Degradation of Carbaryl following Thermal Processing

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Experiments were undertaken to determine if carbaryl underwent further degradation in green beans during storage after thermal processing. Experimental variables were (a) enamel-lined cans vs. glass jars, (b) distilled water vs. 2% brine solution, and (c) presence or absence of beans. Carbaryl (>90%) was degraded during thermal processing. Further significant degradation occurred only in samples containing 2% brine and processed in enamel-lined cans.

Carbaryl has been suggested as a broad spectrum pesticide of supposedly 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 livestock and domestic animals (Johnson and Stansbury, 1965; Kuhr, 1970). Because of its supposedly low toxicity, residue tolerances have been established in the range of 5–12 ppm (Kuhr, 1970).

The possibility of human exposure to trace amounts of carbaryl has been historically considered of little importance. Recent data, however, have shown carbaryl to be carcinogenic. Shimkin et al. (1969) reported carbaryl-induced lung tumors in 40% of mice treated with 6.0 mg of carbaryl (i.e. three 0.5-mg injections per week for 4 weeks). In addition, it has been noted that carbaryl can react with nitrite to form nitrosocarbaryl (Elespuru and Lijinsky, 1973). This derivative is a close analogue of *N*-nitrosomethylurethane, and has been shown to produce local sarcomas in Wistar rats following a single subcutaneous injection of 1000 mg/kg (Eisenbrand et al., 1974). *N*-Nitrosocarbaryl caused transformation of BALB/3T3 cells in culture but carbaryl did not (Quarles and Tennant, 1975).

Another factor which has allowed widespread use and high tolerance limits for carbaryl was its supposed rapid degradation in the environment. Johnson and Stansbury (1965) initially reported the half-life of carbaryl in soil as approximately 8 days. In more recent work, Kazana et al. (1972) observed less than 40% evolution of $^{14}CO_2$ after incubating carbonyl-labeled carbaryl in rice patty soils for 32 days. Caro et al. (1974) have suggested that 135 days are required for 95% of carbaryl to disappear from soil. Therefore, the persistence of carbaryl may be greater than once thought.

Little work has been published concerning persistence of carbaryl on field crops. Gunther et al. (1962) observed a half-life of 28 days in lemons and 42 days in oranges.

The degradation and removal of carbaryl from vegetables by commercial and home preparative procedures have received limited attention. Elkins et al. (1968) reported 89% reduction of carbaryl during commercial canning of green beans and 99% reduction during home canning. Therefore, all carbaryl residues were not eliminated in either procedure.

Since carbaryl may be a carcinogenic hazard and its persistance now appears to be longer than previously thought, a definition of the persistence of residues in processed foods seems desirable. No work has been performed to ascertain whether or not the trace quantities of carbaryl which might persist in contaminated vegetables through the thermal processing steps might degrade during storage.

This study was undertaken to define degradation rates of carbaryl during and following thermal processing as a function of (a) type of container (glass jars vs. enamel-lined tin cans), (b) liquid packing matrix (water vs. 2% brine), and (c) presence or absence of green beans.

Table I. Description of Experimental Treatments Used in This Study[a]

Exptl treatment	Container	Liquid matrix	Green beans
A	#303 enamel can	2% NaCl	+
B	#303 enamel can	HOH	+
C	#303 enamel can	2% NaCl	–
D	#303 enamel can	HOH	–
E	Pint glass jar	2% NaCl	+
F	Pint glass jar	HOH	+
G	Pint glass jar	2% NaCl	–
H	Pint glass jar	HOH	–

[a] All samples contained 20 ppm of carbaryl.

MATERIALS AND METHODS

The green beans used in this study were *Phaseolus vulgaris*, var. Half Runner. Three lots were purchased locally and a 100-g sample randomly selected from each to ascertain background carbaryl levels. The samples contained no carbaryl.

Processing. The beans were prepared for processing by washing, snipping the ends, and cutting into 1.5-in. sections. The cut beans were water blanched for 2 min in a steam kettle and cooled to room temperature with 10 °C water spray.

