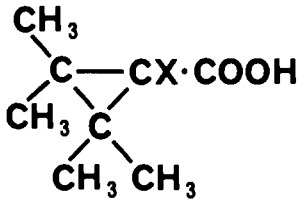

Figure 1. Structure of tetramethylcyclopropanecarboxylic acid, X being H, ^2H, or ^3H

acids they investigated, the reactions were complete in a reasonably short time when conducted in a stainless steel bomb at 150°C. However, even at this elevated temperature, the α proton in cyclopropanecarboxylic acid was not exchanged to a satisfactory degree. In the study reported here, it was found that the exchange of deuterium or tritium for the α proton in a tetramethylcyclopropanecarboxylic acid takes place to a considerable extent in a relatively short time when the reaction temperature is raised to 200°C by using refluxing ethylene glycol as solvent for the base. These convenient conditions may be suitable for deuterium or tritium exchange of protons in other organic acids.

To confirm the course of the reaction, tetramethylcyclopropanecarboxylic acid was treated repeatedly with deuterium oxide under the conditions above and the nmr spectra of the deuterated and undeuterated acids were compared. In pyridine [but not in deuterochloroform or carbon tetrachloride (Barlow *et al.*, 1971)] the signal from the C-1 proton is clearly separate from the signals due to the methyl groups and after deuteration had an intensity relative to them only one-fifth as great as that of the corresponding signal in the undeuterated acid. Further, in the mass spectrum of the undeuterated acid, peaks from M^+ and $M^+ - CH_3$ appeared at 142 and 127. In the deuterated preparation, the corresponding peaks were at 143 and 128, respectively. The relative peak intensities corresponded to a content of 79% of a species in which one proton had been replaced by deuterium. This evidence from the nmr and mass spectra showed that only the C-1 proton is displaced under the reaction conditions, so that, with tritium oxide, an acid labeled specifically at this position would be obtained.

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LITERATURE CITED

Atkinson, J. G., Csakvary, J. J., Herbert, G. T., Stuart, R. S., *J. Amer. Chem. Soc.* **90,** 499 (1968).
Barlow, F., Elliott, M., Farnham, A. W., Hadaway, A. B., Janes, N. F., Needham, P. H., Wickham, J. C., *Pestic. Sci.* **2,** 115 (1971).
Berteau, P. E., Casida, J. E., J. AGR. FOOD CHEM. **17,** 931 (1969).
Elliott, M., *J. Chem. Soc.* 5225 (1964).
Elliott, M., *Bull. Wld. Health Org.* **44,** 315 (1971).
Elliott, M., Casida, J. E., J. AGR. FOOD CHEM. in press (1972).
Elliott, M., Janes, N. F., Pearson, B. C., *J. Chem. Soc. (C)* 2251 (1971).
Fieser, L. F., Fieser, M., "Reagents for Organic Synthesis," Wiley, New York, N.Y., 1967, p 1158.
Maciver, D. R., *Pyrethrum Post* **9**(4), 41 (1968).
Matsui, M., Kitahara, T., *Agr. Biol. Chem.* **31,** 1143 (1967).
Mescheryakov, A. P., Dolgii, I. E., *Izv. Akad. Nauk. SSSR, Otd. Khim. Nauk.* 631 (1960); *Chem. Abstr.* **54,** 24436 (1960).

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Rapid Fluorometric Evaluation of the Deposition and Persistence of Carbaryl in the Presence of an Adjuvant on Bean and Tomato Leaves

A rapid analytical procedure for determining carbaryl on the leaves of bean and tomato plants was developed and used to evaluate the effectiveness of an adjuvant, β-pinene polymer, in spray formulations. Small aliquots of methylene chloride extracts of treated leaves were placed into glass vials and the solvent was allowed to evaporate. Sodium hydroxide solution was then added to hydrolyze carbaryl into 1-naphthol, which is leached out into the clear aqueous solution for measurement of the fluorescence intensity. No significant difference was found in the initial deposit (~1000 ppm) or the chemical lifetime of carbaryl when the spray did or did not contain this adjuvant. The method may readily be applied to other studies with carbaryl.

Stickers, surfactants, and other adjuvants are sometimes added to spray formulations to increase the effectiveness of pesticides on sprayed plants. This method of regulating the rate of loss of a pesticide on sprayed plants could make control less expensive for the farmer by requiring the use of less pesticide. Thus, an adjuvant that increases the effectiveness of degradable organophosphorus and carbamate pesticides would be highly desirable. A comprehensive study of the effect of 74 adjuvants in prolonging the toxicity of two organophosphorus compounds on lima bean foliage was reported by Smilowitz and Dewey (1969), who used a bicassay of two insect species to make their determinations.

Carbaryl, a widely used carbamate insecticide, is relatively insoluble in water and is usually applied as a wettable powder in aqueous spray formulations. Blazquez *et al.* (1970) added an adjuvant, a β-pinene polymer, to this spray mix and reported that it increased the initial deposit of carbaryl on tomato leaves sevenfold and the life of the compound three-

fold; this conclusion was based on a visual comparison of the intensity of spots on a thin-layer chromatogram with a cholinesterase inhibition technique. We have now developed a rapid chemical procedure for the analysis of residues of carbaryl ranging between 20 and 1000 ppm on the leaves of bean and tomato plants and believe this method to be more precise.

EXPERIMENTAL

Extraction. Blend 25 g of leaves and 100 g of anhydrous sodium sulfate with 150 ml of methylene chloride (CH_2Cl_2) in a blender for 3 min. Vacuum filter the slurry through Whatman No. 1 paper, and rinse the filter cake with 100 ml of CH_2Cl_2. Much of the CH_2Cl_2 evaporates during this step. Dilute the filtrate to exactly 200 ml, and store in amber-colored glass bottles.

