An Integrated Method for Trifluralin, Diphenamid, and Paraquat in Soil and Runoff from Agricultural Land

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An integrated method has been developed involving gas chromatographic and colorimetric techniques for the determination of admixed residues of the herbicides trifluralin (α,α,α-trifluro-2,6-dinitro-*N,N*-dipropyl-*p*-toludine), diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide), and paraquat (1,1-dimethyl-4,4-bipyridinum dichloride) in soil and runoff samples. The use of the Coulson electrolytic conductivity detector permits simultaneous gas chromatographic determination of trifluralin and diphenamid, and eliminates the necessity for cleanup of soil and sediment samples. A modification of the standard colorimetric method for paraquat reduces analysis time and minimizes sample attendance. Recoveries from spiked soil and sediment ranged from 86 to 94% for trifluralin, 95–96% for diphenamid, and 91–99% for paraquat. With spiked water samples, recoveries ranged from 82 to 91% for trifluralin, 90–95% for diphenamid, and 83–94% for paraquat. Recoveries from spiked runoff (water and sediment combined) samples ranged from 82 to 84%, 95–97% and 94–107% for trifluralin, diphenamid, and paraquat.

A research program to develop water pollution control technology required an analytical method to measure trifluralin (α,α,α,-trifluro-2,6-dinitro-*N,N*-dipropyl-*p*-toludine), diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide), and paraquat (1,1-dimethyl-4,4-bipyridinum dichloride) in agricultural runoff.

Runoff studies usually require large numbers of samples to be processed in the time available between rainfall events. The data provided can then be used as the basis for program adjustments for the ensuing event. The analytical method therefore had to be conducted on a production line basis, providing a large sample throughput in a minimum amount of time. A rapid analysis procedure also reduces the risk of trifluralin loss by volatilization and degradation.

Existing gas chromatographic methods for determination of trifluralin and diphenamid require cleanup of the soil extract and the use of multiple detection systems (Food and Drug Administration, 1969; Tepe and Scroggs, 1967; Tepe *et al.*, 1967). The colorimetric methods for paraquat are lengthy and require considerable sample attention (Carlestrom, 1971; Chevron Chemical Co., 1970; Food and Drug Administration, 1969).

The integrated method presented herein, involving gas chromatographic and colorimetric procedures, was developed for the admixed herbicides in soil, water, and sediment. The use of the Coulson electrolytic conductivity detector permits simultaneous gc determination of trifluralin and diphenamid and eliminates time-consuming cleanup procedures normally needed for soil and sediment. Paraquat was determined by a modified method which reduces analysis time and requires less sample attendance than previous methods (Pope and Benner, 1974).

APPARATUS

Sediment-Water Separations. A schematic representation of the six-unit sediment-water separation apparatus is presented in Figure 1. The components of the system are (letters referring to the figure): (a) sample container; (b) vacuum pump, Welch 1402 or equivalent; (c) 1000-ml suction flask; (d) 2000-ml suction flask; (e) Coors #3 Buchner funnel; (f) siphon tube and collector column with stopcock (22 mm i.d. × 300 mm); (g) metal stopcock; (h) ¾-in. galvanized tee; (i) ¾-in. × 12 in. threaded galvanized pipe; (j) vacuum gauge; and (k) cutoff valve.

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Gas Chromatography. A Tracor MT-220 gas chromatograph equipped with a Coulson electrolytic conductivity detector (nitrogen mode) was employed for the analysis. The gc column was a 6 ft ¼ in. o.d. glass, cut for off-column injection. A glass trap containing silane-treated glass wool was used in the injection port. The column was packed with 80–100 mesh Gas Chrom Q, coated with 10% DC-200, and conditioned for 72 hr at 235°.

Colorimetric Analysis. A Perkin-Elmer model 202 recording spectrophotometer equipped with an auxiliary recorder and scale expansion accessory was used in the analysis.

Reference Materials and Reagents. Analytical standards of diphenamid were supplied by the Upjohn Co., Kalamazoo, Mich., trifluralin by Eli Lilly and Co., Indianapolis, Ind., and paraquat by Chevron Chemical Co., Ortho Division, Richmond, Calif. Sodium sulfate was reagent grade anhydrous and all solvents were distilled-in-glass (Burdick & Jackson Laboratories, Inc., Muskegon, Mich.). The ion exchange resin used for paraquat extraction was AG-50W ×8, 100–200 mesh, H^+ form (Bio-Rad Laboratories, Richmond, Calif.).

