too low for detection by present methods. We are currently in the process of developing satisfactory analytical methods for these compounds in tissues. Even with a dose of T-2 toxin approaching that which will cause emesis in swine, the resulting radioactive levels in the edible tissues are very small.

LITERATURE CITED

Bamburg, J. R., Marasas, W. F., Riggs, N. V., Smalley, E. B., Strong, F. M., *Biotech. Bioeng.* **10**, 445 (1968).
Bamburg, J. R., Strong, F. M., Smalley, E. B., *J. Agric. Food Chem.* **17**, 443 (1969).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., Burmeister, H. R., *Poultry Sci.* **56**, 103 (1977a).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., *Poultry Sci.* **56**, 306 (1977b).
Chi, M. S., Mirocha, C. J., Kurtz, H. J., Weaver, G., Bates, F., Shimoda, W., *Poultry Sci.* **56**, 628 (1977c).
Chi, M. S., Robison, T. S., Mirocha, C. J., Reddy, K. R., *App. Environ. Microbiol.* **35**, 636 (1978a).
Chi, M. S., Robison, T. S., Mirocha, C. J., Swanson, S. P., Shimoda, W., *Toxicol. Appl. Pharmacol.* **45**, 391 (1978b).
Gilgan, M. W., Smalley, E. B., Strong, F. M., *Arch. Biochem. Biophys.* **114**, 1 (1966).
Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., Kosuri, N. R., Nichols, R. E., *J. Argic. Food Chem.* **18**, 734 (1970).
Hsu, I.-C., Smalley, E. B., Strong, F. M., Ribelin, W. E., *Appl. Microbiol.* **24**, 684 (1972).
Kosuri, N. R., Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., Nichols, R. E., *J. Am. Vet. Med. Assoc.* **157**, 938 (1970).
Petrie L., Robb, J., Stewart, A. F., *Vet. Rec.* **101**, 326 (1977).
Smalley, E. B., Strong, F. M., "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, New York, 1974, pp 199–228.
Wallace, E. M., Pathre, S. V., Mirocha, C. J., Robison, T. S., Fenton, S. W., *J. Agric. Food Chem.* **25**, 836 (1977).
Weaver, G. A., Kurtz, H. J., Mirocha, C. J., Bates, F. Y., Behrens, J. C., Robison, T. S., Gipp, W. F., *Can. Vet. J.* **19**, 72 (1978a).
Weaver, G. A., Kurtz, H. J., Mirocha, C. J., Bates, F. Y., Behrens, J. C., Robison, T. S., *Can. Vet. J.* **19**, 310 (1978b).
Weaver, G. A., Kurtz, H. J., Bates, F. Y., Chi, M. S., Mirocha, C. J., Behrens, J. C., Robison, T. S., *Vet. Rec.* **103**, 531 (1978c).

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Simultaneous Extraction and Analysis of Trifluralin and Nitrofen Residues in Crucifers

A very sensitive analytical method has been developed for determination of trifluralin and nitrofen residues in kohlrabi, radish, rutabaga, and turnip. The method utilizes simultaneous extraction and purification of both herbicides and detection by electron-capture gas chromatography. Recoveries were greater than 90% at fortification levels of 10–40 ppb.

Application of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) and nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether) is an effective treatment for selectively controlling various weeds in kohlrabi, radish, rutabaga, and turnip. Since residue data were not available for registration of this use of herbicides, it was necessary to develop a residue method for quantifying chemical residues in these crops.

Trifluralin and nitrofen are readily detectable in the picogram range by electron-capture gas chromatography. Thus the primary objective of the method development was to provide a single extraction and cleanup procedure which would be suitable for both herbicides.

Simultaneous extraction of more than one pesticide residue has been applied to soil residues of trifluralin and three other herbicides (Smith, 1974) and to various herbicide and insecticide residues in crop plants (Luke et al., 1975). The foregoing methods were not employed because of the necessity of handling toxic solvent or because of the more time consuming procedures required in a screening method. Thus the present paper describes a simplified method for the simultaneous extraction, purification, and analysis of trifluralin and nitrofen residues in crucifer crops.

MATERIALS AND METHODS

Sample Extraction. In order to obtain a representative sample, crucifer tubers were diced into small pieces weighing 2–3 g. After thorough mixing, a 25-g sample of the pieces was macerated with methanol in a food blender. The resulting slurry was poured into a Soxhlet thimble and extracted overnight in a Soxhlet extractor with methanol. After cooling, the extract was concentrated to about 50 mL on a rotary evaporator and poured into a 1-L separatory funnel. A 100-mL portion of hexane was used to rinse the residue in the evaporator flask into the separatory funnel.

Partial purification of the extract was achieved by solvent partitioning. Thus 750 mL of 0.1 M KOH containing 5% KCl was shaken with the hexane–methanol extract. After removal of the hexane phase, the aqueous methanol was reextracted with two additional 100-mL portions of hexane. The combined hexane extracts were boiled down to 25 mL on a steam bath.

Silica Gel Cleanup. Purification of soil extracts was not necessary for trifluralin analysis using the electrolytic conductivity detector (Payne et al., 1974). However, preliminary work on crucifers showed cleanup was necessary for analysis on the electron-capture detector. Silica gel (60–200 mesh, Bakers Analyzed Reagent) was activated by heating overnight at 110 °C and deactivated by the addition of 4.6% water. The cleanup column was prepared by adding 4 g of silica gel to a 1 × 20 cm chromatography column containing a glass wool plug and a column of hexane. The column was wet packed to prevent entrapment of air bubbles.

