A Study To Determine the Feasibility of Using ^{31}P NMR for the Analysis of Organophosphorus Insecticide Residues in Cole Crops

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It is not generally recognized that ^{31}P NMR spectroscopy is now sensitive enough to be used for the analysis of organophosphorus insecticide residues without resorting to the extraction of large samples or the use of prolonged NMR acquisition times. The analysis of pesticides, such as disulfoton, diazinon, dimethoate, parathion, and azinphos-methyl, has been demonstrated to be feasible at 0.5 ppm on broccoli and cabbage.

INTRODUCTION

Organophosphorus chemicals are widely used in agricultural practice as insecticides, herbicides, and, on a more limited scale, fungicides (Brooks, 1989). As a result, traces of these materials may be found in our food supply. Among the 10 organophosphorus insecticides approved for use in Canada on various cole crops, the maximum allowable residue level varies from 0.25 to 6 ppm. In addition, there are five others allowed on a negligible residue basis at 0.1 ppm.

The levels of these residues are monitored by regulatory agencies, principally by gas chromatography. The gas chromatographic detectors used to analyze organophosphorus compounds, such as the flame photometric detector and the nitrogen/phosphorus detector, are sensitive for phosphorus but not specific; they are prone to interferences from non-phosphorus-containing compounds. The residues in cole crops, such as cabbage, broccoli, and turnips, are particularly troublesome to analyze (Luke et al., 1981; Blaha and Jackson, 1985; Andersson and Ohlin, 1986; Ambrus and Thier, 1986; Stan and Kellner, 1989) because of the numerous volatile coextractives containing nitrogen and sulfur that are found in the *Brassica* genus and which are sensed by these detectors (Fenwick et al., 1989).

An analytical technique that is entirely element-specific is nuclear magnetic resonance spectroscopy (NMR). Unfortunately, NMR also has the reputation of being relatively insensitive in comparison with GC or HPLC. In 1976, Gurley and Ritchey determined that the minimum detectable level ($S/N_{rms} = 2$) for a phosphorus-containing compound by ^{31}P FT-NMR was about 10 ppm with a 100-MHz instrument (2.34 T). The acquisition time was limited to 25 min to make the analysis comparable in length to that of gas chromatography. Subsequent applications of ^{31}P NMR to organophosphorus pesticides are few and are limited to the analysis of technical formulations where sample quantity was plentiful (Greenhalgh and Shoolery, 1978; Cochrane et al., 1979; Wayne et al., 1982; Greenhalgh et al., 1983; Miyata et al., 1989).

NMR lends itself well to the analysis of thermally sensitive compounds because it typically operates close to ambient temperature. Problematic organophosphorus insecticides such as methyl parathion, naled, and trichlorfon, which are thermally labile on GC analysis (Betowski and Jones, 1988), should be readily analyzed by ^{31}P NMR. In addition, no chromatographic cleanup of the sample should be needed unless the matrix contains extractable phosphorus compounds of similar structures.

The insecticide NMR signals are well distributed over a chemical shift range of approximately 120 ppm. The four major insecticide groups, phosphorodithioate, phosphorothioate, phosphorothiolate, and phosphate, appear to be conveniently subdivided into four ranges: roughly 90–100, 50–70, 20–30, and 0 to −18 ppm, respectively, relative to phosphoric acid (Miyata et al., 1989; Muller et al., 1956; Ross and Biros, 1970). This may be a useful characteristic, in conjunction with the chemical shift value, in identifying a particular component. Furthermore, as the NMR experiment detects all ^{31}P nuclei equally, all phosphorus compounds, including phosphorus-containing metabolites, will be observed and not just those for which the analysis may be intended.

Since Gurley and Ritchey's work in 1976, NMR instrumentation has improved significantly, particularly the strength and stability of the commercially available magnetic fields. As a result, the sensitivity and resolving power of NMR has increased. The purpose of this work was to determine the feasibility of using ^{31}P NMR for the analysis of trace levels (0.1–10 ppm) of organophosphorus pesticides in food substances, particularly in cole crops, without having to extract an inconveniently large sample and without having to acquire NMR data over a long time.

EXPERIMENTAL PROCEDURES

NMR Instrumentation and Procedures. *On the Bruker AM400.* NMR spectra were recorded for the organophosphorus insecticides and food extracts in 0.6 mL of $CDCl_3$ containing 8–10 mg of chromium acetylacetonate [$Cr(AcAc)_3$] at 296 K on a Bruker AM400 spectrometer equipped with an Aspect 3000 computer and process controller, using DISNMR version 870101. A 5-mm "reverse" broadband probe (i.e., the proton coils on the inside) was used for obtaining the spectra. The proton-decoupled ^{31}P NMR spectra (161.9 MHz) were acquired with a sweep width of 140 ppm, with 16K data points, giving a resolution of 2.9 Hz/point. Zero-filling to 64K appeared to improve the line shape and signal-to-noise ratio to a small degree. For quantitation experiments, 4800 scans were accumulated by using a 90° pulse width with an acquisition time of 0.344 s and no relaxation time (for a total experiment time of about 30 min). To study the effect of $Cr(AcAc)_3$ on chemical shifts, a relaxation delay of 1.0 s and a pulse angle of 15° were used for the sample mixture with no relaxation agent, while the values above were used for the samples after addition of $Cr(AcAc)_3$. A capillary tube containing 85% H_3PO_4 (from a new bottle) was suspended in the middle of

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0021-8561/91/1439-0911$02.50/0 Published 1991 by the American Chemical Society

the 5-mm NMR tube. All shifts in this study were referenced to this external standard (0.00 ppm).

