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CHARACTERIZATION OF CHEMICAL WEAPONS CONVENTION SCHEDULE 3 COMPOUNDS BY QUANTITATIVE ^{13}C NMR SPECTROSCOPY

Terry J. Henderson

RESEARCH AND TECHNOLOGY DIRECTORATE

David B. Cullinan

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
Abingdon, MD 21009

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14. ABSTRACT

A quantitative ^{13}C NMR method has been developed for characterizing hydrogen cyanide, cyanogen chloride, and phosgene, three Chemical Weapons Convention Schedule 3 compounds in common use today. Towards this end, the ^{13}C spin-lattice relaxation behavior (T_1) of the compounds has been assessed at 75 and 126 MHz for temperatures between 5-15 °C, holding them in their liquid states to dramatically improve detection sensitivity. The derived single exponential T_1 values were used to derive relaxation delays for collecting quantitative ^{13}C data sets yielding a signal-to-noise ratio (S/N) exceeding that necessary for certifying the compounds at ≥95 carbon atom % and 99% confidence. At 126 MHz, only a single data acquisition with a high-sensitivity cryogenic probe head exceeded the certifying S/N; however, for analysis at 75 MHz with a conventional probe head, ≥5 acquisitions were necessary for phosgene, and ≥12 acquisitions were necessary for the other two compounds. In terms of accuracy and execution time, the resulting NMR method rivals typical chromatographic methods.

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Nuclear magnetic resonance	Chemical Weapons Convention Schedule 3	^{13}C Quantitation
Spin-lattice relaxation (T_1)	Carbon atom percent	Natural abundance

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PREFACE

The work described in this report was authorized under Project No. 05P-0199. The work was started in January 2005 and was completed in February 2006.

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CONTENTS

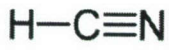
FIGURES

TABLES

CHARACTERIZATION OF CHEMICAL WEAPONS CONVENTION SCHEDULE 3 COMPOUNDS BY QUANTITATIVE ^{13}C NMR SPECTROSCOPY

1. INTRODUCTION

Hydrogen cyanide (1), cyanogen chloride (2), phosgene (carbonyl chloride, 3) and chloropicrin (trichloronitromethane, 4) are all recognized for their very high volatility and toxicity. The characteristics give the chemicals tremendous potential for use as chemical warfare agents, and the four were among the very first chemicals considered for such use during World War I. A total of 100,000 tons of toxic chemicals were deployed against soldiers and civilians alike, resulting in about 92,000 deaths and 1.3 million casualties by the end of the war. The horrors of chemical warfare caused such outrage that the countries of the world resolved to ban the use of toxic chemicals and chemical weapons forever. This culminated in the signing of the 1925 Geneva Protocol for the Prohibition of the Use of Asphyxiating, Poisonous or Other Gases, and Bacteriological Methods of Warfare. Today, the sections of the Protocol specific to chemical weapons are superseded by the Convention on the Prohibition of the Development, Production, Stockpiling, and Use of Chemical Weapons and their Destruction,[1] more commonly referred to as the Chemical Weapons Convention (CWC).



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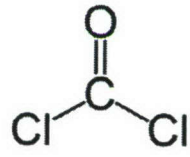

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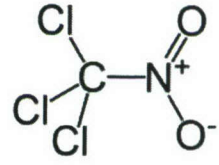

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The CWC classifies toxic chemicals and their precursors into one of three different schedules: Schedule 1, chemicals with few or no uses other than chemical weapons; Schedule 2, chemicals with legitimate, small-scale applications apart from chemical weapons; and Schedule 3, chemicals with large-scale uses apart from chemical weapons. Along with the 13 precursors used for their production, phosgene, cyanogen chloride, hydrogen cyanide and chloropicrin comprise the entire list of Schedule 3 chemicals. Widespread use of the chemicals presents a potential threat not only to those working directly with the chemicals and to emergency responders, but when considering the prevalence of terrorism today, the chemicals pose a threat to US and Allied militaries as well as innocent civilians. Schedule 3 chemicals are attractive to foreign states or terrorists seeking a mass-destruction capability, since they are easily obtained in comparison to most weapons of mass destruction, are relatively inexpensive to produce, and do not require the elaborate technical infrastructure necessary for nuclear weapons. Producers of Schedule 3 chemicals residing in CWC signatory countries are placed under stringent regulatory control. Plants that manufacture more than 30 tons/yr of any Schedule 3 chemical must be declared, and are subject to CWC inspections. Furthermore, there are restrictions addressing the export of Schedule 3 chemicals to countries which are not CWC signatories.

