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A PRELIMINARY INVESTIGATION INTO THE USE OF ^{31}P-NMR SPECTROMETRY TO FOLLOW THE HYDROLYSIS OF SOME CHEMICAL WARFARE AGENTS

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DEFENCE RESEARCH ESTABLISHMENT OTTAWA
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ABSTRACT

The use of ^{31}P-NMR spectrometry as a technique for monitoring the hydrolysis of sarin, soman, tabun and VX in water, in Hoskin buffer, and in Hoskin buffer in the presence of five commercially available enzymes was investigated. Calculated values for half-lives were demonstrated to be in line with experimentally observed times to 100% hydrolysis as well as with values from the literature. This investigation showed that ^{31}P-NMR spectrometry can be used as a semiquantitative technique to follow the hydrolysis hence, the decontamination, of phosphorus-containing chemical warfare agents.

RESUMÉ

Nous avons examiné l'emploi de la spectrométrie RMN du P-31 comme technique pour suivre l'hydrolyse du sarin, soman, tabun et du VX dans l'eau et dans une solution Tampon de Hoskin. La technique RMN du P-31 a été aussi utilisée lors de l'investigation de l'hydrolyse de ces agents avec la solution Tampon en présence de cinq enzymes obtenus commerciallement. Les valeurs calculies pour la demi-vie sont en accordance avec le temps observé expérimentallement pour l'hydrolyse à 100% de même qu' avec les valeurs dans la littérature. Cette recherche montre que la spectrographic RMN du P-31 peut être utilisée comme méthode semi-quantitative pour suivre l'hydrolyse des composés organophosphorés.

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EXECUTIVE SUMMARY

The use of ^{31}P-NMR spectrometry was evaluated as a technique for monitoring the breakdown of the chemical warfare agents sarin, soman, tabun and VX. The time taken to degrade 50% of the material in various water-based environments was utilized as a basis of comparison to established values. The results of the investigation demonstrated that ^{31}P-NMR spectrometry can be used to monitor the breakdown of chemical warfare agents. Only semiquantitative information, however, can be obtained due to the limitations of the instrumental technique. With further development, however, ^{31}P-NMR spectrometry has the potential to become a useful technique in screening for new chemical warfare agent decontaminants as well as to prove useful in the area of detection of chemical warfare agents.

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1.0 INTRODUCTION

Organophosphorus (OP) compounds, including the chemical warfare (CW) agents pinacolyl methylphosphonofluoridate (soman, GD), isopropyl methylphosphonofluoridate (sarin, GB), ethyl N,N- dimethylphosphoramidocyanidate (tabun, GA) and O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX), generally are reactive towards nucleophiles. When water is the nucleophile, the resulting hydrolysis of OP compounds, in general, and of the CW agents soman, sarin, tabun and VX, in particular (Appendix 1.0, Figures 1 and 2), is of importance because it results in their detoxification (1-5). Although the reaction between a phosphorus ester and water, or acid, or base, is known to obey second-order kinetics, in conditions where one reactant is in large excess or where its concentration is held constant, the reaction may be regarded as pseudo first-order (5-6). The rate of reaction in such cases is usually represented by a hydrolysis rate constant (k) and/or a half-life ($t_{1/2}$) value; each being unique for a particular set of reaction conditions. In the past, numerous techniques, including colorimetric, manometric, titrimetric (including pH stat), selective ion monitoring, and biochemical assays, have been employed to study the kinetics of the hydrolysis reaction of OP compounds and/or their simulants (7-11). Each of the techniques, however, has exhibited its own unique drawback(s) and/or limitation(s) thus leading to an ongoing search for alternate means of investigating the rate of hydrolysis of such compounds.

CW agents contain nuclei which produce nuclear magnetic resonance signal(s) which can be measured by high-resolution pulse Fourier transform (FT) techniques. It is generally accepted that high-resolution pulse FT ^{31}P-NMR spectrometry is one of the most useful techniques for organophosphorus compounds. When applied to CW agents, ^{31}P-NMR has exhibited a wide range of applicability: for example, trace analyte detection, systematic identification, and determination of formation rates of some methylphosphonofluoridates by following the reaction of methylphosphonofluorochloridates and various alcohols (12-16).

It was the objective of this work to investigate the use of ^{31}P-NMR spectrometry as a technique for monitoring the rate of hydrolysis of the CW agents: sarin, soman, tabun, and VX; alone, in the presence of water or Hoskin buffer, and in the presence of Hoskin buffer and various commercially-available enzymes. The results of the studies, together with comments on the use of ^{31}P-NMR spectrometry as a technique for monitoring CW agent hydrolysis rates, are presented.

2.0 EXPERIMENTAL

2.1 Materials

Water-white redistilled samples of soman, sarin, tabun and VX were obtained from the Chemical Detection and Decontamination Section of the Protective Sciences Division at the Defence Research Establishment Ottawa. They were shown to be greater than 98% pure, by ^{31}P-NMR spectrometry, prior to use.

Enzymes utilized in the study included alkaline phosphatase (32 units/mL), esterase (114 units/mL), catalase (994 units/mL), inorganic pyrophosphatase (21 units/mL) and PEG-catalase (320 units/mL). They were obtained from the Sigma Chemical Company, St. Louis, Missouri, U.S.A.

All other chemicals were obtained from commercial suppliers, and were reagent grade.

2.2 Methods

2.2.1 Sample Preparation for ^{31}P-NMR Spectrometry

Sample preparation was carried out directly in 10-mm NMR sample tubes. The overall reaction volume was kept constant at 2.5 mL for all experiments. The sample blank consisted of distilled deionized water and 20 mM overall concentration of one of sarin, soman, tabun and VX while the control sample contained Hoskin buffer (400 mM KCl, 50 mM NaCl, 5 mM bistrispropane (1,3-bis[tris hydroxymethyl methylamino] propane), pH 7.2) and one agent. Experimental samples consisted of Hoskin buffer and one each of an enzyme and agent until all combinations of experimental samples were completed.

In all experiments, the reaction was initiated by the addition of the enzyme. A delay time of 2 minutes between reaction initiation and data collection was necessary to allow for transfer of the experimental sample into, and final adjustments of, the NMR spectrometer.

2.2.2 ^{31}P-NMR Spectrometry

Proton decoupled ^{31}P-NMR spectra for all experimental samples were obtained on a Varian XL-200 NMR spectrometer using a DREO-modified technique of Peacock, Pottage and Sellars who have utilized ^{31}P-NMR spectrometry to study the hydrolysis of soman in human blood (personal communication, 1988). All spectra were recorded at room temperature (23 ± 2°C) using a 10-mm switchable V.T. probe tuned to 80.98 MHz. A sealed 5-mm tube containing trimethyl phosphate (1% in C_6D_6) was used as an external standard for integration and was co-run with the experimental samples by virtue of being included in the 10-mm sample tube containing the experimental sample . Spectra were measured over a spectral range specific for each CW agent using a pulse width of 9.5 µsec (45°) and a pulse repetition of 0.5 sec. Sampling time for each spectrum was 5 minutes. A ^{31}P spectrum of the initial sample was taken for subsequent comparative purposes to ^{31}P spectra which were obtained every hour until complete hydrolysis was observed or until 24 hours had elapsed. The ^{31}P chemical shifts reported are relative to the external reference 85% phosphoric acid (H_3PO_4).

Kinetic values were estimated from plots of normalized percent hydrolysis data versus time. The times to 50% and 100% hydrolysis were read off the curves. The k values were calculated from typical first-order reaction kinetics as presented by Moore and Pearson (6). The $t_{1/2}$ values were subsequently calculated according to the equation presented by Small (17).

