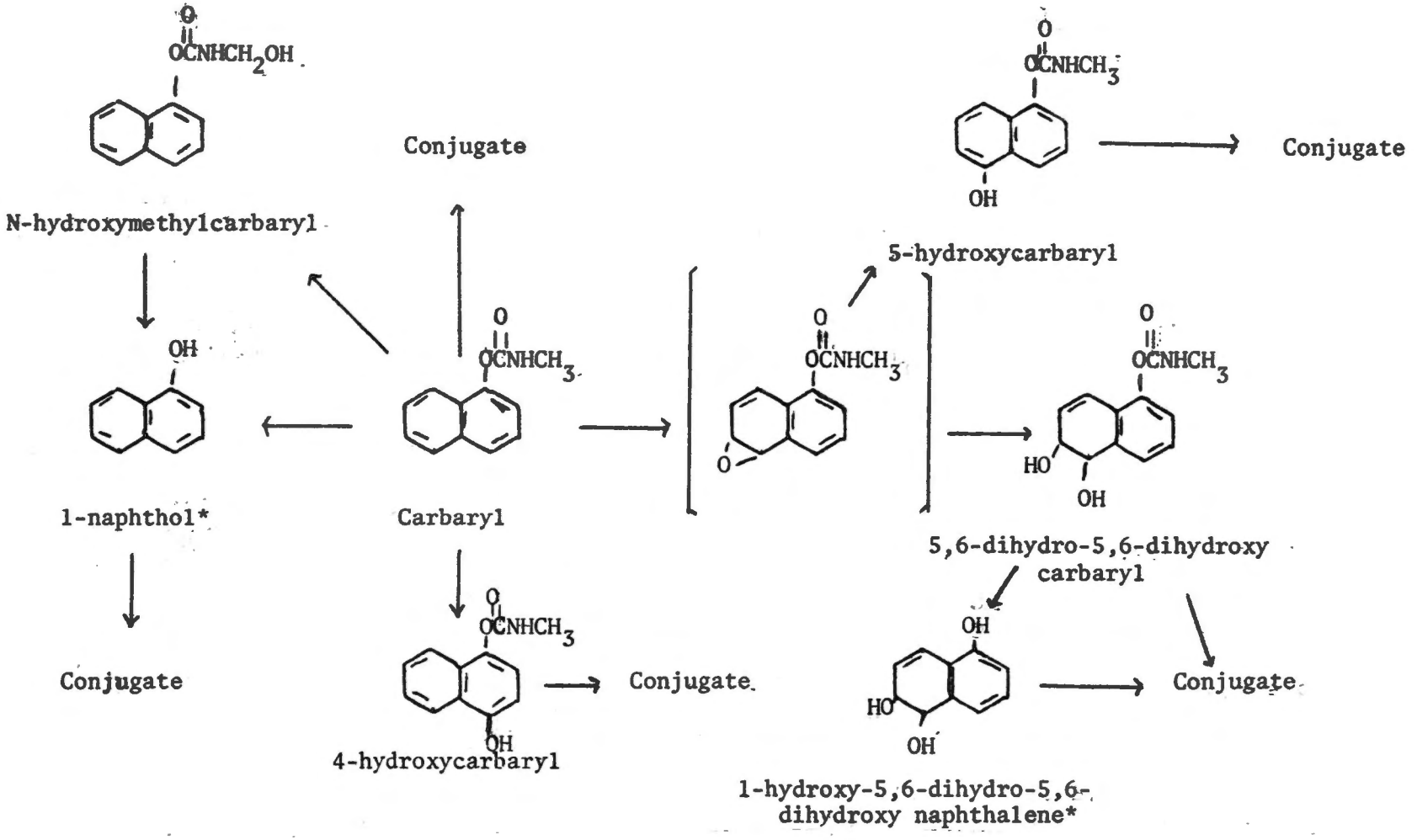


Figure 1. Proposed metabolic pathway for carbaryl in animals (13). Compounds with asterisk are the minor hydrolytic products.

TABLE 2

TOXICITY OF CARBARYL AND SOME OF ITS METABOLITES TO RATS[a]

Compound	Acute oral LD_{50}	7-Day feeding LD_{50}
	mg/Kg	mg/Kg
Carbaryl	430	125-250
4-Hydroxycarbaryl	1190	> 1000
5-Hydroxycarbaryl	297	> 1000
N-Hydroxymethylcarbaryl	5360	250-500
1-Naphthol	2590	500-1000

[a]Dorough (13).

derivative of carbaryl was found to be more toxic to rats than the parent compound when considered on an acute oral basis. Its chronic toxicity, however, was much less than carbaryl, indicating a very rapid detoxication and/or excretion from the animal. The 5,6-dihydro-5,6-dihydroxy derivative, which was not listed in Table 2, was reported by Baron et al. (2) to be the major organic extractable metabolite of carbaryl in the milk and urine of cattle. Although no data was given, this derivative was reported to be less toxic than the parent carbaryl compound.

Prior to 1970, the least defined area in carbaryl metabolism involved the conjugated metabolites. It was not until Cardona and Dorough (5) investigated the glucoside conjugates of the 4- and 5-hydroxycarbaryl derivatives, that any literature concerning the toxicity of the conjugate metabolites was reported. They found that the β-D-glucoside conjugates of the 4- and 5-hydroxycarbaryl derivatives decreased the toxicity of these derivatives 20 to 30 fold (Table 3).

Kingsley (31) reported, that if poisoning did occur, the acetylcholinesterase was reversibly inhibited and that cholinesterase reactivation was spontaneous and rapid. She also stated that clinical signs of carbamate poisoning developed fairly rapidly and dangerous exposures were less likely to occur due to the early incapacitating effect.

Carcinogenesis

Since carbaryl and related compounds have been considered "safe" for widespread use, the possibility of human exposure to trace amounts has been considered of little consequence.

TABLE 3

THE TOXICITY OF 4- AND 5-HYDROXYCARBARYL AND THEIR RESPECTIVE β-D-GLUCOSIDES TO MICE[a]

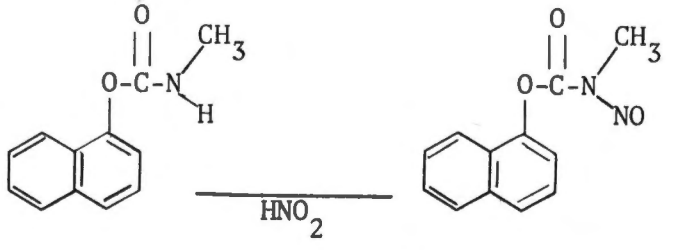
Compound	LD_{50}[b]
	mg/Kg
4-Hydroxycarbaryl	55
4-Hydroxycarbaryl glucoside	1550
5-Hydroxycarbaryl	50
5-Hydroxycarbaryl glucoside	950

[a]Cardona and Dorough (5).

[b]Mortality was recorded after 24 hours.

In 1969, however, Shimkin et al. (47) investigated the carcinogenic potential of a series of carbamates. They reported that carbaryl caused lung tumors in 40% of the mice treated with 6.0 mg of carbaryl (three 0.5 mg injections per week for 4 weeks).

Recently, Elespuru and Lijinsky (15) reported that carbaryl can react with nitrite at moderately acid pH to form a nitrosocarbaryl as follows:



Carbaryl Nitrosocarbaryl

The yields of nitrosocarbaryl after one hour at 37°C are given in Table 4.

