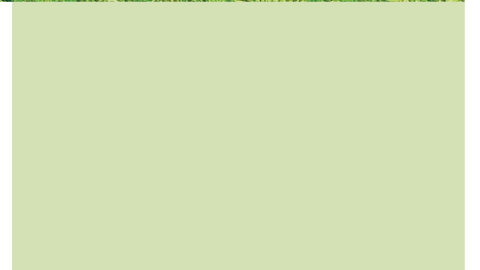


## APPLICATION NOTE

Analysis of Herbicides,  
Insecticides, and  
Fertilizers via  $^{31}\text{P}$   
Benchtop NMR

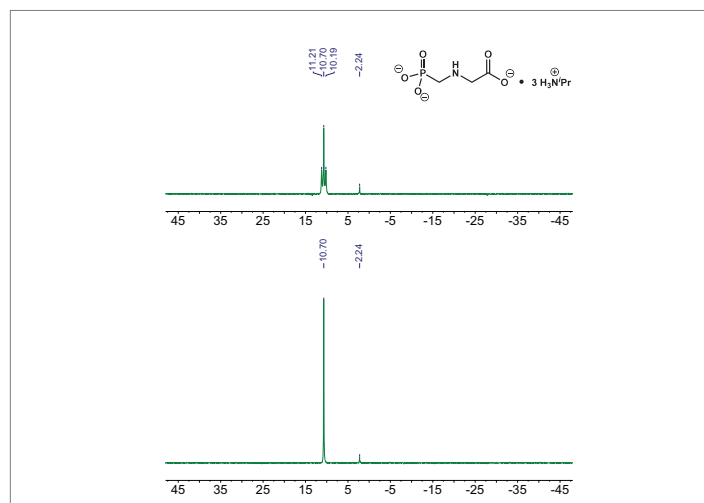


## Analysis of Herbicides, Insecticides, and Fertilizers via $^{31}\text{P}$ Benchtop NMR

Due to their roles as crucial agricultural components, pesticides and fertilizers have been the subject of much study.<sup>1,2</sup> As subclasses of pesticides, herbicides and insecticides have additionally been on the receiving end of much criticism over the years, particularly with respect to their potentially long-lasting environmental impacts and their uptake by living organisms, as these can often make their way into water streams.<sup>3,4</sup> These products are controlled by both health and regulatory agencies, and several methods exist for the qualitative and quantitative analysis of their active ingredients, as well as the rest of their commercial formulations. Often, gas chromatography-mass spectrometry (GC/MS or GC/MS/MS) or liquid chromatography-mass spectrometry (LC/MS or LC/MS/MS) are used to identify and quantify these components, even if they are present in extremely low concentrations.<sup>5</sup>

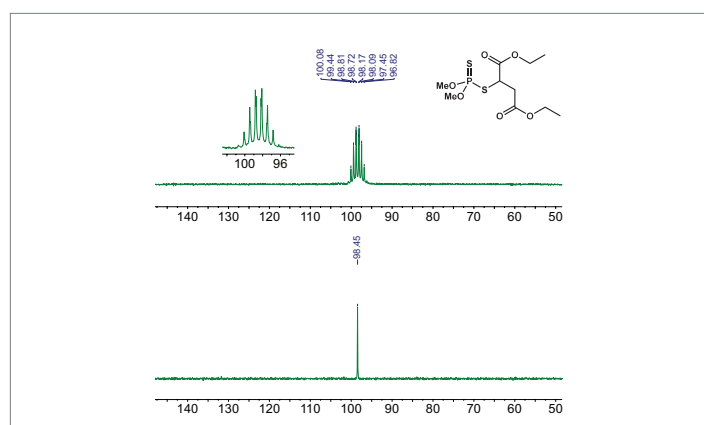
Nuclear magnetic resonance (NMR) is another very powerful tool allowing chemists to analyze these types of products.<sup>6,7</sup> While  $^1\text{H}$  remains the most widely used nuclide for NMR, heteronuclear NMR offers an elegant alternative for these analyses.<sup>8,9</sup> Heteronuclides have much larger chemical shift windows than protons, which minimizes signal overlap and is ideal for peak identification and quantification in mixtures. Organophosphates form an important class of pesticides and fertilizers commonly used by farmers across the world, and while some of these products are available for home-use, many are restricted for large-scale agricultural operations.<sup>10</sup> The active ingredients in these organophosphate products are particularly well-suited for  $^{31}\text{P}$  NMR analysis. Unfortunately, the significant upfront and recurring costs of traditional high-field NMR spectrometers, as well as their space requirements and necessity for routine maintenance, means that these are often prohibitively expensive. The emergence of high-resolution benchtop NMR instruments in the last several years renders this technology more accessible than ever before, allowing for non-expert users to collect NMR spectra quickly and easily. Additionally, NMR is an inherently quantitative technique (qNMR), meaning that the area under the curve is directly proportional to the number of nuclei in each chemical environment giving rise to this signal. This means that specific analytical standards are not required to quantify components in mixtures.<sup>11</sup>

