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Residue Analysis of Carbaryl on Forest Foliage and in Stream Water Using HPLC

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Field tests conducted by the U.S. Forest Service in 1974 and 1975 included the use of carbaryl against defoliating insects. Monitoring the insecticide under forest conditions was part of the program. Preliminary to analysis of carbaryl residues on some forest substrates, several techniques were examined. Available methods based on colorimetry (ANON. 1974, JOHNSON 1964, STANSBURY and MISKUS 1964) or spectrophotofluorometry (ARGAUER and WEBB 1972) were unsatisfactory because of low sensitivity and interfering extractives. Analysis by gas chromatography (LORAH and HEMPHILL 1974, RIVA and CARISANO 1969) was unsuccessful due to thermal degradation of the insecticide. Satisfactory results were obtained with high performance liquid chromatography (HPLC). HPLC for analysis of carbaryl has been reported (COLVIN et al. 1974, ISHII 1975, MOYE 1975) with a UV detector absorbing at 254 nm. A UV detector absorbing at 280 nm, as suggested by DOROUGH and THORSTENSON (1975) was used in this study with HPLC in reverse phase operation. Sample cleanup was mainly by Florisil column chromatography and liquid-liquid partitioning. Foliage samples were surface extracted rather than homogenized (ANON. 1974).

METHODS

Foliage extraction and cleanup. Place 20 g of foliage in a pint jar with a Teflon-lined lid. Add 40 ml of $CHCl_3$ and swirl gently for 3 min. Pass the extract through about 2 g of Na_2SO_4. Collect a 24-ml aliquot in a vial with a Teflon-lined cap and store at -20°C.

For grass and geranium, transfer the 24-ml extract aliquot to a flask and add 1 ml CH_3CN. Reduce the volume to about 2 ml using a rotary evaporator at 37°C. Evaporate to dryness with a stream of air or nitrogen. Transfer the residue to a 60-ml separatory funnel with 5 ml hexane followed by 5 ml of CH_3CN. After shaking and allowing the phases to separate, collect the CH_3CN in a flask. Extract the hexane phase with 5 ml and 2 ml of CH_3CN. Combine all acetonitrile fractions and evaporate to dryness.

Prepare "solvent A" by shaking $CHCl_3$, hexane, and water in a ratio of 65:35:50 (v/v/v): discard the water layer. The water removes ethanol usually added to $CHCl_3$ as a stabilizer. Any ethanol present in solvent A will affect the subsequent elution of carbaryl from the Florisil column.

Deactivate Florisil by adding 10% H_2O (w/w) and allow to stand

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for 1 day in a closed container. Batches of Florisil may differ. Check each batch for the amount of water required.

Pack a 10.5 mm i.d. column with 5.2 g of deactivated Florisil. Use glass wool plugs at both ends. Tap the column lightly so that the Florisil bed will be 10 cm long and wet with 10 ml of solvent A.

Dissolve the residue in 2 ml of solvent A and transfer to the Florisil column. Repeat with additional 2 ml and 15 ml portions of solvent A. Adjust column flow to obtain distinct drops. Allow the solvent to sink into the column bed between additions. Discard the eluate. Add 100 ml of solvent A and evaporate the eluate to dryness in a flask.

Add 3 ml of CH_3CN and cap the flask with aluminum foil. Warm the flask gently to dissolve any film of plant waxes. Cool the flask at 3°C for about 1/2 h and pass the sample through a Millipore Fluoropore^R filter (0.5 μm pore size). Proceed to the HPLC measurement.

For aspen and Douglas-fir an extra step is required to remove waxes and resins present. These substances cause the formation of emulsions during the liquid-liquid partitioning step. Pack a 9 mm i.d. column with 1.6 g of dry Florisil. Tap the column lightly so that the packing is 5 cm long. Pass the initial extract (24 ml) through this column followed by 15 ml $CHCl_3$. Collect all the eluate and proceed as described earlier for the extracts of grass and geranium foliage.

Water extraction and cleanup. In a 1000-ml separatory funnel, shake a 400-ml water sample twice with 70 ml and once with 60 ml of CH_2Cl_2. Pass the CH_2Cl_2 extract through sodium sulfate and evaporate a 150-ml aliquot in a flask. Fill a 10.5 mm i.d. column with 7.8 g (15 cm) of deactivated Florisil. Dissolve the residue in 2 ml of solvent A and transfer the sample to the column. Rinse the flask with 15 ml of solvent A in small amounts. Add the rinses to the column, elute dropwise, and discard the rinses. Pass 100 ml of solvent A through the column and evaporate the eluate to dryness. Dissolve the residue in 3 ml of CH_3CN and filter (Fluoropore). Proceed to HPLC measurement.