3.0 RESULTS

The ^{31}P-NMR spectra of the hydrolysis of GB, GD, GA and VX, respectively, in Hoskin buffer are shown in Appendix 2.0, Figures 1 to 4 and are representative of all the reactions utilizing them as substrates. An increase, with time, in the ^{31}P-NMR signal of the hydrolysis products of GB, GD, GA or VX together with a decrease in the ^{31}P-NMR signal of GB, GD, GA or VX, respectively, can clearly be seen.

The ^{31}P-NMR spectrum of GB (^{1}H decoupled) showed two signals at 40.9 and 28.0 ppm from the external reference (85% H_3PO_4) due to a ^{31}P - ^{19}F coupling of 1044.33 Hz (Appendix 2.0, Figure 1). GB acid (isopropyl methylphosphonic acid, ^{1}H decoupled) was found to occur as a singlet in the ^{31}P-NMR spectrum, which, during the course of the hydrolysis reaction exhibited a shift in its signal from 27.5 to 28.8 ppm from the external reference.

The ^{31}P-NMR spectrum of GD (^{1}H decoupled) showed four signals, from two diastereomers, at 41.4, 40.8, 28.5 and 27.9 ppm from the external reference (85% H_3PO_4) with a ^{31}P - ^{19}F coupling of 1045.29 Hz (Appendix 2.0, Figure 2). The ^{31}P-NMR signal of GD acid (pinacolyl methylphosphonic acid, ^{1}H decoupled), however, was a singlet. As seen with GB acid singlet, the GD acid singlet was found to shift during the course of the hydrolysis reaction from 28.7 to 29.6 ppm from the external reference.

The ^{31}P-NMR spectrum of GA (^{1}H decoupled) showed one signal at -7.2 ppm from the external reference (85% H_3PO_4, Appendix 2.0, Figure 3). The major product of the hydrolysis of GA had its ^{31}P resonance at 0.9 ppm. A smaller but significant product appeared at 9.7 ppm. A third product exhibited a tendency to shift its signal over the range of 3.6 to 5.9 ppm. Differentiation and identification of the hydrolysis products of GA, however, was not carried out.

The ^{31}P-NMR spectrum of VX (^{1}H decoupled) showed one signal at 62.4, which was found to shift slightly to 61.8 ppm from the external reference (85% H_3PO_4) as the hydrolysis proceeded (Appendix 2.0, Figure 4). In the early stages of the hydrolysis of VX, one product was formed and found to appear at 27.3 ppm. As hydrolysis proceeded, the formation of a second hydrolysis product at 43.1 ppm was seen. Differentiation and identification of the hydrolysis products was not carried out.

Detailed kinetic analyses of the results were not possible because the concentrations were estimated by integration of the ^{31}P peaks time averaged over the sampling period of 5 minutes. Resulting values led only to semiquantitative pseudo first-order kinetic constants (k) and half-lives ($t_{1/2}$), and times to 50% and 100% hydrolysis (obtained from plots of experimental data) for each agent. The values are presented in Tables 1 to 4.

Table 1 presents hydrolysis information for GB. In water, GB displayed a k value of 0.17 mM hr^{-1} and a $t_{1/2}$ value (as calculated from the linear portion of the experimental data) of 4.1 hrs, while in Hoskin buffer, they were found to be 0.34 mM hr^{-1} and 2.0 hr, respectively. Alternately, the observed times to achieve 50% hydrolysis, obtained from a plot of experimental data, was 10 hr for both GB in water and in Hoskin buffer while the observed times to 100% hydrolysis were 24 and 18 hr, respectively. Catalase (from Aspergillus niger), inorganic pyrophosphatase (from Baker's yeast) and PEG-catalase (from bovine liver) had no effect on the hydrolysis of GB, exhibiting similar values for k and $t_{1/2}$ when compared to the control sample (Table 1). Alternately, both alkaline phosphatase and esterase were found to prolong the hydrolysis of GB, increasing the calculated $t_{1/2}$ from the control value of 2.0 hr to 11.4 and 18.2 hr, respectively.

Hydrolysis information for GD is presented in Table 2. With the possible exception of esterase (from porcine liver), which appears to prolong the hydrolysis of GD, no significant differences in the k and $t_{1/2}$ values for GD blank, control and experimental samples were found. Blank,

TABLE 1. LITERATURE AND ^{31}P-NMR CALCULATED HYDROLYSIS INFORMATION FOR SARIN

SOURCE	k[1] (mM hr^{-1})	$t_{1/2}$[1] (hr)	TIME TO 50%[2] HYDROLYSIS PRODUCT (hr)	TIME TO 100%[2] HYDROLYSIS PRODUCT (hr)	COMMENTS
EXPERIMENTAL[3]					
SARIN + WATER	0.17	4.1	9.9	24	-
SARIN + BUFFER[4]	0.34	2.0	9.7[5]	18	-
SARIN + BUFFER + ALKALINE PHOSPHATASE (E.COLI, TYPE III, 32 UNIT/mL)	0.06	11.4	19	-	69% HYDROLYZED AT 24 HR
SARIN + BUFFER + ESTERASE (PORCINE LIVER, TYPE I, 114 UNIT/mL)	0.04	18.2	23	-	51.5% HYDROLYZED AT 24 HR
SARIN + BUFFER + CATALASE (ASPERGILLUS NIGER, 994 UNIT/mL)	0.33	2.1	14	-	93% HYDROLYZED AT 24 HR
SARIN + BUFFER + INORGANIC PYROPHOSPHATASE (BAKERS YEAST, 21 UNIT/mL)	0.20	3.5	16	-	91% HYDROLYZED AT 24 HR
SARIN + BUFFER + PEG-CATALASE (BOVINE LIVER, 320 UNIT/mL)	0.28	2.5	11.5	20	-
LITERATURE					
R. TRAPP (2)	-	7.5 (pH 8.0, 25°C)	-	-	-
M.J. SMALL (17)	-	7.3 (pH 8.0, 20°C)	-	-	-
S.M. KENNEY-GARRETT ET AL (18)	-	5.0 (pH 9.0, 25°C)	-	-	-

1 Calculated from the linear portion of experimental data.

2 Determined from graphical data.

3 Concentration of sarin = 20 mM. Overall reaction volume = 2.5 mL.
Starting pH = 7.1 - 7.2 (The pH was not monitored as the reaction progressed.)
Temperature for the experiments = 23 ± 2°C

4 The buffer utilized was Hoskin Buffer (400 mM KCl, 50 mM NaCl, 5 mM Bistrispropane, pH = 7.2)

5 This result does not reflect the expected value when comparison to control (sarin + water) is made. It is noted that in both instances, an initial prolongation of the reaction was observed. The prolongation did not seemingly affect the calculated values of k and $t_{1/2}$ which are carried out on the linear portion of the curve. The authors have no explanation for the anomalous behaviour of sarin in water or buffer at the present time.