One of the most popular herbicides in the world, Roundup®, contains the active ingredient glyphosate, present as the isopropylamine salt. The  $^{31}\text{P}$  NMR spectra of neat Roundup® Super Concentrate collected on a 60 MHz benchtop spectrometer are shown in Figure 1. In the  $^1\text{H}$ -decoupled spectrum, a sharp singlet corresponding to glyphosate is observed at 10.7 ppm, along with a small impurity around 2.2 ppm. In the  $^1\text{H}$ -coupled spectrum, a triplet is observed due to coupling between the phosphorus and the adjacent methylene protons. We suspect that the minor impurity might be phosphorous acid ( $\text{H}_3\text{PO}_3$ ), a possible degradation product of glyphosate, but we have not confirmed this in our lab.<sup>12</sup>



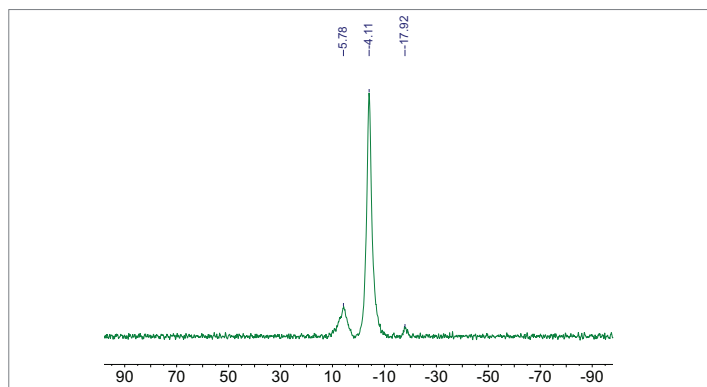
**Figure 1.**  $^{31}\text{P}$  (24.3 MHz) NMR spectra of glyphosate (isopropylamine salt) in neat Roundup® Super Concentrate. Top:  $^1\text{H}$  decoupling off. Bottom:  $^1\text{H}$  decoupling on.

A very common household insecticide, malathion, can also be analyzed using  $^{31}\text{P}$  NMR. The spectra for this product are shown in Figure 2, as a 1:1 solution in toluene- $d_8$ . Although this product is formulated as a 50% mixture in petroleum distillates, a single resonance is observed as we are only observing phosphorus-containing species. When  $^1\text{H}$  decoupling is turned on, a sharp singlet is observed, but when this is turned off, a complex multiplet can be seen, due to coupling between the phosphorus, the two adjacent methyl fragments, and possibly the C-H fragment adjacent to the sulfur.



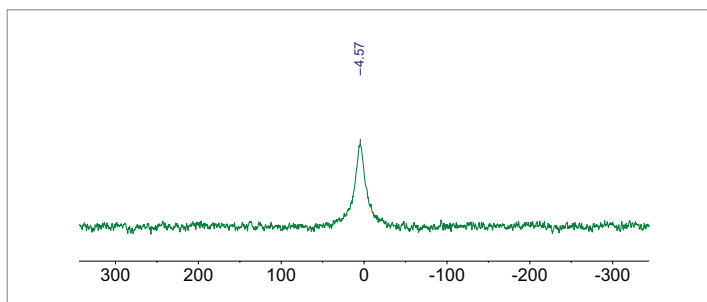
**Figure 2.**  $^{31}\text{P}$  (24.3 MHz) NMR spectra of malathion in 50% Malathion Concentrate Insecticide®, prepared as 1:1 solution in toluene- $d_8$ . Top:  $^1\text{H}$  decoupling off. Bottom:  $^1\text{H}$  decoupling on.

Fertilizers are crucial in ensuring that plants and crops receive proper nutrients. These are typically labeled using the “NPK” notation, with numbers such as 20-20-20, indicating that the fertilizer is composed of 20 wt% nitrogen (N), phosphorus (P), and potassium (K) species. In addition, fertilizers will often contain chelated metals important for plant growth, such as zinc, manganese, iron, and copper. The  $^{31}\text{P}$  NMR spectrum of Schultz™ Liquid Plant Food (10-15-10) is shown in Figure 3, prepared by dissolving 50  $\mu\text{L}$  of the product in 550  $\mu\text{L}$  of  $\text{D}_2\text{O}$ . Due to the presence of paramagnetic chelated metal species in solution, significant signal broadening is observed when too much product is used for analysis. However, a good balance was found using these amounts, allowing for the observation of three distinct broad signals, centered around 5.8 ppm, -4.1 ppm, and -17.9 ppm. While these individual species have not been explicitly identified, they correspond well to known phosphate species in fertilizers, such as orthophosphates (5.8 ppm), pyrophosphates (-4.1 ppm), and polyphosphates (-17.9 ppm).<sup>8</sup>



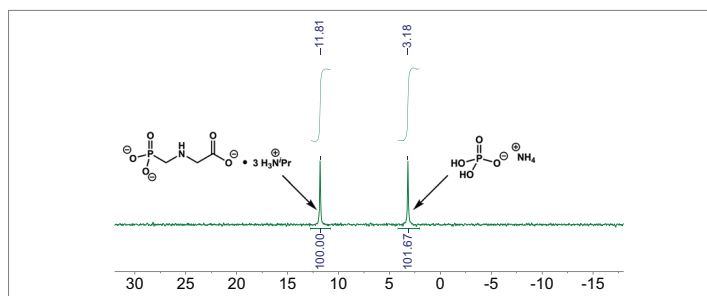
**Figure 3.**  $^{31}\text{P}$  (24.3 MHz) NMR spectrum of phosphorus-containing species in Schultz™ All Purpose Liquid Plant Food (10-15-10), prepared as a dilute solution in  $\text{D}_2\text{O}$ .