This nitroso derivative of carbaryl is a close analogue of N-nitrosomethylurethane and would be expected to have similar carcinogenic and mutagenic properties (14,18). Since nitrites are present in many foods, a carcinogenic hazard to man could exist when food containing residues of carbaryl and nitrite are simultaneously present in the stomach.

III. PERSISTENCE IN THE ENVIRONMENT

Carbaryl was registered for use as a soil insecticide until 1971; however, studies of its persistence and fate in soil have been limited

TABLE 4

FORMATION OF N-NITROSOCARBARYL FROM CARBARYL AND NITRITE IN ONE HOUR AT 37°C[a]

Concentration of Carbaryl	Concentration of Sodium Nitrite	Volume	Acid	pH	Yield of Nitrosocarbaryl[b]	
						Theoretical
(M)	(M)	(ml)			(mg)	%
0.5	2.0	20	Hydrochloric	3.2	130	5.7
0.1	0.4	20	Hydrochloric	2.8	24	5.2
0.5	2.0	20	Acetic	3.9	245	11.0
0.1	0.4	20	Acetic	3.7	29	6.4

[a]Elespuru and Lijinsky (15).

[b]Percentage yields were not much reduced at five times lower concentrations of the reactants.

in scope with contradictory conclusions. Johnson and Stansbury (29) reported the half-life of carbaryl in soil was approximately 8 days. The pesticide was incorporated into a single sandy loam soil and residues below application depth were not measured. Such residues could have been appreciable since carbaryl does move in soil (24). By contrast, Kazano et al. (30), in laboratory experiments, observed only 22 to 37.4% evolution of $^{14}CO_2$ after incubating carbonyl-labeled carbaryl with five moist Japanese rice paddy soils for 32 days at 25°C. These data suggested that carbaryl persisted in these soils considerably longer than the previously reported 8 day half-life.

Caro et al. (7) recently reported the disappearance of carbaryl from the soil did not conform to a first-order reaction. They noted no significant loss during the first 40 days after application to the soil. Their data suggested that about 135 days were required for 95% of the pesticide to disappear, as compared to the 8 day half-life (equivalent to 35 days for 95% disappearance) reported by Johnson and Stansbury (29). The 135 day period reported by Caro et al. (7), was an overall value for their entire watershed. Data for the individual sampling points revealed localized lag periods ranging from 25 days to more than 116 days. However, once degradation began in any one place, it proceeded quite rapidly. Since several common types of soil fungi and bacteria are known to degrade carbaryl (4,49), the lag period is believed to indicate that carbaryl degradation in the soil is primarily microbiological. The time lag represents the time needed for active soil microorganisms to grow and adapt to the introduction of a foreign

chemical. Caro et al. (7) also observed that less than 0.2% of the active carbaryl applied to the field was lost in the runoff water over the entire season and that at no time were metabolites such as 4-hydroxy or 5-hydroxy-carbaryl found in the water, sediment, or soil. The absence of these metabolites agrees with the observation made by Kazano et al (30). Caro et al. (7) found that hydrolisis was the main pathway of degradation of carbaryl in soil.

Guseinov (23) observed that even foilar applications of carbaryl can result in soil residues. He also noted that carbaryl can permeate through the root system of the cotton plant and translocate to the leaves and into the ball, thus contaminating the cotton fiber. At 3.5-4 months after field application, he found carbaryl in the cotton seeds and in trace amounts in cottonseed oil.

As for persistence on crops, Gunther et al. (22) observed that carbaryl had an average half-life of 28 days on and in lemons, and a half-life of 42 days on and in oranges.

IV. REMOVAL OF RESIDUE ON CROPS

Some information is available in the literature pertaining to the removal of pesticides from vegetables by commercial and home preparative procedures (19,20,37).

Elkins et al. (16) investigated the effects of commercial and home canning procedures on the removal of DDT, malathion and carbaryl from green beans. The commercial canning procedure, which included water blanching and thermal processing, reduced DDT 91%, malathion 94%, and

carbaryl 89%. The home canning procedure, which included washing, blanching, and thermal processing, reduced DDT 89%, and malathion and carbaryl were reduced greater than 99%. Carbaryl was not completely degraded in either procedure even though it is unstable at temperatures greater than 70°C, but was reduced more in the home canning procedure.

Only one reference was found relating to the residue levels of carbaryl in processed foods under storage conditions. Elkins et al. (16), in a very limited study utilizing fresh green beans and storing them at 7°C for 11 days, observed the following: (A) that DDT residue remained level, (B) that malathion residue decreased sharply and (C) that carbaryl residue decreased only slightly.

Summarizing, the literature shows:

1. Carbaryl may be a carcinogenic hazard to man, even in trace amounts.
2. It is considerably more persistent in soil than previously thought.
3. Carbaryl may be translocated from the soil to the aerial portions of plants.
4. Carbaryl residue on crops may persist longer than previously thought and be carried into the food supply.
5. Commercial and home canning procedures do not remove all of the carbaryl which may be present.

Considering these recent observations and the fact that literature pertaining to the residue levels of carbaryl in processed foods under storage conditions is practically nonexistent; the present study was conducted in order to obtain answers to the following questions:

1. Does carbaryl undergo degradation following thermal processing during storage?
2. What effect does the type of container have on the degradation of carbaryl?
3. What effect does the type of liquid packing matrix have on the degradation of carbaryl?
4. What effect does the presence of plant material have on the degradation of carbaryl?

CHAPTER II

MATERIALS AND METHODS

I. EXPERIMENTAL DESIGN

The objective of the experiment was to determine the rate of degradation of carbaryl following thermal processing as a function of liquid matrix, presence of plant material, and container. The experimental systems are listed in Table 5.

The green beans used in this study were Phaseolus vulgaris, var. Half Runner. They were purchased locally, and a 100-gram sample was selected randomly to ascertain if any carbaryl residue was present. The beans were prepared for processing by washing, snipping the ends, and cutting into 1.5-inch sections. The cut beans were water blanched for 2 minutes in a steam kettle and cooled to room temperature with a water spray.

All of the containers were sterilized by boiling in water for 10 minutes prior to filling. Seven containers for each experimental system were filled as described in Table 6. The systems which included green beans were packed by adding the beans, applying carbaryl to the beans and then adding salt and water to applicable systems. The systems which did not include green beans were packed by adding the carbaryl, then salt and water where applicable.

TABLE 5

EXPERIMENTAL DESIGN

System	Container	Liquid Matrix	Green Beans
A	#303 enamel can	2% brine solution	present
B	#303 enamel can	distilled water	present
C	#303 enamel can	2% brine solution	absent
D	#303 enamel can	distilled water	absent
E	pint Mason jar	2% brine solution	present
F	pint Mason jar	distilled water	present
G	pint Mason jar	2% brine solution	absent
H	pint Mason jar	distilled water	absent

TABLE 6

QUANTITIES FOR EXPERIMENTAL SYSTEMS

System	Distilled Water (grams)	Salt (grams)	Green Beans (grams)	Net Weight (grams)	Carbaryl (μg)
A	181	4	275	460	9,250
B	185	---	275	460	9,250
C	436	9	---	445	9,000
D	445	---	---	445	9,000
E	147	3	265	415	8,050
F	150	---	265	415	8,050
G	392	8	---	400	8,000
H	400	---	---	400	8,000

Carbaryl was added to the containers in each system in the following manner. The systems were spiked with a 125 µg/µl solution of carbaryl in methylene chloride. The carbaryl solution was prepared from an analytical grade carbaryl (assay 99.9%) obtained from Union Carbide Corporation. The carbaryl was applied to the surface area of the packed green beans with a 50 µl Hamilton syringe and allowed to dry. With the systems which did not include green beans, the carbaryl was deposited into the empty container.