TABLE 2. LITERATURE AND ^{31}P-NMR CALCULATED HYDROLYSIS INFORMATION FOR SOMAN

SOURCE	k[1] ($mM\ hr^{-1}$)	$t_{1/2}$[1] (hr)	TIME TO 50%[2] HYDROLYSIS PRODUCT (hr)	TIME TO 100%[2] HYDROLYSIS PRODUCT (hr)	COMMENTS
EXPERIMENTAL[3]					
SOMAN + WATER	0.15	4.5	7	21	-
SOMAN + BUFFER[4]	0.20	3.5	4	14	-
SOMAN + BUFFER + ALKALINE PHOSPHATASE (E.COLI, TYPE III, 32 UNIT/mL)	NV[5]	NV[5]	NV[5]	NV[5]	39% HYDROLYZED AT 20 HR
SOMAN + BUFFER + ESTERASE (PORCINE LIVER, TYPE I, 114 UNIT/mL)	0.11	6.1	7.5	-	93% HYDROLYZED AT 22 HR
SOMAN + BUFFER + CATALASE (ASPERGILLUS NIGER, 994 UNIT/mL)	0.17	4.0	8.5	21	-
SOMAN + BUFFER + INORGANIC PYROPHOSPHATASE (BAKERS YEAST, 21 UNIT/mL)	0.21	3.3	8.5	21	-
SOMAN + BUFFER + PEG-CATALASE (BOVINE LIVER, 320 UNIT/mL)	0.20	3.5	7.8	-	93% HYDROLYZED AT 17.45 HR
LITERATURE					
R. TRAPP (2)	-	21.78 (pH 7.6, 20°C)	-	-	-
S.M. KENNEY-GARRETT ET AL (18)	-	45 (pH 6.65, 25°C)	-	-	-

1 Calculated from the linear portion of experimental data.

2 Determined from graphical data.

3 Concentration of soman = 20 mM. Overall reaction volume = 2.5 mL.
Starting pH = 7.1 - 7.2 (The pH was not monitored as the reaction progressed.)
Temperature for the experiments = 23 ± 2°C

4 The buffer utilized was Hoskin Buffer (400 mM KCl, 50 mM NaCl, 5 mM Bistrispropane, pH = 7.2)

5 NV = no value, reaction stopped before reaching 50% hydrolysis.

control, and experimental samples all appear to have an approximate k value of 0.17 mM hr^{-1} and an approximate $t_{1/2}$ of 4.2 hr. The times to 50% hydrolysis for all samples was approximated to be 8 hr, while to achieve 100% hydrolysis it was approximated to be 21 hr.

The hydrolysis information for GA is presented in Table 3. The k and $t_{1/2}$ for the blank (water) and control (Hoskin buffer) samples were similar, exhibiting an average k of 0.26 mM hr^{-1} and an average $t_{1/2}$ of 2.7 hr. In the presence of alkaline phosphatase, esterase, catalase and inorganic pyrophosphatase, k values for GA were slightly higher than the blank or control values, averaging 0.37 mM hr^{-1}. The $t_{1/2}$'s for the same samples were correspondingly lower than the blank and control. In the presence of PEG-catalase, however, k was found to be approximately twice that of the blank or control, 0.51 mM hr^{-1}, while the $t_{1/2}$ was correspondingly approximately one-half of the values, 1.4 hr. Further experimentation is necessary to determine the significance of this difference in kinetic values for GA in the presence of PEG-catalase.

It was determined, for all of the experimental samples containing GA, that the average time to 50% hydrolysis was 2.7 hr while for 100% hydrolysis it was 12 hr.

The hydrolysis information for VX is presented in Table 4. With the possible exception of the PEG-catalase experimental sample, it is seen that k, for VX in the presence of water, Hoskin buffer or the four enzymes, is similar and of the order of 1.0×10^{-2} mM hr^{-1}. Correspondingly, the $t_{1/2}$'s for the same samples are similar and are, on average, 75 hr. No experimentally-determined time to 50% or 100% hydrolysis product was obtained, since these studies were terminated at 24 hr.

4.0 DISCUSSION

In this preliminary investigation, we sought to examine the use of ^{31}P-NMR spectrometry as a tool for monitoring the rate of hydrolysis of the chemical warfare agents sarin, soman, tabun and VX, alone, in the presence of water or Hoskin buffer, and in the presence of Hoskin buffer and various commercially-available enzymes. Of the many factors that could have affected the use of ^{31}P-NMR spectrometry as a tool for monitoring the rate of hydrolysis of chemical warfare agents, as well as both the non-enzymatic and enzymatic reaction kinetics themselves, three main factors appear to predominate. The three factors are:

TABLE 3. LITERATURE AND ^{31}P-NMR CALCULATED HYDROLYSIS INFORMATION FOR TABUN

SOURCE	k[1] (mM hr^{-1})	$t_{1/2}$[1] (hr)	TIME TO 50%[2] HYDROLYSIS PRODUCT (hr)	TIME TO 100%[2] HYDROLYSIS PRODUCT (hr)	COMMENTS
EXPERIMENTAL[3]					
TABUN + WATER	0.25	2.8	2.8	15	-
TABUN + BUFFER[4]	0.27	2.5	3.5	12	-
TABUN + BUFFER + ALKALINE PHOSPHATASE (E.COLI, TYPE III, 32 UNIT/mL)	0.32	2.2	2.7	-	84% HYDROLYZED AT 20 HR
TABUN + BUFFER + ESTERASE (PORCINE LIVER, TYPE I, 114 UNIT/mL)	0.40	1.8	2.0	12	-
TABUN + BUFFER + CATALASE (ASPERGILLUS NIGER, 994 UNIT/mL)	0.39	1.8	2.1	12	-
TABUN + BUFFER + INORGANIC PYROPHOSPHATASE (BAKERS YEAST, 21 UNIT/mL)	0.32	2.2	2.8	15	-
TABUN + BUFFER + PEG-CATALASE (BOVINE LIVER, 320 UNIT/mL)	0.51	1.4	3.0	-	93% HYDROLYZED AT 6 HR
LITERATURE					
FORSMAN ET AL (4)	-	2 (pH 9.0, 25°C)	-	-	-
S.M. KENNEY-GARRETT ET AL (18)	-	8.5 (pH 7.0, 20°C)	-	-	-

[1] Calculated from the linear portion of experimental data.

[2] Determined from graphical data.

[3] Concentration of tabun = 20 mM. Overall reaction volume = 2.5 mL.
Starting pH = 7.1 - 7.2 (The pH was not monitored as the reaction progressed.)
Temperature for the experiments = 23 ± 2°C

[4] The buffer utilized was Hoskin Buffer (400 mM KCl, 50 mM NaCl, 5 mM Bistrispropane, pH = 7.2)

TABLE 4. LITERATURE AND ^{31}P-NMR CALCULATED HYDROLYSIS INFORMATION FOR VX

SOURCE	k x 10^{-3} [1] ($mM\ hr^{-1}$)	$t_{1/2}$ [1] (hr)
LITERATURE		
M.J. SMALL (17)	-	900 (pH 7.0, T= NV[2])
S.M. KENNEY-GARRETT ET AL (18)	-	40 (pH 7.0, 25°C)
EXPERIMENTAL[3]		
VX + WATER	8.3	83.6
VX + BUFFER[4]	12.0	57.3
VX + BUFFER + ALKALINE PHOSPHATASE (E.COLI, TYPE III, 32 UNIT/mL)	7.9	88.3
VX + BUFFER + ESTERASE (PORCINE LIVER, TYPE I, 114 UNIT/mL)	13.0	52.9
VX + BUFFER + CATALASE (ASPERGILLUS NIGER, 994 UNIT/mL)	7.5	92.0
VX + BUFFER + INORGANIC PYROPHOSPHATASE (BAKERS YEAST, 21 UNIT/mL)	NV	NV
VX + BUFFER + PEG-CATALASE (BOVINE LIVER, 320 UNIT/mL)	4.2	167.2

[1] Calculated from the linear portion of experimental data.