EXPERIMENTAL SECTION

Refer to Figure 2 for a schematic diagram of the procedures involved in the extraction and analyses.

Sample Handling. The following analytical procedure assumes a minimum sample volume of 2000 ml containing 10 g or more of sediment. Calcium chloride was added to the samples in the field to aid in flocculation and its presence had no apparent effect on residue extraction.

Trifluralin is very difficult to analyze under production-line conditions because it is extremely volatile and readily degraded by ultraviolet radiation (Probst and Tepe, 1969); however, samples refrigerated in small-mouth amber glass jugs with Teflon-lined caps for 48 hr showed no apparent residue loss.

Sediment-Water Separation. After the sediment settled under refrigeration for a minimum of 12 hr, the sample jug was marked for determination of the initial sample volume and carefully moved to the separation rack so the sediment layer was undisturbed (Figure 1). A tared Whatman no. 42 (15.0 cm) filter paper was wetted, placed in a Buchner funnel (e), and the siphoning action started by blowing into the priming tube. When the collector column (f) filled to 3–4 in., it was capped with the siphon tube stopper, the sample flow started dropwise, and the vacuum adjusted so the filtrate did not degas. The siphon tube was adjusted periodically to protrude just below the sam-

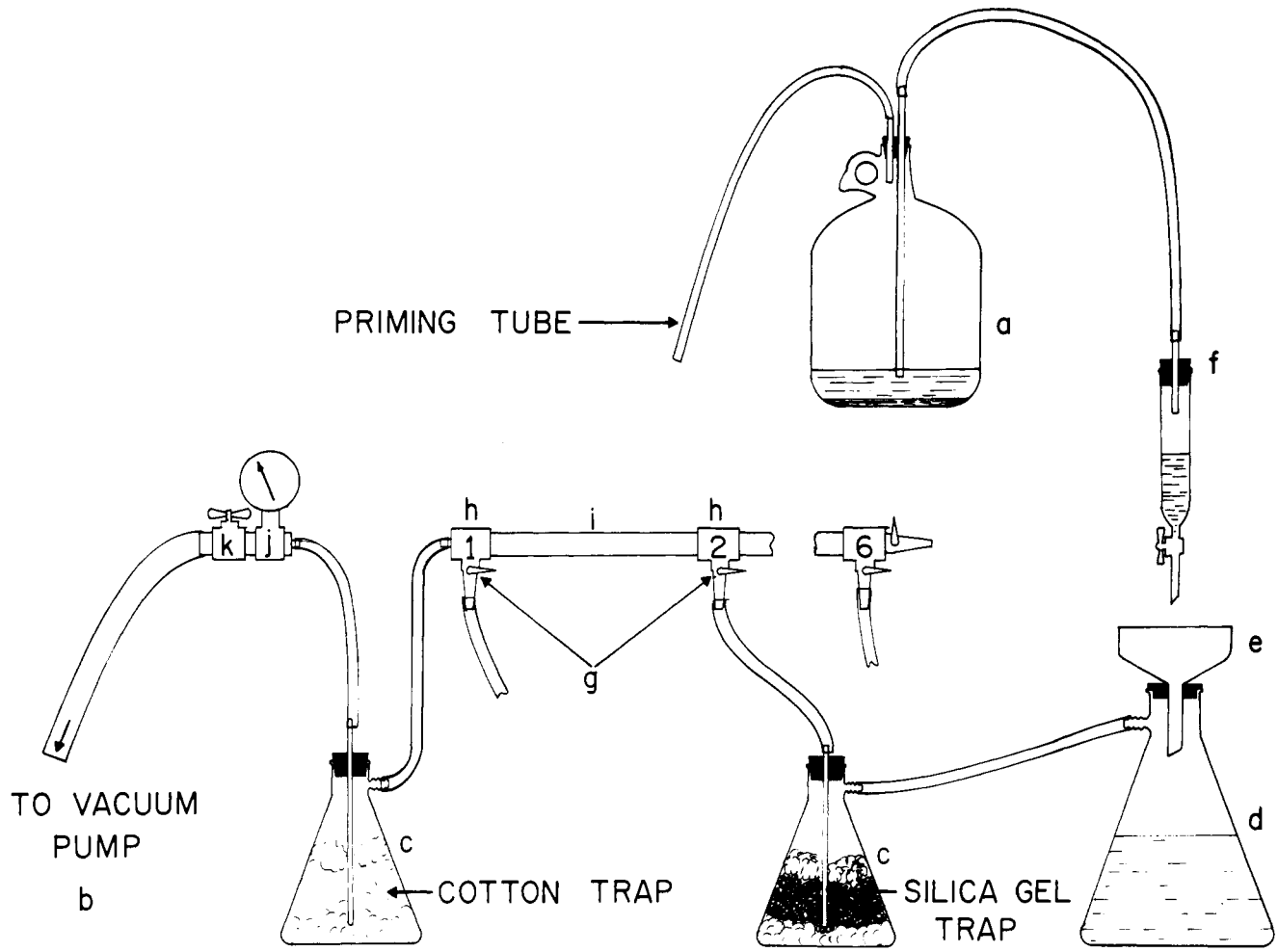

Figure 1. Sediment–water separation apparatus.

