effect in preventing the washing off of carbaryl from the wettable powder-treated bean leaves when skinner-line irrigation was used to simulate rain. Since the amount of carbaryl found on the plant leaves 8 days after application nearly equaled the initial deposits, both with and without adjuvant present, greenhouse conditions may not be severe enough to demonstrate the adjuvant's merit as an extender of pesticide lifetime in the field.

A common problem in making sensitive measurements of fluorescence for the analysis of pesticides is the difficulty in removing interfering materials that absorb the excitation radiation or the emitted fluorescence or fluoresce strongly themselves. For example, when carbaryl is to be determined by fluorescence in samples of honeybees or in alfalfa, the amount of such materials must be reduced by column chromatographic cleanup (Argauer *et al.*, 1970). Also, with fruit and vegetables, steam distillation is used as a cleanup step (Ott *et al.*, 1971). A method developed for carbaryl in spray formulations that contained additional pesticides required only sample dilution for analysis, since the effect of strong absorption of incident radiation by interferences in the formulations was eliminated (diluted out) by a 10,000-fold or greater dilution by adjusting the concentration of carbaryl to less than a 1 ppm before the measurement of fluorescence (Argauer and Bontoyan, 1970). The normal time that elapses from addition of the sodium hydroxide solution to 20 vials to the subsequent reading of the fluorescence intensity is about 30 min. No column chromatography or extensive chemical or mechanical procedures are required.

The method described is particularly well-suited to studies of the effects produced by other adjuvants and of the distribution characteristics and efficiencies of spray equipment in field studies where the effects of wind, morning dew, transpiration, and other factors should be greater than those existing in a greenhouse.

LITERATURE CITED

Argauer, R. J., Bontoyan, W., *J. Ass. Offic. Anal. Chem.* **53,** 1166 (1970).
Argauer, R. J., Shimanuki, H., Alvarez, C. C., J. AGR. FOOD CHEM. **18,** 688 (1970).
Blazquez, C. H., Vidyarthi, A. D., Sheehan, T. D., Bennett, M. J., McGrew, G. T., J. AGR. FOOD CHEM. **18,** 681 (1970).
Ott, D. E., Ittig, M., Friestad, H. O., *J. Ass. Offic. Anal. Chem.* **54,** 160 (1971).
Smilowitz, Z., Dewey, J. E., *J. Econ. Entomol.* **62,** 1347 (1969).

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An Improved Gas Chromatographic Method for the Analysis of 5,6-Dichloro-2-trifluoromethylbenzimidazole in Soil

An improved method was developed for the extraction of 5,6-dichloro-2-trifluoromethylbenzimidazole (NC-2983) from soil. This chemical is a major degradation product in the soil of the acaricide fenazaflor, 5,6-dichloro-1-phenoxycarbonyl-2-trifluoromethylbenzimidazole (Lovozal). NC-2983 was extracted with a mixture of methylene chloride and isopropyl alcohol. Interfering soil materials were removed by means of alkaline saline washing of the organic phase. Electron capture gas chromatography employing a 3% OV-17 column was used to determine the quantity of NC-2983 in the final sample preparations. This modified procedure is faster and simpler to perform than a more entailed acidic methanolic extraction procedure, but is equal to it in sensitivity and reliability.

Lovozal is the trade name of the new acaricide which has 5,6-dichloro-1-phenoxycarbonyl-2-trifluoromethylbenzimidazole as its active ingredient in commercial formulations. Fenazaflor is the accepted common name for this chemical. It is a nonsystemic acaricide which is highly effective in controlling all stages of phytophagous mites, including eggs, and organophosphorus-resistant strains (Saggers and Clark, 1967). Fenazaflor hydrolyzes rapidly in moist soil to 5,6-dichloro-2-trifluoromethylbenzimidazole (NC-2983), phenol, and carbon dioxide. Thus within about 24 hr after the acaricide comes into contact with moist soil the major residual product thereafter is NC-2983 (Fisons, 1968). Since this acaricide has shown potential usefulness for the control of European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus urticae* (Koch), on apples (Asquith, 1968), it became of interest to study the persistence of fenazaflor's major degradation product, NC-2983, in orchard soils.

