Determination of Trichlorfon [*O,O*-Dimethyl (2,2,2-Trichloro-1-hydroxyethyl)phosphonate] in Forest Environmental Samples

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A gas chromatographic method is described for the determination of trichlorfon [*O,O*-dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate] in various forest environmental samples such as leaves, twigs, forest litter, soil, mud, water, aquatic vegetation, and animal tissues. Trichlorfon residues were removed with chloroform. Animal extracts were processed through hexane-water and water-chloroform partition steps to remove lipid materials. Forest extracts were cleaned up with Nuchar C-190N activated carbon. Determination of trichlorfon was made with a gas chromatograph equipped with a flame photometric detector in the phosphorus mode. Recovery from the various types of samples averaged 96%. The method is sensitive to 0.002 ppm for water and 0.05 ppm for all other sample types.

Since the accidental release of the gypsy moth [*Porthetria dispar* (L.)] in this country over a century ago, its growth and destruction have been exponential. In recent years, the defoliation of forest land in the Northeast due to this insect has been over 1 million acres per year. Since the use of persistent insecticides such as DDT has been curtailed, control of the gypsy moth has been accomplished with less persistent chemicals.

In 1971, several test plots were sprayed with trichlorfon for the control of the gypsy moth. An environmental impact study was performed to detect any harmful ecological effects. An important aspect of such a study is the extent of contamination and the persistence of the chemical. Forest samples of leaves, twigs, litter, and soil as well as aquatic samples of water, mud, vegetation, fish, and frogs were collected for analysis. A residue method by Thornton (1970) would have been adequate for the forest samples, but because of the large number of samples a shorter method was developed. Analysis of animal tissues was similar to but shorter than a method reported by Olson (1969). This report describes the residue methods which were developed and employed for analysis of the various types of environmental samples.

EXPERIMENTAL SECTION

Reagents and Apparatus. All solvents and chemicals were reagent grade. The activated carbon was Nuchar C-190N.

A Tracor MT-220 gas chromatograph equipped with a flame photometric detector (Brody and Chaney, 1966) in the phosphorus mode (526-nm filter) was employed for analysis. A 6 ft × 3/16 in. i.d. glass column was packed with 16% XF-1150 on 60/80 mesh acid-washed Chromosorb W. Five inches of the upstream end of the column were left unpacked. The inlet temperature was 260°, the column was 125°, and the detector was 180°. The carrier gas flow rate was 60 ml/min, hydrogen was 150 ml/min, oxygen was 20 ml/min, and air was 20 ml/min.

Storage of Samples. Samples were stored in heavy plastic bags in a freezer at −10° until they were ground and extracted. Water and mud samples were acidified in the field with several drops of concentrated hydrochloric acid and kept in Mason jars at −10° until they were extracted.

Extraction. Water samples were allowed to thaw before a 100-ml subsample was transferred to a 500-ml separatory funnel. A 25-g portion of sodium chloride was added to the water and dissolved by shaking. The water was then extracted with three 50-ml portions of chloroform, shaking for 30 sec each time. The extracts were passed through approximately 30 g of anhydrous sodium sulfate into a round-bottomed flask. The sodium sulfate was rinsed with a final 20-ml portion of chloroform. The combined extracts were evaporated just to dryness on a rotary vacuum evaporator with a water bath maintained at 40°. The residue was quantitatively transferred to a graduated centrifuge tube with several rinses of acetone. If necessary, further evaporation was performed with the aid of a beaker of warm water and a stream of air. With a final volume of 1.0 ml and a 10-μl injection, a sensitivity of 2 ppb was obtained. This sensitivity gave a peak height of approximately 10% FSD.

Forest and aquatic vegetation samples were ground to a fine consistency in a Hobart food chopper. A 50-g subsample was blended in a Waring blender for 3 min with 300 ml of chloroform and 100 g of sodium sulfate. A 400-ml portion of chloroform was used for forest litter samples. The filtered extract was then stored in a cold room at 5° until an aliquot was treated with activated carbon. Soil samples were extracted in a similar manner, while extracts of mud were passed through additional sodium sulfate to remove any small traces of water before storing in a cold room.