[2] NV = no value

[3] Concentration of VX = 20 mM. Overall reaction volume = 2.5 mL. Starting pH = 7.1 - 7.2 (The pH was not monitored as the reaction progressed.). Temperature for the experiments = 23 ± 2°C

[4] The buffer utilized was Hoskin Buffer (400 mM KCl, 50 mM NaCl, 5 mM Bistrispropane, pH = 7.2).

i) changes in the pH of the reaction mix;
ii) the process of reversible inhibition; and,
iii) the type and concentration of substrate.

It is generally accepted that the rate of non-enzymatic hydrolysis of phosphorus esters is influenced by:

i) variations in the substituents of the phosphorus compounds, i.e. electron-withdrawing substituents making the compounds more susceptible to OH^- attack and electron-donating substituents conferring greater stability;

ii) the presence of various catalytic agents;

iii) variations in the solvent used, i.e. hydrolysis is only a special case of solvolysis; and,

iv) variations in temperature and pH (1).

Equally, it is acknowledged that reactions involving enzymes are more sensitive to changes in conditions than most non-enzymatic reactions (19). Typically, this is because:

i) products of the reaction may inhibit the enzyme;

ii) the degree of saturation of the enzyme with substrate falls because of a fall in substrate concentration as the reaction proceeds;

iii) the reverse reaction becomes more important as the concentration of products increase;

iv) the enzyme(s) undergoes inactivation at the temperature or pH of the reaction; and/or,

v) a reversible inhibitor combines with the enzyme to temporarily give an inactive complex.

A complete study of enzyme kinetics, therefore, usually involves the investigation of variation of enzyme concentration, substrate concentration, temperature, pH, and the presence of activators or inhibitors. However; in this preliminary investigation, not all variables were studied. It is recommended, that future work include studies of all variables affecting kinetics, which consequently would give rise to knowledge of the mechanism of the enzymatic reaction(s).

Both non-enzymatic and enzymatic reactions are susceptible to variations in pH. In this experimental design, however, there were no special attempts made to keep pH constant over the total reaction time. Consequently, as hydrolysis progressed, the acids of the respective agents were formed thus changing the initial pH conditions. In this experiment,

it was observed, in some instances, that the change in pH was significant enough to denature the enzymatic material and precipitate it out of solution. Although directly affecting the results of the experimental data, the variations in pH over the reaction did not negate the use of ^{31}P-NMR spectrometry as a tool in determining how the reaction was affected. On the contrary, the calculated $t_{1/2}$'s, gained from the evaluation of data obtained by following the hydrolysis of sarin, soman, tabun and VX by ^{31}P-NMR spectrometry, were found to correlate well to the observed times to 100% hydrolysis seen in this investigation. It is suggested that the influence of pH in further experiments be controlled by use of a more powerful buffering system or that samples be titrated between sampling times to maintain as stable a pH as possible.

The second factor found to be predominating, particularly in the enzymatic reactions, is the process of reversible inhibition. This process involves the temporary combining of a reversible inhibitor with an enzyme to give an inactive complex. In most instances, this is not a problem. If, however, the time for the reversible process is too long or too short, the reaction never really goes forward consequently making the determination of reaction kinetics difficult to impossible. In this work, sarin, soman, tabun and VX exhibited varying degrees of reversible inhibition towards the five enzymes utilized. Although having occurred, this did not appear to interfere with the subsequent determination of $t_{1/2}$'s by ^{31}P-NMR spectrometry as mentioned previously.

The third factor influencing both the use of ^{31}P-NMR spectrometry as a tool for monitoring reaction kinetics, as well as the reaction kinetics themselves (particularly those involving enzymes), is the type of substrate as well as its concentration. One of the well-known limitations of NMR is a relatively low level of sensitivity, thereby necessitating the use of relatively concentrated samples. In monitoring non-enzymatic kinetics, this is not normally a problem. This work was no exception and using ^{31}P-NMR spectrometry to monitor the non-enzymatic hydrolysis of relatively high concentrations of sarin, soman, tabun and VX did not pose a problem. In following the enzymatic hydrolysis, however, both the substrate and substrate concentration can have an effect on an enzyme's activity (i.e. it is standard belief in enzymology that a particular enzyme may not have the ability to breakdown, may only partially breakdown or be totally specific for a given compound which may/may not be concentration dependent, or, alternately, a given compound can sometimes activate, exhibit no effect on or inhibit a particular enzyme based solely on its concentration in the reaction mixture, 19).

In this work, it is suggested that no large differences were seen in the reaction kinetics between the enzymatic and non-enzymatic control samples because either the enzymes could not utilize sarin, soman, tabun and VX as substrates and/or the initial concentration of them was too high, and thus, the enzymes were inhibited by sarin, soman, tabun and VX.

Apart from the three predominating factors discussed above, which are suggested to affect the reaction kinetics, two secondary concerns result from this investigation and are also included for general discussion. Firstly, in this investigation, the chemical shift of some of the hydrolysis products was found to vary with time, moving downfield from H_3PO_4 as the hydrolysis reaction progressed. The shifting of the resonance signal, in some cases, for the hydrolysis product to overlap with the resonance signal for the original starting material had a minimal effect with respect to the determination of the reaction kinetics. In future experiments, however, it is suggested that reaction conditions be maintained over the course of the reaction in order to minimize/eliminate this potential source of error.

The second concern for general discussion is that both the non-enzymatic and enzymatic hydrolysis of the initial compounds are known to undergo secondary hydrolysis (i.e. secondary hydrolysis being largely dependent on reaction conditions). However, only hydrolysis of the fully esterified phosphorus compound(s) is of usual concern since it is known that hydrolysis of the first ester constitutes detoxification (1). In some instances, in this investigation, some secondary hydrolysis was observed. This did not pose a problem for two reasons:

i) firstly because we were interested in the disappearance of initial materials; and,

ii) secondly because even if secondary hydrolysis occurs, under properly chosen measuring conditions, the intensity of the ^{31}P-NMR peak(s) is directly proportional to the amount of phosphorus in the compound. Total, as well as the amount of phosphorus due to primary or secondary hydrolysis, can be obtained and related to the disappearance of initial materials.

Utilizing normalized data, reaction kinetics information could subsequently be calculated. Although not of specific interest to this work, the results of this work do suggest that, under appropriately chosen conditions, the secondary hydrolyses of phosphorus-containing compounds could also be followed by ^{31}P-NMR spectrometry.

5.0 CONCLUSIONS AND RECOMMENDATIONS

The results of this preliminary investigation have demonstrated that ^{31}P-NMR spectrometry can be used as a technique, albeit as a semiquantitative technique, to follow the kinetics of hydrolysis of the phosphorus-containing compounds sarin, soman, tabun and VX.

It is recommended, that further work be carried out to establish firmly the calculated and observed values for the kinetic constants and half-lives for both non-enzymatic and enzymatic hydrolyses of sarin, soman, tabun and VX. For the non-enzymatic reactions with sarin, soman, tabun, and VX, it is suggested that control of the reaction conditions (particularly pH) and appropriate replication of experiments would help to establish acceptable limits of error. For the enzymatic reactions, it is suggested that a series of experiments using a wide range of enzyme and substrate concentrations, temperature, and pH, as well as some investigation of activators/inhibitors are necessary before enzymatic reaction kinetics and specific activities can be firmly established by this technique.