Crofts and Whiteoak (1969) developed two equally effective methods for the analysis of NC-2983 in soil. One method involved a time-consuming extraction from the soil with acidified methanol and a subsequent alkaline digestion and cleanup procedure. In the second method NC-2983 was extracted with a mixture of methylene chloride and isopropyl alcohol, and interfering soil constituents were removed by washing the extract with water. In both methods quantification was achieved by electron capture gas chromatography using SE-30 or OV-7 columns. The latter method, though much more

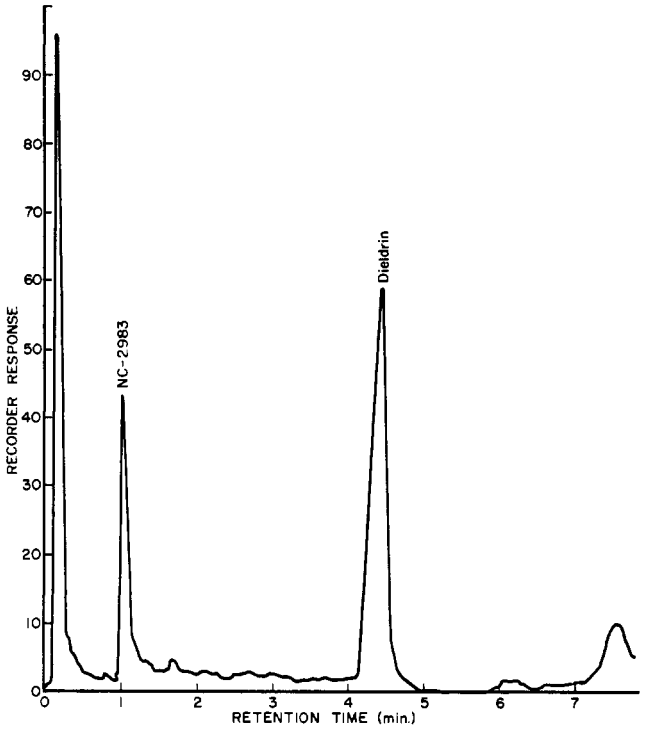

Figure 1. Glc tracing of 0.32 nl/μl of NC-2983 in an extract of field soil diluted 50,000× in benzene and fortified with 0.25 ng/μl of dieldrin as an internal standard

convenient to use for the analysis of NC-2983 in soil, was objectionable because of unpredictable emulsion formations. This study was made, therefore, to improve the reliability and efficiency of the simpler method of extraction of NC-2983 from soil with the co-solvent methylene chloride and isopropyl alcohol.

EXPERIMENTAL PROCEDURE

Extraction. Twenty grams of thoroughly air-dried soil were weighed into a 250-ml Erlenmeyer flask to which 50 ml of a mixture of methylene chloride and isopropyl alcohol (3:1) were added by means of a Re-pipet (Labindustries, Berkeley, California). The flask was closed with a Teflon encapsulated rubber stopper (Chemical Rubber Co., Cleveland, Ohio) and, after being shaken for 20 min on a Burrell Wrist-Action Shaker, the supernatant was filtered through prefolded Whatman No. 510 filter paper into a 250-ml separatory funnel. The soil was reextracted a second time in the same manner. The second supernatant was recovered after filtration into the same separatory funnel, and the soil remaining in the flask was shaken manually with an additional 20 ml of the extraction solvent. The supernatant was collected after filtration into the separatory funnel, and the extraction with 20 ml of solvent was repeated once more. Twenty milliliters of saturated sodium chloride were next added to the separatory funnel, followed by the addition of 30 ml of water and 10 drops of 2 *N* ammonium hydroxide. The funnel was then shaken three times for periods of 1 min and allowed to stand for 2 hr to permit the complete separation of the aqueous and organic phases. The organic phase was then collected into a 250-ml Erlenmeyer flask which contained approximately 10 g of anhydrous sodium sulfate. The aqueous solution remaining in the funnel was extracted twice more with 25-ml portions of methylene chloride, and the organic phases were pooled with the original fraction.

