Pesticide Residue Analysis in Foods by NMR. 3. Comparison of ^{19}F NMR and GC-ECD for Analyzing Trifluralin Residues in Field-Grown Carrots

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Carrots were grown in trifluralin-treated soil and sampled over a 6-week period beginning 6 weeks after seeding. The residue of pesticide and principal metabolite in the carrot was measured by both ^{19}F NMR and GC-ECD. The trifluralin concentration was observed to decline from 0.3 to 0.03 mg/kg. Although there was excellent agreement between the two methods at all levels for the parent compound, in general, only the GC method was sensitive enough to measure the low concentrations of the metabolite.

Keywords: *Trifluralin; NMR; residue; pesticide; analysis; GC-ECD; carrots; metabolite; cleanup; foods; extract*

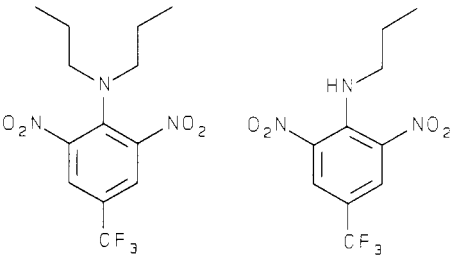
INTRODUCTION

We have previously demonstrated the feasibility of using NMR for determining organophosphorus and fluorine-containing pesticides at the trace level (≤1 mg/kg) in spiked food samples (Mortimer and Dawson, 1991a,b). At the time, the lack of samples known to us to contain a pesticide residue incurred during cultivation prevented us from demonstrating this technique in a real application. We have now completed a field trial in which carrots were grown in soil treated with the pre-emergent herbicide, trifluralin (**I**) (see Figure 1). As this pesticide contains a trifluoromethyl group, we knew from our previous work that the limit of detection (LOD) in the NMR would be ~0.01 mg/kg using the extract from 25 g of sample and 30 min of instrument time. In addition, the principal metabolite **II** formed in carrots (Probst et al., 1967) and roughly 5% of the trifluralin residue also contains the trifluoromethyl group and would have a similar LOD. As the maximum allowed level (MRL) of trifluralin residue in the carrot is 0.5 mg/kg in Canada, the gap between the MRL and the expected LOD provides a good range in which to demonstrate the value of the NMR technique to pesticide residue analysis. The purpose of this brief paper is to compare the results from the analyses of these field-grown carrots by NMR with those by GC-ECD.

EXPERIMENTAL PROCEDURES

Pesticide Standards. A sample of the metabolite of trifluralin, α,α,α-trifluoro-2,6-dinitro-*N*-(1-propyl)-*p*-toluidine, was generously donated by DowElanco.

Field Trial. Two plots of sandy loam soil, 20 × 50 ft, at the Ottawa Research Station, Agriculture Canada, were seeded with carrots (Imperator variety) on May 26, 1993. Twelve days prior to seeding, one of these plots had been sprayed with Treflan (trifluralin, 545 g/L ai) at the recommended application rate of 1.5 L/hta and the top 3 in. of soil tilled. Composite samples of soil were taken from both plots at the time of seeding and again at the end of sampling, and soil moisture was determined by oven drying of subsamples at 110 °C overnight. Carrot samples were taken from both plots at 41, 48, 55, 63, 70, and 77 days after seeding. The foliage was removed with the top 2–3 mm of the root and discarded, and then the root was rubbed by hand under flowing tap water until free of soil. The total carrot sample was then chopped in a Waring blender and sampled in duplicate (50 g) prior to storage at −20 °C.



I II

Figure 1. Structures of trifluralin (**I**) and its metabolite (**II**).