Animal samples such as fish and frogs were ground in a Hobart meat grinder and extracted as previously described for the forest samples. A 200-ml aliquot was then taken for cleanup by the partition steps. Because of the small size of some of the animal tissues (5–20 g), the entire frozen sample was cut up and weighed into a small Waring blender bowl. The sample was blended for 30–60 sec with 25 g of sodium sulfate to disintegrate the tissues. The samples was then extracted with three 100-ml portions of chloroform, blending for 2 min each time. The filtered extracts were combined and then processed through the partition steps for cleanup.

Activated Carbon Treatment. After allowing the extract to come to room temperature, a 200-ml aliquot was evaporated just to dryness on a rotary evaporator. The residue was dissolved in 50 ml of acetone and 5 g of activated carbon was added. The slurry was swirled for 1 min and was then filtered through a fine porosity fritted Büchner funnel with the aid of vacuum. The sample flask and funnel were rinsed with two 50-ml portions of acetone. The filtrate was evaporated just to dryness and the residue was taken up in an appropriate amount of acetone for analysis. With a final volume of 5 ml and a 10-μl injection, a sensitivity of 0.05 ppm was obtained.

Partition Cleanup Steps. Extracts from the animal samples were evaporated just to dryness. The residue was transferred to a 500-ml separatory funnel with two 100-ml rinses of hexane. The hexane was extracted with two 100-ml portions of a saturated sodium chloride solution, shaking for 30 sec each time. The hexane was discarded and

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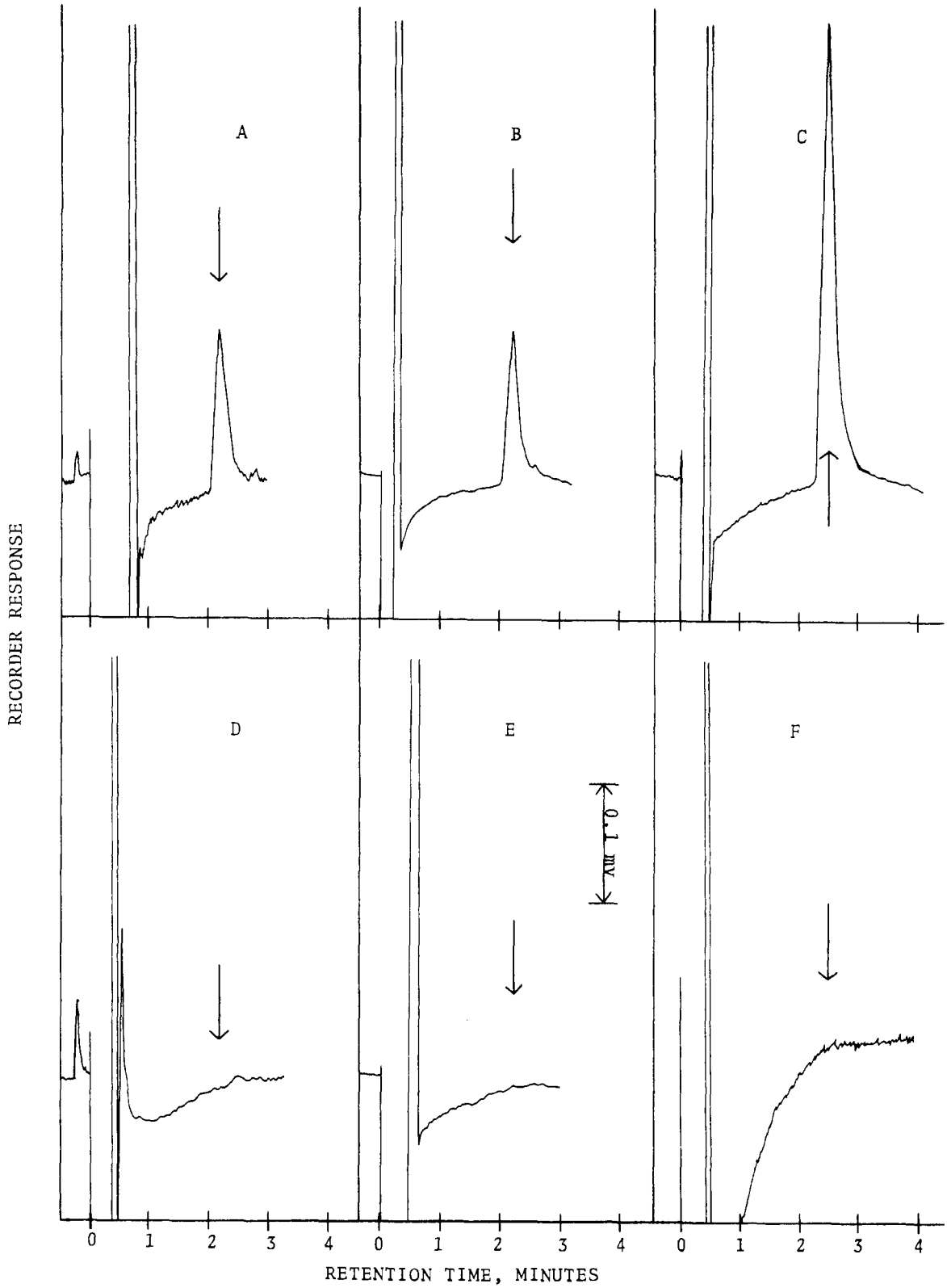