Table I. Recoveries of Trifluralin, Diphenamid, and Paraquat from Spiked Water, Soil, and Simulated Runoff

	% recoveries from spiked water											
	Trifluralin, ppb				Diphenamid, ppb				Paraquat, ppm			
	0.5	1.0	5.0	10.0	0.5	1.0	5.0	10.0	0.1	0.5	1.0	2.0
Avg	82	91	91	91	94	90	92	95	83	84	90	94
Std dev	±3.6	±2.7	±6.1	±2.6	±1.6	±6.0	±1.0	±5.2	±5.0	±3.5	±4.7	±3.2

	% recoveries from spiked soil							
	Trifluralin, ppm		Diphenamid, ppm		Paraquat, ppm			
	0.05	1.0	0.15	1.0	0.5	1.0	2.5	5.0
Avg	86	94	96	95	95	99	97	91
Std dev	±3.1	±2.9	±2.3	±4.6	±2.5	±6.7	±1.9	±3.2

	% recoveries from spiked simulated runoff						
	Trifluralin, ppb		Diphenamid, ppb		Paraquat, ppm		
	5.0	10.0	5.0	10.0	1.0	2.5	5.0
Avg	84	82	97	95	107	94	94
Std dev	±2.3	±2.1	±4.9	±1.6	±2.6	±4.5	±3.1

ple water level because the filtering rate slows considerably when the tube penetrates the sediment layer.

The first 500 ml of filtrate was discarded, the next 1000 ml taken for trifluralin and diphenamid analysis, and the next 500 ml for paraquat analysis. When the siphon tube reached the bottom of the jug and siphoning ceased, the tube and column were rinsed with distilled water onto the filter paper. The sediment remaining in the jug was then transferred to the filter paper with distilled water, which flushed most of the residual sample water from the sediment cake. The wash volume was held to a minimum so the adsorbed pesticide-sediment equilibrium was not dis-

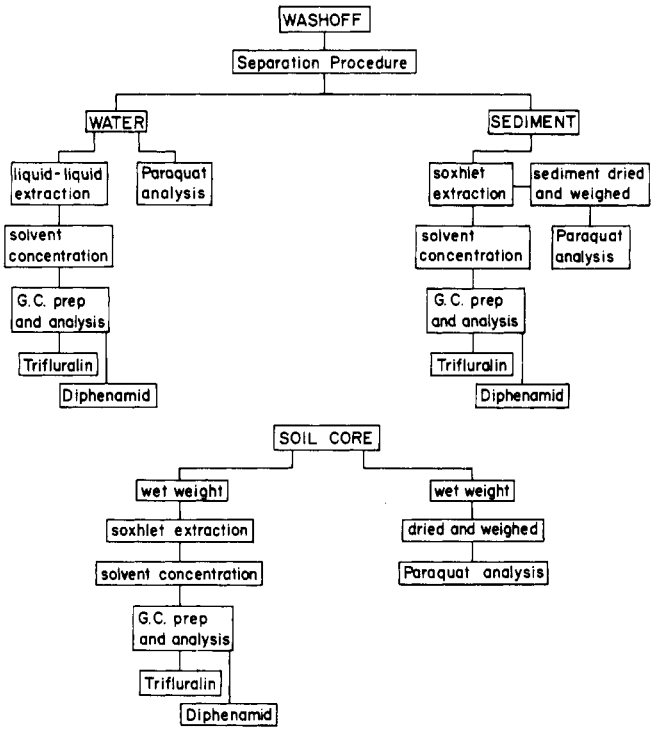

Figure 2. Analysis flow chart.