The filled containers were exhausted in a water-bath for 10 minutes at 93°C (43,48). All containers were sealed immediately postexhausting. Samples contained in the #303 enamel cans were thermally processed in a retort at 116°C for 25 minutes (43). They were then water-cooled and the outside of the can allowed to air dry. Samples contained in the pint Mason jars were thermally processed in a home pressure canner at 116°C for 20 minutes (48). The jars were allowed to air-cool upon completion of processing. All samples were stored at ambient temperature and the nondegraded carbaryl remaining in each system was measured at zero time (same day as processed), one day, one week, two weeks, one month, and three months postprocessing.

This procedure was twice repeated with different lots of green beans, carbaryl, salt, and water at two day intervals to obtain three replicate samples of each system.

II. EXTRACTION AND CLEANUP OF SAMPLES

Gas chromatography is generally an unsatisfactory method for analysis of monomethylcarbamates. The carbamates undergo on-column

decomposition, and detector response is weak unless the carbamate contains a group, e.g. halogen, or is converted to a derivative, e.g. 2,4-Dinitrophenal Ether (26,27). Consequently, thin layer chromalography was chosen as the method of analysis.

A rapid qualitative and semiquantitative method for the determination of carbaryl has been reported by Finocchiaro and Benson (21). Their method involves blending, extracting, cleanup, and determination by thin layer chromatography (TLC). Because of its specificity, speed, and improved sensitivity over colorimetric methods by Benson and Finocchiaro (3) and Johnson (8,28), this TLC procedure has been used by the Food and Drug Administration as part of its monitoring and surveillance programs.

The method employed in this study was basically that of Finocchiaro and Benson (21). Slight modifications were made to improve the recovery of carbaryl residues, and a spectrodensitometric method was developed to quantitatively delineate the amount of carbaryl on thin layer plates.

Reagents

Acetone—Redistilled, analytical reagent grade from Fisher Scientific Company.

Alcoholic Potassium Hydroxide Solution—1.0 N potassium hydroxide in methanol.

Benzene—Analytical reagent grade from Fisher Scientific Company.

Carbaryl (1-Naphthyl N-methylcarbamate)—Reference standard was provided by Union Carbide Corporation, Chemicals Division, Technical Center, South Charleston, W. Va.

Chromogenic Reagent—Made by stirring a solution of 10% diethylene glycol in ethanol (95%) for 2 minutes with sufficient p-nitrobenzenediazonium tetrafluoroborate for saturation (ca. 25 mg/100 ml). Filter and keep cold during use. Store the reagent in a referigerator and discard after three days.

Coagulating Solution—Dissolve 0.5 grams ammonium chloride in 400 ml distilled water containing 1 ml phosphoric acid (85%).

Diethylene Glycol—Analytical reagent grade from Fisher Scientific Company.

Diethylene Glycol Solution—Dilute 10 ml diethylene glycol to 100 ml with methylene chloride.

Hyflo Super-Cel—Fisher Scientific Company.

Methylene Chloride—Fisher Certified from Fisher Scientific Company, or redistilled analytical reagent grade.

P-Nitrobenzenediazonium tetrafluoroborate—Analytical reagent grade from Eastman Company.

Sodium Sulfate—Analytical reagent grade, anhydrous, granular, from Fisher Scientific Company.

Apparatus

Buchner Funnels—9 in.

Chromatographic Tank—lined with blotting paper.

Filter Flasks—500 ml.

Filter Paper—No. 2 Whatman.

Fritted Glass Funnels—medium porosity, ca. 30 ml.

Graduated Laboratory Cylinders—100 ml with 24/40 ℧ glass stoppers.

Hamilton Syringe—10 µl.

Mason Jars—pint size with Osterizer blending heads.

Mills Concentrator Tubes—10 ml, graduated.

Osterizer Blender

Oven—forced air.

Pipets—1 ml, 5 ml, and 10 ml.

Rubber Tubing

Separatory Funnels—500 ml with teflon stopcocks and glass stoppers.

Steam Distillation Apparatus

Thin Layer Plates—precoated aluminum oxide with fluorescent indicator from Eastman Company. Neutralize and heat activate prior to use as directed by manufacturer.

Vacuum Take-Off Adapter—24/40 ℧.

Procedure

A 15-gram sample of drained green beans was selected randomly and transferred to a pint Mason jar. Ten grams of the drained liquid were also added to the jar. Twenty-five grams of thoroughly mixed liquid were used in those systems which did not contain green beans.

One hundred and fifty grams of anhydrous sodium sulfate and 150 ml of methylene chloride were added to the blender jars containing the sample. An Osterizer blending head was affixed to the jar and the sample was blended for 2 minutes at low speed. The sample was allowed to settle ca. 2 minutes before decanting.

A 9 cm buchner funnel containing No. 2 Whatman filter paper was attached to a 500 ml filter flask. The filter paper was covered with ca. 0.25-inch layer of Hyflo Super-Cel prepared as a paste in methylene chloride. The sample was decanted into the buchner and vacuum was applied cautiously. The blender jar was rinsed with 50 ml of methylene chloride which was filtered. The residue remaining in the jar was reextracted with another 150 ml of methylene chloride and decanted into the buchner. The jar was again rinsed with 50 ml of methylene chloride and filtered.

One milliliter of diethylene glycol solution was added to the filter flask. The flask with buchner and filter pad was placed on a steam bath and vacuum applied. When the volume in the flask had been reduced to ca. 5 ml, the flask was removed and swirled until dry. The vacuum was then released, the buchner removed, and the flask allowed to cool.

The side of the flask was rinsed down with 3 ml of acetone dispensed from a pipet, and the flask swirled to dissolve the residue. Fifteen milliliters of coagulating solution was added to the flask with gentle swirling to precipitate the plant waxes and other water-insoluble extractives. The flask was then allowed to stand for 10 minutes with occasional swirling.

The residue and precipitate were filtered with a small fritted glass funnel containing ca. 0.25-inch layer of Hyflo Super-Cel. The funnel was attached to a vacuum take-off adapter and a 100 ml g-s graduated laboratory cylinder. The precipitate was washed with three

2 ml portions of acetone-water solution (1 + 9). Each washing remained in contact with the precipitate for ca. 15 seconds before the vacuum was applied. The filtrate and washings were then diluted to a final volume of 25 ml with the acetone-water solution. The cylinder containing the residue was stoppered and set aside for determination.

III. THIN LAYER CHROMATOGRAPHIC ANALYSIS OF SAMPLES

The sample solution in the stoppered, graduated cylinder was thoroughly mixed and a 10-ml aliquot transferred to a 500-ml separatory funnel. The solution was extracted with two 5-ml portions of methylene chloride, shaking 5 to 10 seconds each time. The extracts were combined in a 10-ml Mills concentrator tube and evaporated to 0.1 ml in a forced air oven at 60°C.

