Using ^{19}F NMR for Trace Analysis of Fluorinated Pesticides in Food Products

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Fluorine nuclear magnetic resonance spectroscopy is sufficiently sensitive for the analysis of fluorinated pesticides in food sample extracts at the low parts per billion level without manipulation of large samples and lengthy instrument time. This sensitivity also permits the direct analysis of fluorine-containing compounds in liquid food products such as vegetable oil and wine at ≥1.0 mg/L with a 5 mm diameter probe.

INTRODUCTION

Roughly 10% of the several hundred pesticides listed in the *Agricultural Handbook* (2nd ed., Royal Society of Chemistry, 1987) contain fluorine, and it is increasingly common to find this atom in more recently synthesized structures. In Canada, five fluorinated chemicals, fluazifop-butyl, trifluralin, flamprop-methyl, diflubenzuron, and ethalfluralin, are registered for control of weeds and insects on a wide variety of food produce. Trifluralin, for example, is commonly used on the prairies to control broad-leaved weeds and wild oats in oilseed and cereal crops (Darwent, 1990). Although the maximum residue level for fluazifop-butyl in strawberries and soyabeans is 1 mg/kg and for trifluralin in carrots is 0.5 mg/kg, the residue level for the majority of the applications is set at the negligible residue level of 0.1 mg/kg.

Mazzola et al. (1984) examined the feasibility of using nuclear magnetic resonance spectroscopy (NMR) for the analysis of fluorinated pesticides in foods. With the instrumentation available to them at the time, they found that relatively large samples (100 g) and long instrument times (8 h) were required to analyze residues at the 0.1 mg/kg level. Recently, we demonstrated that it was feasible to use NMR for the screening of organophosphorus insecticides on cole crops at residue levels down to 0.1 mg/kg (Mortimer and Dawson, 1991) with only 30 min of instrument time, albeit with a more powerful instrument than that available to Mazzola et al. As the receptivity of the fluorine nucleus is 12.5 times greater than that of phosphorus, it should be possible to measure residue levels of fluorinated pesticides at ≤0.01 mg/kg under similar conditions. In addition, as many fluorinated pesticides contain multiple equivalent fluorine atoms, e.g., the trifluoromethyl group in trifluralin and in the insecticide bifenthrin, a further sensitivity gain would be expected in these compounds. This level of sensitivity even raises the possibility of analyzing pesticides such as trifluralin *directly* in liquids and eliminating time-consuming procedures like extraction and chromatographic cleanup.

The purpose of this paper was to establish the minimum detectable level of representative fluorinated pesticides in some food products while maintaining a convenient sample size (25 g) and an instrument time period comparable to that of a GC analysis (30 min). In addition, the factors that influence sensitivity in a NMR analysis are discussed.

EXPERIMENTAL PROCEDURES

NMR Analysis. All ^{19}F NMR spectra were obtained on a Bruker AM400 (9.4 T) spectrometer operating at 376.50 MHz with a dedicated 5-mm $^{19}F/^{1}H$ probe and a ^{19}F-specific preamplifier. A Bruker 400 MHz band-pass filter and a 376.5-MHz band-stop filter were used in the proton decoupler channel, and a 376.5-MHz band-pass filter and a 400-MHz band-stop filter were used in the fluorine observe channel for all acquisitions. Proton decoupling was achieved by using low power (composite phase decoupling). Relaxation times were measured by using microprograms from the standard Bruker library (version DISR89). Chemical shifts are reported relative to trichlorofluoromethane ($CFCl_3$) at 0.00 ppm. Spectra were acquired with 16K data points, processed with 5-Hz line broadening, and zero-filled to 64K.

Pesticide Standards. Fluorinated pesticides were taken from the collection of analytical standards (purities typically ≥96%) maintained in the Food Research Division of the Health Protection Branch (stored at −20 °C).

Analytical Solutions. A stock solution was prepared by dissolving 14.17 mg of trifluralin, 12.69 mg of bifenthrin, 25.73 mg of diflubenzuron, and 44.25 mg of flamprop-methyl in 10 mL of $CDCl_3$ (MSD Isotopes, Pt. Claire, PQ). An aliquot (100 μL) was diluted to 10 mL in $CDCl_3$ and, then, serially diluted by transferring 5.0 mL to a 10-mL volumetric flask and filling to the mark with $CDCl_3$. An aliquot (3.0 mL) of each serial dilution and 1.0 mL of $CDCl_3$ containing 0.375 mg of trifluoromethoxy acetanilide (Aldrich, Milwaukee, WI), the internal standard, was transferred to a 5.0-mL volumetric flask which was then filled to the mark with $CDCl_3$.