Soil and sediment extraction (ANON.-2) and cleanup. To 40 g of sample material add 80 ml of a mixture of acetone and water (1:1). Allow to stand for 1/2 h and shake vigorously for 5 min. Allow to stand for 2 min and decant the extract into two 50-ml centrifuge tubes, distributing the weight equally. Centrifuge and filter the supernatant through a fluted filter. Collect 40 ml and transfer the aliquot to a 125-ml separatory funnel. Shake with 20 ml of CH_2Cl_2 and drain the lower layer into a flask. Repeat this extraction twice and evaporate the combined CH_2Cl_2 extracts. Transfer the residue to a Florisil column (see cleanup for grass) with 2 ml of solvent A. Repeat with 2 ml and 20 ml of solvent A. Discard the eluate. Add 100 ml of solvent A and evaporate the eluate in a flask. Dissolve the residue in 3 ml of CH_3CN and proceed to HPLC analysis.

HPLC: Instrument. A Waters Associates liquid chromatograph, equipped with a pump Model 6000A, a 61 cm x 2 mm i.d. Bondapak C_{18}-Corasil^R (37-50 µm) column, a Model 440 UV detector absorbing at 280 nm and a U6K injector. Solvent: 40% CH_3CN/60% water (degassed); flowrate: 0.3 to 0.6 ml/min; sensitivity: 0.005 AUFS; recorder: 10 mV.

After proper dilution of the sample, inject from 1 to 10 µl and determine the amount of carbaryl by measuring the peak height against a standard curve with carbaryl standards ranging from 5 to 20 ng. Approximately 1/3 full-scale deflection is produced with 10 ng of carbaryl.

After 3 to 5 sample injections, check for changes in sensitivity of the HPLC system by injecting a carbaryl standard. The residue in ppm can be calculated taking into account amount of injected carbaryl found, injected and final volumes, aliquot (cleanup) and recovery factor.

DISCUSSION OF THE METHOD

Extraction. Surface extraction of carbaryl from foliage avoids many natural plant products which are released by homogenization and may interfere with subsequent cleanup or measurement by UV absorption. The method of extraction was developed by the manufacturer, Union Carbide Corporation (ANON. 1974). Experience with a variety of plants for periods of up to 3 mo after treatment indicates that the carbaryl is essentially a surface residue and significant amounts do not penetrate the plant tissue (ROMINE 1975-1976).

Controls. Control samples of grass, geranium, Douglas-fir, aspen and water were free of interfering peaks.

Recoveries. At the 5-ppm fortification level (100 µg of carbaryl in 1 ml of $CHCl_3$ added to 20 g untreated foliage), the following average percent recoveries were obtained: Grass, 89.5; geranium, 86.5; aspen 75.0; Douglas-fir 49.8.

Other mean percent recoveries were: From stream water fortified at the 0.1 ppm level, 99.7; from soil fortified at the 0.1, 0.5 and 1.0 ppm level, 103.0, 106.5 and 110.0 respectively. Total avg: 106.0. From sediment fortified at the 0.1, 0.5 and 1.0 ppm level: 101.0, 105.5 and 113.0 respectively. Total avg: 106.5.

The recovery values greater than 100% found for soil and sediment point to the limits of the technique. Such recoveries are not uncommon, however, and are reported in the literature.

Recoveries from freshly fortified Douglas-fir foliage were 100 percent. The low recoveries given above for Douglas-fir may be due to degradation of carbaryl during the 7 mo of storage.

RESIDUES OF CARBARYL IN FIELD SAMPLES

Carbaryl was applied aerially in a test conducted on the Beaverhead National Forest, Montana, in 1975. As part of the experiment, foliage and water samples were collected to study the persistence of carbaryl under existing conditions.

The insecticide was applied as SEVIN-4-OIL and was diluted with an equal volume of diesel oil. The material was sprayed by helicopter at a rate of 1 lb/0.5 gal/acre on three 1000-acre plots. One plot was sampled for residues.