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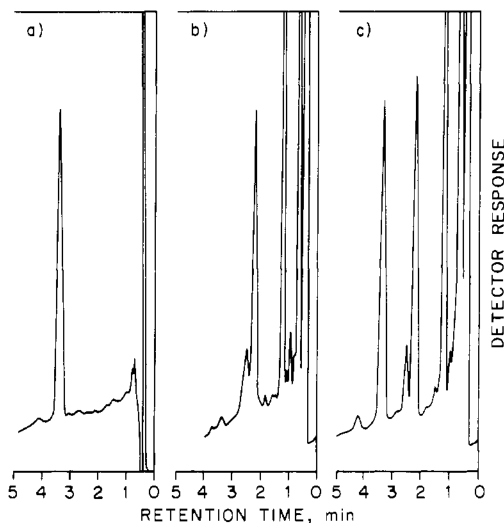

Figure 1. Chromatogram a, 40 pg of trifluralin; chromatogram b, control rutabaga, 5 μL/25 mL of extract; chromatogram c, 20 ppb fortification of control rutabaga, 2 μL/25 mL of extract.

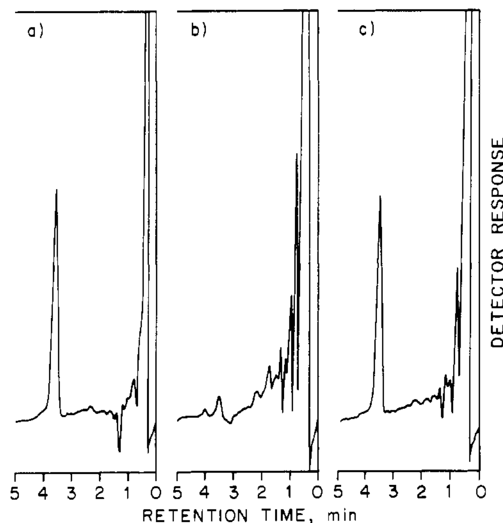

Figure 2. Chromatogram a, 20 pg of nitrofen; chromatogram b, control rutabaga, 4 μL/25 mL of extract; chromatogram c, 20 ppb fortification of control rutabaga, 1 μL/25 mL of extract.

When the excess hexane had dropped to the top of the silica gel, the 25 mL of hexane extract was poured onto the column. After passage of the extract, the column was washed with 25 mL of hexane. The initial 50 mL of hexane was discarded and trifluralin was eluted with 100 mL of hexane. Nitrofen was eluted with 125 mL of 5% benzene in hexane. The extracts were concentrated to 25 mL on a steam bath and analyzed by gas chromatography.

Fortification. Recovery of herbicides was determined by adding an acetone solution of both chemicals to the pieces of tuber prior to blending. The acetone was allowed to evaporate before adding methanol. Fortification levels ranged from 10 to 40 ppb.

Gas Chromatography. GLC analyses were performed on a Varian 2100 gas chromatograph equipped with a scandium EC detector. The 1.65 m x 2 mm i.d. glass column was packed with low level Carbowax 20M on 100/120 mesh Chromosorb W (Aue et al., 1973). The flow rate of nitrogen carrier gas was 15 mL/min. At a column temperature of 145 °C, the retention time of trifluralin was about 3.5 min. The retention time of nitrofen was also about 3.5 min at a column temperature of 220 °C. At an attentuation of 32×, a 40-pg injection of either chemical gave about 50% full-scale deflection. Injection size varied from 1 to 5 μL. Appropriate dilutions were made when the herbicide gave more than 50% full-scale deflection at an attenuation of 32×.

RESULTS AND DISCUSSION

Only a minimal amount of background was carried through the purification procedure. In the trifluralin analysis, only 2 of 35 untreated samples contained more than 2 ppb of "apparent" trifluralin. Background values of nitrofen were a little higher, but did not interfere with detection of significant residues of the herbicide. Most interference values were at or below 5 ppb.

The recovery of both compounds was very good and was not significantly different between crops or between fortification levels of 10–40 ppb. The average recovery of trifluralin was 95% with a standard deviation of 6%. Average recovery and standard deviation of nitrofen was 93% and 7%, respectively. The recoveries were corrected for background interference on an individual basis rather than subtracting the average interference of all controls.

The good recovery of trifluralin during evaporation of hexane extracts appears to be at variance with the results of Payne et al. (1974). They encountered significant losses upon evaporating acetone or chloroform solutions of trifluralin. Although hexane's boiling point is comparable to chloroform and acetone, we did not experience any significant loss of trifluralin. This was probably due to the fact that their method involved concentration of the extracts to a very low volume (1 mL).

Typical chromatograms of standards, control samples and recoveries are shown in Figure 1 and 2. In both sets of chromatograms the recovery was about 95%. The background values for both herbicides was about 1 ppb.

Trifluralin residues were quite low in crucifers from plots treated with 0.85 kg/ha, preplant incorporation. Residue levels ranged from less than 2 ppb up to 15 ppb. Use of nitrofen, applied preemergence at 6.7 kg/ha or postemergence at 4.5 kg/ha, resulted in somewhat higher residues. Maximum nitrofen residues in kohlrabi and rutabaga were less than 100 ppb. However, the maximum residue in turnips was a little over 500 ppb, and some radish samples contained over 1 ppm. The higher residues in radish were probably due to the rapid, early-season development of this plant when soil residues were highest.

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LITERATURE CITED

Aue, W. A., Hastings, C. R., Kapila, S., *J. Chromatogr.* **77**, 299 (1973).

Luke, M. A., Froeberg, J. E., Masumoto, H. T., *J. Assoc. Off. Anal. Chem.* **58**, 1020 (1975).

Payne, W. R., Pope, J. D. Benner, J. E., *J. Agric. Food Chem.* **22**, 79 (1974).

Smith, A. E., *J. chromatogr.* **97**, 103 (1974).

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