Food Extracts. Thawed frozen peas (50 g) and diced fresh carrot (50 g) were each homogenized in 100 mL of acetone and partitioned with methylene chloride according to Steinwandter's (1985) procedure. Ground soyabean (10 g) was first soaked in water (45 mL) for 45 min at room temperature and then homogenized and extracted as above. The resulting extracts were evaporated to near dryness under vacuum at 37 °C and the residues taken up in 25 mL of methylene chloride; these solutions were dried with anhydrous sodium sulfate. After filtration, the extracts were made up to 40 mL, split into two equal parts, and evaporated just to dryness under vacuum. The residues were redissolved in 0.4 mL of $CDCl_3$ and 0.1 mL of $CDCl_3$ containing 37.5 μg of trifluoromethoxy acetanilide. One of each pair of extracts was spiked with 0.5 μL of the pesticide stock solution. For a 25-g sample, the spike represented 0.025, 0.028, 0.051, and 0.088 mg/kg of bifenthrin, trifluralin, diflubenzuron, and flamprop-methyl, respectively.

RESULTS AND DISCUSSION

Chemical Shift Range. Figure 1 includes 11 structures that are representative of the fluorine-substituted functional groups that are encountered in fluorinated pesti-

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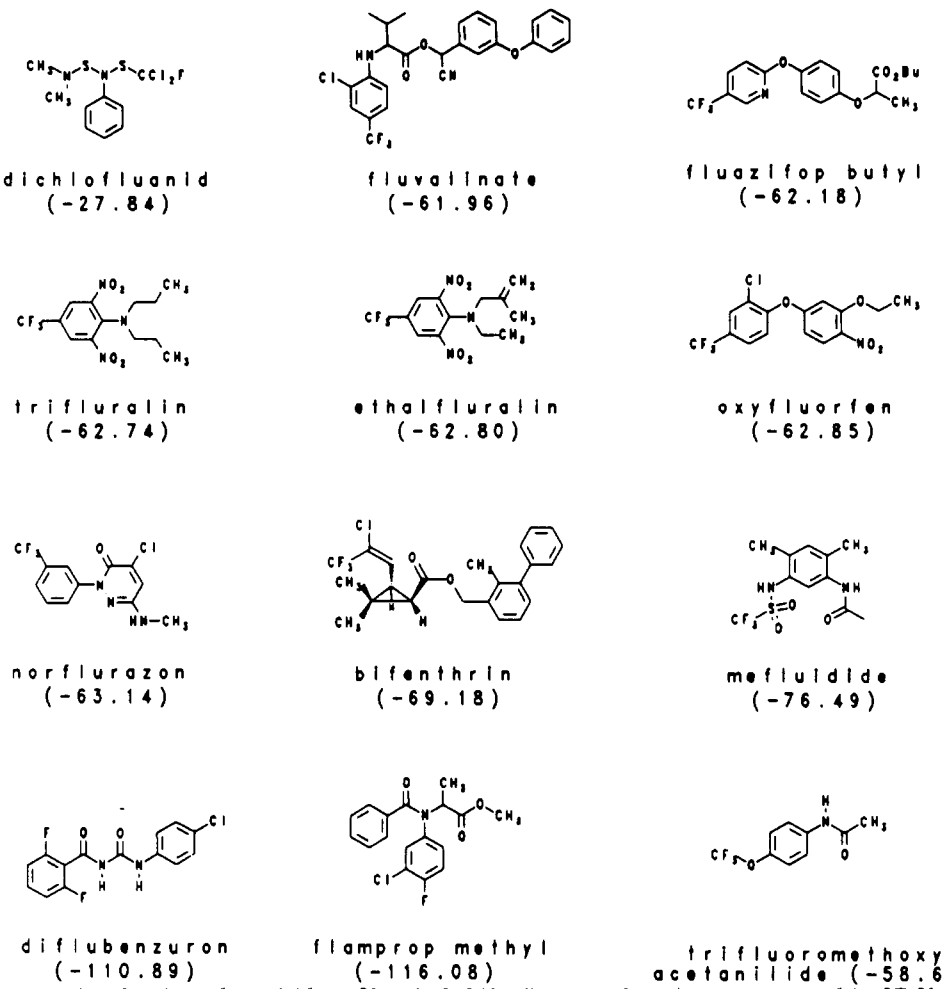