Approximately 500 g of foliage per sample were collected for each of the following plant species: Grass (species unknown), geranium *(Geranium viscosissimum)*, Douglas-fir *(Pseudotsuga menziesii)*, and aspen *(Populus tremuloides)*. Each sample was a composite of different collections; e.g., from 5 trees sampled at different heights. The same trees were sampled on different dates. The samples were stored in plastic bags at -18°C. One quart of water per sample was collected and stored at 1 to 3°C. After extraction all samples were stored at -18°C.

Two analyses were performed on each foliage field sample and results corrected for percent recovery (Table 1). Each water sample was analyzed once (Table 2).

TABLE 1

Residues of Carbaryl on Foliage at Various Time Intervals after Application, Beaverhead National Forest, Montana, 1975 (ppm)

	Time after spray (days)							
Foliage and replication	0[1/]	1	3	7	14	28	47	63
Grass 1	89	51	76	52	17	0.3	<0.1	<0.1
2	51	61	54	40	25	0.3	0.1	<0.1
Avg.	70	56	65	46	21	0.3	<0.1	<0.1
Geranium 1	227	93	110	73	36	3.6	0.2	0.1
2	108	104	97	45	12	2.0	0.2	<0.1
Avg.	168	99	104	59	24	2.8	0.2	<0.1
Aspen 1	31	40	17	5.4	11	3.4	2.5	0.5
2	29	20	16	17	22	9.9	2.2	0.4
Avg.	30	30	17	11	17	6.7	2.4	0.5
Douglas-fir 1	103	134	49	66	18	3.5	5.4	0.6
2	173	113	39	68	39	4.4	3.6	7.6
Avg.	138	124	44	67	29	4.0	4.5	3.8

[1/] 1 to 2 hours after spray.

TABLE 2

Residues of Carbaryl in Stream Water on Day of Application, Beaverhead National Forest, Montana, 1975

Spray plot	No. of samples	Collection period after spray (hrs)	Range (ppb)
Daisy Creek	6	1.5	3- 6
Lenard Creek	6	1.6	3-175
Warm Springs	7	2.8	2-260
Ruby Creek	5	1.3	4-108

Since a surface extraction technique was adopted, the foliage could not be homogenized before division into subsamples. This may explain some of the large differences between residues found in replicate analyses.

At the application rate of 1 lb/acre, carbaryl residues dropped below the 1 ppm level by 28 days for grass, 47 days for geranium and 67 days for aspen. On Douglas-fir, 3.8 ppm were found after 67 days. On the basis of the results, the half-life values in days were estimated from empirically derived decay curves, as follows: Grass 8, geranium 3, aspen 8, and Douglas-fir 4.5. Residues in water samples taken during the first 3 h after the spray showed an irregular pattern. High carbaryl content in some water samples may have resulted from local accumulations of the floating oil-based spray.

In addition to analysis of carbaryl residues we found that HPLC, without a change in operating conditions, can also successfully be used to determine the amount of the insecticide in formulations (unpublished data).

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REFERENCES

ANONYMOUS-1: Report: A method for the determination of residues of carbaryl on plant foliage. Union Carbide Corp. Agric. Prod. R&D Dep., S. Charleston, W. VA. (June 1974).

ANONYMOUS-2: Report: Determination of carbaryl residue in soil. Union Carbide Corp. Agric. Prod. R&D Dep. S. Charleston, W. VA. (no date).

ARGAUER, R.J. and R.E. WEBB: J. Agric. Food Chem. 20, 732 (1972).

COLVIN, B.M., B.S. ENGDAHL and A.R. HANKS: J. Assoc. off. Anal. Chem. 57, 648 (1974).

DOROUGH, H. W. and J. H. THORSTENSON: J. Chromatogr. Sci. 13, 212 (1975).

ISHII, Y.: Varian Instruments Applications: 9, 10 (1975).

JOHNSON, D. P.: J. Assoc. off. Anal. Chem. 47, 283 (1964).

LORAH, E. J. and D. D. HEMPHILL: J. Assoc. off. Anal. Chem. 57, 570 (1974).

MOYE, H. A.: J. Chromatogr. Sci. 13, 268 (1975).

RIVA, M. AND A. CARISANO: J. Chromatogr. Sci. 42, 464 (1969).

ROMINE, R. R.: Personal communications (1975-1976).

STANSBURY, H. A. and R. MISKUS: In Analytical Methods of Pesticides, G. Zweig, ed., 437 (1964).