Extraction Efficiency Determinations of Labeled Systemic Parathion Residues[1]

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Past methodologies of extracting parathion residues (5,7) were often validated by fortifying the sample just prior to extraction and analysis. These methods, therefore, were primarily designed for the extraction and analysis of loosely bound residues of the parent compound. Extraction efficiencies were not determined on 'grown-in' or field-treated residues, and confirmation of structural integrity was rarely provided. This report describes the progress in analytical methodologies pertinent to the extraction and positive identification of systemic parathion residues.

EXPERIMENTAL

Bean (Phasedus vulgaris L. 'Tenderbest') seeds were surface-sterilized by a 0.5% sodium hypochlorite solution for 10 min, soaked in running water for 3 hr and germinated in vermiculite. Plants were grown under 750 foot candles of fluorescent light (Plant-Aid, KEN-RAD) with a 14-hr photoperiod/24 hr at 21 $\pm$ 2°C and 50 $\pm$ 5% relative humidity. After three weeks, individual bean plants were transferred to foil-wrapped 125 ml erlenmeyer flasks, each containing 0.3 μ Ci of 1,2-^{14}C-ring-labeled parathion (International Chemicals and Nuclear Corp.) dissolved in 100 ml of a modified Hoagland nutrient solution (3). The plants were allowed to grow in the fortified medium for 6 days and then subjected to residue analysis.

Autoradiography of whole plants and developed thin-layer plates was carried out according to CRAFTS and YAMAGUCHI (1964) using Kodak No screen X-ray film. Radiocarbon content was determined by combustion analysis (4), and structural confirmation was verified by conventional chromatographic and spectroscopic procedures. Thin-layer chromatography was performed using Polyamide II precoated plastic sheets (Brinkmann Instruments, Inc.) and a solvent system comprised of 5% ethyl ether in benzene. Extraction efficiencies were calculated from data obtained through the utilization of: blender, sonifier, Polytron (2), soxhlet, tur-

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boshear, tissue grinder, sublimation, dry ice and liquid nitrogen.

RESULTS AND DISCUSSION

Autoradiography was used to establish the incorporation of labeled residues into all plant parts (Figure 1). The heavily labeled plants were then divided into 8 groups of 12 plants each and subjected to various maceration and extraction techniques presented largely in Table I. Acetonitrile was the solvent of choice (6,8). Recoveries were determined from the total ^{14}C count of the extractive solvent and the remaining plant pulp.

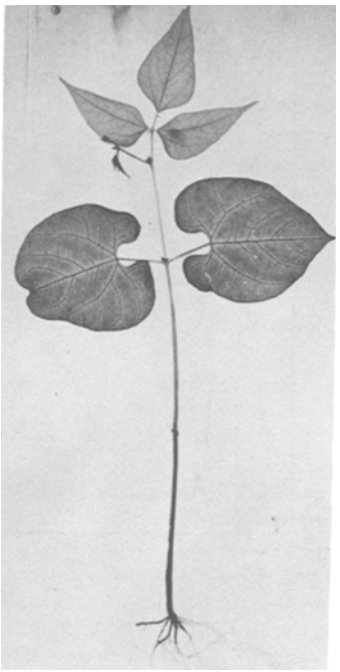

Fig. 1. Autoradiogram of ^{14}C-parathion in bean plant tissues.

In general, the extraction efficiencies appear to be comparable. Sonification alone resulted in less than adequate results. More important, however, is the time factor involved in each extraction method, e.g. the Polytron possessing a resonator equipped with a sawtooth cutting head employed only a 30-sec extraction, whereas the soxhlet method required an 8-hr extraction time.

Characterization of the extractable residues was effected to determine any chemical alterations resulting from exposure to ultrasonic waves, heat, vacuum, or cryogenic temperatures inherent in any one of the extraction procedures. Figure 2 represents an autoradiogram of a thin-layer plate containing reference standards and ^{14}C-residues from bean plants.

The results clearly indicated that parathion in fact was the major radioactive component. Radioactive conversion products such as S-ethyl parathion, paraoxon and p-nitrophenol did not appear in any higher concentration in the plant extracts than in the original parathion formulation. Structural confirmation of the major radioactive component as parathion was made by infrared spectroscopy (Figure 3).

SUMMARY

These results demonstrate the total reliability and efficiency of the Polytron extraction method. Differences in recovery of the Polytron extraction method and the soxhlet method were small, but differences in extraction times were considerable. The only limiting factor in the number of residue samples extracted by the Polytron would be those imposed by limitations in associated glassware.