turbed. Suction was discontinued when the final wash water had seeped into the filter cake. The sample jug was then filled to the mark and the initial sample volume recorded.

Trifluralin and Diphenamid Analysis. *Sediment Extraction.* The damp sediment and filter were transferred to a Soxhlet extractor and refluxed for 4 hr with methylene chloride, which selectively extracts trifluralin and diphenamid from paraquat. The extract was then transferred to a beaker for the solvent concentration step. When sediments were not extracted immediately after separation, they were held in a freezer. No herbicide loss was noted for controls held frozen for 3 days. The extracted sediment and filter were transferred to a beaker for paraquat analysis.

Water Extraction. The 1000-ml portion of filtrate was transferred to a 2000-ml separatory funnel with 100 ml of methylene chloride and shaken vigorously for 2 min. The sample was extracted three more times with 50-ml portions of solvent and the extracts were combined in a beaker for sample concentration.

Soil Core Extraction. Each core sample was thoroughly mixed and two 25-g portions were taken. One portion was weighed onto a tared Whatman no. 42 (15.0 cm) filter paper, placed in a Soxhlet extractor, and refluxed 4 hr with methylene chloride to extract trifluralin and diphenamid. This extract was transferred to a beaker for solvent concentration. When immediate concentration of the extracts was not possible, the beakers were covered with aluminum foil and held under refrigeration for a maximum of 48 hr.

The other portion was weighed into a tared aluminum pan (Fisher no. 8-732) and dried to constant weight at 105° for determination of the moisture content. The dried sample was then analyzed for paraquat.

Solvent Concentration. The beakers containing the water, soil, and sediment extracts were placed on a water bath (~40°), a few boiling chips were added, and the sample was concentrated to 7.5–10 ml. The samples were cooled and transferred to 15-ml conical test tubes. If gas chromatographic analysis was to be delayed, the tubes were capped with corks wrapped in aluminum foil and refrigerated. The tubes were placed in small beakers filled with tepid (~21°) water and evaporated with cool, dry air to 0.5–1.0 ml. This small volume of solvent was evaporated by hand, using a squeeze bulb and a drawn glass tube. One milliliter of benzene was immediately added to the tube; however, this initial volume was usually adjusted, depending upon the sample concentration.

Gas Chromatographic Analysis. The following conditions were used. Temperature: injection port, 230°; oven, 220°; transfer line, 240°; block, 240°; furnace, 820°. Gases: helium (carrier) 100 ml/min; hydrogen, 80 ml/min.

Under the conditions described, trifluralin eluted in approximately 2 min and diphenamid in 6 min. Minimum detectable amounts at an attenuation of 1× were 5 ng for trifluralin and 20 ng for diphenamid.

Trifluralin and diphenamid concentrations were calculated by comparing sample peak heights to those of appropriate standards. Calibration curves were run daily and were linear over the range 5–35 ng for trifluralin and 20–120 ng for diphenamid.

Paraquat Analysis. Filtered runoff water and dried sediment and core samples were analyzed by the method of Pope and Benner.

A portion of the filtered runoff water was analyzed directly without prior concentration or cleanup.

The methylene chloride extracted sediments and core samples were dried to a constant weight at 105°. Sediments of 25 g or more were mixed thoroughly and a portion was taken for analysis. Sediments less than 25 g were used in their entirety, including the filter.

RESULTS AND DISCUSSION

Recovery experiments were run by adding known amounts of the pesticides to soil core, water, sediment, and runoff samples and extracting them as described. Simulated runoff samples contained 10.0 g of soil in 3000 ml of water, together with 15 g of $CaCl_2$. Table I presents the recovery data.