Figure 1. Representative fluorinated pesticides. Chemical shifts (in parentheses) were measured in $CDCl_3$ containing an internal reference ($CFCl_3$, 0.0 ppm). The negative sign indicates a signal that is upfield from the reference.

cides. Their chemical shift range is nearly 90 ppm. Although the trifluoromethylaryl examples are clustered in a much narrower range (1.2 ppm), on the 400-MHz (9.4-T) instrument, this is a 450-Hz window which provides plenty of space for peaks whose width at half-height is typically 2 Hz. For example, trifluralin and ethalfluralin, whose chemical shifts differ by only 0.06 ppm, are baseline-separated peaks.

Relaxation Times. Signal intensity in an NMR experiment is, in part, proportional to the number of accumulated scans. The number of scans that can be taken in a unit time is dependent on the relaxation time (T_1), which is proportional to the time needed to re-establish equilibrium in the spin states after each pulse (ca. $5T_1$). This relaxation can be accelerated by the addition of paramagnetic compounds to the sample. We have found chromium acetylacetonate [$Cr(AcAc)_3$] to be an effective relaxation agent for the fluorinated pesticides. The T_1 values of trifluralin, diflubenzuron, flamprop-methyl, and trifluoromethoxy acetanilide in $CDCl_3$ were reduced from 1.64, 1.71, 2.14, and 2.45 to 0.31, 0.18, 0.20, and 0.13 s, respectively, when 2 mg of $Cr(AcAc)_3$ was added to the sample (0.5 mL). The T_1 values decreased further when more relaxation agent was added; however, the signals broadened unacceptably. For example, the width at half-height for the internal standard increased from 2 to 4 to 7.2 Hz when 1.6 and 4.2 mg of $Cr(AcAc)_3$ were added to 0.5 mL of solution.

Mazzola et al. (1984) did not add a relaxation agent but instead used a shorter pulse width (the Ernst angle, 40°) to reduce the time to recover spin equilibrium. Although this maximized the available scan time (0.5 s/scan), it reduced the potential signal intensity per scan by 36%. The shorter relaxation times brought about by $Cr(AcAc)_3$ allowed us to reduce the repetition time to 0.28 s and use a 90° pulse for better signal intensity. As a result of adding $Cr(AcAc)_3$, we were able to achieve S/N ratios for trifluralin and flamprop-methyl in 30 min equal to those observed in 2 h without the relaxation agent. Because the relaxation times are slightly different for each pesticide, the use of a single pulse width may not provide optimum signal strength for every pesticide but, what is more important, it will provide a reproducible signal for each compound.

As we observed previously with the chemical shift of the organophosphorus insecticides (Mortimer and Dawson, 1991), $Cr(AcAc)_3$ moved the chemical shifts of the fluorinated pesticides downfield in comparison to an external reference (in this case, $CFCl_3$). The magnitude of the change in chemical shifts was linearly related (R values ≥ 0.9998) to the amount of $Cr(AcAc)_3$. When referenced to internal $CFCl_3$, however, no change appeared to have taken place as the magnitudes of the changes were very similar for all the pesticides as well as the internal reference. For example, the slopes of the linear regressions [chemical shift vs $Cr(AcAc)_3$ concentration] for trifluralin, diflubenzuron, and flamprop-methyl were 0.183, 0.189, and 0.189, respectively.