Today, hydrogen cyanide, cyanogen chloride, phosgene and chloropicrin are all produced in large volumes comparable to any industrial chemical. The US production of hydrogen

cyanide for instance, exceeded 1,600 million pounds in the year 2000, and worldwide phosgene production is roughly two million tons annually. The four chemicals have uses as herbicides, fungicides, insecticides or other pesticides, or as fumigants for rodent extermination. Additionally, they all are used as a raw material for the synthesis of other chemicals, especially pesticides, dyestuffs and polymers. Hydrogen cyanide in particular is used to produce many valuable products, including adiponitrile, the precursor to Nylon 66; acetone cyanohydrin, a precursor to methyl methacrylate, and the essential amino acid methionine. Cyanogen chloride has specific uses in ore refining and as a metal cleaner.

The Army is responsible for the testing and evaluation of all chemical agent protective equipment manufactured for the US Department of Defense. The equipment includes filters and filtering systems, air-purifying respirators (gas masks), and a variety of different types of protective clothing. Chemical agent standard analytical reference material is an Army requirement for all testing and evaluation purposes using chemicals. For CWC Schedule 3 compounds, chemical agent standard analytical reference materials are lots of phosgene, cyanogen chloride or hydrogen cyanide at ≥95 carbon atom % (chloropicrin is presently not used for testing). As expected, one of the most critical aspects in the testing and evaluation of chemical agent protective equipment is the certification of agent purity for each lot of reference material. NMR spectroscopy has proven to be a reliable method for the determination of agent purity and identification of contaminants, and is one of the major analytical techniques used by the Army to certify chemical agent standard analytical reference materials.

NMR spectroscopy is an accurate quantitative technique when conducted under appropriate conditions, a potential demonstrated conclusively almost 30 years ago.[2-3] Quantitative NMR analyses are typically reported for ^{1}H,[4-6] ^{13}C [7-9] and ^{31}P[10-12] spectroscopy, however, quantitative spectroscopy for other nuclei[13-19] can also be found. Further, the accuracy and precision of NMR signal intensity measurements have recently been reviewed,[20] and more recently, Maniara *et al.*[21] reported a systematic validation of the quantitative NMR method. Their validation demonstrated that when carefully implemented, the purity of major components in a complex mixture can be determined with accuracy and precision better than 1%, and impurities comprising ≤0.1% of the sample mass can be quantified. On the basis of these results, a quantitative ^{13}C NMR method has been developed for validating the purity of hydrogen cyanide, cyanogen chloride and phosgene to be used for the testing and evaluation of chemical agent protective equipment. This report details and discusses a natural abundance, quantitative, ^{13}C NMR method for analyzing these CWC Schedule 3 chemicals.

2. EXPERIMENTAL SECTION

2.1 Gases and Supplies

Two lots of hydrogen cyanide were purchased from the Louisiana Millennium Technology Group, LLC (Many, LA), and eight lots of cyanogen chloride and two lots of phosgene were purchased from Semiconductor Resources (Toledo, OH) and Scott Specialty Gases, Inc. (Plumsteadville, PA)respectively; all were supplied at ≥95 wt % in pressurized gas cylinders. Tetramethylsilane was purchased from Sigma-Aldrich (St. Louis, MO) for use as a NMR chemical shift reference compound. All NMR sample tubes, including 5 mm pressure/vacuum valve NMR sample tubes (Catalog number 528-PV-9), were purchased from Wilmad-Labglass (Buena, NJ).

2.2 Sample Preparation

Hydrogen cyanide, cyanogen chloride, and phosgene were prepared for NMR spectroscopy by condensing their respective gases into pressure/vacuum valve NMR sample tubes. The tubes were necessary to contain the boil-off from the volatile samples, as well as the associated pressure build-up. A slow, steady stream of each gas was led separately from its pressurized cylinder directly into a sample tube held in an ice water bath (**Warning**: Hydrogen cyanide, cyanogen chloride and phosgene are very toxic and must be handled in a closed system or a fume hood with a minimum air flow velocity of 100 ft/min.). The gas stream was stopped, and the pressure/vacuum valve closed tightly after 1.0-1.5 mL of liquid condensed into the tube. Closed tubes were found to hold all liquids, without loss, for at least 2 months when held at either 8 °C or room temperature.

2.3 NMR Spectroscopy

NMR spectroscopy was conducted at 7.06 or 11.75 T, using respectively, a Bruker-Biospin Corp. (Billerica, MA) Avance DRX-300 spectrometer fitted with a conventional (room temperature) QNP probe head (observe configuration), or an Avance DRX-500 spectrometer fitted with a cryogenic TCI probe head (inverse configuration), unless specified otherwise. Both spectrometers used XWIN-NMR software (Bruker-Biospin Corp.) for data acquisition and processing, and were fitted with a B-CU 05 probe head pre-cooling unit for precise temperature stabilization. All experiments were conducted unlocked with the sample spinning at 20 Hz and held at one of three different temperatures between 5-15 °C (see Table 1). Sample temperatures were carefully calibrated for different probe head gas flow rates and gas temperatures against the methyl and hydroxyl ^{1}H signal chemical shift values for neat methanol.[22] Calibrations were specific to each probe head and probe head gas flow rate to ensure very accurate temperature determinations.