Table I. Recovery of NC-2983 by Modified Methylene Chloride Isopropyl Alcohol Extraction Method from Soil Fortified with Varying Amounts of Chemical

ppm of NC-2983 added	Number of analyses	Percent NC-2983 recovered
0.5	12	95 ± 5
1.0	15	96 ± 8
1.5	4	96 ± 2
2.0	11	96 ± 8
3.0	4	97 ± 8
4.0	11	100 ± 8

The solvent was next separated from the sodium sulfate by filtering through E&D No. 615 filter paper, collecting the eluate into a dry 250-ml Erlenmeyer flask. The flask was rinsed with 25 ml of methylene chloride, and after this washing was collected through the filter, an additional 15 ml of methylene chloride were passed through the residue in the filter. The sodium sulfate residue in the filter was pressed with a small vial to remove the last traces of solvent from it. The filtrate was then evaporated to dryness on a hot plate at 40°C and under a gentle stream of filtered compressed air. After all of the solvent was removed, the residue in the flask was taken up with 10.0 ml of benzene and then transferred into an appropriately marked vial and sealed with an aluminum-lined cap. An aliquot of this solution was subsequently diluted with benzene, to which dieldrin was added as an internal standard, to yield samples containing approximately 0.1 to 0.40 ng/μl of NC-2983 and exactly 0.25 ng/μl of dieldrin. All sample dilutions were made with Eppendorf microliter pipets (Brinkmann Instruments Inc., Westbury, N. Y.).

Determination. The amount of NC-2983 in the extract was determined by injecting a 1.0-μl "plug" of the final diluted samples into a gas chromatograph with a series 7005N Hamilton syringe under the conditions given below. Instrument: Microtek Model 220 equipped with a ^{63}Ni detector operated at 30 V DC mode. Recorder: Honeywell Model 9348W strip chart, 1 mV. Output attenuation: 4. Input attenuation: 10^2. Chart speed: 1 in./min. Column: Glass, 6 ft × 1/8-in. i.d., packed with 3% OV-17 on silanized 80–100 mesh Supelcoport. Injection temperature: 250°C. Column temperature: 219°C. Dectector temperature: 275°C. Carrier gas: Nitrogen at 70 ml/min. Purge gas: Nitrogen at 20 ml/min. Retention time of NC-2983: 65 sec. Retention time of NC-2983 relative to dieldrin: 0.28.

A standard curve was plotted each day, using standards containing 0.01, 0.05, 0.25, 0.50, and 1.00 ng/μl of NC-2983, each having been fortified with 0.25 ng/μl of dieldrin. A linear relationship was obtained when the peak heights of NC-2983 were plotted for concentrations of NC-2983 up to 0.50 ng/μl.

$$\frac{\text{Pk. Ht.}_{\text{(NC-2983 sample)}}}{\text{Pk. Ht.}_{\text{(dieldrin sample)}}} \times \frac{\text{Pk. Ht.}_{\text{(dieldrin, standard)}}}{\text{Pk. Ht.}_{\text{(NC-2983, standard)}}} \times \text{ng}/\mu\text{l}_{\text{(NC-2983 standard)}} \times \text{Dilution factor} \div \text{Sample weight, mg} = \text{ppm}$$

RESULTS AND DISCUSSION

The limit of sensitivity of this method when gas chromatographing a 1.0-μl aliquot representing 20 g of soil sample in 20.0 ml of final solution was 0.01 ppm. Injection of larger volumes of extract or more concentrated extracts to increase detectability would have entailed a commensurate increase in