A 4-µl aliquot was spotted on an aluminum oxide thin layer plate with a Hamilton 10 µl syringe. Standard carbaryl solutions (in methylene chloride) corresponding to 0.1, 0.2, and 0.3 µg were spotted on the same thin layer plate containing the sample. The thin layer plate was placed in a saturated chromatographic tank containing 50 ml of acetone-benzene solution (1 + 4). The plate was developed until the solvent front reached a line 10 cm from the origin. The plate was then removed and allowed to dry at room temperature ca. 15 minutes. The dry plate was then sprayed with 1.0 N alcoholic-potassium hydroxide solution until wet. The moist plate was then sprayed with chromogenic solution and carbaryl appeared as blue spots.

Quantitation

Quantitation was achieved with the use of a Schoeffel spectrodensitometer (SD3000) with Schoeffel density computer (SDC300). The spectrodensitometer was operated as a double beam reflectance instrument. The following settings were found to give an optimum response: (A) Wavelength, 500 nm; (B) Slit width, 1.0 mm; (C) Beam width, 0.5 mm; (D) Beam length, 1.00 cm; (E) O. D. units range, 0.40; and (F) Density computer, log function.

After chromatographing, the plate was scanned with the spectrodensitometer. A regression equation obtained from the standards on each plate was used to calculate the quantity of residue in samples developed on the same plate. The micrograms of residue found were multiplied by 62.5 (multiplication factor used to correct for dilution of samples due to aliquots taken in cleanup and extraction procedure), to obtain the quantity of residue in the original 25-gram sample. The micrograms of residue found in the sample was converted to parts per million and also to percent carbaryl loss.

IV. VALIDITY OF ANALYTICAL METHOD

Finocciaro and Benson (21) have reported acceptable carbaryl recoveries in the concentration range of 0.05 to 0.2 ppm with this method. In a collaborative study by Palmer and Benson (45), satisfactory results were reported by most of the collaborators at levels of 0.1 ppm and above. Farrow et al. (19) however, reported that recoveries of carbaryl by this method were low at low residue levels but increased in

proportion to the increase in residue level. Recoveries were found to range from 60% at 0.2 ppm to 90% at 4 ppm. Therefore in the present investigation the accuracy, precision, and sensitivity of this method had to be defined before data collection could begin.

Method Evaluation

Standard curve. Aluminum oxide plates were spotted with standard carbaryl solutions at concentrations ranging from 0.05 to 1.0 µg. The mean peak areas of two replicates were used to obtain a standard curve and a regression analysis of the standard curve was performed to define the regression equation.

Concentrations greater than 0.4 µg resulted in spots which were too large to be scanned by the spectrodensitometer. Concentrations ranging between 0.1 and 0.4 µg produced a standard curve (Figure 2) with a correlation coefficient of 0.995 and a regression equation (Table 7) of:

$$Y = 0.0018(X) - 0.0204$$

where X is the area under the curve and Y is µg of carbaryl. Although a spot containing 0.05 µg was faintly visible to the human eye, it caused no response on the spectrodensitometer. This was because of the peak areas registered by the thin layer plate background. At concentrations $\leq$ 0.05 µg, the background response was equal to, or exceeded the sample.

The results indicated that the optimum range for quantitative detection was 0.1 to 0.4 µg. This agrees with the range reported by Palmer and Benson (45).

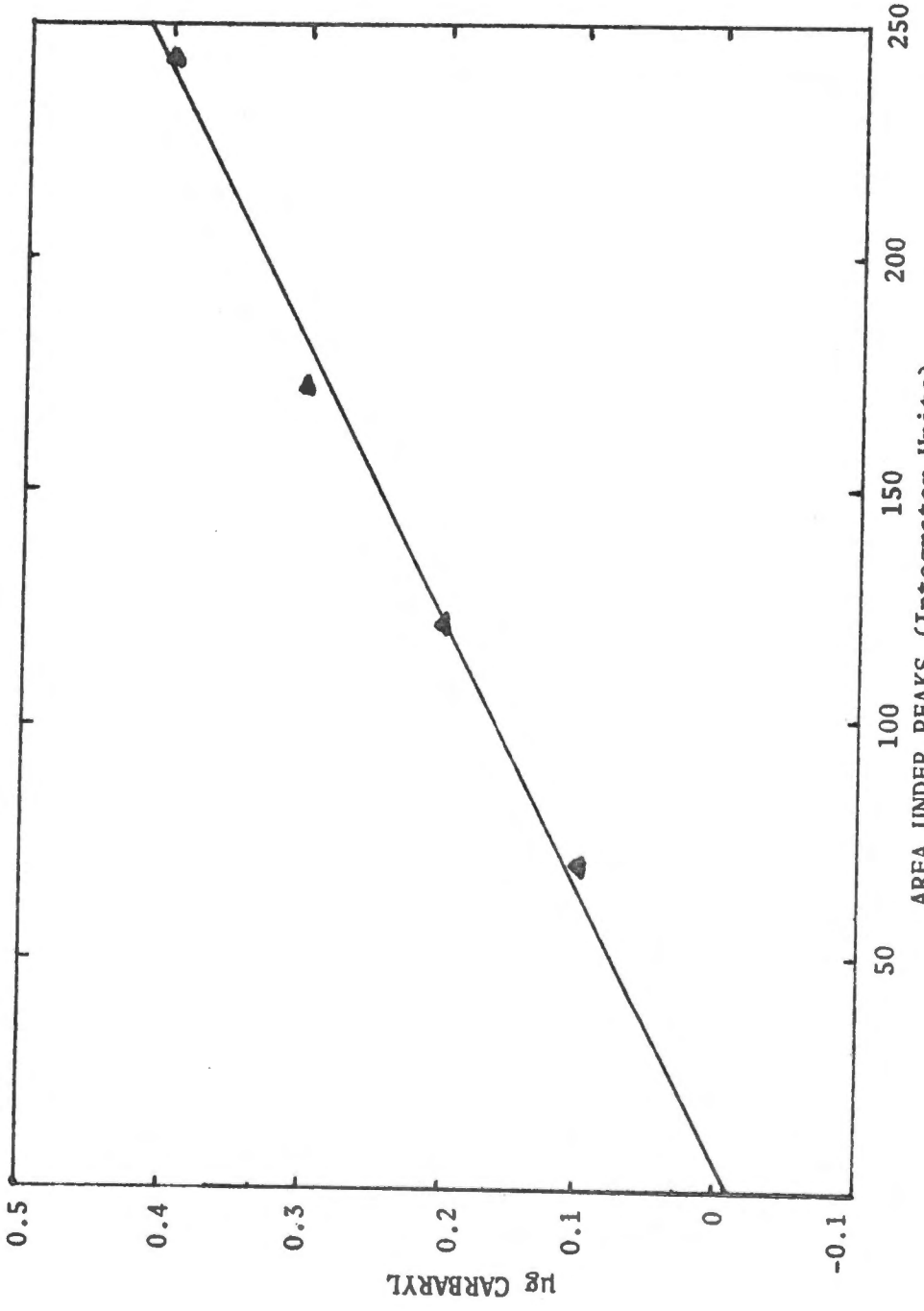


Figure 2. Standard curve obtained from the mean peak areas of two replicates containing 0.1, 0.2, 0.3, and 0.4 µg of standard carbaryl solution.

TABLE 7

REGRESSION ANALYSIS OF STANDARD CURVE OBTAINED FROM THE MEAN PEAK AREAS OF TWO REPLICATES CONTAINING 0.1, 0.2, 0.3, AND 0.4 µg OF STANDARD CARBARYL SOLUTION

Concentration (µg)	Peak Area Plate #1	Peak Area Plate #2	Mean Peak Area
0.1	70	70	70
0.2	125	115	120
0.3	170	160	165
0.4	240	230	235

r = 0.9950.

m = 0.0018.

b = -0.0204.