Preparation of Soil Extracts. Soil (100 g, oven-dry equivalent) was soaked in methanol (200 mL) for 48 h, filtered, and then soaked again in methanol (100 mL) for 5 h before a second filtering. The combined methanol filtrates were evaporated to the aqueous residue under reduced pressure at 40 °C. Sodium chloride-saturated phosphate buffer (pH 7, 20 mL) was added to this residue and the mixture extracted with ethyl acetate (20 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and taken to dryness under reduced pressure at 40 °C. The residue was taken up in 5.0 mL of 5% ethyl acetate in hexane, and an aliquot (0.25 mL) was prepared for GC analysis as outlined for carrots below. The remaining solution was evaporated to dryness under N_2 and the residue dissolved in 1.0 mL of $CDCl_3$ containing 4.0 mg of chromium(III) acetylacetonate and 37.6 μg of the internal standard, 4-(trifluoromethoxy)acetanilide, for ^{19}F NMR analysis (Mortimer and Dawson, 1991b).

Preparation of Carrot Extracts. A thawed sample of carrot (50 g, in duplicate) was homogenized with acetone (100 mL) and filtered through two Whatman No. 7 filter papers. The pad of carrot fiber and the top filter paper were homogenized again with acetone (100 mL) and then filtered through the bottom filter paper. The pad of carrot was rinsed with acetone (20 mL × 2), and the combined filtrates were transferred to a separatory funnel with NaCl (4 g) and CH_2Cl_2–

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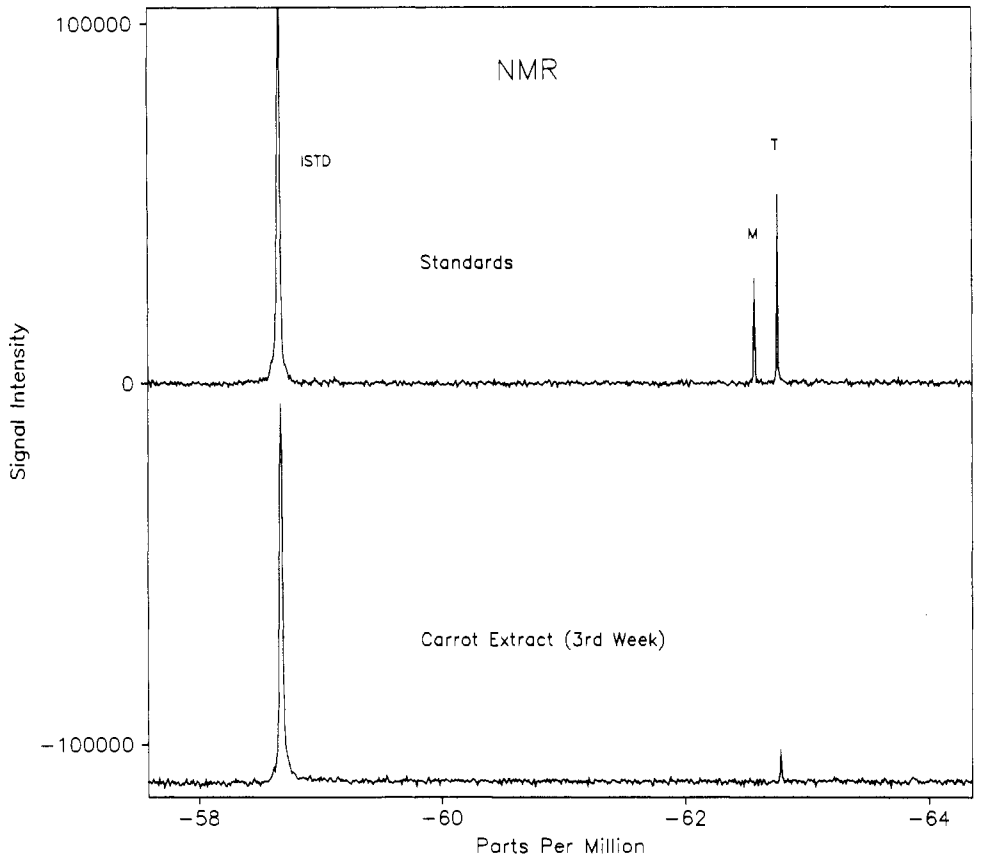

Figure 2. Comparison of ^{19}F-NMR spectra of a standard of 5.4 μg of trifluralin (T) and 2.8 μg of metabolite (M) and of a carrot extract from the treated plot on the third week of sampling [ISTD is 4-(trifluoromethoxy)acetanilide, 37.6 μg].

