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Simplified Determination of Carbaryl in Rainbow Trout Liver Tissue

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Since discovered in 1953, carbaryl has become established worldwide as a broad-spectrum insecticide. Carbaryl is presently used to protect vegetable and fruit crops and forest timber resources and to dermally treat domestic animals and livestock (Johnson et al. 1963; Johnson and Stansbury 1965). Wide usage of carbaryl necessitates the development of residue analysis methodology to determine its persistence in the environment.

Quantification of carbaryl residues in animal tissues by direct gas-liquid chromatographic (GLC) analysis presents considerable difficulties. Under typical GLC conditions, carbaryl tends to dissociate to 1-naphthol and N-methyl carbamyl moieties (Zielinksi and Fishbein 1965). With short GLC columns of high liquid phase load and silanized support, thermal breakdown of the carbamate is greatly reduced. Possible enzymatic hydrolysis of the parent compound (Kuhy and Dorough 1976) and the presence of lipids also complicate GLC analysis.

Several general methods have been developed to quantify carbaryl residues (Magallona 1975). Several approaches to GLC analysis of carbamates are based on the derivation of the parent compound to a more GLC stable entity. Such methodologies have been developed by Argauer (1969), Butler and McDonough (1968), Mount and Oehme (1980), Paulson et al. (1970), and Tilden and Van Middelem (1970). Also, successful GLC analyses of carbamates have been reported in which microcoulometric (Cook et al. 1969) or KCl-thermionic (Riva and Carisano 1969) nitrogen detector systems were used.

This paper reports methodology for a sensitive GLC residue analysis procedure with which a large number of small volume samples could be processed expeditiously. The basic extraction/cleanup methodology was developed by Mount and Oehme (1980). In their methodology, ball and mill extraction of carbaryl is achieved in acetone. Samples are cleaned using freeze-out columns (FOC)(Fig. 1) developed by Solomon and Lockhart (1977) and Florsil column

chromatography. The carbaryl is then derivatized with heptafluorobutric anhydride (HFBA) in the presence of trimethylamine (TMA). GLC analysis uses an electron-capture detection system.

MATERIALS AND METHODS

A Hewlett-Packard model 5790A GLC is equipped with a nitrogen-phosphorus detector and a Hewlett-Packard 3390A integrator. The GLC glass column (0.6 m long, 3 mm ID) contains 2% OV-101 on 100/120 mesh Chromosorb W-HP support. GLC operating conditions are as follows: oven temperature, 160°C; detector temperature, 300°C; injector temperature, 250°C; air flow rate, 90 mL/min; and hydrogen flow rate, 3 mL/min. Bead current is adjusted daily for optimum response. A Brinkman Instruments model PCU-2 polytron equipped with a PT-105T generator homogenizes the samples. A Büchii roto-evaporator is used for solvent evaporation.

All solvents are nanograde quality except for the acetone in the freeze-out column bath. Carbaryl (N-methyl-1-naphthyl, >99.4% purity) was obtained from Union Carbide Corporation. Granular anhydrous sodium sulfate and 60/100 mesh Florsil are dried in an oven at 100°C for a minimum of 24 h prior to usage.

Approximately 1 g of frozen liver tissue of rainbow trout (Salmo gairdneri Richardson) is completely thawed and placed in a glass vial (29 x 94 mm). Standard solutions of 0.5 ng/µL, 1.0 ng/µL, 5 ng/µL, 10 ng/µL and 100 ng/µL are prepared by solubilizing carbaryl in acetone. Tissue samples are fortified to one of three concentrations--0.5, 1.0, or 10.0 µg/g (ppm)--with carbaryl. Spiking volumes range from 100 to 500 µL. The fortified tissue then stands at room temperature for 3 h prior to extraction.

To the shell vial containing the fortified tissue, 5 g of anhydrous sodium sulfate and 10 mL of nanograde acetone are added. The mixture is then homogenized using a Brinkman polytron for 30 sec. The resultant slurry is centrifuged at approximately 1000 rpm and -10°C for 10 min.

While the sample is centrifuging, the freeze-out apparatus (FOA) (Fig. 1) is assembled. A Dewar flask is filled with acetone and cooled to approximately -90°C by the addition of dry ice. When the acetone has cooled, the FOC is placed in the Dewar flask.

A 5-mL solvent aliquot is taken from the centrifuged sample and placed in the FOC. To the FOC 5 mL of acetone are added and the pressure head is attached. The pressure head is filled with 10 mL of acetone. After complete solvent elution through the FOC, the eluent is transferred to a 250-mL round-bottom flask. The eluent collection vial is rinsed three times with 1 to 2 mL of acetone and added to the round-bottom flask. The acetone is evaporated under a vacuum to near-dryness with a Büchii roto-evaporator. The roto-evaporator's water bath temperature is maintained at 32°C.