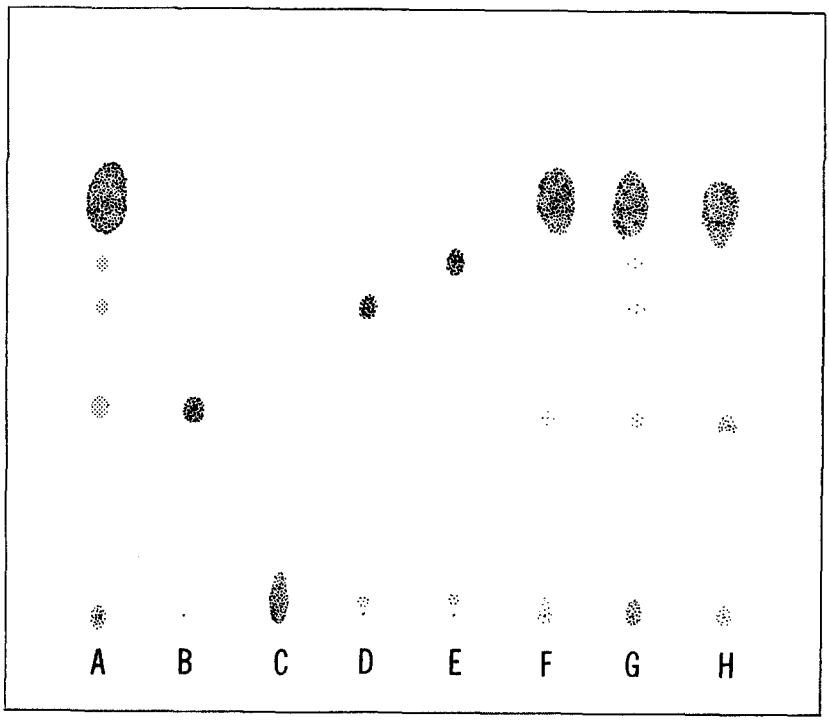

Fig. 2. Autoradiogram representation of a Polyamide thin-layer plate containing parathion reference standards and associated ^{14}C-residues extracted from root-treated bean plants. Thin-layer plate was developed in benzene:ethyl ether (95:5).

A. Parathion
B. Paraoxon
C. Nitrophenol
D. S-ethyl parathion
E. S-phenyl parathion
F. Extract from bean roots
G. Extract from bean shoots
H. Extract from nutrient solution

TABLE I

Summary of grown-in ^{14}C-parathion extraction studies.

Extraction Method	Plant #	Combustion Analysis (dpm) Macerate	Extract	Total	Recovery %	Ave Recovery %
Turbo-shear Virtis	Control	54	60	114	--	
	1	450	5495	5945	92	
	2	672	6882	7554	91	90
	3	783	5590	6373	88	
Polytron	Control	59	110	169	--	
	1	818	8460	9278	91	
	2	588	6510	7098	92	92
	3	779	6489	7268	93	
Soxhlet	Control	76	71	147	--	
	1	530	7977	8507	94	
	2	181	7206	7387	98	96
	3	344	7151	7495	95	
Sonifier	Control	71	84	155	--	
	1	960	1497	2457	61	
	2	891	1356	2247	60	61
	3	720	1172	1892	62	
Waring Blendor	Control	67	69	136	--	
	1	572	6554	7126	92	
	2	800	5750	6550	88	90
	3	820	7235	8055	90	

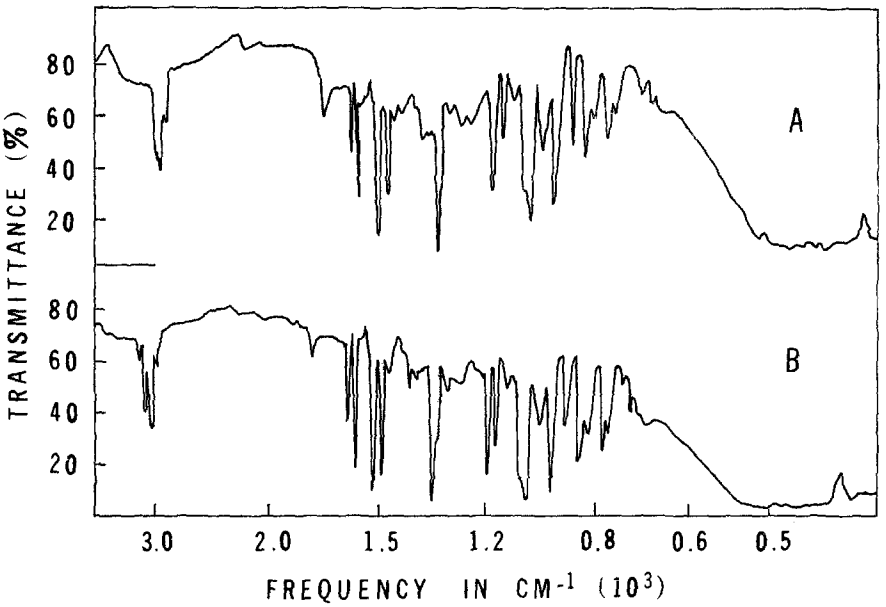

Fig. 3. Infrared spectra of standard parathion (A) and parathion (B) isolated from bean plant extract by vacuum sublimation.

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