On the Bruker AM250 and Bruker AM500. Spectroscopic conditions for the Bruker AM250 and Bruker AM500 were similar to those for the Bruker AM400 spectrometer. For the AM250 spectra, 5-mm tubes were used in a 10-mm broadband probe. For the AM500 instrument, a 5-mm triple-tuned probe (^{13}C, ^{31}P, and ^{1}H) was used for the acquisitions. The parameters for both instruments were adjusted so as to obtain the same number of scans (4800) in the same amount of time (30 min) to get a good comparison of the sensitivity at different field strengths.

Insecticide Standards. Organophosphorus insecticides 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 of nearly equimolar reference insecticides was prepared by dissolving phosalone (15.59 mg), phosmet (13.51 mg), parathion (13.30 mg), and malathion (14.60 mg) in $CDCl_3$ (MSD Isotopes, Pt. Claire, PQ) in a 10-mL volumetric flask. Serial dilution was done by transferring 5.0 mL of stock solution to a 10-mL volumetric flask and diluting to the mark with $CDCl_3$. An aliquot (3.0 mL) of each dilution and 1.0 mL of $CDCl_3$ containing 10.07 mg of triphenylphosphine (Aldrich, Milwaukee, WI) was transferred to 5-mL volumetric flasks which were then filled to the mark with $CDCl_3$ (see Table III for concentrations). These solutions were analyzed on the 400-MHz instrument.

A second stock solution for analysis on the 250- and 500-MHz instruments was prepared by using 6.59 mg of phosalone, 6.18 mg of phosmet, 6.44 mg of parathion, and 5.75 mg of malathion. The stock solution was serially diluted as above. An aliquot (1.0 mL) of each dilution and 1.0 mL of $CDCl_3$ containing 10.1 mg of triphenylphosphine were mixed and diluted as before (concentrations listed in Table III).

Solutions of insecticides for determination of chemical shifts or for determination of the temperature effect were prepared in $CDCl_3$ (2–6 mg/mL).

Food Extracts. Broccoli (100 g) and red cabbage (200 g) were each homogenized in acetone and the resulting extracts partitioned into methylene chloride according to Steinwandter's (1985) procedure. Each extract was split into two equal parts, concentrated at reduced pressure at 40 °C, and taken up in 0.5 mL of $CDCl_3$ containing 2.03 mg of triphenylphosphine. One broccoli extract was diluted with 0.1 mL of $CDCl_3$ containing 50 μg each of azinphos-methyl, naled, and dimethoate and the other with 0.1 mL of $CDCl_3$ only. One red cabbage extract was diluted with 0.1 mL of $CDCl_3$ containing 50 μg each of parathion, diazinon, and disulfoton and the other, to be used as the control, with 0.1 mL of $CDCl_3$ only.

Additional extracts of broccoli (50 g) and red cabbage (100 g) were prepared as above and, after evaporation, taken up in 0.5 mL of $CDCl_3$ containing 2.21 mg of triphenyl phosphate (Aldrich). The solution of the broccoli extract was diluted with 0.1 mL of insecticide sample as above, whereas the solution of the red cabbage extract was diluted with 0.1 mL of $CDCl_3$. All samples were stored in the refrigerator prior to analysis by NMR.

RESULTS AND DISCUSSION

Chemical Shifts. Initially, a series of organophosphorus insecticides were analyzed on a 400-MHz (9.36-T) instrument to determine if any of their chemical shifts were overlapping. The measured values (relative to the signal of 85% phosphoric acid) are listed in Table I. Of the 10 pesticides registered for use on cole crops in Canada, azinphos-methyl and malathion were the closest with a difference in chemical shift of 0.23 ppm. At this field strength, the difference is 37.2 Hz (1 ppm = 161.9 Hz). As typical signal width at half-height is less than 3 Hz, the available resolution is more than adequate to differentiate these two signals.