Sensitivity and Quantitation. Trifluoromethoxy acetanilide (Figure 1) was chosen as the internal standard (ISTD) for quantitation studies. It is a neutral, crystalline solid which is commercially available in high purity and

Table I. Concentrations, Peak Ratios, and S/N Ratios for Serially Diluted Pesticide Samples

concn, μg/mL	peak ratio[b]	S/N	concn, μg/mL	peak ratio[b]	S/N
	Trifluralin			Bifenthrin	
8.50	0.0721	45.4	7.61	0.0518	39.2
8.50	0.0709	49.2	7.61	0.0535	39.1
4.25	0.0413	28.4	3.81	0.0297	20.5
4.25	0.0420	27.6	3.81	0.0307	23.6
2.13	0.0197	12.4	1.90	0.0131	8.1
2.13	0.0185	ND	1.90	0.0138	ND
1.06	0.0088	5.1	0.95	0.0064	4.6
1.06	0.0104	6.3	0.95	0.0058	3.0
0.53	0.0049	3.0	0.48	0.0041	2.7
0.53	0.0054	3.3	0.48	0.0042	2.4
	Diflubenzuron			Flamprop-Methyl	
15.4	0.0985	69.5	26.6	0.0726	41.2
15.4	0.0976	68.9	26.6	0.0743	45.6
7.72	0.0502	33.4	13.3	0.0407	24.5
7.72	0.0501	28.9	13.3	0.0418	26.6
3.86	0.0245	12.0	6.64	0.0179	9.3
3.86	0.0260	ND	6.64	0.0173	ND
1.93	0.0120	6.9	3.32	0.0112	4.7
1.93	0.0133	9.8	3.32	0.0091	6.4
0.96	0.0064	4.1	1.66	0.0041	2.3
0.96	0.0061	4.6	1.66	0.0047	3.3

[a] Peak height sample/peak height ISTD.

readily soluble in organic solvents. Its fluorinated functional group (CF_3O) is not presently found in any pesticides, and its fluorine chemical shift (−58.67 ppm) is separate from any others examined.

A series of solutions containing decreasing concentrations of trifluralin, bifenthrin, diflubenzuron, and flamprop-methyl and a fixed concentration of the ISTD and $Cr(AcAc)_3$ were repeatedly scanned for 30 min at 293 K. The calculated ratios (see Table I) of pesticide peak heights and ISTD peak height were plotted against the corresponding concentrations of pesticides. Linear regressions gave R^2 values ≥0.992 for all four pesticides. From these results and assuming a minimum usable sample volume of 0.4 mL, the minimum detectable amounts (S/N_{rms} = 3) under our conditions are approximately 0.2 μg for trifluoromethyl-containing pesticides, 0.3 μg for difluoro pesticides, and 0.7 μg for pesticides with a single fluorine atom. For a 25-g sample, these limits would represent 0.008, 0.012, and 0.028 mg/kg, respectively.

It was also evident from this set of samples that the chemical shift values were independent of concentration. Standard deviations (n = 10) were less than or equal to ±0.003 ppm.

Temperature Effect. Measurement of the chemical shifts of trifluralin, bifenthrin, diflubenzuron, and flamprop-methyl at 20 and 30 °C showed a small upfield shift with temperature, 0.100, 0.006, 0.022, and 0.063 ppm, respectively. As the temperature of the probe is controlled within ±0.05 °C, stray fluctuations in ambient temperature would not affect the chemical shift values.

Solvent Effect. Solvent, however, can affect the chemical shift. Table II lists the chemical shifts of four fluorinated pesticides in a number of deuterated solvents. Severe broadening of the diflubenzuron signal and a corresponding reduction in its S/N ratio were observed in acetone and dimethyl sulfoxide. As no other signals were affected, this broadening may be a result of increased hydrogen bonding in these solvents between one of the two ortho fluorine atoms and the relatively acidic proton of the imide nitrogen. This could give rise to two magnetically nonequivalent fluorine atoms which might be slow to interconvert (on the NMR time scale). If a polar solvent is needed to dissolve extracted material, methanol would be preferred to acetone to avoid this line broadening of the diflubenzuron signal and possibly other like compounds. The magnitude of the change in chemical shift with solvent underlines the importance of running standards and samples in the same solvent when structures are assigned by the chemical shifts.

Table II. Influence of Solvent on Chemical Shift

	chemical shift,[a] ppm			
solvent[b]	flamprop-methyl	diflubenzuron	bifenthrin	trifluralin
$CHCl_3$	−116.06	−110.91	−69.19	−62.75
acetone	−117.90	−113.63[c]	−68.82	−62.33
benzene	−116.50	−111.69	−68.95	−62.41
DMSO	−117.23	−113.43[c]	−67.68	−63.80
methanol	−117.69	−114.48	−69.99	−63.48
pyridine	−116.94	−113.01	−68.66	−62.08

[a] Versus internal $CFCl_3$. [b] Perdeuterated. [c] Broadened badly.