Detection limit. The detection limit was defined as twice the standard deviation of the thin layer plate background. Five random background areas were chosen from each of three replicate thin layer plates to obtain the detection limit of the spectrodensitometer. This level of response was substituted into regression equations to delineate carbaryl levels which could be differentiated from zero levels.

The detection limits for measuring carbaryl as a function of thin layer plate background are shown in Table 8. The mean detection limit ± standard error of the mean for the three replicates was 0.0286 ± 0.0120 µg. This would indicate that at the 99% confidence level detection of 0.0646 µg or less of carbaryl is not possible. This confirms previous observations when trying to detect 0.05 µg for establishment of a standard curve.

Percent Recovery

The percent carbaryl recovered from green beans was determined by adding 1, 5, 9, 13, and 17 ppm carbaryl to green bean samples and then extracting and measuring the carbaryl content of the sample.

The percent recovery of carbaryl was initially determined without any modification of the extraction and cleanup procedure of Finocchiaro and Benson (21). The results are shown in Table 9. The low percent recoveries at the low residue levels and the higher percent recoveries at the high residue levels appears to substantiate the observation made by Farrow et al. (19).

Further examination of the extraction and cleanup procedure revealed that some of the Hyflo-Super-Cel filtered through to the bottom

TABLE 8

DETECTION LIMIT FOR MEASURING CARBARYL AS A FUNCTION OF THIN LAYER PLATE BACKGROUND. THE DETECTION LIMIT IS DEFINED AS TWICE THE STANDARD DEVIATION OF THE THIN LAYER PLATE BACKGROUND

Plate #1		Plate #2		Plate #3	
Peak Area	µg	Peak Area	µg	Peak Area	µg
70	0.0106	35	-0.0125	50	-0.0895
50	-0.0127	58	0.0122	45	-0.0958
50	-0.0127	38	-0.0093	30	-0.1151
70	0.0125	35	-0.0125	42	-0.0997
60	-0.0010	80	0.0360	40	-0.1022
Detection limit (µg)		Detection limit (µg)		Detection limit (µg)	
0.0234		0.0424		0.0200	

Mean detection limit = 0.0286 ± 0.0120.

TABLE 9

PERCENT RECOVERY OBTAINED USING A THIN LAYER OF HYFLO SUPER-CEL PREPARED AS A SLURRY IN CH_2Cl_2

Actual Concentration (ppm)	Calculated Amount (ppm)	% Recovery
1.00	0.54	54.00
5.00	4.17	83.40
9.00	8.56	95.11
13.00	12.65	97.31
17.00	16.51	97.12

Mean % recovery ± standard error of the mean = 85.39 ± 18.462.

of the filter flask. When the filtrate was evaporated to dryness, the Hyflo-Super-Cel formed a cake which would not loosen when acetone was added to dissolve the residue. Some of the residue might have been trapped within the cake resulting in the low recoveries.

The problem was eliminated by preparing the Hyflo-Super-Cel as a paste (ca. 50 g/200 ml) in methylene chloride rather than as a slurry (ca. 50 g/350 ml) and by applying ca. a 0.25-inch layer to the filter paper rather than a thin coat.

The percent recoveries obtained with the modification are shown in Table 10. A significant improvement in the percent recovery at the lower residue levels was obtained by this new procedure. At the 1 ppm residue level, the recovery increased from 54% to 95%. The mean percent recovery ± standard error of the mean for the five residue levels was 97.30 ± 1.723% (Table 10). At the 99% confidence level, greater than 92% recovery was obtained for residue levels $\geq$ 1 ppm.

All standard curves upon which percent recovery determinations were made were highly linear with none having a correlation coefficient < 0.994 (Table 11).

Precision

Precision was evaluated by determining the standard error of the mean of measurements of three replicate bean samples containing 1, 5, 9, 13, and 17 ppm carbaryl.

The precision with which carbaryl was measured with this method is shown in Table 12. At no concentration was the standard error of the mean greater than 3.3% of the mean. The mean standard error of the mean

TABLE 10

PERCENT RECOVERY OBTAINED USING A 1/4 INCH LAYER OF HYFLO SUPER-CEL PREPARED AS A PASTE IN CH_2Cl_2

Actual Concentration (ppm)	Calculated Amount (ppm)	% Recovery
1.00	0.95	95.00
5.00	4.90	98.00
9.00	8.64	96.00
13.00	12.88	99.08
17.00	16.73	98.41

Mean % recovery ± standard error of the mean = 97.30 ± 1.723.

TABLE 11

REGRESSION ANALYSIS OF STANDARD CURVES USED AS A BASE FOR DELINEATING DETECTION LIMIT, PERCENT RECOVERIES, PRECISION AND ACCURACY OF CARBARYL

Plate #	r[a]	m[b]	b[c]
1	0.997	0.0017	-0.2323
2	0.999	0.0020	-0.3083
3	0.994	0.0017	-0.2915
4	0.998	0.0011	-0.0712
5	0.999	0.0010	-0.0503
6	0.998	0.0012	-0.1537

[a] r = Correlation coefficient.

[b] m = Slope of curve.

[c] b = Y-intercept.

TABLE 12

PRECISION OF MEASUREMENT OF CARBARYL. VALUES ARE THE MEAN OF TRIPLICATE MEASUREMENTS OBTAINED FROM REGRESSION EQUATIONS STANDARD ERROR OF THE MEAN

Actual Concentration (ppm)	Mean Values	Standard error of the mean as a % of the mean
1.00	0.94 ± 0.014	1.48
5.00	4.81 ± 0.159	3.30
9.00	8.64 ± 0.106	1.22
13.00	12.87 ± 0.052	0.24
17.00	16.64 ± 0.164	0.98

Mean standard error of the mean as a percent of the mean = 1.44 ± 1.136.

as a percent of the mean was 1.44 ± 1.136%. At the 99% confidence level, greater than 95% precision can be expected with this method.

Accuracy

Accuracy was determined by plotting a standard curve, determining the correlation coefficient and regression equation for the curve, and determining the percentage error in measurement of duplicate bean samples containing 1, 5, 9, 13, and 17 ppm carbaryl.

The accuracy with which carbaryl can be measured with this method is shown in Table 13. At no concentration was the error greater than 5%. The mean error ± standard error of the mean for the five residue levels was 2.70 ± 1.722%. At the 99% confidence level, less than 8% error can be expected with this method.

All standard curves upon which percent error determinations were made were highly linear with none having a correlation coefficient < 0.994 (Table 11, page 33).

Summary

The thin layer chromatographic method of Finocchiaro and Benson (21), with the modification suggested, can measure carbaryl residues accurately and precisely. It is capable of achieving greater than 92% recovery with less than a 5% error in precision at the 99% confidence level.

The use of a spectrodensitometer allowed simultaneous qualitative and quantitative analysis of the carbaryl extracts. Quantitative analysis was highly linear in the range of 0.10 to 0.40 µg carbaryl with a lower detection limit of 0.0286 ± 0.0120 µg.

TABLE 13

ACCURACY OF CARBARYL MEASUREMENT

Actual Concentration (ppm)	Calculated Amount (ppm)	% Error
1.00	0.95	5.00
5.00	4.90	2.00
9.00	8.64	4.00
13.00	12.88	0.92
17.00	16.73	1.59

Mean error = 2.70 ± 1.722.