Additional compounds have been included in Table I to demonstrate that minor structural changes in the phosphorus substituents can have an appreciable effect on the ^{31}P chemical shift and to show that the likelihood of finding two insecticides with the same chemical shift is probably small. Changing ethoxy groups to methoxy groups shifts the parathion signal downfield by 3.60 ppm and the azinphos signal downfield by 5.27 ppm. The two geometrical isomers of mevinphos are separated by 0.58 ppm and those of chlorfenvinphos by 0.73 ppm. A comparison of azinphos-methyl and phosmet or of azinphos-ethyl and phosalone shows that changing the heterocyclic ring produces shifts of 0.91 and 0.52 ppm, respectively, even though the ring is three atoms removed from the phosphorus nucleus. The signals for phorate and disulfoton, whose structures differ only by a methylene group in the side chain, are separated by 1.94 ppm.

Of the 24 insecticides in Table I, a number of which differ only by the substituents or by the substituent pattern on the aromatic ring, methyl parathion and fenitrothion were the closest signals. These two structures differ by a methyl group on the aromatic ring, and their ^{31}P shifts differ by only 0.04 ppm [increases to 0.06 ppm with Cr(AcAc)$_3$; see later]. Nevertheless, Figure 1 shows that they are distinguishable peaks and almost baseline separated once zero-filling is done (the ringing baseline pattern in the zero-filled case is due to the truncated FID).

Maple et al. (1989) noted that the ^{31}P chemical shifts of many phosphorus-containing compounds exhibit a larger temperature dependence than that of ^{13}C chemical shifts and reported a value of 0.024 ppm/°C for triphenylphosphine. A single solution of 16 insecticides in $CDCl_3$ was run at 289 K and at 296 K and the change in chemical shifts calculated. The differences varied from 0.01 to 0.14 ppm, consistent with the value reported by Maple et al., and showed some dependence on molecular structure. The six phosphorodithioates showed the largest shift increases

Table I. Measured ^{31}P Chemical Shifts of Organophosphorus Insecticides[a]

insecticide	shift, ppm	insecticide	shift, ppm
dimethoate[b]	99.38	methyl parathion	66.27
azinphos-methyl[b]	96.50	dichlofenthion	63.41
malathion[b]	96.27	fensulfothion	63.18
phosmet	95.59	parathion[b]	62.67
disulfoton[b]	95.45	chlorpyrifos	61.53
phorate	93.51	diazinon[b]	60.98
terbufos	93.22	methamidophos[b]	36.04
phosalone	91.75	omethoate	31.17
azinphos-ethyl	91.23	oxydemeton-methyl	30.14
demeton[b]	68.21	naled[b]	−2.15
fenthion	67.39	mevinphos[b]	−5.27, −5.85
fenitrothion	66.31	chlorfenvinphos	−5.91, −6.64

[a] In $CDCl_3$ at 296 K. Chemical shifts are relative to 85% phosphoric acid in a sealed capillary centered in the NMR tube. Triphenyl phosphate has a chemical shift of −17.09 ppm. All shifts were repeatable within ±0.003 ppm (n = 2). [b] Registered pesticide in Canada (1990).

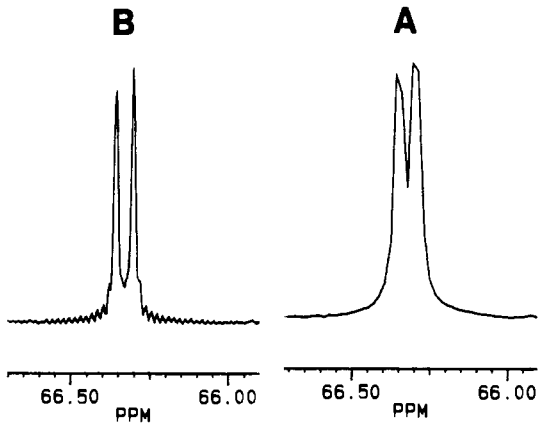

Figure 1. ^{31}P NMR spectrum (AM400) of a mixture of methyl parathion and fenitrothion standards: (A) without zero-filling; (B) with zero-filling.

Table II. Change in ^{31}P Chemical Shift of Various Organophosphorus Compounds by Addition of Chromium Acetylacetonate[a,b]

compound	$Cr(AcAc)_3$ concn, mg/mL			
	5.2	10.6	20.6	42.2
dimethoate	0.261	0.659	1.419	2.632
phosmet	0.258	0.617	1.317	2.447
phosalone	0.241	0.602	1.300	2.415
demeton	0.244	0.603	1.284	2.396
methyl parathion	0.245	0.621	1.320	2.451
diazinon	0.256	0.623	1.314	2.433
omethoate	0.266	0.644	1.375	2.542
oxydemeton-methyl	0.260	0.619	1.317	2.431
naled	0.251	0.623	1.310	2.424
mevinphos (*E*)	0.252	0.615	1.311	2.421
mevinphos (*Z*)	0.251	0.610	1.294	2.404
triphenyl phosphate	0.253	0.613	1.295	2.398

[a] The chemical shift change is the chemical shift with $Cr(AcAc)_3$ added minus chemical shift without any $Cr(AcAc)_3$. [b] All samples run in 0.5 mL of $CDCl_3$ with an 85% phosphoric acid sample in a capillary tube centered in the NMR tube.