^{1}H free induction decay data comprising 8,192 or 16,384 complex points were summations of 32 acquisitions recorded with 10 ppm spectral windows, 90° pulse widths, and 5-9 s relaxation delays. These were Fourier transformed directly into spectra, and manually phase-corrected into pure absorption mode. All ^{13}C free induction decay data

were recorded at natural abundance with 65,536 complex points, using 90° pulse widths, 220 ppm spectral windows and 8-112 acquisitions. ^{13}C spin-lattice relaxation time (T_1) values were measured with the inversion recovery pulse sequence[23] [180°-τ-90°-acquisition] incorporating seven randomized τ delays. Cyanogen chloride and phosgene experiments used relaxation delays $\geq 5T_1$ to allow complete spin-lattice relaxation for quantitative spectra, while hydrogen cyanide experiments incorporated inverse-gated ^{1}H decoupling with a low-power composite pulse sequence[24] and relaxation delays of $\geq 8T_1$ to ensure quantitative results.[25] Recorded ^{13}C and $^{13}C\{^{1}H\}$ data were multiplied by an exponential window function with a line-broadening factor of 2 Hz before Fourier transformation into spectra and manual phase correction into pure absorption mode. Chemical shift values for all spectra were referenced to external tetramethylsilane.

^{13}C signal intensities were determined by electronic integration of expanded regions around the signals for T_1 measurements and the quantitation of CWC Schedule 3 chemicals containing impurities. Spectral regions chosen for integration included all spinning sideband signals originating from the parent signal. T_1 values were derived from fitting the measured signal intensities as a function of τ to a single exponential expression.[23] For each compound, T_1 values were typically measured several times and found to differ by <2%. All purity values were reported as carbon atom %.

2.4 Method Specificity, Limits of Detection and Quantitation

Method specificity was evaluated for hydrogen cyanide, cyanogen chloride and phosgene by demonstrating the absence of interference between each of their respective ^{13}C signals and impurity signals. Limits of detection and quantitation are not strictly applicable to this method; however, both are addressed below in a general discussion.

2.5 System Suitability

The suitability of the spectrometers for the method was demonstrated by periodically measuring the ^{13}C spectral line shape (signal symmetry) and routinely measuring spectrometer ^{13}C sensitivity (signal-to-noise ratio) using 1.0% ethylbenzene in $CDCl_3$.

3. RESULTS AND DISCUSSION

3.1 Sample Volatility and Analysis Temperature

In contrast to many compounds, hydrogen cyanide, cyanogen chloride and phosgene are found in their gaseous state under typically encountered conditions of temperature and pressure. This can be problematic for NMR analyses, because substantially less analyte molecules are present per unit volume in this state relative to the liquid or solid state, severely limiting detection sensitivity. Further, solid state NMR analyses of the compounds can be challenging, not only because of its inherent low sensitivity and resolution, but such analyses require the maintenance of low experimental

temperatures to hold the compounds in their solid state. Table 1 reveals for example, that the solid state analysis of phosgene requires experimental temperatures below -118 °C. The ideal situation therefore, would be to analyze the compounds as liquids. The table shows the three compounds to have boiling points between 8-26 °C, temperatures well within the range of commercial NMR spectrometers when using liquid nitrogen boil-off for cooling or a refrigerated probe head pre-cooling unit. The table shows that our analysis temperatures for hydrogen cyanide, cyanogen chloride and phosgene are 10.6±0.2, 3.8±0.2 and 3.2±0.2 °C, respectively, lower than their corresponding boiling points by ensuring their liquid state during data acquisition.

Table 1. Physical and Experimental Parameters for CWC Schedule 3 Compounds

Compound	Molecular Weight (g/mole)	Melting Point (°C)[a]	Boiling Point (°C)[a]	Analysis Temperature (°C)[b]
Hydrogen cyanide	27.03	-13.4	25.6	15.0±0.2
Cyanogen chloride	61.46	-6.0	13.8	10.0±0.2
Phosgene	98.90	-118.0	8.2	5.0±0.2

a Values at atmospheric pressure.
b Values reported ± spectrometer temperature stability.