This method can therefore be used to measure the nondegraded carbaryl residue remaining in the experimental samples.

V. STATISTICAL METHODS

The experimental data from which all statistical analysis were calculated are listed in Tables A-1 and A-2 in the Appendix. The data were analyzed by a two way analysis of variance and the analyses were performed in two parts: the effect of time on replicates for each system, and the effect of the systems on replicates for each time period. Significant differences between means were determined by Duncan's Multiple Range Test.

A student t-distribution was employed to determine significance between the mean residue values and the zero detection limit of the carbaryl anlaysis method.

CHAPTER III

EXPERIMENTAL RESULTS

I. EFFECTS OF STORAGE TIME ON CARBARYL CONTENT OF GREEN BEANS

In the experimental systems utilizing glass containers and enamel lined cans without brine solution, thermal processing reduced the average carbaryl content (zero time) by ca. 97% (from 20 ppm to ca. 0.6 ppm) (Appendix A-2). An analysis of variance of the data indicated no further significant reduction of carbaryl in these systems during three months of storage following thermal processing (Table 14). Conversely, the carbaryl content of the experimental systems utilizing enamel lined cans and brine solution (with and without green beans) were significantly reduced during storage after thermal processing (Table 14). Evaluation of the means by Duncan's Multiple Range Test indicated that in system A (green beans, 2% brine, enamel lined cans) there was a significant decrease in residue level (97.02 to 98.56% loss) between zero time and one day in storage, but no significant change after that (Table 15). In system C (2% brine, no beans, enamel lined cans) the residue levels at zero time and one day postprocessing were not significantly different. A significant drop in residue (98.28 to 100.00% loss) did occur between one day and one week in storage with no further significant change after that (Table 15). System C was the

TABLE 14

SUMMARY OF ANALYSIS OF VARIANCE OF CARBARYL RESIDUES: TIME VERSUS REPLICATIONS

Experimental[a] System	Time F-Ratio	Replications F-Ratio
A	6.300[b]	0.646
B	1.335	0.104
C	5.052[b]	1.121
D	0.599	1.704[b]
E	0.827	0.036
F	2.148	0.929
G	1.726	6.046[b]
H	0.712	0.049

[a]Experimental systems: A, enamel can, green beans, 2% brine; B, enamel can, green beans, distilled water; C, enamel can, 2% brine; D, enamel can, distilled water; E, Mason jar, green beans, 2% brine; F, Mason jar, green beans, distilled water; G, Mason jar, 2% brine; H, Mason jar, distilled water.

[b]F-ratios are significant at the 0.05 level of probability.

TABLE 15

SUMMARY OF MEANS AND DUNCAN'S MULTIPLE RANGE TEST: TIME[1] IN STORAGE

Experimental[2] Systems	Means[3]					
	Zero Time	One Day	One Week	Two Weeks	One Month	Three Months
A	97.02[a]	98.56[b]	100.00[b]	99.71[b]	100.00[b]	99.75[b]
B[4]	96.52	97.98	97.18	97.51	98.98	99.07
C	98.24[c]	98.28[d]	100.00[e]	99.76[e]	100.00[e]	100.00[e]
D[4]	97.75	97.87	97.63	97.32	98.37	98.53
E[4]	96.39	97.47	97.47	98.12	98.38	98.85
F[4]	97.10	96.28	97.44	96.70	98.41	98.48
G[4]	98.08	97.70	99.14	98.72	98.71	98.84
H[4]	98.18	97.28	97.53	97.46	97.23	98.38

[1]Mean values represent the percent loss of carbaryl.

[2]Experimental system: A, enamel lined can, green beans, 2% brine; B, enamel lined can, green beans, distilled water; C, enamel lined can, 2% brine, D, enamel lined can, distilled water; E, Mason jar, green beans, 2% brine; F, Mason jar, green beans, distilled water, G, Mason jar, 2% brine; H, Mason jar, distilled water.

[3]Means in the same horizontal row followed by the same letter are not significantly different at the 0.05 level of probability.

[4]As the f-value from the analysis of variance was not significant, no DMR was performed on the data in this horizontal row.

only system in which the carbaryl residue was reduced to a nondetectable level during and after three months storage.

The analysis of variance indicated a significant difference between the three replicates for systems D (distilled water, enamel lined can) and G (2% brine, Mason jar) during storage (Table 14, page 39). Evaluation of the means by Duncan's Multiple Range Test indicated that in system D, replicate one (97.203%) was significantly different from replicates two (98.387%) and three (98.303%), and in systems G, replicate two (99.347%) was significantly different from replicates one (98.185%) and three (98.070%) (Table 16).

II. EFFECT OF EXPERIMENTAL TREATMENTS ON THE CARBARYL CONTENT OF GREEN BEANS AFTER A GIVEN TIME IN STORAGE

The carbaryl content in all of the experimental systems was reduced by ca. 97% (20 ppm to ca. 0.6 ppm) as a result of thermal processing (zero time) (Appendix A-2). An analysis of variance of the data indicated no significant difference among the treatments between zero time and one day in storage (Table 17). There was a significant difference in residue levels between one day and one week in storage. Evaluation of the means by Duncan's Multiple Range Test indicated that systems A (green beans, 2% brine, enamel lined can) and C (2% brine, enamel lined can) were signiificantly different from the other experimental systems after one week in storage (Table 18). Residue was detectable in the other experimental systems but not in systems A and C. At the end of three months storage, residue was detectable in system A, but not in system C, as a result of lowering the analytical

TABLE 16

SUMMARY OF MEANS AND DUNCAN'S MULTIPLE RANGE TEST: REPLICATIONS[1]

Experimental[2] System	Means[3] Rep #1	Rep #2	Rep #3
A[4]	98.93	99.46	99.12
B[4]	97.75	98.10	97.76
C[4]	99.33	99.69	99.12
D	97.20[a]	98.39[b]	98.30[b]
E[4]	97.86	97.74	97.79
F[4]	97.09[g]	97.71[g]	97.24
G	98.18[c]	99.35[d]	98.07[c]
H[4]	97.77	97.76	97.85

[1]Mean values represent the percent loss of carbaryl.

[2]Experimental system: A, enamel can, green beans, 2% brine; B, enamel can, green beans, distilled water; C, enamel can, 2% brine; D, enamel can, distilled water; E, Mason jar, green beans, 2% brine; F, Mason jar, green beans, distilled water; G, Mason jar, 2% brine; H, Mason jar, distilled water.

[3]Means in the same horizontal row followed by the same letter are not significantly different at the 0.05 level of probability.

[4]As the f-value from the analysis of variance was not significant, no DMR was performed on the data in this horizontal row.

TABLE 17

SUMMARY OF ANALYSIS OF VARIANCE OF CARBARYL RESIDUES: TREATMENT VERSUS REPLICATIONS

Time	Treatment F-Ratio	Replications F-Ratio
Zero	0.665	0.575
One Day	1.462	5.319[a]
One Week	3.951[a]	4.492[a]
Two Weeks	3.089[a]	0.358
One Month	6.020[a]	0.487
Three Months	9.744[a]	4.043[a]

[a]F-ratios are significant at the 0.05 level of probability.