To illustrate the differences in phosphorous-containing species present in different fertilizers, a sample of Miracle Gro® Water Soluble Plant Food (20-20-20) was analyzed by dissolving a 60 mg sample in  $\text{D}_2\text{O}$ . Like the Schultz fertilizer, the presence of chelated metal species leads to some important signal broadening in the  $^{31}\text{P}$  spectrum, shown in Figure 4. In fact, the peak width at half-height was measured to be around 300 Hz, which makes it difficult to ascertain whether other phosphate species are hidden underneath this resonance, or whether this sample only contains orthophosphates.



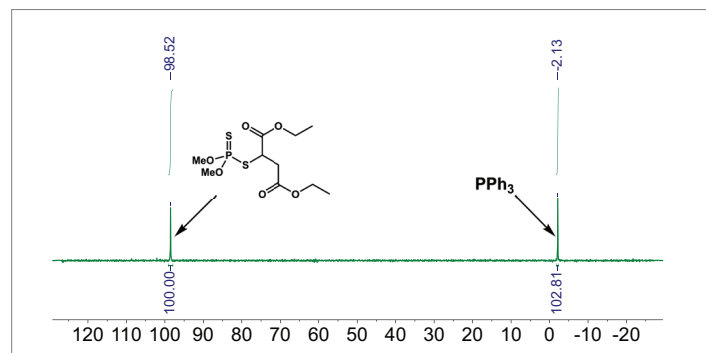
**Figure 4.**  $^{31}\text{P}$  (24.3 MHz) NMR spectrum of phosphorus-containing species in Miracle Gro® Water Soluble Plant Food (20-20-20), prepared as a dilute solution in  $\text{D}_2\text{O}$ .

The quantification of phosphorus using  $^{31}\text{P}$  NMR is straightforward, provided that the internal calibrant used has a known purity,<sup>13</sup> is soluble and stable in the medium of analysis, and that its signals do not overlap with the others in the sample.<sup>14–16</sup> The glyphosate content in Roundup® Super Concentrate is listed as 356 g/L, with the other primary component being water. As such, water-soluble ammonium phosphate monobasic (APM) was used as the internal calibrant to quantify the glyphosate content in this product. A stock solution of APM in  $\text{D}_2\text{O}$  was prepared, and an accurate amount was added to a precisely measured volume of Roundup® Super Concentrate (47.4  $\mu\text{L}$ , 16.9 mg). The sample was analyzed in triplicate using a scan delay of 20 seconds to ensure that all spins of interest had fully relaxed between scans. The qNMR results of this analysis provided an average glyphosate mass of 16.6 mg, matching the theoretical amount of 16.9 mg very closely. In addition, the triplicate values of 16.5 mg, 16.4 mg, and 17.0 mg are very similar. One of the  $^{31}\text{P}$  spectra used for qNMR is presented in Figure 5.



**Figure 5.**  $^{31}\text{P}$  (24.3 MHz) NMR spectrum of Roundup® Super Concentrate and ammonium phosphate monobasic in  $\text{D}_2\text{O}$ , used for quantification of glyphosate.

This same fundamental approach can be used to quantify the 50% Malathion Concentrate Insecticide®, but a different internal calibrant must be used due to the aliphatic nature of the mixture. For this study, a stock solution of triphenylphosphine in toluene- $d_6$  was prepared and a precise amount was added to an accurately weighed solution of the malathion product. The sample was analyzed in triplicate using a scan delay of 10 seconds. An average composition of 48.9% malathion (wt%) was calculated, which matches the composition on the label very closely (50%). In addition, the triplicate values of 48.9%, 49.0%, and 48.9% are very similar. One of the  $^{31}\text{P}$  NMR spectra used for qNMR is presented in Figure 6.



**Figure 6.**  $^{31}\text{P}$  (24.3 MHz) NMR spectrum of 50% Malathion Concentrate Insecticide® and triphenylphosphine in toluene- $d_6$ , used for quantification of malathion.

As demonstrated in this application note, the use of heteronuclear NMR is a very powerful way of analyzing molecules that might otherwise exhibit complex  $^1\text{H}$  spectra, or whose spectra might be overwhelmed by the other components in the mixture, such as water or petroleum distillates. This is particularly applicable to many herbicides, insecticides, and fertilizers as these often contain heteroatoms like phosphorus or fluorine. This study showcases only a small portion of the types of products in these areas which can be analyzed using benchtop NMR spectroscopy. If you have any questions about the analyses presented herein using benchtop NMR, or about how you could incorporate our instruments into your workflow, please don't hesitate to reach out to us!

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