3.2 Nuclear Magnetic Relaxation and Quantitative Spectra

As is the general case with quantitative NMR measurements, nuclear magnetic relaxation must be considered thoroughly before developing rigorous experimental protocols. Most importantly, experimental relaxation delay times must be sufficient in length to allow the complete spin-lattice relaxation of all signals. To derive such relaxation delay times specifically for hydrogen cyanide, cyanogen chloride and phosgene, their ^{13}C spin-lattice relaxation behavior was assessed at the temperatures listed in Table 1. Inversion recovery experiments were conducted at 7.06 and 11.75 T, corresponding to ^{13}C Larmor frequencies of 75 and 126 MHz, respectively. The relaxation behavior observed for each compound is shown graphically in Figure 1, which also shows the calculated, single-exponential inversion recovery behavior best describing the results in each case. The figure illustrates clearly that the observed behaviors are accurately described with single-exponential expressions. Moreover, this is found for each compound at both magnetic field strengths, suggesting that ^{13}C relaxation is dominated by a single mechanism for each compound. Mechanisms can be inferred for compounds simply by considering their molecular structures and ^{13}C relaxation mechanisms identified for structurally similar ^{13}C nuclei in other compounds. Except in cases of methyl groups relaxing by spin rotation[26-27] or bromine-substituted ^{13}C nuclei relaxing by scalar coupling to ^{79}Br,[26] relaxation of ^{13}C nuclei in CH_n groups (n>0) dominated by the dipolar interaction with their attached protons.[26-27] It is reasonable to assume , therefore, that relaxation of the hydrogen cyanide ^{13}C nucleus is dominated by the ^{1}H-^{13}C dipolar interaction. For

nonprotonated ^{13}C nuclei on the other hand, the most important relaxation mechanism almost always results from the modulation of local fields produced by the tumbling of a molecule with chemical shift anisotropy (CSA).[26-27] The CSA mechanism is significant for these nuclei at all magnetic field strengths,[27] and its contribution to ^{13}C relaxation increases proportionally to the square of the field strength.[28-29] It is likely, therefore, that the CSA mechanism contributes to a large degree to the relaxation of cyanogen chloride and phosgene ^{13}C nuclei, especially at the higher, 11.75 T magnetic field. However, because the ^{13}C-^{14}N dipolar interaction can also be significant for nonprotonated carbon atoms directly bonded to nitrogen atoms,[30] a sizable contribution from this mechanism to cyanogen chloride ^{13}C relaxation cannot be ruled out.

^{13}C T_1 values derived from fitting our inversion recovery results (Figure 1) to single-exponential expressions are reported in Table 2 along with their 95% confidence intervals. 75 MHz values measured at 5 °C for hydrogen cyanide and cyanogen chloride are also listed along with that for phosgene to allow direct comparisons of T_1 values measured under identical conditions. Narrow confidence intervals are found throughout the table, and are a direct reflection of the close agreement between the measured and calculated data. The table also reveals that T_1 values for hydrogen cyanide are significantly shorter than the other values at both magnetic field strengths, and are the shortest 75 MHz values measured at 5 °C. These observations are consistent with our assumptions of a strong CSA contribution to cyanogen chloride and phosgene ^{13}C relaxation, and a dominant ^{1}H-^{13}C dipolar interaction for hydrogen cyanide. However, there are some trends in the table, which are theoretically discordant with these mechanisms, and suggest that other mechanisms contribute to ^{13}C relaxation as well. Hydrogen cyanide for instance, not only displays a magnetic field strength dependency for T_1, but its 75 MHz values appear to decrease as temperature increases. A rotational correlation time (τ) of *ca.* 1 ps was estimated for hydrogen cyanide at 5 °C, using the Stokes-Einstein equation for a rigid isotropic rotor:

$$\tau = \frac{4}{3}\pi r^3 \frac{\eta}{kT}$$

In the equation, η is viscosity (0.224 mPa s at 5 °C), extrapolated from the reported viscosities for hydrogen cyanide at 0 and 25 °C,[31] r is the radius of the hydrogen cyanide molecule, calculated from its 1.064×10^{-8} cm C-H distance and 1.156×10^{-8} cm C-N distance measured by microwave spectroscopy,[32] k is Boltzmann's constant, and T is the absolute temperature. Our estimated τ value places hydrogen cyanide at 5 °C deep into the extreme narrowing regime for the ^{1}H-^{13}C dipolar interaction, far removed from τ values with a T_1 dependency on magnetic field strength and even farther from values displaying an inverse relationship between T_1 and temperature. Cyanogen chloride and phosgene also show a direct correlation between T_1 and magnetic field strength, in contrast to the inverse relationship expected for CSA relaxation.