Measurement of Fluorescence. Transfer 100-μl aliquots of the 200-ml solution to two 4-dram vials, and add 50 μl of a 100 μg/ml carbaryl in CH_2Cl_2 standard to one of the vials. (Also, add 50 μl of standard to a clean vial to permit comparison by both the method of standard addition and comparison with a standard.) The small volume of CH_2Cl_2 in these vials evaporates quickly at room temperature. Next, add exactly 10.0 ml of 0.25 *N* aqueous sodium hydroxide to each vial. The dried colored extract adheres to the bottom of the vial as the carbaryl hydrolyzes to yield the sodium salt of 1-naphthol, which is leached out into the clear aqueous sodium hydroxide. Cap and shake each vial several times. Decant a portion of the sodium hydroxide solution into a 1-cm^2 quartz cuvette, and measure the fluorescence intensity at wavelengths of 340 nm excitation and 460 nm emission in a spectrofluorometer. Since the absorbance of the solution is less than 0.05 units at 340 nm, it is sufficiently dilute to permit determination of the fluorescence intensity without further dilution. The excitation and emission spectra and the relationship between the concentration of carbaryl (after hydrolysis to 1-naphthol) and fluorescence intensity has been reported elsewhere (Argauer *et al.*, 1970).

The following equation can be used to calculate the amount of carbaryl present

$$\text{ppm on leaves} = \frac{A}{B-A} \times 5 \times \frac{2000}{25} \cong \frac{A}{C} \times 5 \times \frac{2000}{25}$$

where A = fluorescence intensity of aliquot of sample, B = fluorescence intensity of aliquot to which 5 μg of carbaryl were added, and C = fluorescence intensity of 5 μg standard. For other crops the equation may be modified to take into account the need to use a larger or smaller aliquot of the methylene chloride extract. This equation serves as a check for substances that may coextract into the sodium hydroxide solution and interfere with the determination, particularly when large aliquots are used.

The possibility that the carbaryl hydrolyzes to 1-naphthol on the plant leaves and hence the method would give erroneously high values for the amount of carbaryl analyzed was eliminated as follows. Five-milliliter aliquots of various samples were diluted to 150 ml with CH_2Cl_2 and extracted with 25 ml of 0.25 *N* NaOH to remove free 1-naphthol. None of the aqueous phases exhibited significant fluorescence. Furthermore, when 3-ml aliquots of the CH_2Cl_2 phases were added to vials the solvent evaporated, and the vials were treated as before, substantially the same results were obtained as with the original 100-μl aliquots.

Spray Application. Manapal tomato and Henderson bush lima bean plants were grown in a 50:50 peat moss–vermiculite mixture for study in the greenhouse. The adjuvant studied was Nu-Film 17, a β-pinene polymer, which was applied at a rate of 1 qt per 100 gal per acre.

Two sources of carbaryl, a liquid formulation and an 80% wettable powder, were mixed with water and applied with and without the adjuvant to both top and bottom surfaces of the leaves of the beans and tomato plants when the bean plants were in the first fully expanded trifoliate stage and the tomato plants were in the 8–10 leaf stage. Then at 1 hr and at 1, 2, 4, 8, and 16 days after the application, duplicate samples, about 20 leaves per sample, were picked at random from each plot and stored at −18°C prior to analysis.

Recoveries of carbaryl from samples fortified in the blender prior to extraction at levels of 100, 500, and 1000 ppm were 90–110%.

Table I. Carbaryl (ppm) on Greenhouse Manapal Tomato and Lima Bean Leaves

Time lapse after application	Liquid formulation		Wettable powder	
	Without adjuvant	With adjuvant	Without adjuvant	With adjuvant
	(1.56 lb active/acre) Tomato leaves		(1.5 lb active/acre)	
1 hr	1050	1055	1250	1210
1 day	1040	770	1010	1150
2 days	1260	880	1220	1210
4 days	980	920	1190	1050
8 days	750	910	800	960
16 days	940	805	797	800
Rain simulated on 2nd day[a]				
4 days	474	790	540	780
8 days	344	790	310	770
16 days	386	777	376	796
Check	5	...	5	...
	(1.56 lb active/acre) Lima bean leaves		(1.0 lb active/acre)	
1 hr	874	1010	590	695
1 day	864	870	617	740
2 days	1050	970	620	595
4 days	882	903	660	490
8 days	850	800	522	352
Rain simulated on 2nd day[a]				
4 days	278	618	316	270
8 days	254	700	210	173
Check	4	...	4	...

[a] Liquid formulation: fine spray 10 sec to runoff. WP formulation: skinner line irrigation, 1 cm water/2 hr.

RESULTS AND DISCUSSION

Table I gives the results obtained when carbaryl was applied to tomato leaves. No significant difference was apparent in the initial deposit when the spray formulation contained the adjuvant and when it did not. The levels of these deposits, both with and without adjuvant, are approximately twice those reported for the field studies with adjuvant and nearly 14 times the field studies without adjuvant (Blazquez *et al.*, 1970) and may indicate that we have obtained superior spray coverage. After the samples were taken on day 2, half the remaining leaves were sprayed on each side for 1 sec with a fine stream of water, and the adjuvant appeared to aid somewhat in the retention of carbaryl with this simulation of mild rain. The results obtained with bean leaves in most part paralleled those obtained with tomato leaves. No significant increase was apparent in the initial deposit or in the lifetime of the chemical when the adjuvant was included. The adjuvant had little

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