Determination of Zectran Residues in Aerial Forest Spraying

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The persistence of Zectran, sprayed to control spruce budworm, was investigated. Residues on Douglas fir and five common browse species of plants were determined. The cleanup procedure preceding gas chromatographic analysis is described. Zectran residue levels, followed over a period of 4 weeks, showed a rapid initial decline.

Zectran (4-dimethylamino-3,5-xylyl methylcarbamate, Dow Chemical Co.) was used in pilot tests for control of spruce budworm [*Choristoneura fumiferana* (Clemens)] larvae in Montana in 1966. Application was made at the rate of 0.15 pound of the 95% technical material per acre. Because of growing concern for possible side effects of pesticides on forest wildlife, the persistence of the insecticide was investigated on Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and five common browse species of plants.

The methods of analyses available were not completely satisfactory. A gas chromatographic method of analysis using electron capture for the detection of Zectran as described by Eberle and Gunther (1965) did not provide the desired degree of sensitivity.

A colorimetric method of determining Zectran based on the reaction of the xylenol with luteoarsenotungstic acid has been described by Marquardt and Luce (1963). Their method was unsatisfactory because of its low sensitivity and the presence of interfering chromogenic materials in the plant extractives. However, by using a modification of their cleanup procedure in combination with gas-liquid chromatography and an electrolytic conductivity detection system, it was possible to determine 10 to 20 ng. of Zectran at the 0.1-p.p.m. level. In this system, the Zectran-nitrogen was catalytically reduced at about 800° C. to NH_3, which was subsequently measured in the conductivity detector cell. The resulting change in electrolytic conductivity of the water was recorded and is a function of the amount of Zectran introduced into the gas chromatograph.

EXPERIMENTAL

Equipment and Materials. A gas chromatograph (Coulson Instruments Co., Model 1) equipped with a 4-foot column packed with 80- to 100-mesh Gas Chrom-Q coated with 10% Dow Corning 200 silicone oil, a reduction furnace, and an electrolytic conductivity detector cell for the detection of nitrogen (Coulson, 1966) was used.

Sample Preparations. Homogenize 60 grams of foliage in 300 ml. of 0.5*N* H_2SO_4 in a Waring Blendor and store at about 0° C. Re-extract an aliquot of the foregoing homogenate representing 20 grams of foliage with 50 ml. of 0.5*N* H_2SO_4 and filter through four layers of cheesecloth. Re-extract the residue with 150 ml. of 0.5*N* H_2SO_4.

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Wash the combined extract three times with 50-ml. portions of ether in a 1-liter separatory funnel. Discard the ether fractions. Wash the extract once more with 50 ml. of chloroform. Discard the chloroform fraction. Neutralize the aqueous fraction to about pH 7.3 (pH paper) with at least 255 ml. of saturated $NaHCO_3$.

Extract the resulting solution three times with 50-ml. portions of benzene. Discard the aqueous phase. Rinse down possible emulsion and foam remaining in the separatory funnel with a small amount of water. Whenever emulsions occur, centrifuge to obtain better separation of the phases. After most of the aqueous fraction has been removed, treat any remaining emulsion with anhydrous sodium sulfate and decant the benzene. Wash the residual sodium sulfate with about 20 ml. of benzene. The combined benzene extract can be washed once with 25 ml. of water; however, in most cases, this step can be omitted. Filter the benzene extract through a small amount of anhydrous sodium sulfate to remove remaining water.

Reduce the volume of the benzene extract to about 3 ml. in a rotary vacuum evaporator. Carefully transfer the concentrate to a graduated and stoppered tube and make up to a known volume with benzene.

Gas Chromatography. After proper dilution, if necessary, inject a 50-μl. aliquot of the final extract onto the column of the gas chromatograph. Conditions for gas chromatography are as follows:

Inlet temperature, 225° C.

Programmed column temperatures, 2.3 minutes isothermal at 170° C. and a linear temperature increase thereafter until after 11 minutes a temperature of 250° C. is reached. Reduction furnace temperature, 800° C. Carrier gas flow (He), 83 ml. per minute (30 p.s.i.). Hydrogen gas flow, 128 ml. per minute (5 p.s.i.). Attenuation, 1.

Retention time under these conditions is 3 to 4 minutes.

Table I. Recovery of Zectran from 20-Gram Fortified Foliage Samples

Plant Species	Zectran Added, P.P.M.	Recovery, %
Pseudotsuga menziesii	0.1	81
	0.3	72
Abies grandis	0.3	63, 81
Balsamorhiza sp.	0.1	95
	0.3	75
Ceanothus sp.	0.3	45, 51
Taraxacum sp.	0.3	70, 70
Fragaria sp.	0.3	42, 53
Tragopogon sp.	0.3	78, 82

Table II. Zectran Found after Period Indicated (P.P.M. by Species)

Species	Days								Weeks			Recovery Factor, %
	0	1	2	3	4	5	6	8	2	3	4	
Pseudotsuga menziesii	2.86	0.19	0.22	0.15	0.14	0.17	0.14	0.17	...	0.13	...	77
Balsamorhiza sp.	7.85	0.47	0.33	0.28	0.94	0.82	0.94	0.13	0.22	0.04	0.0	85
Ceanothus sp.	1.29	1.56	0.94	1.25	1.52	1.56	1.00	0.67	2.01	...	0.19	48
Fragaria sp.	6.25	4.17	4.58	2.19	5.73	2.71	3.10	...	0.73	0.94	0.38	48
Taraxacum sp.	2.29	0.44	0.11	0.11	0.08	0.04	0.01	0.13	0.07	...	0.0	70
Tragopogon sp.	0.75	0.56	0.29	0.06	0.04	...	0.04	0.01	0.01	...	0.11	80

Determine the Zectran by comparison of the peak height with a previously constructed standard curve.

Establish points for the standard curve by injecting 50 μl. of solutions containing 20 to 80 ng. of Zectran in benzene.

Recoveries. Add known amounts of Zectran in 5 ml. of 0.5*N* H_2SO_4 to an homogenate containing 20 grams of foliage in 145 ml. of 0.5*N* H_2SO_4. Treat this mixture as in the foregoing extraction procedures. Table I shows recovery data.

Calculations.

$$\text{P.p.m.} = \frac{\text{nanograms of Zectran found} \times 10^3}{\text{dilution factor} \times \text{recovery} \times \text{sample weight in grams}}$$

DISCUSSION

Preparation of a homogenate of foliage in 0.5*N* H_2SO_4 allows for a conveniently long period of time in which the samples can be further analyzed for Zectran. Thin-layer and gas-liquid chromatography showed that Zectran remains stable for at least 2 months when dissolved in 0.5*N* H_2SO_4 and kept at about 0° C. The method does not require an excessive cleanup procedure or large quantities of organic solvents.

Zectran residue levels drop rapidly in most of the plants investigated after the first day (Table II). The amount left after 1 week is negligible except perhaps in the *Fragaria* and *Ceanothus* species. This condition indicates that the rate of disappearance was mediated by the plant substrate.

The gas chromatographs of the browse plant species often showed the presence of an unidentified material, with a retention time about one-half minute longer than that of Zectran. Under the above conditions, three major breakdown products of Zectran gave peaks with about the same retention time and could therefore not be distinguished from each other (Abdel-Wahab *et al.*, 1966).

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