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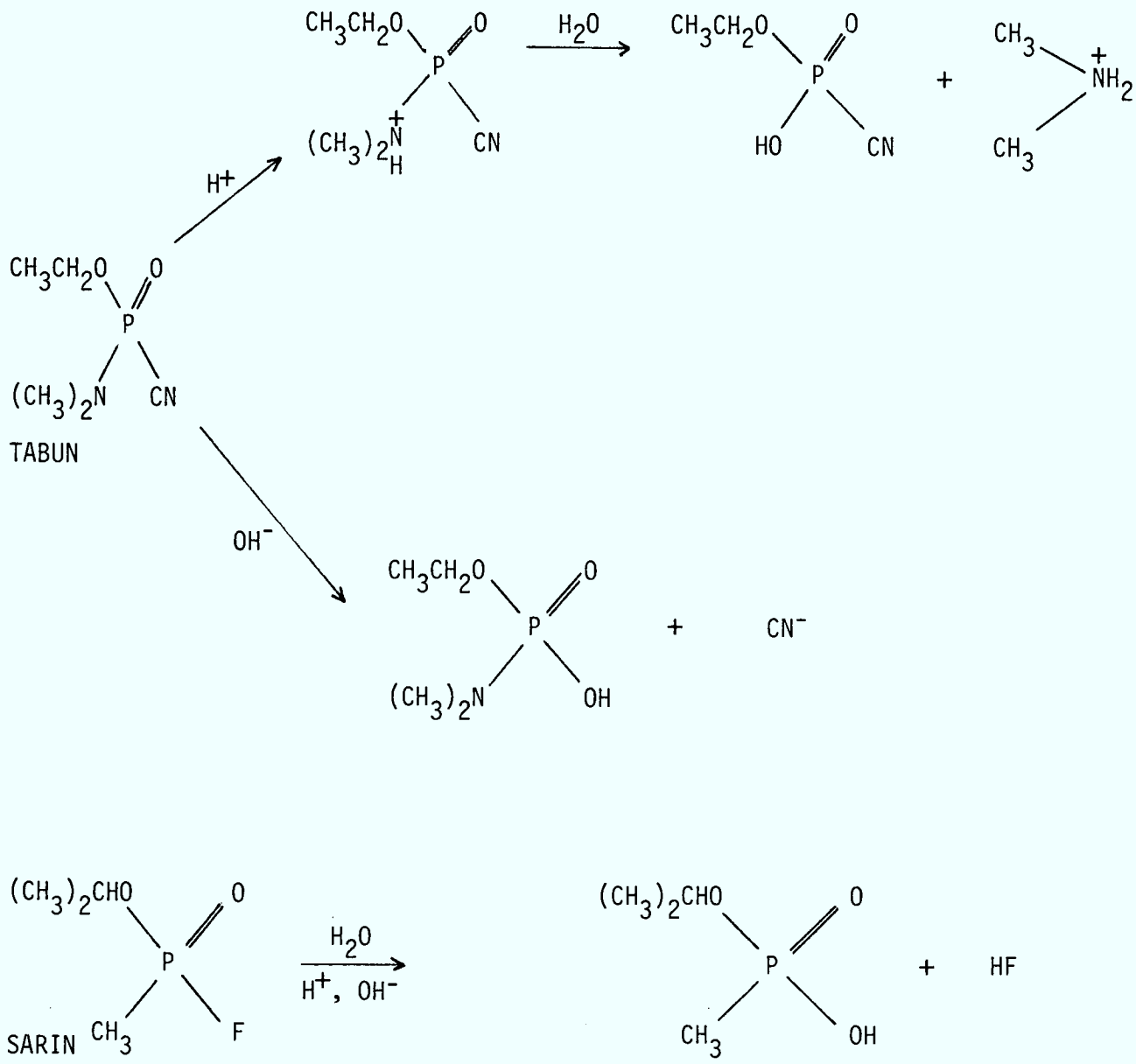
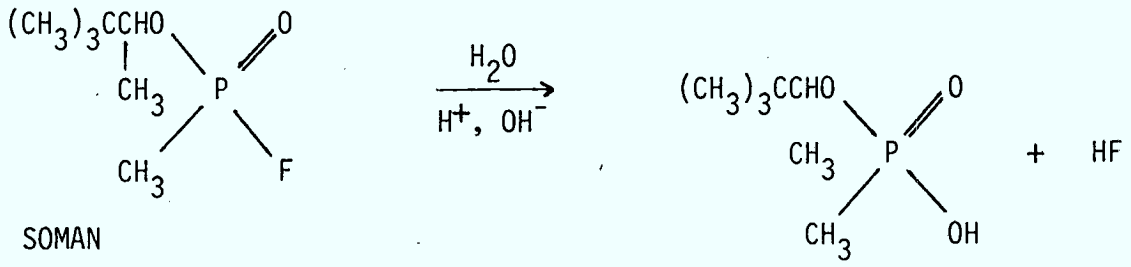
APPENDIX 1.0

FIGURE 1: HYDROLYSIS OF G AGENTS



APPENDIX 1.0 (CONT'D)

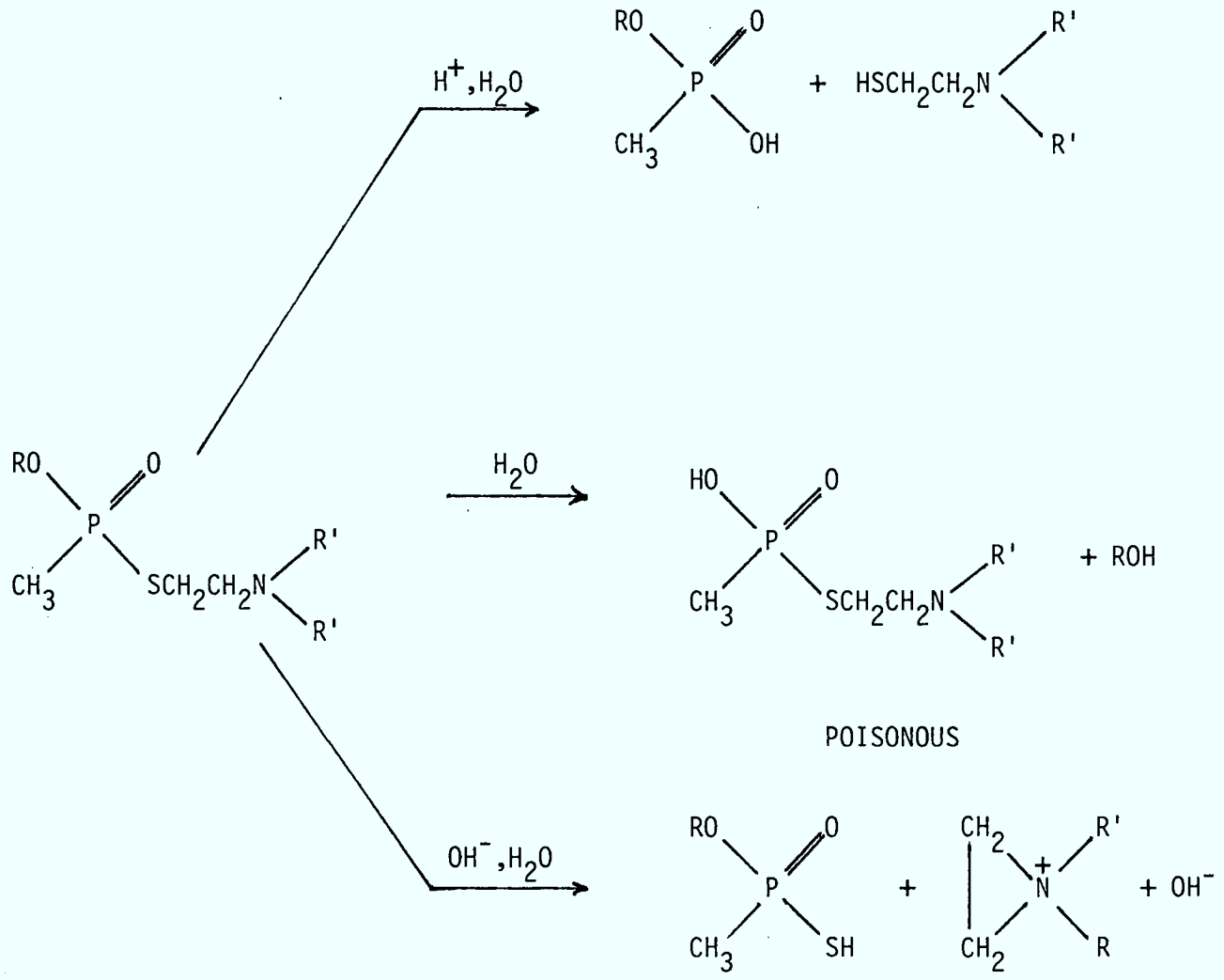
FIGURE 1 (CONT'D)