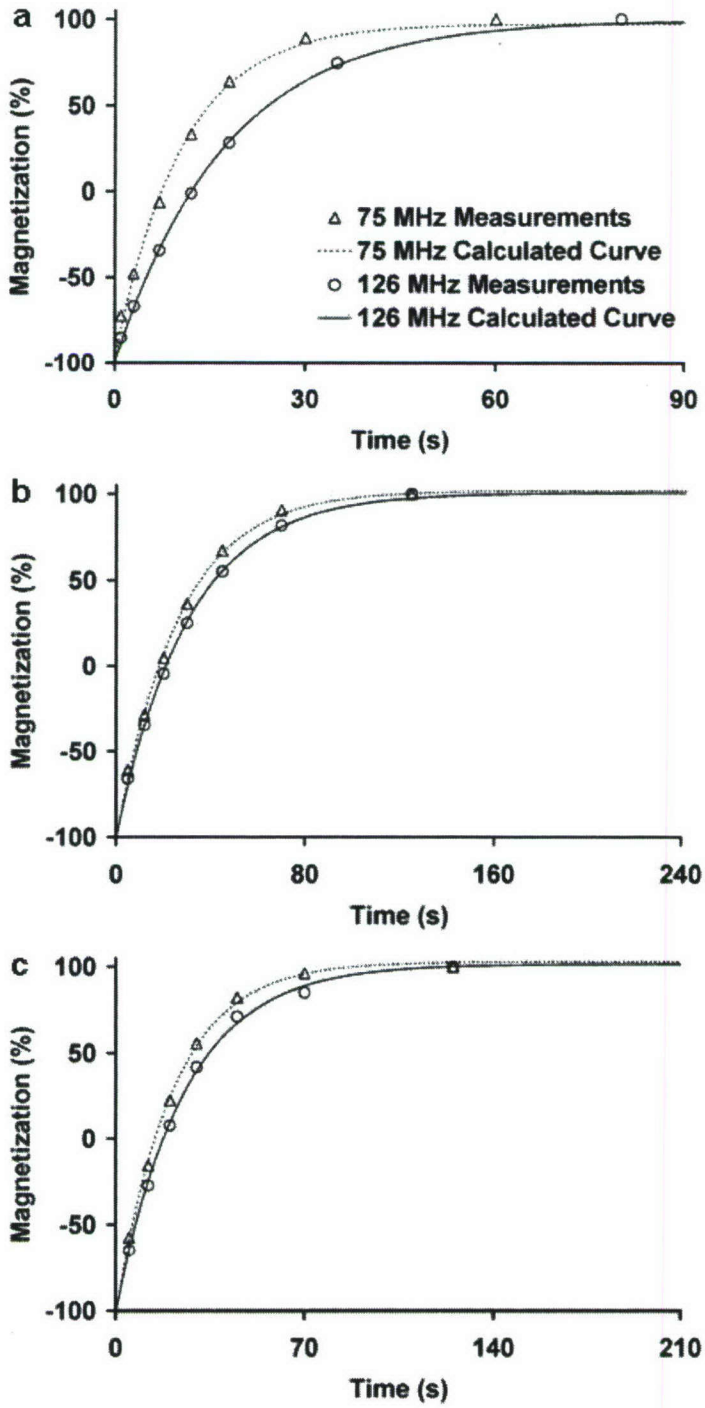

Figure 1. Measurements of Bulk ^{13}C Magnetization along the Static Magnetic Field Following Inversion with a 180° Pulse as a Function of Time. Measurements at both 75 and 126 MHz are shown for (a) hydrogen cyanide at 15 °C, (b) cyanogen chloride at 10 °C and (**c**) phosgene at 5 °C for each compound, recovery of the bulk magnetization calculated from derived T_1 values are also shown for both resonance frequencies.

Table 2. ^{13}C T_1 Measurements for CWC Schedule 3 Compounds

Compound	Temperature (°C)	T_1[a] 75 MHz (s)	T_1[a] 126 MHz (s)
Hydrogen cyanide	15	10.5±0.1	17.3±0.0
	5	15.0±0.1	**b**
Cyanogen chloride	10	26.1±0.1	30.2±0.0
	5	24.5±0.0	**b**
Phosgene	5	20.8±0.0	25.3±0.1

a Values reported ±95% confidence intervals.
b Not determined.

For nuclei experiencing single-exponential relaxation behavior, quantitative spectra are acquired by using pulse sequences incorporating relaxation delays $\geq 5T_1$ for all signals of interest. The only exception is the case of cross-relaxation arising from continuous decoupling, where very different nuclear Overhauser effect (NOE) enhancements can occur from one signal to the next. In the specific case of ^{13}C $\{^1H\}$ spectroscopy, Opella and coworkers[25] have suggested the use of an inverse-gated decoupling scheme and an additional $3T_1$ for relaxation delays ($\geq 8T_1$ for all signals) to allow complete elimination of NOE enhancements. These conditions were used for acquiring quantitative ^{13}C $\{^1H\}$ spectra for hydrogen cyanide, since the decoupled experiment gives twice the sensitivity of the ^1H coupled experiment. In contrast, the ^{13}C nuclei of cyanogen chloride and phosgene are not scalar coupled to protons, and a conventional ^{13}C pulse sequence with relaxation delays $\geq 5T_1$ were used to acquire quantitative data sets. Typical results for the three compounds at >99 carbon atom % are presented in Figure 2, where 75 and 126 MHz spectra from eight acquisitions each illustrate differences between data acquired with a conventional probe head at 7.06 T and a cryogenic probe head at 11.75 T, respectively. The lower sensitivity of the conventional probe head is immediately apparent in the 75 MHz hydrogen cyanide spectrum (Figure 2a) when compared to the other spectra in the figure, demonstrating the need for more acquisitions at this magnetic field strength (see below).