(0.07–0.14 ppm) with the increased temperature, the six phosphorothioates and the three phosphates increased by 0.01–0.05 ppm, while the shift of the single phosphoramidate examined (methamidophos) decreased (!) by 0.09 ppm. As the temperature of the NMR probe is controlled to ±0.05 °C, the influence of stray ambient temperature changes on the reported chemical shifts is negligible.

Relaxation Agent. The sensitivity of the FT-NMR analysis is determined, in part, by the number of scans that can be accumulated in a given time. This is dependent on the relaxation time (T_1) of the particular nucleus after the radio-frequency pulse. As phosphorus T_1 values can be long, e.g., that for triphenylphosphine is 19.9 s (Maple et al., 1989) and that for fenitrothion is 5 s (Greenhalgh and Shoolery, 1978), the resulting delay between pulses can be appreciable, thereby reducing the number of accumulated scans in a given time. Fortunately, certain inorganic complexes can accelerate this relaxation and shorten the delay between scans. Although Gurley and Ritchey (1976) found $Cr(AcAc)_3$ to be ineffective in reducing phosphorus relaxation times, Greenhalgh and Shoolery (1978) observed that an equimolar charge of this complex reduced the T_1 of fenitrothion from 5.0 to 0.15 s. We have also found $Cr(AcAc)_3$ to be an effective relaxation agent for the phosphorus signals.

On the other hand, Greenhalgh and Shoolery (1978) reported that $Cr(AcAc)_3$ did not disturb the chemical shifts. We have observed that this is not precisely the case. The results in Table II, from a solution of representative insecticides to which an increasing quantity of $Cr(AcAc)_3$ was added, show that the chemical shifts of all the compounds moved to lower field in linear fashion ($R^2 \geq 0.996$) as the amount of relaxation agent increased. This change was only evident, however, when the external reference (H_3PO_4) was present. In the absence of the external reference, there appeared to be little or no significant shift induced, as observed by Greenhalgh and Shoolery, because the magnitude of the effect was the same for most of the compounds present as well as the internal reference to which the other chemical shifts were compared (triphenyl phosphate).

Dimethoate and omethoate (its oxygen analogue), however, are clearly exceptions; their phosphorus signals suffer a greater shift (slopes of the linear plots are significantly higher). The reason for this is not known but presumably reflects stronger complexation of the chromium atom by these molecules, perhaps via the amide carbonyl group.

These results show that the influence of the $Cr(AcAc)_3$ cannot be overlooked; its concentration must be carefully controlled if the chemical shifts are to be assigned accurately.

Table III. Concentrations of Calibration Standards and Resulting *S/N* Ratios[a]

sample	concn, μg/mL (*S/N* ratio)			
	malathion	parathion	phosmet	phosalone
A[b]	867 (47.6)	798 (67.9)	811 (47.2)	935 (49.9)
B[b]	438 (26.4)	399 (33.9)	405 (27.8)	468 (27.6)
C[b]	219 (15.1)	200 (20.7)	203 (15.8)	234 (13.5)
D[b]	110 (8.6)	99.8 (9.5)	101 (9.0)	117 (8.5)
E[b]	54.8 (4.3)	49.9 (4.1)	50.7 (4.5)	58.5 (3.3)
F[b]	27.4 (3.9)	24.9 (2.5)	25.3 (2.6)	29.2 (2.5)
1[c]	115 (3.1)	129 (5.5)	124 (4.1)	132 (3.3)
2[d]	28.8 (7.8)	32.2 (9.9)	31.0 (7.3)	33.0 (7.2)
3[d]	14.4 (3.0)	16.1 (6.2))	15.5 (3.7)	16.5 (3.7)

[a] S/N_{rms} calculated by the Aspect 3000 computer from 4800 scans. [b] Run on the 400-MHz instrument. [c] Run on the 250-MHz instrument. [d] Run on the 500-MHz instrument.

Calibration Curves. To quantify the insecticide concentration, an internal reference standard (ISTD) is needed. The ratio of the insecticide signal and the ISTD signal must give a reproducible, preferably linear, response with the concentration of the insecticide.

Triphenylphosphine was chosen initially as the ISTD (see later), because of its ready availability in high purity, and calibration curves were prepared for phosalone, phosmet, parathion, and malathion. The concentrations of these insecticide solutions (coded A–F) and the resulting signal-to-noise ratios of the ^{31}P signals measured on the 400-MHz instrument after 30 min (4800 scans) are given in Table III. The Aspect 3000 calculates the S/N ratio using root-mean-square noise; the resulting values were 2.5 times larger than those calculated from measured peak-to-peak noise, as predicted by theory (Derome, 1987). The peak heights of the insecticide signals and the triphenylphosphine signal were determined by the Aspect 3000 and the ratios calculated from them. Samples A and E were run five times (30 min each time), while the other samples were only repeated twice to reduce the workload.