SOMAN

APPENDIX 1.0 (CONT'D)

FIGURE 2: HYDROLYSIS OF ALKYLPHOSPHONOTHIOLATE COMPOUNDS

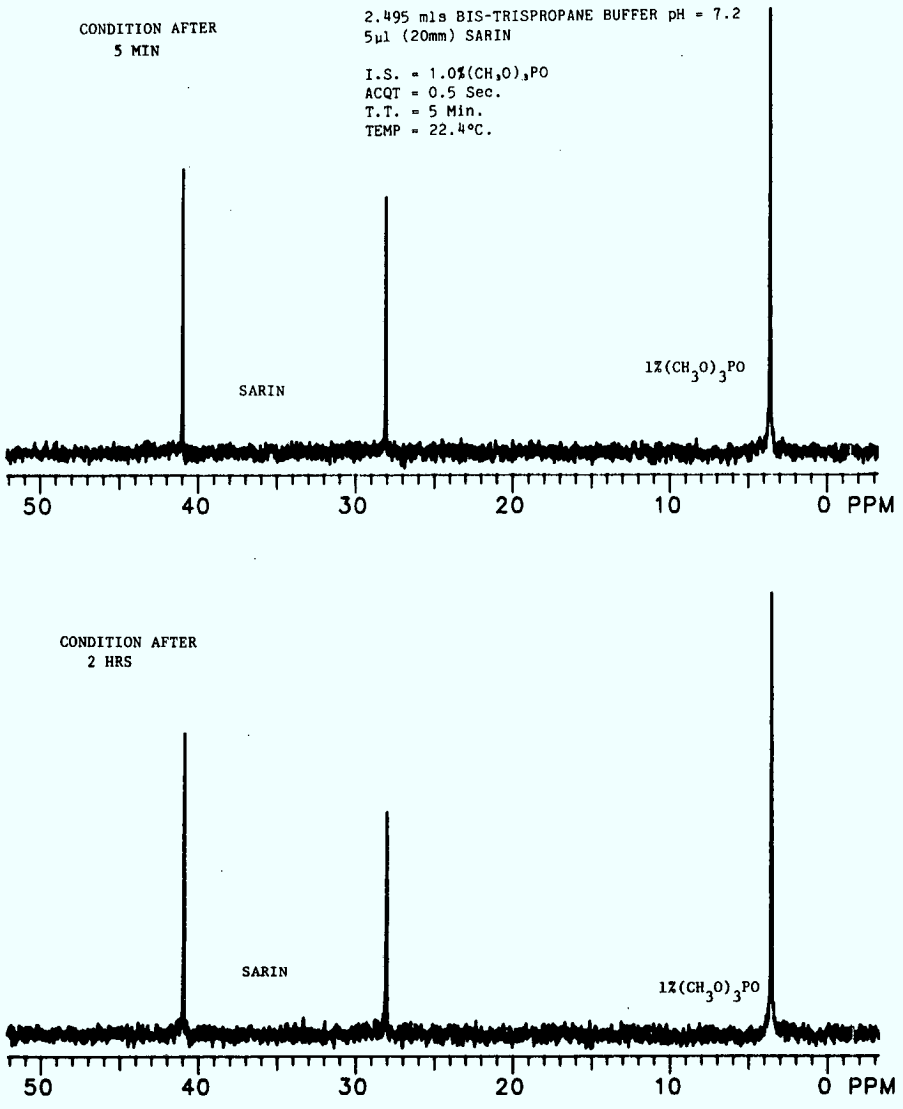


[1] Source: S. Franke et al (3)