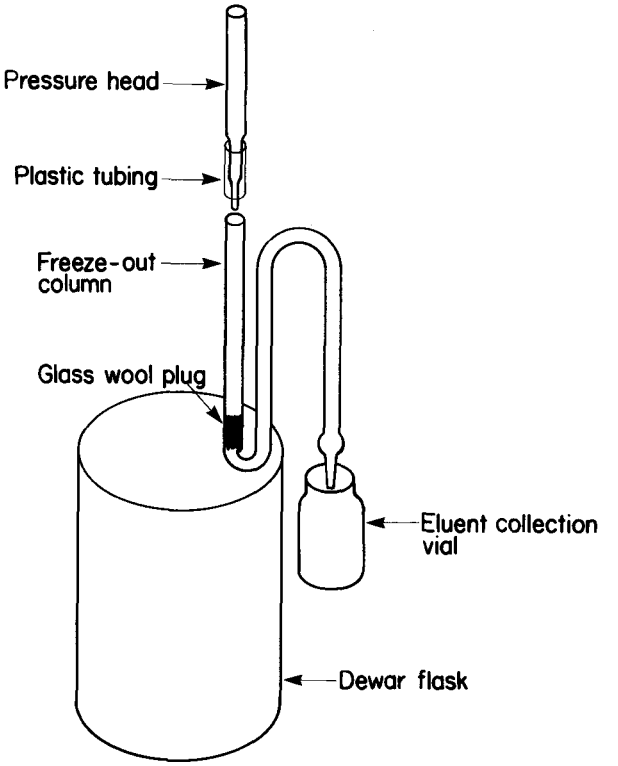

Figure 1. Freeze-out apparatus (FOA). Glassware designed by Solomon and Lockhart (1979).

One g of Florsil and 1 g of anhydrous sodium sulfate are placed in a Bio-Rad polypropylene econo-column. Approximately 1.5 to 2.0 mL of hexane are poured into the econo-column to prime the Florsil and sodium sulfate. The dried sample is reconstituted in 4 mL of nanograde hexane and eluded through a prepared Florsil column. The round-bottom flask is rinsed twice with approximately 3 mL of hexane. The rinses are poured into the column. Next, 5 mL of 6% ethyl acetate (V/V) in hexane are eluded through the column. Then, 30 mL of 20% ethyl acetate (V/V) in hexane are eluded through the column. The eluent is evaporated just to dryness using a Büchii roto-evaporator. The residues are reconstituted in 5 mL of nanograde hexane. The evaporation flask is rinsed with 2 mL of nanograde hexane. Prior to GLC analysis, the sample is stored in an 8 mL (17 x 57 mm) scintillation vial at 0°C.

Each morning the Hewlett-Packard integrator is calibrated with carbaryl standard solutions. Once calibrated, 1 to 5 µL of the processed sample are injected into the GLC. Each sample was injected at least twice and averaged to estimate residue recovery. Standard solutions are run every third injection to check for changes in detector response. If a change in detector

response is noted, the integrator is recalibrated to adjust for the change.

RESULTS AND DISCUSSION

Carbaryl recoveries are listed in Table 1. Liver samples were also fortified to 0.1 ppm and processed. However, at this level we were unable to obtain a consistent estimate of carbaryl recovery. The lower limit of detection was 0.1 ng/μL of injected sample. Control samples showed no interfering peaks.

Table 1. Recovery statistics for carbaryl from fortified rainbow trout liver tissue samples.

Initial fortification (ppm)	N	Mean recovery (%)	Standard deviation
0.5	9	81.9	12.3
1.0	9	82.8	7.1
10.0	9	85.3	15.5

This procedure, adapted from the methodology of Mount and Oehme (1980), contains several distinct modifications. First, Mount and Oehme use a ball and mill extraction. This procedure uses a Brinkman Polytron for extraction. Combining the ultrasonic and mechanical shearing capabilities of the polytron enable the extraction time for six samples to be reduced from 25 min to approximately 5 min. Second, twice within this procedure relatively large amounts of solvents are evaporated off the sample. Carbaryl is a volatile pesticide (Mount and Oehme 1980). The Büchii roto-evaporator's controlled water bath temperature and vacuum control capabilities allow the rate of evaporation to be closely regulated. This ensures that carbaryl evaporation is minimized. Third, Mount and Oehme derivatize the carbaryl with HBA in the presence of TMA to a more GLC stable compound. Although derivatization of the parent compound is common in carbamate analysis, it has several inherent problems. First, the carbamate phenol could occur in the substance under analysis by chemical and metabolic processes (Dorough 1970). Secondly, hydroxylation of the ring and N-methyl group are common metabolic pathways for carbamates. These compounds may or may not react to form GLC-detectable derivatives. Thirdly, derivatizing agents are reactive compounds susceptible to attack by polar coextractives. Interfering materials can be created through these chemical interactions. For example, phenols occur ubiquitously in nature and often survive cleanup procedures to form derivatives which complicate quantification of the insecticide (Dorough and Thorstenson 1975). Finally, derivatization is time consuming. The TMA-HFBA derivatization as employed by Mount and Oehme (1980) requires a 30 min reaction time. The nitrogen-phosphorous specific detector combined with a short column allow the troublesome derivatization

step to be eliminated. The changes in the GLC methodology also greatly reduced the time required for GLC analysis. Retention time for the carbaryl is 2.31 ± .05 min and runs are completed by 5.5 min.

Although utmost care was taken to standardize treatment of individual samples, variations in reproducibility occurred. With a volatile insecticide like carbaryl, losses due to evaporation must account for some of this variation. The Büchii roto-evaporator's controlled-temperature water bath and vacuum greatly enhanced the ability to minimize evaporation. Another source of variation is change in detector response. Detector sensitivity increased throughout the day and variations occurred between days. Compensations for changes in detector sensitivity were made by running carbaryl standard solutions after every two sample runs. Also, samples were analyzed a minimum of two times to check for consistency.

The method described for quantifying carbaryl residues offers relative accuracy and sensitivity. Modifications to the procedure developed by Mount and Oehme (1980) substantially reduce the time required to process each sample. This method is especially suitable where numerous small volume samples need to be rapidly analyzed. Larger volume samples can also be analyzed by increasing the amounts of solvents throughout the procedure.

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