Lots of CWC Schedule 3 compounds are not always supplied at high purity. An example is shown in Figure 3, a quantitative ^{13}C spectrum of a cyanogen chloride sample containing a single impurity identified as CCl_4 (data not shown). The ratio of intensities for the cyanogen chloride and CCl_4 signals is 10.2:1, giving a cyanogen chloride purity of 90.2 carbon atom %. For impure samples, the spin-lattice relaxation of impurity nuclei must be accounted for in addition to that of the analyte signal for deriving purity values. Probably the simplest approach is to use inversion recovery experiments to determine whether the impurity signal relaxes faster than the analyte signal. In such cases, a quantitative relaxation delay can be calculated directly from the analyte signal T_1 value. Conversely, when the impurity signal relaxes slower than the analyte signal, the length of the relaxation delay must be calculated from the impurity signal T_1 value. This value will have to be estimated, at least, to derive an appropriate relaxation delay for recording quantitative data sets. Once again, the inversion recovery experiment provides the simplest and fastest means for estimating T_1 values.

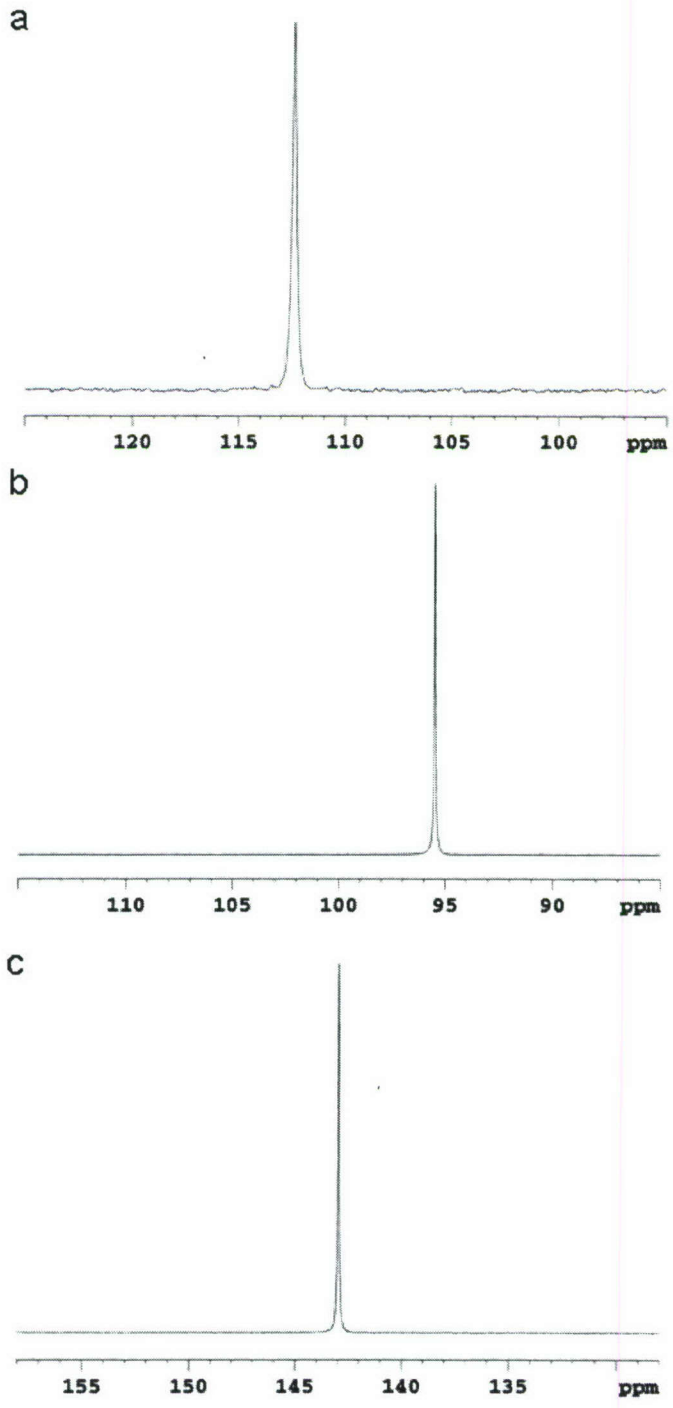

Figure 2. Typical ^{13}C Spectra of CWC Schedule 3 Compounds with Measured Chemical Shift (δ_C) and $^{1}J_{CH}$ Values; (**a**) 75 MHz hydrogen cyanide spectrum acquired at 15 °C, (**b**) 126 MHz cyanogen chloride spectrum acquired at 10 °C and (**c**) 126 MHz phosgene spectrum acquired at 5 °C.