All containers were sterilized with boiling water for 10 min prior to filling. Seven containers for each experimental system were filled with the contents described in Table I. Experimental systems which contained green beans were packed by placing the beans in the container, applying a methylene chloride suspension of carbaryl (125 $\mu g/\mu l$) to the beans, and allowing this to dry 30 min and then adding salt and water. The beans contained ca. 20 000 ppm of carbaryl after treatment. In those systems which did not include green beans, the carbaryl was deposited into the empty container and the liquid matrix immediately added.

The filled containers were exhausted in a water bath for 10 min at 93 °C and immediately sealed. Samples packed in enamel-lined cans were thermally processed in a still retort at 116 °C for 25 min. They were then water cooled and the outsides of the cans allowed to air dry. Samples contained in pint glass jars were thermally processed in

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a home pressure canner at 116 °C for 20 min and the jars allowed to air cool.

All samples were stored at ambient temperature and the nondegraded carbaryl remaining in each system was measured at zero time (i.e. same day as processed), 1 day, 1 week, 2 weeks, 1 month, and 3 months after processing.

This procedure was repeated in duplicate with three different lots of green beans, carbaryl, salt, and water to obtain three replicates with two observations per replicate.

Extraction of Carbaryl. A 15-g sample of drained green beans was selected randomly from each container and transferred to a pint glass blender jar. Ten grams of the drained liquid was also added to the jar. In those systems which did not contain green beans, 25 g of liquid was used.

Anhydrous Na_2SO_4 (150 g) and 150 ml of methylene chloride were added to the blender jars containing samples. The samples were blended for 2 min at 1200 rpm, and allowed to settle for 2 min 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 a 0.25 in. 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 then filtered in the same manner as above. 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 the rinse liquid 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 about 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 with 3 ml of acetone dispensed from a pipet and the flask swirled to dissolve the residue. Coagulating solution (15 ml) 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 min with occasional swirling.

The residue and precipitate were filtered through a small fritted glass funnel containing a 0.25-in. 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 15 s 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.

Thin-Layer Chromatographic Analysis of Samples. The sample solution in the stoppered, graduate cylinder was thoroughly mixed and transferred to a 500-ml separatory funnel. The solution was extracted with two 12.5-ml portions of methylene chloride, shaking 5 to 10 s each time. The extracts were combined in a 50-ml graduated beaker, evaporated to about 5 ml in a forced air oven at 60 °C, and quantitatively transferred to 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 samples. 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 for 15 min. The dry plate was then sprayed with a 1.0 N alcoholic–potassium hydroxide solution until wet. The moist plate was then sprayed with chromogenic solution (prepared by stirring a solution of 10% diethylene glycol in ethanol (95%) for 2 min with sufficient *p*-nitrobenzenediazonium tetrafluoroborate for saturation, 25 mg/100 ml) and carbaryl appeared as a blue spot.

Table II. Effect of Thermal Processing on Amount of Carbaryl Extractable from Green Beans[a]

Exptl treatment	% carbaryl degraded	ppm of carbaryl remaining
A	97.02	0.603
B	96.52	0.701
C	98.24	0.356
D	97.75	0.446
E	96.39	0.718
F	97.10	0.579
G	98.08	0.384
H	98.18	0.365

[a] Each value is the mean of three replicates with two observations/replicate. Experimental treatments are defined in Table I.

Quantification. Quantification 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 nm; (d) beam length, 1.00 cm; (e) optical density units range, 0.40; and (f) density computer, log function.

After chromatographing, the plate was scanned with the spectrodensitometer. A regression equation obtained from three 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 25 (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-g sample. The micrograms of residue found in the sample was converted to parts per million and also to percent carbaryl degraded.

Validity of Analytical Method. The method had a detection limit (background response or analytical zero) of 0.029 ± 0.012 μg of carbaryl. Recovery at concentrations as low as 1.0 ppm of carbaryl was 97.3 ± 1.7%. The variation in precision of measurement as measured by the coefficient of variation was at no time greater than 3.3%.

RESULTS AND DISCUSSION

Effects of Thermal Processing on Carbaryl Content of Green Beans. There was a 96–98% decrease in extractable carbaryl residues in all experimental systems following thermal processing (Table II). No significant differences among the experimental treatments could be defined by an analysis of variance. This suggested destruction of carbaryl during thermal processing is more likely a function of the increased temperature and pressure associated with processing than the type of container, liquid matrix, or product.