TABLE 18

SUMMARY OF MEANS AND DUNCAN'S MULTIPLE RANGE TEST: TREATMENTS[1]

Time	Means[2]							
	A	B	C	D	E	F	G	H[3]
Zero[4]	97.02	96.52	98.24	97.75	96.39	97.10	98.03	98.18
One Day	98.56	97.98	98.28	97.87	97.47	96.28	97.70	97.28
One Week	100.00^{a}	97.18^{b}	100.00^{a}	97.62^{b}	97.47^{b}	97.44^{b}	99.14^{ab}	97.53^{b}
Two Weeks	99.71^{c}	97.51^{d}	99.76^{c}	97.32^{d}	98.12^{cd}	96.70^{d}	98.72^{cd}	97.23^{d}
One Month	100.00^{e}	98.98^{e}	100.00^{e}	98.37^{fg}	98.38^{fg}	98.41^{fg}	98.71^{f}	97.23^{g}
Three Months	99.75^{h}	99.07^{i}	100.00^{h}	98.85^{ij}	98.63^{ij}	98.48^{ij}	98.84^{ij}	98.38^{j}

[1]Mean values represent the percent loss of carbaryl.

[2]Means in the same horizontal row followed by the same letter are not significantly different at the 0.05 level of probability.

[3]Experimental system: A, enamel can, green beans, 2% brine; B, enamel can, green beans, distilled water; C, enamel can, 2% brine; D, enamel can, distilled water; E, Mason jar, green beans, 2% brine; F, Mason jar, green beans, distilled water, G, Mason jar, 2% brine; H, Mason jar, distilled water.

[4]As the f-value from the analysis of variance was not significant, no DMR was performed on the data in this horizontal row.

zero residue level (0.1315 ppm to 0.0526 ppm) for the method of analysis. This was achieved by extracting all of the filtrate rather than a 10 µl aliquot.

The analysis of variance also indicated a significant difference among the replicates at one day, one week and three months of storage. Evaluation of the means by Duncan's Multiple Range Test indicated that replicate three was significantly different at one day postprocessing, replicate one was significantly different at one week postprocessing, and at three months postprocessing, replicates one and two were significantly different from each other but not significantly different from replicate three (Table 19).

III. MEAN RESIDUE VALUES

The results of the Student-t test of data presented in Appendix A-1 are shown in Table 20. In these t-tests the mean parts per million of carbaryl remaining in the experimental systems were tested against an analytical zero residue level to ascertain if the remaining carbaryl was significantly different from the analytical zero residue level. The analytical zero residue level was established at 0.1315 ppm during the zero time to one month storage period and at 0.0526 ppm at the three month period.

In the experimental systems utilizing glass containers and enamel lined cans without brine solution, the mean carbaryl residue levels during three months of storage were significantly different from the analytical zero residue levels at the 0.20 level of probability. The

TABLE 19

SUMMARY OF MEANS AND DUNCAN'S MULTIPLE RANGE TEST: REPLICATIONS[1]

Time	Means[2] Rep #1	Rep #2	Rep #3
Zero Time[3]	96.92	97.65	99.67
One Day	98.25[a]	98.04[a]	96.74[b]
One Week	97.42[c]	98.92[d]	98.56[d]
Two Weeks[3]	98.02	98.44	98.03
One Month[3]	98.75	99.30	98.61
Three Months	98.74[e]	99.18[f]	99.09[ef]

[1]Mean values represent the percent loss of carbaryl.

[2]Means in the same horizontal row followed by the same letter are not significantly different at the 0.05 level of probability.

[3]As the f-value from the analysis of variance was not significant, no DMR was performed on the data in this horizontal row.

TABLE 20

STUDENT-t DISTRIBUTION OF THE MEAN CARBARYL RESIDUE VALUES AROUND THE ZERO DETECTION LIMIT

Time	Experimental System[c]							
	A	B	C	D	E	F	G	H
Zero	2.159[b]	2.102[b]	3.202[b]	3.434[b]	2.210[b]	2.941[b]	1.770[b]	2.461[b]
One Day	7.861[b]	1.883[b]	1.251[b]	1.610[b]	2.069[b]	35.794[b]	2.494[b]	2.893[b]
One Week	a	2.742[b]	a	1.788[b]	2.092[b]	1.906[b]	1.506[b]	3.190[b]
Two Weeks	1.007	2.278[b]	1.000	2.115[b]	2.299[b]	4.397[b]	1.831[b]	3.655[b]
One Month	a	1.998[b]	a	6.110[b]	2.060[b]	2.282[b]	1.515[b]	3.755[b]
Three Months	1.013	4.363[b]	a	2.488[b]	3.166[b]	4.122[b]	37.082[b]	10.697[b]

[a]No carbaryl residue was detected.

[b]Student-t values are significant at the 0.20 level of probability.

[c]Experimental systems; A, enamel can, green beans, 2% brine; B, enamel can, green beans, distilled water; C, enamel can, 2% brine; D, enamel can, distilled water; E, Mason jar, green beans, 2% brine, F, Mason jar, green beans, distilled water; G, Mason jar, 2% brine; H, Mason jar, distilled water.

mean residue levels for the systems utilizing enamel lined cans and brine solution were also significantly different from the analytical zero residue levels between zero time and one day in storage. After one week, the mean residue levels of systems A (green beans, 2% brine, enamel lined can) and C (2% brine, enamel lined can) were not signficantly different from the analytical zero residue levels.

CHAPTER IV

DISCUSSION

The experimental data in Table A-2 in the Appendix indicated that although carbaryl was readily broken down during thermal processing, there was not a complete breakdown of the parent compound. The mean percent loss ranged from 96.39 to 98.24% with no significant difference among the experimental systems. Although the initial concentration of carbaryl (20 ppm) prior to thermal processing was high compared to what may be expected under field conditions, earlier investigators reported reductions in carbaryl ranging from no change to almost 100% from initial levels of 10 ppm or less (16,19,20,37). This would indicate that the amount of breakdown during thermal processing was not solely a function of the initial carbaryl concentration in the system.

The Analysis of Variance (Table 14, page 39) indicated no significant reduction of carbaryl in the experimental systems utilizing glass containers and enamel lined cans without brine solution during the three months of storage following thermal processing. The mean residue levels of these systems were found to be significantly different from the analytical zero residue levels. This would indicate that carbaryl was persistent under conditions utilizing glass containers and enamel lined cans without brine solution.

The carbaryl content in the experimental systems utilizing enamel lined cans and brine solution was found to be significantly reduced

during storage. The residue levels in systems A (green beans, 2% brine, enamel lined can) and C (2% brine, enamel lined can) were significantly reduced between zero time and one day (97.02 to 98.56% loss), and between one day and one week (98.28 to 100.00% loss) respectively, with no significant change after that. The mean residue levels for systems A and C were significantly different from the analytical zero level at zero time and one day, but not after one week in storage. This would indicate that further breakdown of the carbaryl residue was caused by the presence of an enamel lined can and salt.

DDT can decompose by an oxidation reaction utilizing iron as a catalyst in an enamel lined can (6,25). Possibly iron has a similar effect on carbaryl. If iron is responsible for the breakdown of carbaryl during storage, then the presence of residue in system A (green beans, 2% brine, enamel lined can) and not in system C (2% brine, enamel lined can) after three months in storage may possibly be attributed to the presence of green beans in system A. The iron may form a complex with the plant material or the carbaryl may be embedded in a mass of plant material. The iron would have unrestricted access to the carbaryl in system C which contained only brine solution.

The presence of brine solution was also necessary for a significant breakdown of carbaryl during storage. The presence of salt possibly may have enhanced the leaking of iron into the liquid matrix of the system.