Table II. Comparative Recovery of NC-2983 from Samples of Field-Treated Soil by Two Different Analytical Procedures

Sample	ppm Recovered: MeOH/HCl method[a]	MeOH/HCl method[b]	Modified method[c,d]
1	1.22	1.16	1.10
2	0.91	0.89	0.92
3	1.73	1.84	1.96
4	2.10	1.94	2.01
5	1.78	1.91	2.04
6	3.66	3.84	3.66
7	4.14	4.40	4.33
8	3.83	3.68	3.89
9	0.92	0.98	0.92
10	0.94	0.91	0.87
11	1.66	1.83	2.00
12	2.67	2.50	2.46
13	4.07	3.93	3.75
14	4.24	3.88	3.76

[a] Analyzed by the Fisons Agrochemical Division, Chesterford Park, England, using methanol:HCl extraction procedure. [b] Analyzed by the Pesticide Research Laboratory, Pennsylvania State University, using methanol:HCl extraction procedure. [c] Analyzed by the Pesticide Research Laboratory, Pennsylvania State University, using modified methylene chloride:isopropyl alcohol extraction procedure. [d] $t_{0.05} = 2.160$.

deterioration of the column and contamination of the detector, since these extracts were subjected to a minimum of cleanup. For this reason, if a large number of samples are to be analyzed, 1/20,000th of the final soil extract (equivalent to 1 mg of soil) is the maximum amount which can be injected repetitively into the glc column with loss of efficiency.

The recovery of NC-2983 from control soil samples which had been fortified with various amounts of the chemical, from 0.5 to 4.0 ppm, ranged from 88 to 108% (Table I). The average recovery value for 57 fortified samples was 97%.

Figure 1 is a tracing of the glc recorder response of 1.0 μl of a benzene solution, diluted 50,000×, and fortified with 0.25 ng/μl of dieldrin as an internal standard, of the residue extracted from 20 g of field soil containing 0.80 ppm of NC-2983. Gas chromatograms of numerous control samples of untreated soil did not show any significant peaks (peak heights less than 0.5 cm) which interfered with that of NC-2983.

The effectiveness of this modified procedure was determined by comparing the results obtained with it to those obtained in our and another laboratory using the more lengthy methanol extraction procedure on split samples from field-treated plots. An analysis of the data in Table II by the Student's t-distribution test revealed no significant difference at the 5% level for the 14 samples analyzed by the two different methods, thus demonstrating that this modified method is as efficient as the more exhaustive methanolic method for the analysis of NC-2983 residues in soil. This modified method, therefore, can be used effectively to analyze field samples of soil for residues of NC-2983 because it overcomes the limitations of emulsion formation often experienced in the original method as proposed by Crofts and Whiteoak (1969). It is comparable in sensitivity and reliability to the more entailed methanolic procedure and has the added advantage that it is considerably faster and simpler to conduct.

LITERATURE CITED

Asquith, D., *J. Econ. Entomol.* **61,** 1044 (1968).
Crofts, M., Whiteoak, R. J., Chesterford Park Research Station, Nr. Saffron Walden, Essex, England, private communication, 1969.
Fisons Ltd., Chesterford Park Research Station, Nr. Saffron Walden, Essex, England, unpublished data, 1968.
Saggers, D. T., Clark, M. L., *Nature* **215,** 275 (1967).

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Correction

EVALUATION OF THE PROTEIN QUALITY OF MILLED RICES DIFFERING IN PROTEIN CONTENT

In this article by Ricardo Bressani, Luiz G. Elias, and Bienvenido O. Juliano [J. AGR. FOOD CHEM. **19**(5), 1028 (1971)], the title incorrectly appeared as "Evaluation of the Protein Quality *and* Milled Rices Differing in Protein Content."