The sediment loss on the overall procedure was less than 0.1%, based on a 10-g sample. When the sediment weight was less than 25 g, the entire sample was extracted to eliminate the loss of "fines" (silt and clay particles) embedded in the filter fibers and also to eliminate the

mixing required to obtain a representative portion from a small sample.

Acetone and chloroform were effective extracting solvents for trifluralin and diphenamid in soils and sediments. However, the high boiling points of these solvents (56.6° and 61°) caused trifluralin losses as great as 70% during evaporation. Methylene chloride with a boiling point of 40° effectively extracted trifluralin and diphenamid but not paraquat from the soil. Sample concentration and final solvent evaporation procedures did not result in trifluralin loss.

These data were determined using Cecil soil and sediment samples. Extraction efficiencies can vary for other soil types, particularly those of high organic content, and longer extraction periods may be required for efficient pesticide recovery.

The use of the Coulson detector in the nitrogen mode, while not as responsive to trifluralin as the electron capture detector, permitted simultaneous detection of both trifluralin and diphenamid. Time-consuming cleanup procedures were eliminated because the Coulson detector was not as responsive to extraneous extracted material as were the electron capture and flame ionization detectors.

Although two separate instruments are used for the final determinations, the method integrates separation and extraction techniques so that all three herbicides may be measured in the same sediment or soil sample. This is especially important for runoff samples from cropped areas, since sediment load usually decreases as the crop canopy increases.

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Herbicide Residues in Air-Cured Burley Tobacco

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Mature cured leaves from three stalk positions of tobacco (*Nicotiana tabacum*, cv. Ky 14) treated in the field with *N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine (benefin), *N,N*-dimethyl-2,2-diphenylacetamide (diphenamid), or *S*-propylbutylethylthiocarbamate (pebulate) were analyzed for herbicide residues using glc. Benefin was detected in trace amounts (2 to 4 ng/g of dry wt) in the upper leaves from treated plants. Slightly higher amounts occurred in leaves from the bottom of the plant. Diphenamid was detected in amounts as high as 0.16 μg/g of dry wt. The highest levels were from leaves taken from the middle portion of treated plants. Two peaks consistently appeared in chromatographs from treated plants that were not present in the chromatographic record of untreated (control) plants. One of the peaks was tentatively identified as *N*-methyl-2,2-diphenylacetamide. Pebulate was not present in the leaves from treated plants at the lower limit of detection (0.02 μg/g of dry wt).

N-Butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine (benefin), *N,N*-dimethyl-2,2-diphenylacetamide (diphenamid), and *S*-propylbutylethylthiocarbamate (pebulate) are three herbicides registered for use on burley (*Nicotiana tabacum* L.) tobacco. They are applied at planting and are persistent in the soil throughout the growing season, probably remaining available for continued uptake by the tobacco plant. Both diphenamid and pebulate are readily absorbed by many of the crops in which they are used (Bingham and Shaver, 1971; Fang and Fallin, 1965). While residues of these herbicides are not found in significant amounts in the fruits or edible portions of most crops (Fang and Fallin, 1965; Golab *et al.*, 1970; Lemin, 1966), they may be retained in some nonedible portions (*e.g.*, leaves, roots). The present study was initiated to determine if residues were present in mature, air-cured leaves of tobacco plants grown in soils treated with the above-mentioned herbicides.

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MATERIALS AND METHODS

Plant Material and Treatment. Burley tobacco (*Nicotiana tabacum* L. cv. Ky 14) was planted June 7, 1971, in rows 96.5 cm apart in soil fertilized with 165 kg/ha of N. Herbicide treatments were 1.65 and 3.3 kg/ha of benefin and 4.4 and 8.8 kg/ha of pebulate applied preplant and incorporated into the soil approximately 5 cm deep. Diphenamid at 6.6 and 13.2 kg/ha was applied immediately after transplanting tobacco seedlings to the field. The lower rate is the recommended rate of application for each of the three herbicides. Tobacco was also transplanted into untreated soil. Experimental design was randomized complete block with four replications.

When the plants reached maturity they were harvested, cured, stripped, and graded. Leaf samples were collected from the top, middle, and bottom stalk position on the plant. The leaf samples were dried at 43° for 96 hr and