hexane (400 mL, 1:1). The mixture was shaken until the salt dissolved, and then the organic layer was separated and dried over anhydrous sodium sulfate. The extract was filtered and split into two equal parts. One part was evaporated to dryness under reduced pressure at 40 °C and the residue dissolved in $CDCl_3$ solution as above for NMR analysis (the final volume was adjusted to 1.0 mL under N_2). The other part was evaporated to dryness and the residue dissolved in 5% ethyl acetate in hexane to give a final volume of 5.0 mL. An aliquot (0.25 mL) was applied to a minicolumn of silica (500 mg, Davisil, grade 643, 200–425 mesh, 4% moisture, Aldrich) and eluted with the same solvent. When the sharp, orange band was ~5 mm from the bottom of the silica column, eluent was collected for a total of 1 mL. This fraction was then diluted to 5.0 mL and used for GC analysis.

Standard Solutions. A stock solution of trifluralin (2.17 mg) and its metabolite (1.13 mg) was prepared in 2.0 mL of $CDCl_3$. NMR standard solutions were prepared by transferring 1, 2, 5, 10, and 20 μL of stock solution to individual vials, evaporating the solvent under a gentle flow of N_2 at room temperature, and dissolving the residue in 1.0 mL of $CDCl_3$ solution (see Preparation of Soil Extracts). For GC, an aliquot of the stock solution (2 μL) was first diluted to 10.0 mL with 5% ethyl acetate in hexane and then 20-, 40-, 100-, 200-, and 1000-μL aliquots were transferred to 1.0-mL volumetric flasks and made up to the mark with the same solvent.

NMR Analysis. NMR spectra were acquired as reported previously (Mortimer and Dawson, 1991b) except that the standard Bruker library of software was updated to version DISRVK01 (spectral width, 71428 Hz; 2256 scans; pulse width, 5.3 μs; no delay time; acquisition time, 0.115 s; pulse angle, 90°; resolution, 2.18 Hz/point; 16K data points; zero-filled to 64K and broadband proton decoupling). The signals for trifluralin, its metabolite, and the internal standard appeared at −62.77, −62.58, and −58.67 ppm, respectively, relative to an external reference of $CFCl_3$ (see Figure 2).

GC Analysis. GC analyses were done on a HP5890 Series II GC with an on-column injector equipped with a retention gap (1 m, 0.53 mm i.d.) and an electron capture detector set at 325 °C and He carrier gas flow set at 1.0 mL/min. The DB-5 capillary column (30 m, 0.25 mm i.d., 0.25-μm film, J&W Scientific) was attached to the retention gap by a deactivated press-fit connector. After injection, the initial oven temperature of 60 °C was maintained for 0.5 min then raised to 150 °C at 25 °C/min and held at this temperature for 18 min. The injector temperature was initially 63 °C and was raised at the same rate as the column temperature to 153 °C. Trifluralin and its metabolite eluted at 18.7 and 17.9 min, respectively (see Figure 3). Sample volume injected was 1 μL.

RESULTS AND DISCUSSION

Treflan herbicide, reportedly 545 g/L, was measured by NMR as 603 g/L using the extrapolated calibration curve and a diluted Treflan solution. No dealkylated metabolite signal (≤1%) was found in the NMR spectra of the commercial herbicide.

In the concentration ranges of interest, 1.08–21.7 μg/mL for trifluralin and 0.65–11.3 μg/mL for the metabolite, the NMR calibration curves were excellent linear fits for the two standards; the correlation coefficients were 0.9999 and 0.9997, respectively. On the other hand, the GC-ECD calibration curves were typically shallow curves better fit by a second-order polynomial than a linear equation. With such a polynomial, the correlation coefficients were 0.9998 and 0.9999 for trifluralin and the metabolite, respectively.