The calculated ratios and the standard deviations are listed in Table IV. At the highest concentrations (sample A) the standard deviations in the peak height ratios are ±2–4%. These increase to ±2–8.5% in sample E. The range is wider (1–15%) at the lowest concentration (sample F); however, this sample was only run twice. Linear regressions of the peak height ratios vs sample concentrations show that the calibration curves are excellent linear plots for malathion, parathion, and phosalone (R^2 values are 0.9996, 0.9969, and 0.9927, respectively) but not as good for phosmet (0.9865).

In addition, the chemical shifts of the four insecticides are highly reproducible. For a particular sample, the standard deviation for the repeatability of the chemical shift was typically ±0.002 ppm. There was a slightly greater standard deviation (±0.005) observed between samples. Importantly, there is no concentration dependence observed for the ^{31}P shifts of the four insecticides.

Minimum Detectable Levels. Subsequently, a second set of insecticide standards was run on 250- and 500-MHz instruments (magnetic field strengths 5.85 and 11.7 T, respectively). The concentrations used (coded 1–3) are also summarized in Table III along with the recorded S/N_{rms} ratios. The S/N ratios for sample 1 were collected on the 250-MHz machine and those for samples 2 and 3 on the 500-MHz instrument. The minimum detectable limit is defined as the blank value plus three standard deviations. In NMR terms, this is $S/N_{rms} = 3$. It is evident from studying Figure 2 that at $S/N_{rms} = 3$ the peaks are

Table IV. Signal Ratios[a] and Standard Deviations for NMR Results of Insecticide Reference Standards

sample	signal ratio ± SD			
	malathion	parathion	phosmet	phosalone
A	0.3627 ± 0.0144	0.4915 ± 0.0123	0.3643 ± 0.0089	0.4187 ± 0.0123
B	0.1774 ± 0.0078	0.2594 ± 0.0124	0.2236 ± 0.0049	0.2356 ± 0.0136
C	0.0940 ± 0.0019	0.1009 ± 0.0017	0.0909 ± 0.0006	0.0880 ± 0.0008
D	0.0474 ± 0.0024	0.0528 ± 0.0018	0.0536 ± 0.0000	0.0448 ± 0.0011
E	0.0248 ± 0.0009	0.0235 ± 0.0020	0.0308 ± 0.0006	0.0194 ± 0.0010
F	0.0156 ± 0.0019	0.0119 ± 0.0004	0.0142 ± 0.0001	0.0140 ± 0.0021

[a] Ratio of peak heights of the insecticide ^{31}P signal to the triphenylphosphine signal after 4800 scans using non-zero-filled data. [b] Samples A and E were repeated five times, other samples twice.

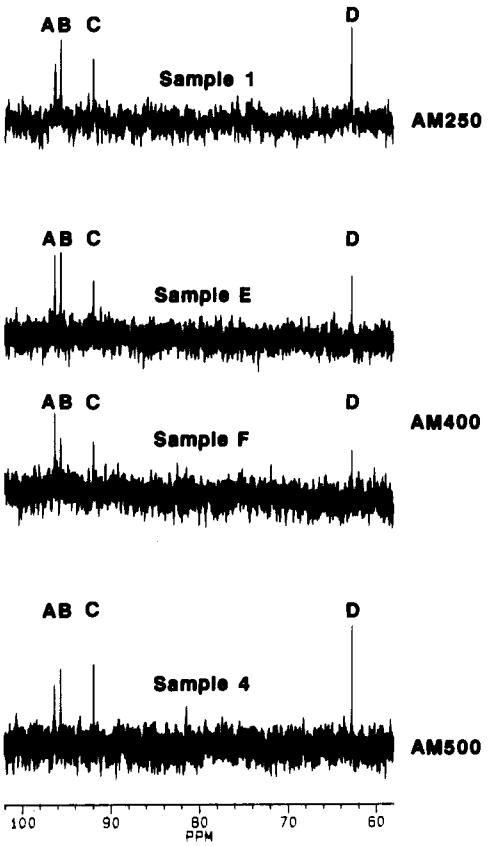

Figure 2. ^{31}P NMR spectra of insecticide solutions at approximately the lowest detectable concentrations at three different field strengths after 30 min of accumulated scans: (A) malathion; (B) phosmet; (C) phosalone; (D) parathion.

Table V. Approximate Minimum Detectable Concentrations[a] at Various Field Strengths

field strength, T	concn, μg/mL (S/N ratio)			
	malathion	parathion	phosmet	phosalone
5.85	115 (3.1))	129 (5.5)	124 (4.1)	132 (3.3)
9.36	27.4 (3.9)	24.9 (2.5)	25.3 (2.6)	29.2 (2.5)
11.7	14.4 (3.0)	16.1 (6.2)	15.5 (3.7)	16.5 (3.7)

[a] Based on 4800 scans (30 min); S/N_{rms} ratios in parentheses.

clearly discernible above the noise. Table V lists the approximate minimum detectable concentrations for the four insecticides at the three different field strengths. The minimum volume required to fill the coil space in a probe designed for a 5-mm NMR tube is ~0.4 mL. As a result, the minimum detectable levels (assuming a 50-g sample, 30-min acquisition time, and all the extract dissolved in 0.4 mL) are roughly 1, 0.3, and 0.12 ppm using 250-, 400-, and 500-MHz instruments, respectively.