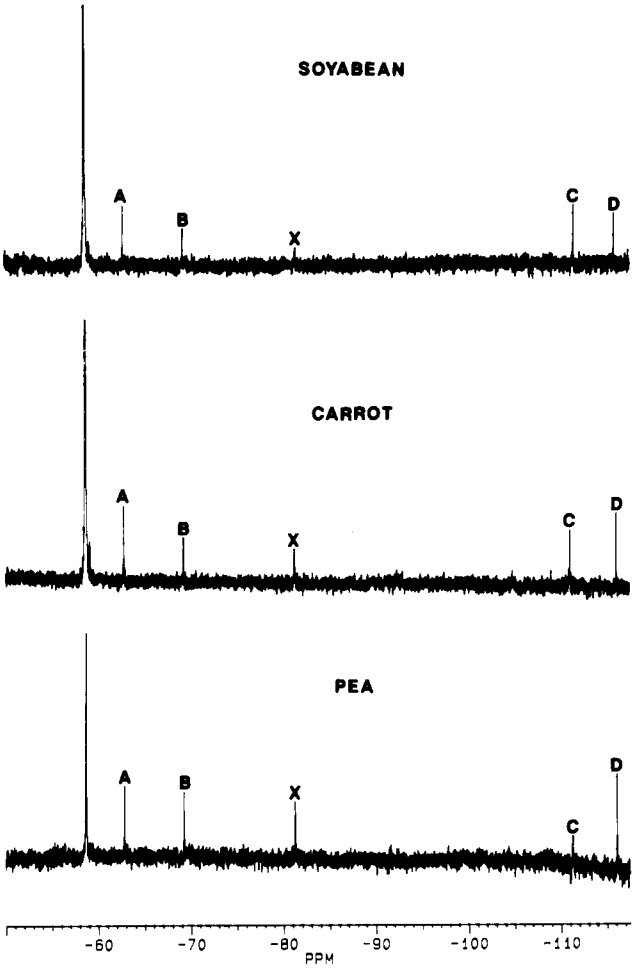

Figure 2. ^{19}F NMR spectra of extracts of pea, carrot, and soyabean spiked with (A) trifluralin, (B) bifenthrin, (C) diflubenzuron, and (D) flamprop-methyl at low ppb levels. X is an unknown contaminant.

Analysis of Plant Extracts. The spectra of the unspiked pea, carrot, and soyabean extracts showed no fluorine signals other than that of the ISTD. The four pesticides in the spiked samples, however, were clearly detectable in all three extracts (see Figure 2). Diflubenzuron posed a problem in the pea extract. The concentrated extract was slightly cloudy so the sample was filtered through a cotton wool plug in a Pasteur pipet. The spectrum of this filtered sample showed no peak for diflubenzuron. If the sample was spiked after filtering in this way, the diflubenzuron signal was evident but appreciably reduced in height due to broadening. Ap-

Table III. Effect of Plant Extractives on Chemical Shifts[a]

extractive	trifluralin	bifenthrin	diflubenzuron	flamprop
none	-62.74	-69.18	-110.89	-116.08
pea	-62.81	-69.24	-111.27	-116.08
veg oil	-62.60	ND	ND	ND
soyabean	-62.76	-69.22	-111.43	-115.80
carrot	-62.76	-69.22	-111.02	-116.02

[a] All chemical shifts referenced to the ISTD (-58.67 ppm).

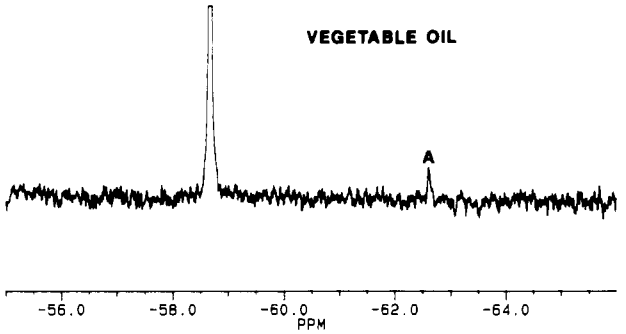

Figure 3. ^{19}F NMR spectrum of vegetable oil spiked with 1 mg/L of (A) trifluralin and containing $Cr(AcAc)_3$ in acetone.

parently, there is a component in peas, possibly paramagnetic ions such as iron and copper, that complexes with this pesticide. This may be a problem in other pesticides with a similar β-diketo structure.