[2] For VX, R = $-CH_2CH_3$; R' = isopropyl

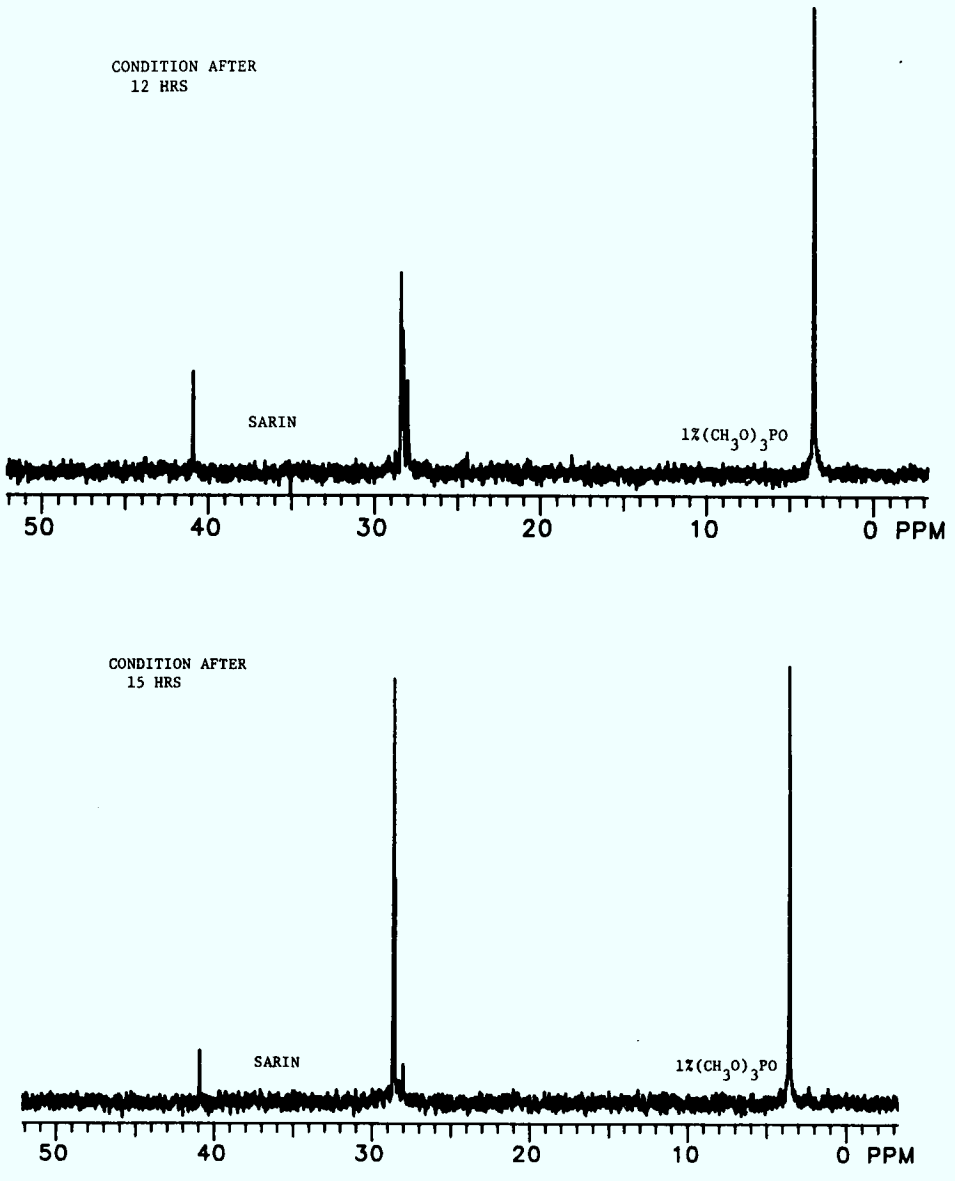
APPENDIX 2.0

FIGURE 1: The ^{31}P-NMR Spectra of Sarin at Various Time Intervals.



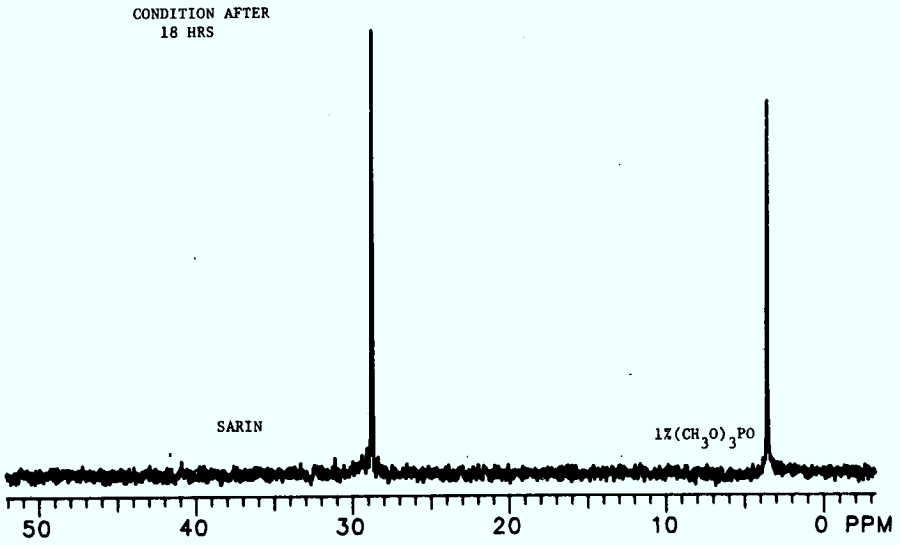
APPENDIX 2.0

FIGURE 1: (Cont'd)



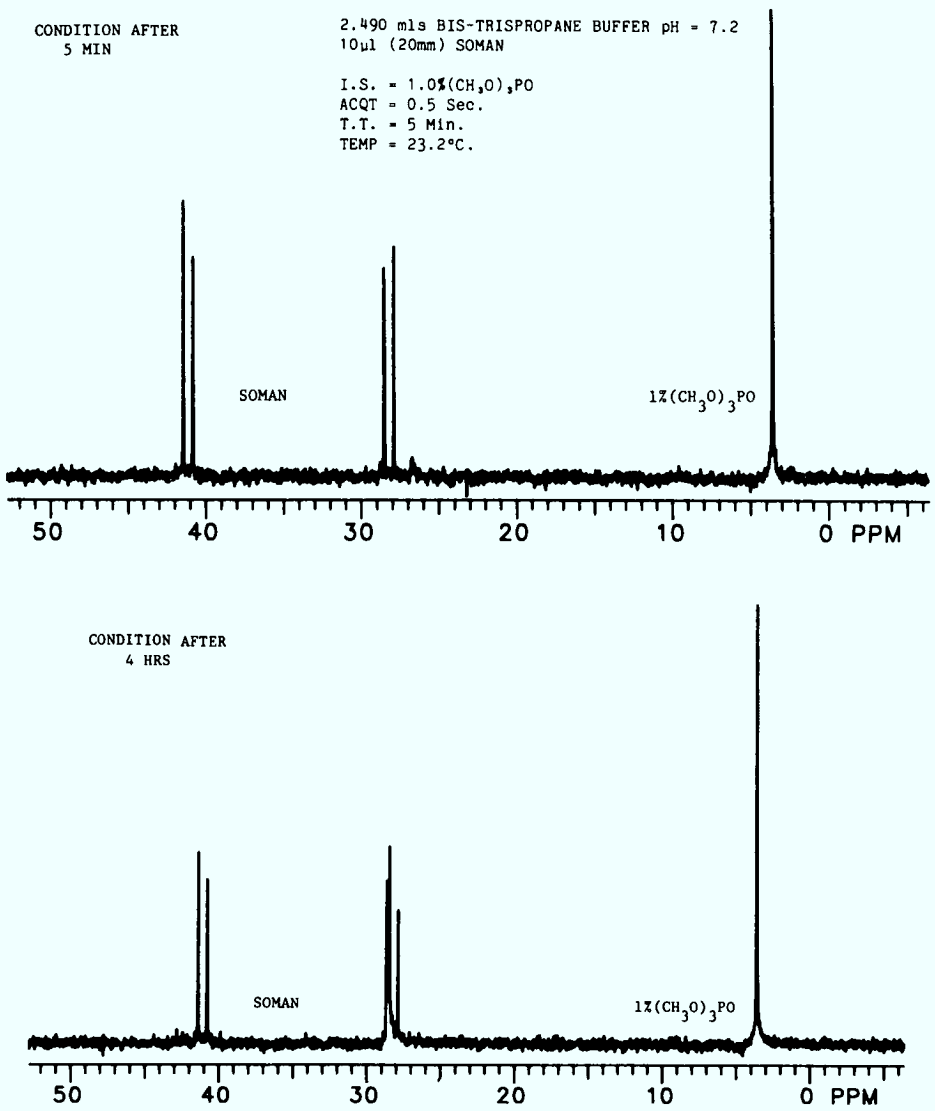
APPENDIX 2.0

FIGURE 1: (Cont'd)



APPENDIX 2.0 (CONT'D)

FIGURE 2: The ^{31}P-NMR Spectra of Soman at Various Time Intervals



APPENDIX 2.0 (CONT'D)

FIGURE 2: (Cont'd)