The large decrease in extractable carbaryl indicated a low persistence of carbaryl during thermal processing and

Table III. Percentage Degradation of Carbaryl during Storage of Experimental Samples following Thermal Processing[a]

Exptl treatment	1 day	1 week	2 weeks	1 month	3 months
A	98.56[a]	100.00[b]	99.71[b]	100.00[b]	99.75[b]
B	97.98	97.18	97.51	98.88	97.07
C	98.28[d]	100.00[e]	99.76[e]	100.00[e]	100.00[e]
D	97.87	97.63	97.32	98.37	98.53
E	97.47	97.47	98.12	98.38	98.85
F	96.28	97.44	96.70	98.41	98.48
G	97.70	99.14	98.72	98.71	98.84
H	97.28	97.53	97.46	97.23	98.38

[a] Each value is the mean of three replicates with two observations/replicate. Experimental treatments are defined in Table I. There were significant differences in the degradation of carbaryl during storage in treatments A and C. Data values in the same horizontal row with different superscripts were significantly different ($P < 0.05$).

Table IV. Carbaryl Residues (ppm) during Storage of Experimental Samples following Thermal Processing[a]

Exptl treatment	1 day	1 week	2 weeks	1 month	3 months
A	0.290[b]	0	0.058	0	0.025
B	0.406[b]	0.567[b]	0.502[b]	0.205[b]	0.188[b]
C	0.350[b]	0	0.049	0	0
D	0.432[b]	0.481[b]	0.542[b]	0.331[b]	0.233[b]
E	0.504[b]	0.504[b]	0.374[b]	0.323[b]	0.273[b]
F	0.740[b]	0.509[b]	0.657[b]	0.317[b]	0.304[b]
G	0.461[b]	0.169[b]	0.256[b]	0.257[b]	0.234[b]
H	0.544[b]	0.494[b]	0.508[b]	0.544[b]	0.324[b]

[a] Each value is the mean of three replicates with two observations/replicate. Experimental treatments are defined in Table I. [b] This value was significantly greater than the analytical zero level ($P < 0.20$).

confirmed earlier findings (Elkins et al., 1968). However, because carbaryl has been shown to be a carcinogen, information regarding the fate of the small amounts of residue remaining after thermal processing could be of considerable importance.

Degradation of Carbaryl during Storage. Only those experimental treatments combining enamel-lined cans and 2% brine showed further significant ($P < 0.05$) decreases in extractable carbaryl after thermal processing during storage (Table III). Partitioning of the data means by Duncan's multiple range test indicated a significant decrease ($P < 0.05$) in extractable carbaryl (97.02–98.56% loss) in the treatment consisting of enamel-lined cans, 2% brine, and the presence of beans during the first day of storage. No additional significant changes occurred after that time. When the experimental system consisted of only the enamel-lined can and 2% brine, there was a significant decrease in the amount of extractable carbaryl between 1 day and 1 week in storage with no further significant change after that time.

Comparison of Carbaryl Residue Levels with Analytical Detection Limits. In developing methodology for these experiments a low detection limit of 0.0526 ppm was established. This figure represents the upper level of the mean ± 99% confidence interval for spectrodensitometric scanning of the background of TLC plates used for carbaryl analysis. Student's t tests were employed to determine whether or not the residues of carbaryl remaining in beans during storage were significantly different from this analytical zero level (0.0526 ppm).

In experimental treatments utilizing either glass containers or enamel-lined cans without brine solution, carbaryl residues were significantly greater than analytical zero residue levels at the 0.20 level of probability throughout the time course of the experiment (Table IV). Experimental treatments utilizing enamel-lined cans and brine solutions were not significantly different from analytical zero levels after 1 week in storage.

These data indicate that carbaryl breaks down more readily following industrial processing techniques than home canning.

COMMENT

Previous literature and these data indicate that residues of carbaryl are not completely removed or broken down by current processing methods and that the probability of complete degradation during storage is a function of type of container and nature of the liquid packing matrix. This confirms that effective control of the carbaryl content of processed foods must be applied at the field level.

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