It is also evident in Figure 2 that there is considerable difference in the peak heights of the four signals. The signal intensity should depend principally on the concentration of the insecticide and the relative amount of phosphorus in the molecule as the nuclear Overhauser effect is small for phosphorus compounds (Gurley and Ritchey, 1976; Greenhalgh and Shoolery, 1978). The top spectrum in Figure 2 (AM250) shows the correct relative signal intensities: parathion > phosmet > malathion ~ phosalone. The bottom spectrum in Figure 2 (AM500) should show the same order, but the phosalone signal is slightly larger than expected. The center two spectra from the AM400 instrument were recorded by using solutions of slightly different concentrations from those run on the other instruments and should show a relative signal intensity of parathion > phosmet ~ malathion > phosalone. In this case, the parathion signal is much lower than expected.

The reason for these inconsistencies remains to be clarified. Although the three probes used in this study are not identical, their differences are unlikely to account for the apparent variation in signal intensity. The 500-MHz instrument was used with a triple-tuned probe (each of fixed frequency; ^{31}P was second coil), the 400-MHz spectrometer was used with a broadband inverse probe, while the 250-MHz machine had a broadband probe for 10-mm tubes (we used 5-mm tubes). Clearly the sensitivity of all three instruments would benefit from the use of a phosphorus-specific probe of appropriate tube size, but the improvement would be especially noticeable with the 250- and 400-MHz machines.

Vegetable Extracts. Because of the difficulty associated with the analysis of organophosphorus insecticides on some cole crops, broccoli and red cabbage were chosen as representative examples for the NMR analysis. The broccoli extract was spiked with azinphos-methyl, naled, and dimethoate at the equivalent of 1 ppm. Figure 3 shows the spectrum after 30 min of accumulated scans on the 400-MHz instrument. Dimethoate (A) and azinphos-methyl (B) are the two sharp spikes at low field, but naled (C) is barely evident as a small spike on the right shoulder of a broad hump centered around 0 ppm. A similar broad hump was observed in the ^{31}P NMR of whole olive leaves (Lang and Martin, 1986) and attributed to inorganic phosphate and glucose 6-phosphate. There are two additional signals (29 and 44 ppm) subsequently identified as triphenylphosphine sulfide and oxide. The triphenylphosphine is apparently reactive in the presence of broccoli extractives (the speed of the reaction is not known as the samples were stored for 7 days at 4 °C prior to NMR analysis).

The spectrum of the unspiked broccoli sample (not illustrated, but Figure 6 represents a typical background) showed only the broad hump, the ISTD signal, and the two triphenylphosphine artifacts. The S/N ratios of dimethoate (8.3) and azinphos-methyl (6.4) at 1 ppm indicate that 0.5 ppm of these insecticides could easily be analyzed in broccoli, or the sample size could be reduced by half at the 1 ppm target. Increasing the analysis time to 2 h doubled the S/N ratios, 16.7 and 13.3, respectively, as expected (sensitivity is proportional to the square-root of the number of scans).

For parathion, diazinon, and disulfoton on cabbage, the

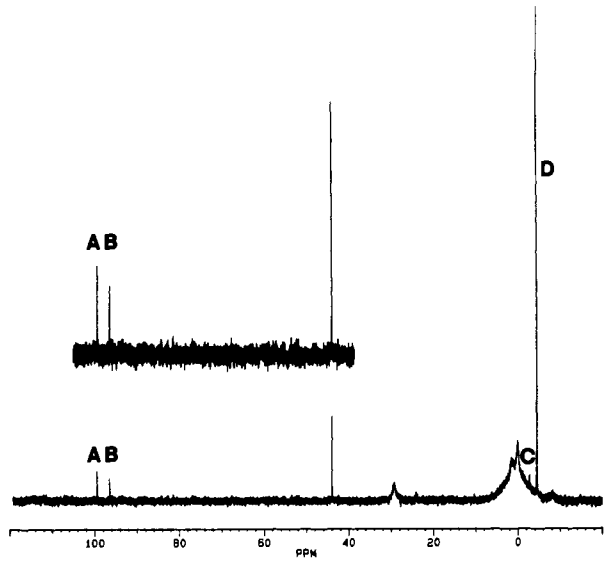

Figure 3. ^{31}P NMR spectrum (AM400) of a broccoli extract spiked with three insecticides, (A) dimethoate, (B) azinphosmethyl, and (C) naled, at 1 ppm and using (D) triphenylphosphine as the internal reference standard after 30 min of accumulated scans. The insert is a portion of the spectrum amplified 3-fold.