Figure 1. Typical chromatograms for trichlorfon analysis. A. Untreated water fortified with 0.002 ppm of trichlorfon, 1 g injected, 96% recovered, 1.6×10^{-7} afs sensitivity. B. Untreated litter fortified with 1.0 ppm of trichlorfon, 8.33 mg injected, 84% recovered, 6.4×10^{-7} afs sensitivity. C. Untreated leaves fortified with 10 ppm of trichlorfon, 1.2 mg injected, 114% recovered, 3.2×10^{-7} afs sensitivity. D. Untreated water, 1 g injected, nondetectable residue, less than 0.002 ppm of trichlorfon, 3.2×10^{-7} afs sensitivity. E. Untreated litter, 66.7 mg injected, nondetectable residue, less than 0.05 ppm of trichlorfon, 3.2×10^{-7} afs sensitivity. F. Untreated leaves, 66.7 mg injected, nondetectable residue, less than 0.05 ppm of trichlorfon, 1.6×10^{-7} afs sensitivity.

the combined aqueous phases were extracted with three 50-ml portions of chloroform. The chloroform was dried with sodium sulfate and the combined extracts were evaporated just to dryness on a rotary evaporator at 40°. The residue was taken up in an appropriate amount of acetone to obtain a sensitivity of 0.05 ppm.

Gas Chromatographic Analysis. Aliquots of the final extracts were injected into a gas chromatograph equipped with a flame photometric detector in the phosphorus mode. Appropriate dilutions were made if the concentration was too high. The area of the sample peak, determined by peak height × width at half-peak height, was