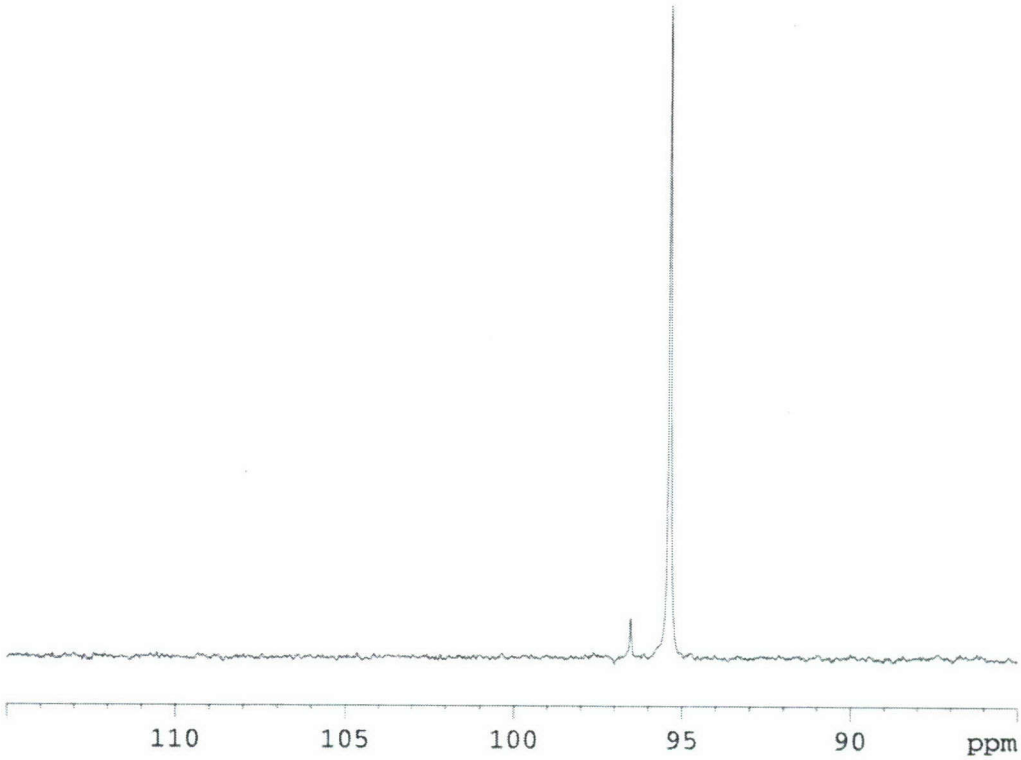

Figure 3. 126 MHz ^{13}C Spectrum of Cyanogen Chloride at 10 °C, Revealing a Single Impurity Signal at 96.4 ppm. Data were collected using a conventional (room temperature) inverse probe head.

3.3 Method Specificity

Specificity is the ability of a method to completely resolve an analyte signal from all impurity signals and spectral artifacts. Accurate results from quantitative NMR spectroscopy require a high degree of specificity to ensure that analyte and impurity signal intensities are derived properly. The broad chemical shift ranges inherent to nuclei such as ^{15}N, ^{19}F, ^{31}P, and ^{13}C used in this method, predispose analytical methods based on their observation for high spectral resolution and method specificity, especially at higher magnetic field strengths. It is possible, however, for an impurity signal to converge or even superimpose with the analyte signal. This is typically observed in analyses of complex mixtures, technical grade materials, and munitions grade materials in the case of chemical warfare agents, but can easily be circumvented by using other impurity and analyte signals to derive quantitative results. This is not possible, however, for hydrogen cyanogen, cyanogen chloride or phosgene, as they all contain a single carbon atom site. The careful treatment of converging or superimposing signals can be of great value in the ^{13}C quantitation of these compounds.

In the event that an impurity signal overlaps an analyte signal, the intensity of the former needs to be carefully determined and subtracted from the total intensity of the converged signals. Two situations exist when impurity and analyte signals overlap and cannot be resolved. When the presence of the impurity is not known, its signal intensity will be added to that of the analyte signal, biasing the analyte concentration toward a higher concentration. On the other hand, if the identity of the impurity has been verified and its concentration accurately determined from quantitative NMR spectroscopy by using a different nucleus or another analytical technique, the NMR-derived purity value may be adjusted to correct for the impurity. Some recent developments in data set processing, including linear prediction with single value decomposition,[33] detection estimation,[34] filter diagonialization,[35-36] and others[37-40] have focused on deconvoluting overlapping and superimposed signals, but there use is far from routine. Spinning sidebands can be similar sources of error in the intensity determination of the analyte signal. Because these signals derive from the actual signal itself, they should be included in the corresponding analyte signal intensity determination.