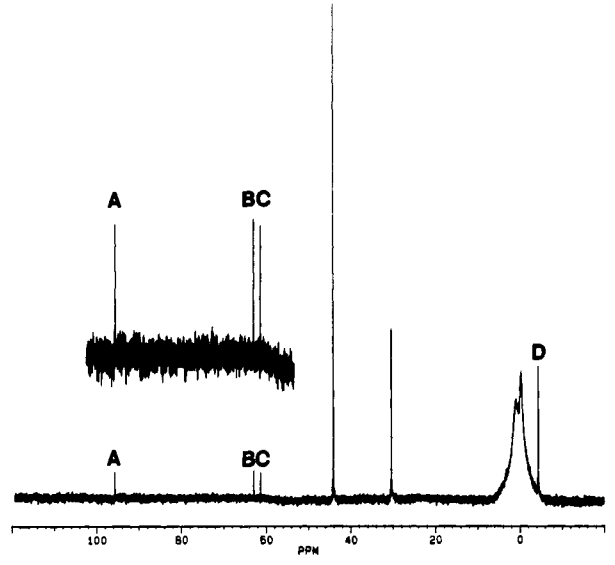

Figure 4. ^{31}P NMR spectrum (AM400) of red cabbage extract spiked with three insecticides, (A) disulfoton, (B) parathion, and (C) diazinon, at 0.5 ppm and using (D) triphenylphosphine as the internal reference standard after 30 min of accumulated scans. The insert is a portion of the spectrum amplified 5-fold.

sample was increased to 100 g and the extract was spiked at the 0.5 ppm level. Figure 4 shows the spectrum after 30 min of accumulated scans. All three insecticides are clearly evident: disulfoton at 95.44, parathion at 62.66, and diazinon at 61.02 ppm. The broad hump around 0 ppm observed in the broccoli extracts is again evident in the red cabbage sample as are the peaks for the triphenylphosphine sulfide and oxide. In this sample and in the unspiked cabbage sample, however, nearly 80% of the triphenylphosphine was consumed. The S/N ratios (6.9, 7.3, and 5.9 for disulfoton, parathion and diazinon, respectively) suggest that a 50-g sample would be sufficient for this analysis. As before, increasing the analysis time to 2 h approximately doubled the S/N ratios (13.5, 12.1, and 9.2). A comparison of the chemical shifts of the three insecticides above with those in Table I reveals small differences that are probably attributable to the Cr(AcAc)$_3$ effect discussed earlier.

Choice of ISTD. In addition to being consumed by

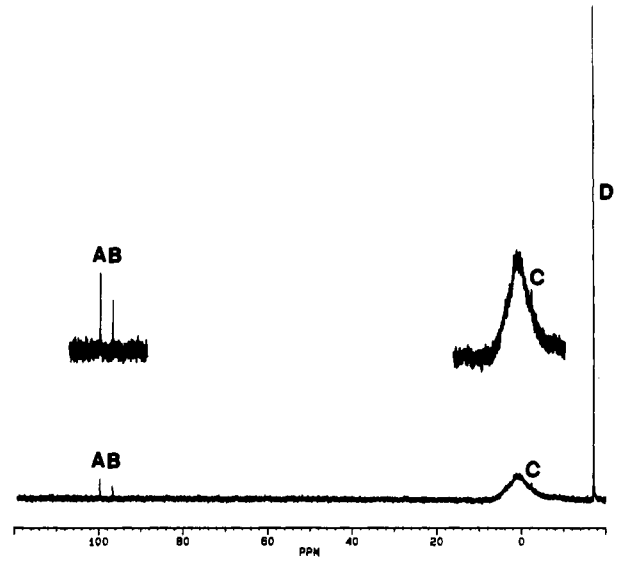

Figure 5. ^{31}P NMR spectrum (AM400) of a broccoli extract spiked with three insecticides as in Figure 3 but using triphenyl phosphate (D) as the internal reference standard after 30 min of accumulated scans. The insert is a portion of the spectrum amplified 4-fold.

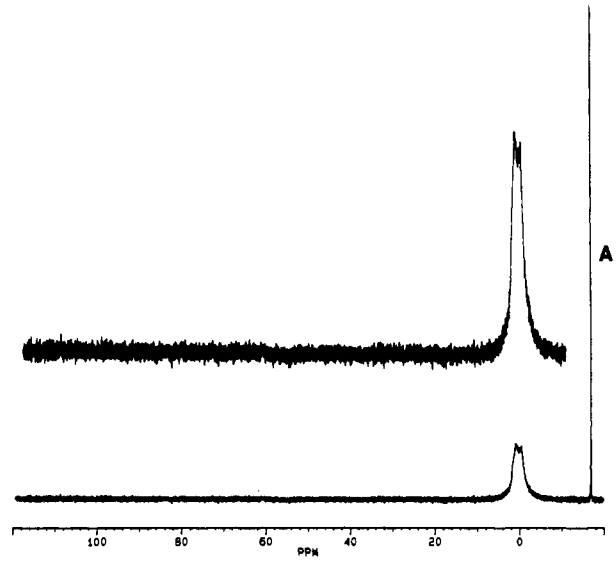

Figure 6. ^{31}P NMR spectrum (AM400) of an unspiked red cabbage extract using triphenyl phosphate (A) as the internal reference standard after 30 min of accumulated scans. The insert is a portion of the spectrum amplified 4-fold.