The signals in the soyabean sample are slightly smaller than in the other two extracts because the larger volume of the oily coextractives caused greater dilution. If a larger sample of soyabean was used, it would be preferable to use a 10-mm probe to approach the limits of detection possible in the other two extracts.

The chemical shifts of the four pesticides (relative to the internal standard) in the various extracts are listed in Table III. It is evident that there are small movements in the values of these shifts in the presence of the different coextractives. To confirm a structural assignment, it would be necessary to spike the actual sample with the known standard.

Direct Analysis of Liquids. Consideration of the detection limits above suggested that liquid samples containing trifluralin could be analyzed directly at 1 mg/L in a 5-mm probe and possibly as low as 0.25–0.3 mg/L in a 10-mm probe (see Influences on Sensitivity). $Cr(AcAc)_3$ is insoluble in vegetable oil, and although addition of the relaxation agent in 0.1 mL of $CDCl_3$ gave an initially clear solution, within 24 h the $Cr(AcAc)_3$ crystallized out. Using 0.1 mL of acetone, however, gave a stable mixture with 0.3 mL of vegetable oil and 2 mg of $Cr(AcAc)_3$ (relative proportions have not been optimized). A sample of vegetable oil spiked with trifluralin at 1 mg/L and analyzed in this way clearly showed the pesticide signal at -62.60 ppm (see Figure 3). The small downfield change in chemical shift from that observed in pure $CDCl_3$ (-62.74) is consistent with the direction of change observed in pure acetone (see Table II).

As $Cr(AcAc)_3$ is also insoluble in water, we found it necessary to use a cosolvent when analyzing aqueous samples with this relaxation agent. Pyridine was superior to others (acetonitrile, acetone, methanol, DMSO, THF, ethylene glycol, methyl cellosolve); a mixture of 0.3 mL of red wine and 0.1 mL of pyridine containing 2 mg of $Cr(AcAc)_3$ remained homogeneous at room temperature for weeks (50 μL was the minimum volume of pyridine for this mixture). Figure 4 shows the spectrum for red wine after it was spiked with the ISTD and different concentrations of trifluralin (1, 2, and 5 mg/L). Fluorinated

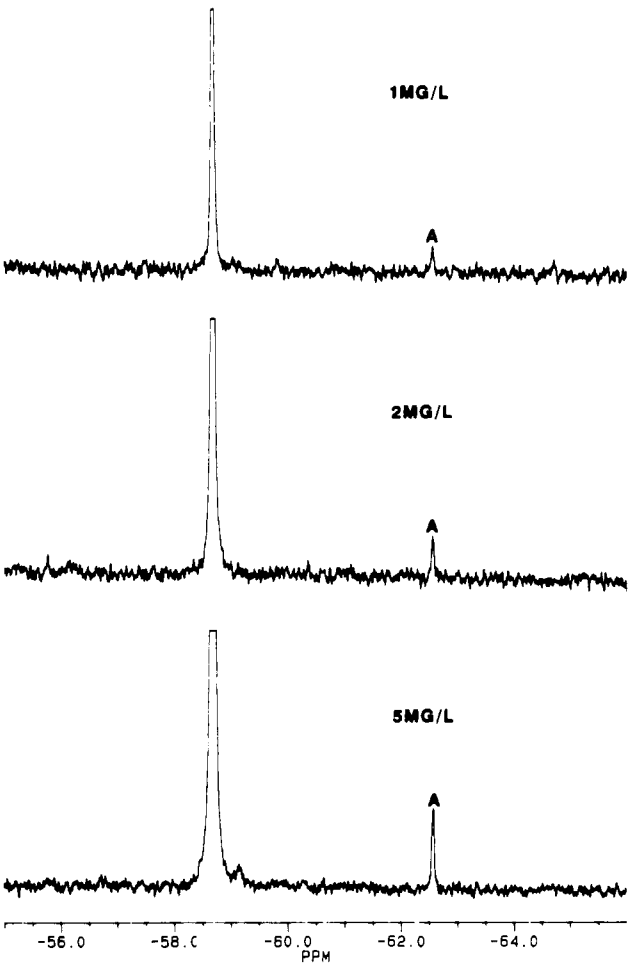

Figure 4. ^{19}F NMR spectra of red wine spiked at 1, 2, and 5 mg/L with (A) trifluralin and containing $Cr(AcAc)_3$ in pyridine.