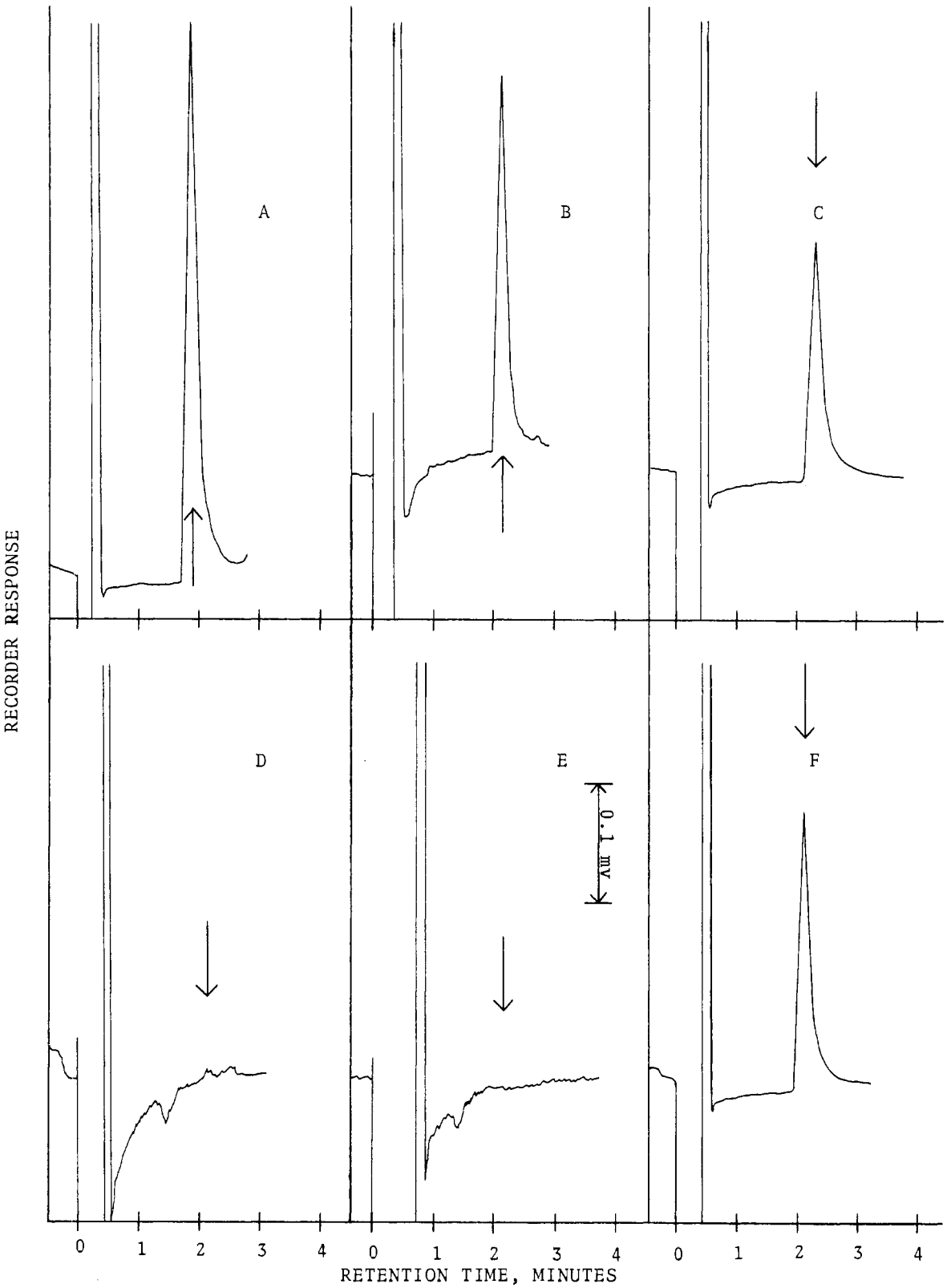

Figure 2. Typical chromatograms for trichlorfon analysis. A. Treated leaves, 0.67 mg injected, 67.4 ppm of trichlorfon found, 6.4×10^{-7} afs sensitivity. B. Trichlorfon standard, 5 ng injected, 3.2×10^{-7} afs sensitivity. C. Treated twigs, 6.67 mg injected, 2.12 ppm of trichlorfon detected, 3.2×10^{-7} afs sensitivity. D. Treated mud, 135 mg injected, nondetectable residue, less than 0.05 ppm of trichlorfon, 3.2×10^{-7} afs sensitivity. E. Treated soil, 66.7 mg injected, nondetectable residue, less than 0.05 ppm of trichlorfon, 1.6×10^{-7} afs sensitivity. F. Treated litter, 5 mg injected, 3.50 ppm of trichlorfon found, 3.2×10^{-7} afs sensitivity.

compared directly with the area of a trichlorfon standard peak to determine the amount of residue present. The peak area was linear at the sensitivities employed for analysis. The response was checked periodically but it generally remained constant during a day's run.