coextractives from both broccoli and red cabbage, the triphenylphosphine also reacted with the naled reference standard. Although all of the other insecticide standards gave single NMR peaks, the naled sample with triphenylphosphine gave four peaks of roughly equal intensity. As a result, the broccoli and red cabbage extracts and the naled standard were rerun by using triphenyl phosphate as the ISTD (Greenhalgh et al., 1983).

As demonstrated in Figures 5 and 6, not only was the phosphorus chemical shift of the ISTD (−17.09 ppm) further away from the broad hump but there was no interaction between this ISTD and the coextractives from either broccoli or red cabbage. Dimethoate and azinphosmethyl in the broccoli extract (Figure 5) are evident at 99.71 and 96.73 ppm, respectively. Again, naled is only just visible on the broad hump at about −2.4 ppm. The unspiked red cabbage extract (Figure 6) showed no signals other than those of the extractable phosphate (broad hump) and the triphenyl phosphate internal standard. The naled reference sample with triphenyl phosphate as

the ISTD gave a single peak. Once again, the small differences between the chemical shifts of the two insecticides above and those in Table II were probably due to the effect of the added $Cr(AcAc)_3$.

Future Work. Before ^{31}P NMR can be used as an alternative to GC or HPLC for the analysis of organophosphorus insecticide residues, an improvement is desirable in the present sensitivity level. This would either allow a reduction in the sample size (e.g., to 10 g) or make the 0.1 ppm level accessible by the less expensive, lower field instruments (e.g., 200 MHz). Polarization transfer techniques such as the INEPT pulse sequence (Morris and Freeman, 1979; Doddrell et al., 1981) may offer a signal enhancement of 3–4-fold for these organophosphorus compounds.

The broad hump found in the broccoli and red cabbage samples should be eliminated so that the phosphate insecticides could be analyzed more satisfactorily. Although the naled peak was evident on the side of the broad hump in the broccoli extract, its S/N ratio (1.5) was poorer than those of the other two insecticides (8.0 and 5.2) of similar concentration which were free of interference at lower field.

The influence of solvent on the chemical shifts must be clarified as it may not be possible to analyze all organophosphorus insecticides in just $CDCl_3$. Triphenylphosphine resonates 0.5 ppm further downfield in methanol than in deuteriochloroform, and the methamidophos signal is shifted nearly 6 ppm in going from $CDCl_3$ to methanol.

Finally, a practical strategy for using $Cr(AcAc)_3$ must be developed so that the chemical shift values may be used reliably for identifying the particular insecticide. These and related problems are the subjects of our present work.

CONCLUSIONS

Of the 10 organophosphorus insecticides registered for use in Canada on various cole crops, 8 may be analyzed without additional cleanup of the extract at or below the minimum allowable residue level by ^{31}P NMR using a 50-g sample or less and 30 min of acquisition time on a 400-MHz spectrometer.

At present, the phosphates naled and mevinphos are the two exceptions. Both are found in the same region of the spectrum as a broad absorbance due to phosphorus-containing coextractives. In addition, mevinphos, which has the lowest allowable residue limit (0.25 ppm) of this group, has two isomers in roughly equal proportions, which reduces the detectability and makes a further improvement in sensitivity desirable.

ACKNOWLEDGMENT

We acknowledge the cooperation of Dr. B. Blackwell, Agriculture Canada, Ottawa, for allowing us to use the 5.85- and 11.7-T instruments and Ms. L. Panopio and Mr. R. Graham for maintaining the pesticide standard collection.

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Received for review August 24, 1990. Revised manuscript received November 19, 1990. Accepted December 10, 1990.

Registry No. $Cr(AcAc)_3$, 21679-31-2; dimethoate, 60-51-5; azinphos-methyl, 86-50-0; malathion, 121-75-5; phosmet, 732-11-6; disulfoton, 298-04-4; phorate, 298-02-2; terbufos, 13071-79-9; phosalone, 2310-17-0; azinphos-ethyl, 2642-71-9; demeton, 8065-48-3; fenthion, 55-38-9; fenitrothion, 122-14-5; methyl parathion, 298-00-0; dichlorofenthion, 97-17-6; fensulfothion, 115-90-2; parathion, 56-38-2; chlorpyrifos, 2921-88-2; diazinon, 333-41-5; methamidophos, 10265-92-6; omethoate, 1113-02-6; oxydemeton-methyl, 301-12-2; naled, 300-76-5; meninphos, 7786-34-7; chlorfenvinphos, 470-90-6; mevinphos (*E*), 298-01-1; mevinphos (*Z*), 338-45-4.