A partial cleanup of the carrot extracts, sufficient to remove polar extractives, was effected by using a minicolumn of Davisil silica. The slightly deactivated surface prevented the trifluralin and the metabolite from separating on elution and conveniently kept them together with the visible, orange carotene band. Studies with standard solutions had shown that recovery from the column was quantitative. The resulting chromato-

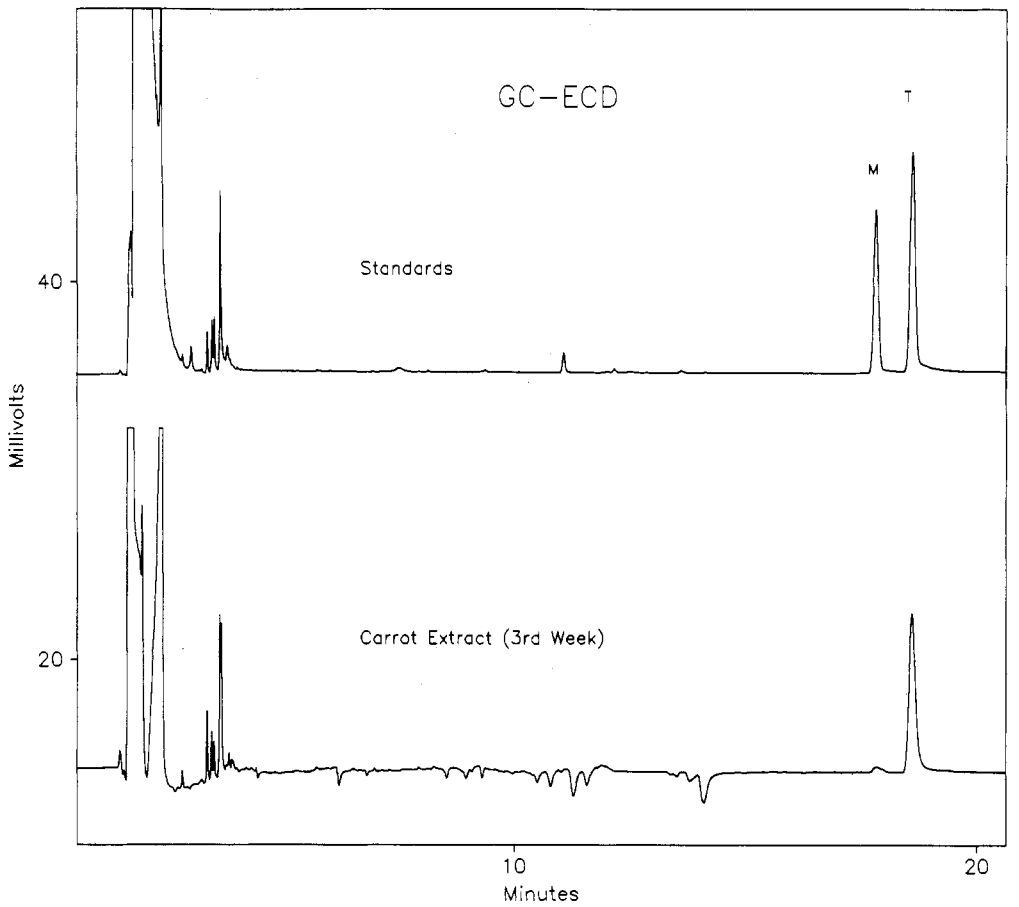

Figure 3. Comparison of gas chromatograms of a standard of 109 pg of trifluralin (T) and 57 pg of metabolite (M) and of a carrot extract from the treated plot on the third week of sampling.

Table 1. Comparison of Results from NMR and GC Analyses (S/N ≥ 3)