RESULTS AND DISCUSSION

With the chromatographic conditions employed for analysis, trichlorfon did not chromatograph intact. It was thermally broken down to dimethyl phosphite (Thornton, 1970) and it was this compound which was detected by

Table I. Percent Recovery of Trichlorfon

Fortification level, ppm	Leaves	Twigs	Litter	Animal tissues
50.0	105			
10.0	95	101	80	
10.0	114	78	110	
1.0			87	85
0.50			97	80
0.20	99	70	91	
0.20	100			
0.10	89	97	99	80
0.10		73		
0.05	123	98	122	86
0.05	77	118		77
Average	99	90	98	82
	Water	Soil	Mud	
0.25	116			
0.20		110	102	
0.10		95	105	
0.10		77		
0.05	97	111	118	
0.05	98	71	92	
0.05		99		
0.005	110			
0.002	100			
Average	104	94	104	

the phosphorus specific detector. The retention time for dimethyl phosphite was approximately 2 min. To help promote this thermal breakdown, the inlet temperature was high and the first 5 in. of column was left unpacked. Several percent of dichlorvos was produced during chromatography of trichlorfon, but its retention time was 5–6 times greater than that of dimethyl phosphite and so did not interfere with the analyses.

Depending on what type of sample was being analyzed, the gas chromatographic response would become erratic after several weeks or so of continuous analysis. By changing the glass wool plug in the inlet section of the gc column and injecting several large (mg) amounts of trichlorfon, the response returned to normal. By keeping a spare conditioned gc column in the oven, a delay in conditioning was avoided. Over 1100 residue samples were analyzed during this study and yet only two gc columns were used.

To determine the effect of storage of water and soil samples upon trichlorfon residues, stability studies were performed. Acidified water was fortified with trichlorfon at the 0.05-ppm level and untreated soil was fortified at 0.1 ppm. These samples were frozen and aliquots were analyzed at various intervals. Trichlorfon was found to be stable for at least 21 days. All water and soil samples were extracted within 7 days of collection.

Table II. Comparison of Exhaustive Extraction and the Described Method

Sample	ppm of trichlorfon	
	Exhaustive[a]	Blending
Leaves	6.20	5.83
Litter	3.60	3.22

[a] A 20-g subsample was extracted for 18 hr in a Soxhlet extractor with 250 ml of a 10% methanol in chloroform solution.

Because of the large number of samples, extraction was performed as soon as possible to prevent breakdown of residues during storage in the freezer. Fortified control extracts were stored at 5° and aliquots were analyzed periodically. After 72 days, trichlorfon was still found to be stable. Since most sample extracts were processed within 4 weeks of extraction (60 days was the longest interval), integrity of the residues was established.

To support the procedure, untreated samples were fortified with aliquots of an acetone solution of trichlorfon before extraction. Table I presents the recovery data, with fortification levels ranging over the expected residue levels. Recovery of trichlorfon averaged 96% for the various types of samples. Figures 1 and 2 show typical chromatograms for trichlorfon analysis. Figure 2B, 5 ng of trichlorfon, shows a peak height of approximately 30% FSD.

To determine the extraction efficiency for removing trichlorfon residues, samples of leaves and forest litter were exhaustively extracted for 18 hr in a Soxhlet extractor with a 10% methanol in chloroform solution (Bowman *et al.*, 1968). The extracts were passed through sodium sulfate and processed through the activated carbon treatment step. The results, as shown in Table II, indicate that extraction by blending gives comparable residues to exhaustive extraction techniques.

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