The results of this study indicated that system C (2% brine, enamel lined can) was the only experimental system in which carbaryl was not detected after three months storage. Residues of carbaryl were present in all of the experimental systems containing a food product.

In view of these results, carbaryl may present a problem to the food industry. If future research corroborates the recent finding that carbaryl may be a carcinogenic hazard to man (15), the Delaney Clause might be evoked and a zero tolerance level for carbaryl established (10).

The literature and this study show that residues of carbaryl will not completely be removed or broken down by current processing methods, and that complete degradation does not occur during storage. This would suggest that any effective control would have to be applied at the field level. This however may not be a feasible approach. Recent literature has indicated that a waiting period of greater than 90 days may be necessary between final pesticide application and harvest to ensure residue free produce (6,20,21).

CHAPTER V

SUMMARY

This experimental study was undertaken to determine if carbaryl underwent further degradation in green beans during storage following thermal processing. Experimental variables consisted of: (1) enamel lined can versus glass container, (2) distilled water versus 2% brine solution, and (3) presence or absence of plant material. The nondegraded residue remaining after thermal processing was measured over a three month storage period.

The results of this study indicated that although carbaryl was partially broken down during thermal processing, complete degradation of the compound did not occur. After three months storage, carbaryl was still detected in all of the experimental systems containing green beans (98.48% to 99.75% loss).

In view of these results, carbaryl may present a problem to the food industry if a zero tolerance level is established. Effective control, short of banning the use of carbaryl, may not be feasible at the field or processing levels.

It would appear that further research is needed to ascertain if trace amounts of carbaryl in the food supply actually are carcinogenic to man. Until this question is answered, the safety of carbaryl residue in the food supply remains questionable.

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APPENDIX

TABLE A-1

PARTS PER MILLION OF CARBARYL RESIDUE IN EXPERIMENTAL SYSTEMS[a]

Time		A	B	C	D	E	F	G	H
Zero	(1)	1.007	1.232	0.467	0.617	0.235	0.505	0.670	0.237
	(2)	0.257	0.529	0.226	0.436	1.151	0.360	0.238	0.551
	(3)	0.545	0.342	0.376	0.286	0.770	0.871	0.245	0.308
One Day	(1)	0.252	0.083[c]	0.202	0.272	0.159	0.769	0.521	0.550
	(2)	0.321	0.658	0.150	0.220	0.588	0.710	0.208	0.294
	(3)	0.298	0.478	0.697	0.805	0.767	0.741	0.654	0.788
One Week	(1)	d	0.826	d	0.851	0.813	0.838	0.192	0.618
	(2)	d	0.278	d	0.528	0.503	0.153	d	0.267
	(3)	d	0.597	d	0.065[c]	0.196	0.536	0.316	0.596
Two Weeks	(1)	d	0.262	0.149	0.926	0.554	0.488	0.320	0.477
	(2)	d	0.432	d	0.297	0.188	0.888	d	0.700
	(3)	0.175	0.813	d	0.404	0.381	0.595	0.448	0.347
One Month	(1)	d	0.089[c]	d	0.359	0.449	0.449	0.232	0.427
	(2)	d	0.266	d	0.266	0.139	0.169	0.109[c]	0.779
	(3)	d	0.259	d	0.369	0.382	0.332	0.432	0.457
Three Months[b]	(1)	0.039[c]	0.229	d	0.375	0.346	0.424	0.243	0.374
	(2)	0.076	0.127	d	0.188	0.134	0.261	0.234[e]	0.309
	(3)	0.039[c]	0.209	d	0.136	0.341	0.226	0.226	0.290

[a]Numbers in parenthesis indicate replicates. Letters indicate experimental systems: A, enamel lined can, green beans, 2% brine solution; B, enamel lined can, green beans, distilled water; C, enamel lined can, 2% brine solution; D, enamel lined can, distilled water; E, Mason jar, green beans, 2% brine solution; F, Mason jar,

green beans, distilled water; G, Mason jar, 2% brine solution; H, Mason jar, distilled water.

[b]The analytical zero limit was lowered from 0.1315 ppm to 0.0526 ppm by extracting all of the filtrate rather than a 10 µl aliquot.

[c]Residue level is less than the analytical zero level established at the 95% confidence level.

[d]Residue was not detected.

[e]Sample was lost, average of remaining two replicates was used.

TABLE A-2

PERCENT LOSS OF CARBARYL IN EXPERIMENTAL SYSTEMS[a]

Time		A	B	C	D	E	F	G	H
Zero	(1)	95.03	93.88	97.69	96.95	98.82	97.47	96.66	98.82
	(2)	98.73	97.37	98.89	97.71	94.21	98.20	98.81	97.25
	(3)	97.29	98.30	98.14	98.59	96.13	95.62	98.78	98.47
One Day	(1)	98.75	99.59	99.01	98.66	99.21	96.14	97.40	97.25
	(2)	98.41	96.73	99.26	98.92	97.05	96.43	98.97	98.54
	(3)	98.52	97.63	96.56	96.02	96.15	96.28	96.74	96.06
One Week	(1)	100.00	95.90	100.00	95.80	95.92	95.78	99.01	96.91
	(2)	100.00	98.62	100.00	97.39	97.48	99.24	100.00	98.67
	(3)	100.00	97.03	100.00	99.69	99.02	97.31	98.42	97.02
Two Weeks	(1)	100.00	98.70	99.27	95.43	97.22	97.55	98.41	97.62
	(2)	100.00	97.86	100.00	98.53	99.06	95.54	100.00	96.50
	(3)	99.13	95.96	100.00	98.01	98.09	97.01	97.76	98.26
One Month	(1)	100.00	99.56	100.00	98.23	97.75	97.75	98.84	97.87
	(2)	100.00	98.67	100.00	98.69	99.31	99.16	99.46	96.11
	(3)	100.00	98.71	100.00	98.18	98.07	98.33	97.84	97.72
Three Months[b]	(1)	99.81	98.87	100.00	98.15	98.27	97.87	98.79	98.14
	(2)	99.63	99.37	100.00	99.08	99.33	98.69	98.84	98.46
	(3)	99.81	98.96	100.00	99.33	98.29	98.87	98.88	98.55

[a]Numbers in parenthesis indicate replicates. Letters indicate experimental systems: A, enamel lined can, green beans, 2% brine solution; B, enamel lined can, green beans, distilled water; C, enamel lined can, 2% brine solution; D, enamel

lined can, distilled water; E, Mason jar, green beans, 2% brine solution; F, Mason jar, green beans, distilled water; G, Mason jar, 2% brine solution; H, Mason jar, distilled water.

[b]The analytical zero level, at the 95% confidence level, was lowered from 0.1315 ppm to 0.0526 ppm by extracting all of the filtrate rather than a 10 µl aliquot.

VITA

John Stephen Pretanik was born in Salem, Massachusetts, on January 18, 1945. He attended elementary school in Salem and was graduated from the Essex Agricultural and Technical Institute in Hathorne, Massachusetts, in 1962. The following September he entered the University of Massachusetts in Amherst, receiving a Bachelor of Science degree in Food Technology in February, 1967.

In April, 1967, he was commissioned a Lieutenant in the United States Air Force and served as Base Officer's Open Mess Secretary. He was released from active duty in January, 1972, and entered the University of Tennessee Graduate School in June, 1972. While in Graduate School he held a departmental graduate research assistantship.

He and his wife, the former Miss Dianne Ella Griffin, were married on August 27, 1966, and they have one daughter, Bethany Anne, age four years.