	trifluralin, ppm		metabolite, ppm	
sample[a]	NMR	GC-ECD	NMR	GC-ECD
soil, control, 1	0.040	0.037	nd[b]	nd
soil, treated, 1	0.389	0.288	nd	0.004
soil, control, 2	0.029, 0.025	0.029	nd	nd
soil, treated, 2	0.529, 0.488	0.514	0.015, 0.013[d]	0.016
control carrot, 1	i.s.[c]	0.014	nd	nd
treated carrot, 1	0.324	0.306, 0.281	nd	0.005, 0.004
control carrot, 2	nd	0.005, 0.006	nd	nd
treated carrot, 2	0.185	0.186, 0.185	nd	0.004, 0.004
control carrot, 3	nd	0.004, 0.004	nd	nd
treated carrot, 3	0.087, 0.092	0.081, 0.080	nd	0.005
control carrot, 4	nd	0.003	nd	nd
treated carrot, 4	0.069, 0.068	0.062, 0.061	nd	0.006
control carrot, 5	nd	0.007	nd	nd
treated carrot, 5	0.037, 0.035	0.040, 0.041	nd	0.003
control carrot, 6	nd	0.004	nd	nd
treated carrot, 6	0.024, 0.027[d]	0.025, 0.025	nd	nd

[a] Number beside carrot entry refers to week of sampling. [b] nd, not detected. [c] i.s., insufficient sample. [d] S/N = 2–2.6.

grams showed very few peaks (see Figure 3) on the electron-capture detector. In spite of this apparent cleanliness, the peak heights of the two analytes soon deteriorated after a few injections. The metabolite, in particular, was very sensitive to accumulated material on the retention gap. Not only did the peak width broaden but the symmetry of the peak became skewed. As a result, frequent changes of the retention gap and a strategy of alternately injecting standard and sample were needed to obtain reliable results.

At a signal-to-noise ratio (S/N) of 3 (peak-to-peak), the limits of detection for trifluralin and its metabolite in the NMR were 0.60 and 0.53 μg/mL, respectively. In contrast, the GC-ECD limits of detection were 0.6 and 1.1 pg/μL, respectively. This 3 orders of magnitude greater sensitivity of ECD over NMR is compensated in part by the much larger sample that can be used in the NMR detector. As a result, in the carrot samples, the limit of detection was 0.024 mg/kg for trifluralin by NMR and 0.002 mg/kg by GC-ECD. The LOD for the NMR is higher than that achieved before (Mortimer and Dawson, 1991b) because this time the extract was taken up in 1.0 mL of solvent for convenience, whereas previously we had used 0.5 mL (twice the concentration). In principle, the LOD for the NMR could be reduced by increasing the pulsing time (set at 30 min), but this is a matter of compromise. Likewise, the sample size is limited by the amount of extractives. As

the volume of extracted material from soil was very small, the limit of detection could have been lowered significantly by using a larger sample of soil. For example, the S/N for the trifluralin peak (0.025 mg/kg) in the second control soil sample was 10 (see Table 1) with 100 g of soil.

Table 1 lists the measured values for the soil and carrot extracts for both trifluralin and its metabolite using both NMR and GC-ECD (see Figures 2 and 3 for representative GC and NMR results from a carrot extract). The correlation of results from the two techniques was excellent (the GC and NMR operators performed the two analyses independently). The NMR was, in general, unable to analyze the metabolite because of its very low concentration. In the second treated soil sample the signal corresponding to the metabolite peak was evident, but the signal-to-noise ratio was only 2.5. In contrast, the metabolite was observed by GC-ECD in many of the treated, carrot samples. Quantitation of the GC results at very low concentrations, however, was made less accurate in the case of the carrots by the error in the calibration curve (~0.004 mg/kg). A second calibration curve, at a lower concentration range, would have been necessary to accurately quantify these low concentrations of metabolite.

The difference in trifluralin concentration in the two soil samples is more likely to reflect the variation in the trifluralin distribution in the soil rather than any variation in the analysis. We were surprised to find trifluralin in the control soil as the field records had shown no Treflan use in this plot for the previous 4 years. The residue appears to be longer lived in this particular soil (or more easily extracted) than in other soils that have been used for degradation studies (Gerwing and McKercher, 1992; Golab et al., 1979), where less than 1.5% of the originally applied trifluralin was extractable with methanol after 3 years.

In summary, ^{19}F NMR is a valuable addition to the arsenal of techniques available for determining certain pesticide residues in foods at very low concentrations (<1 ppm). Its most significant advantage is the elimination of the need to clean up extracts prior to analysis and, therefore, ^{19}F NMR will be most useful in those samples where cleanup is difficult.

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