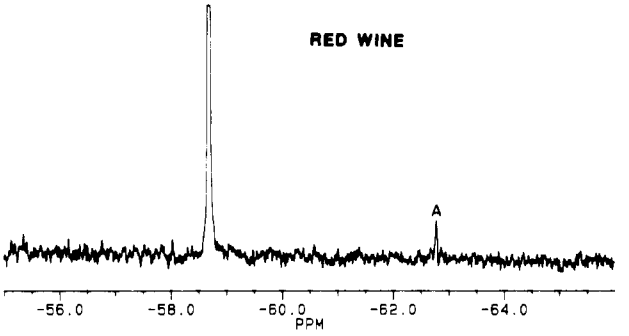

Figure 5. ^{19}F NMR spectrum of red wine spiked with 2 mg/L of (A) trifluralin and using $MnCl_2$ (1 mM) as the relaxation agent.

compounds in wine were effectively relaxed by the $Cr(AcAc)_3$, but those in milk were not observed. It appears that the pesticides partition into the fat phase but the relaxation agent remains in the aqueous pyridine phase.

Three additional signals were observed in the sample of wine which eventually were traced to the pyridine-d_5. The precursor of deuterated pyridine is perfluoropyridine, and apparently small amounts ($\sim$0.01%) of the three monofluoropyridines are still present.

Alternatively, one can use $MnCl_2$ as the relaxation agent for aqueous solutions (Derome, 1987). We found concentrations from 0.1 to 10 mM to be effective in red wine, but 100 mM caused line broadening (lower concentrations were not examined). Figure 5 shows 0.5 mL of red wine spiked with trifluralin at 2 mg/L and 1 mM of $MnCl_2$. The signal is comparable with that in Figure 4 at the same concentration but relaxed with $Cr(AcAc)_3$.

Influences on Sensitivity. There are three principal reasons for the greater sensitivity of our analyses in comparison with Mazzola's (1984) work: availability of a stronger magnetic field and of a smaller diameter probe and use of a relaxation agent. Mazzola used a 80-MHz (1.9-T) magnet with a 10-mm probe, whereas we have used a 400-MHz (9.4-T) magnet with a 5-mm probe. As the signal-to-noise ratio increases with the field strength to the power of 1.5, the stronger magnetic field provides a factor of about 11 in improved sensitivity.

The smaller diameter probe (smaller volume) gives a further improvement of roughly 3 when the same *quantity* of sample is used. Although a larger diameter probe (larger volume) is an improvement when a greater *volume* of sample can be analyzed as in the case of the direct analysis of liquids, the improvement is not directly proportional to the increase in volume because of the inherently lower sensitivity of the larger probe. Only an increase of roughly 3-fold can be expected when the 10-mm probe is used for direct analysis in comparison with the 5-mm probe even though the volume increase is approximately 9-fold. Little or no further gain is expected with a 15-mm probe.

Our use of a fluorine-specific probe rather than a broadband probe was a minor factor in increased sensitivity as this is reported to improve the S/N ratio only by 10–15%.

The use of $Cr(AcAc)_3$ was important, providing a 2-fold increase in S/N for the same scanning time as noted previously.

Finally, zero-filling and other FID manipulations can also improve the S/N ratio (Derome, 1987). In preparing calibration curves for the four pesticides, zero-filling to 64K appreciably increased the R^2 values of the linear regressions (signal ratio vs concentration) for three of them: 0.9194 to 0.9921, 0.9907 to 0.9996, and 0.9808 to 0.9938. The R^2 value for bifenthrin declined slightly from 0.9945 to 0.9922.

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Registry No. Trifluralin, 1582-09-8; flamprop-methyl, 52756-25-9; diflubenzuron, 35367-38-5; ethalfluralin, 55283-68-6.