3.4 Method Limits of Detection and Quantitation

This is a major component method applied to materials which are not sample limited, and the concept of limits of detection and quantitation are not applicable in a strict sense. These limits are, for example, important in analyses of low concentration components present in a technical grade material, analyses of formulated materials where the technical grade active ingredient is diluted, or for the analysis of trace components in a material. Quantitative NMR spectroscopy may be applied to such systems by adjusting experimental parameters to give a signal-to-noise ratio ≥3:1 for detection and ≥10:1 for quantitation of any component in a system.[10,21] Variations of this quantitative method were used for the analysis of such materials, especially the munitions grade nerve agents (sarin, soman and VX). A $^{31}P\{^{1}H\}$ variant using an internal reference standard[10] routinely yields a precision and accuracy of <1%, and signals can be detected for impurities at concentrations as low as 25 μg/mL with acquisition times <25 m. Other military-specific chemicals, as well as a variety of complex mixtures, were also analyzed with similar results. Specifically for ^{13}C detection, substantial decreases in acquisition time have been realized without any loss of precision or accuracy by using the quantitative-DEPT (or Q-DEPT) pulse sequence[41] to enhance sensitivity. For CWC Schedule 3 compounds unfortunately, Q-DEPT experiments are usually not an option, since a proton directly bonded to a carbon atom is essential for polarization transfer. Hydrogen cyanide is the only exception, and even in this case, impurities without a C-H bond cannot be detected. For this method at 126 MHz, a single data acquisition recorded with the cryogenic probe head gave signal-to-noise ratios of 466:1, 490:1 and 677:1 for hydrogen cyanide, cyanogen chloride and phosgene, respectively. Corresponding ratios at 75 MHz using the conventional probe head were 79:1 for hydrogen cyanide, 83:1 for cyanogen chloride and 137:1 for phosgene. These data demonstrate that the sensitivity required for detection and quantitation is clearly exceeded with only a single data acquisition. Probably a more meaningful measure of method detection is reflected by the number of data acquisitions necessary to certify CWC Schedule 3 compounds at ≥95% purity and 99% confidence, requiring a signal-to-noise ratio ≥285:1 (a signal-to-noise ratio of ≥95:1 for 95% purity

multiplied by three for 99% confidence). At 126 MHz, this ratio is realized for each compound with only a single data acquisition recorded with the cryogenic probe head, however, a summation of acquisitions is necessary at 75 MHz using the conventional probe head. Phosgene for example, requires ≥5 data acquisitions, while hydrogen cyanide and cyanogen chloride require ≥12 acquisitions. In practice, ≥8 data acquisitions at 126 MHz and ≥32 acquisitions at 75 MHz were routinely collected to ensure the detection of impurities at low concentrations. Andimpurity signal intensities were not measured until an adequate number of acquisitions are collected to reach a signal-to-noise ratio ≥10:1.

4. CONCLUSIONS

Hydrogen cyanide, cyanogen chloride, phosgene, and chloropicrin are all produced in tremendously large quantities by the chemical industry. In the US, hydrogen cyanide and phosgene are considered high volume chemicals with productions far exceeding 20 million pounds annually. The bulk of the four chemicals are used as precursors and industrial feedstocks for the synthesis of many pesticides, dyestuffs, polymers and a large number other chemicals, and accurate quantitative analysis for chemical characterizations and purity determinations can be expected to be critical in each application. Although on a much smaller scale but equally as important, the quantitative analysis of the chemicals is also a critical component of the US Army's purity certification of military chemical agents to be used as standard analytical reference materials. The certification is extremely important in the case of CWC Schedule 3 chemicals, as they are required throughout the United States for instrument calibrations used in the testing and certification of protective equipment and the certification of lots of chemical resistant materials to be used in the manufacture of protective clothing. Moreover, many costly and complex research programs are dependent upon the proper characterization of the materials. CWC Schedule 3 compounds have been typically used in metabolic, toxicology, and ecotoxicology studies, and the research and development of protective respirators for soldiers and emergency first responders.

The purity of reference compounds has traditionally been determined by combining a number of analytical techniques, and these usually include gas or liquid chromatography. Because chromatographic detector responses vary widely from compound to compound, chromatographic techniques cannot directly measure the absolute purity of a compound. High purity reference standards, therefore, are required for every compound to be evaluated, and their preparation is often time-consuming and expensive. Particularly for CWC Schedule 3 compounds and military nerve agents,[10] this has been eliminated with the use of quantitative NMR spectroscopy. Our NMR-derived results agree closely to those from gas chromatography with thermal conductivity detection, and required roughly the same time or less for data collection. This is especially the case when collecting data with our cryogenic probe head at 126 MHz, which requires <20 m to achieve signal-to-noise ratios of ~2000:1. The many reports in the literature addressing quantitative NMR spectroscopy, some of which are referenced herein,[2-21] support the applicability of NMR spectroscopy for quantitative chemical analysis. Of special note are the United States

Pharmacopoeia[42] and the British Pharmacopoeia,[43] where the general quantitative NMR methodology is a compendium requirement.

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