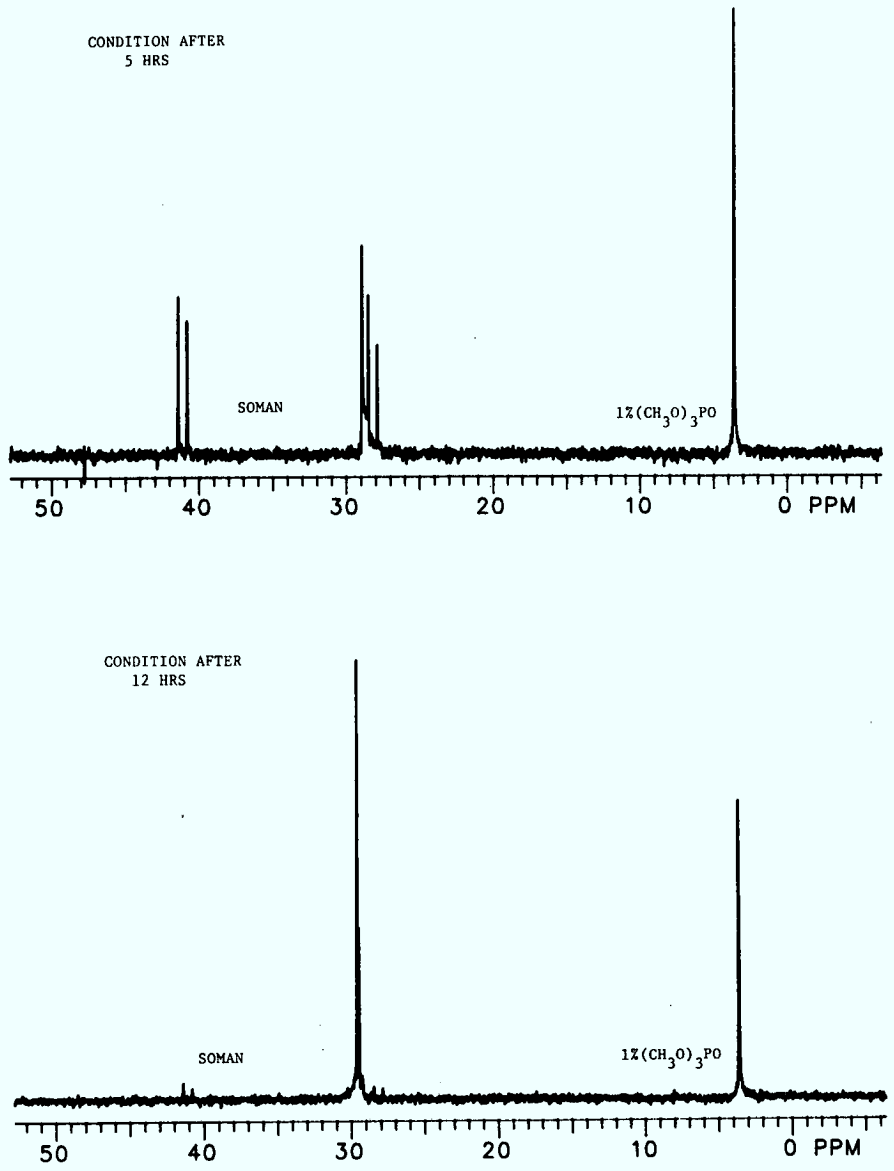


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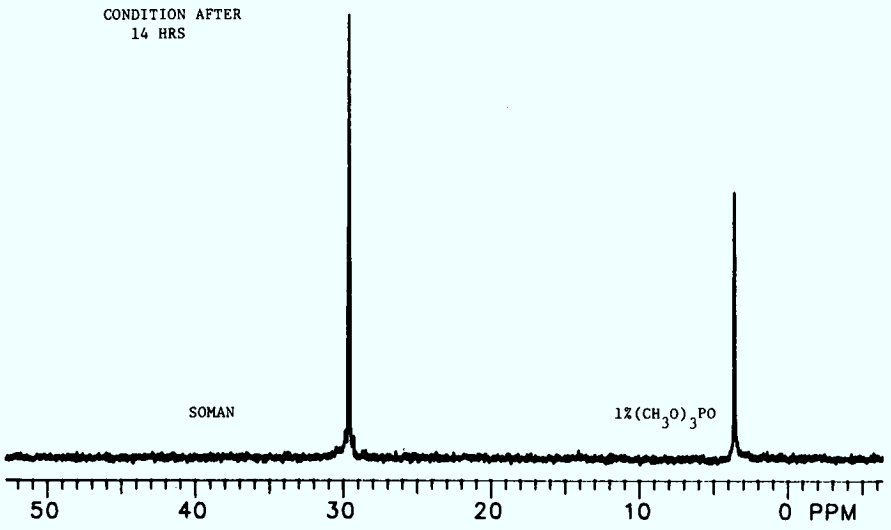
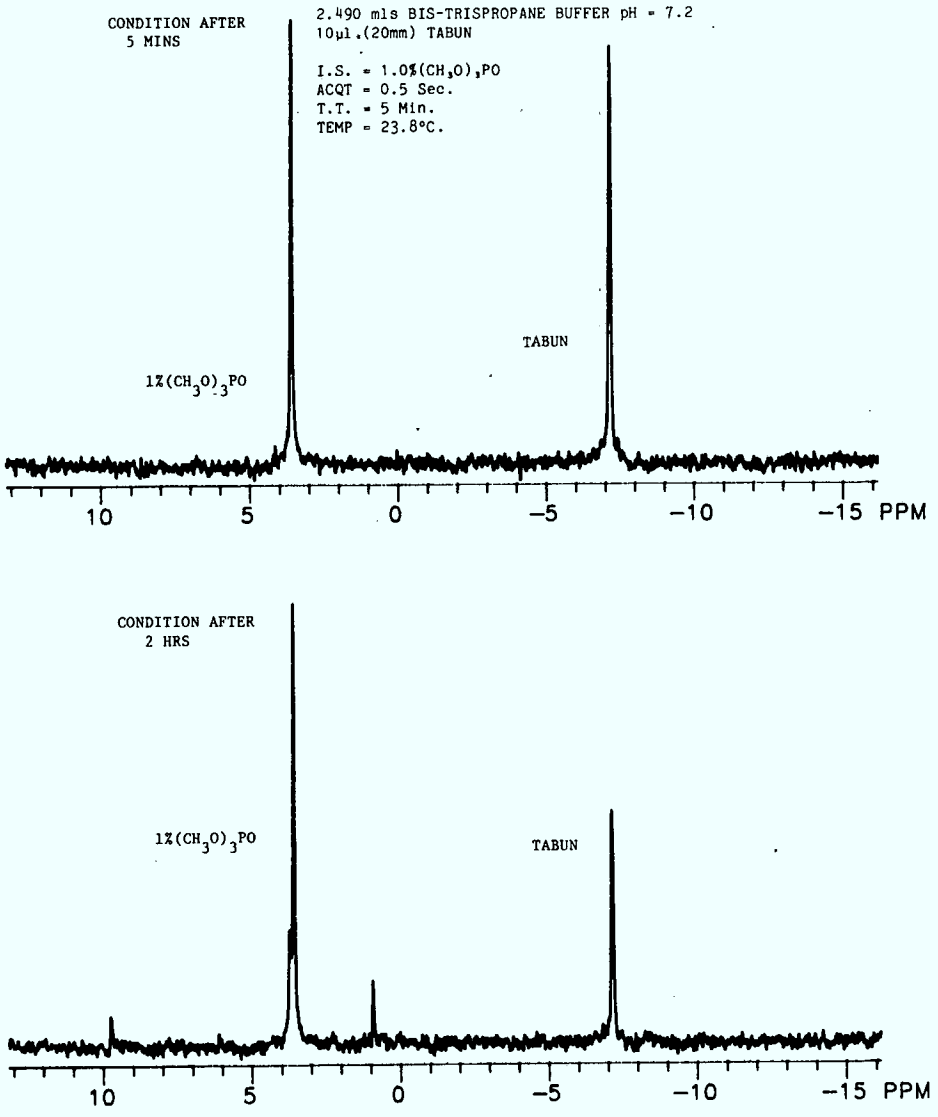
FIGURE 2: (Cont'd)



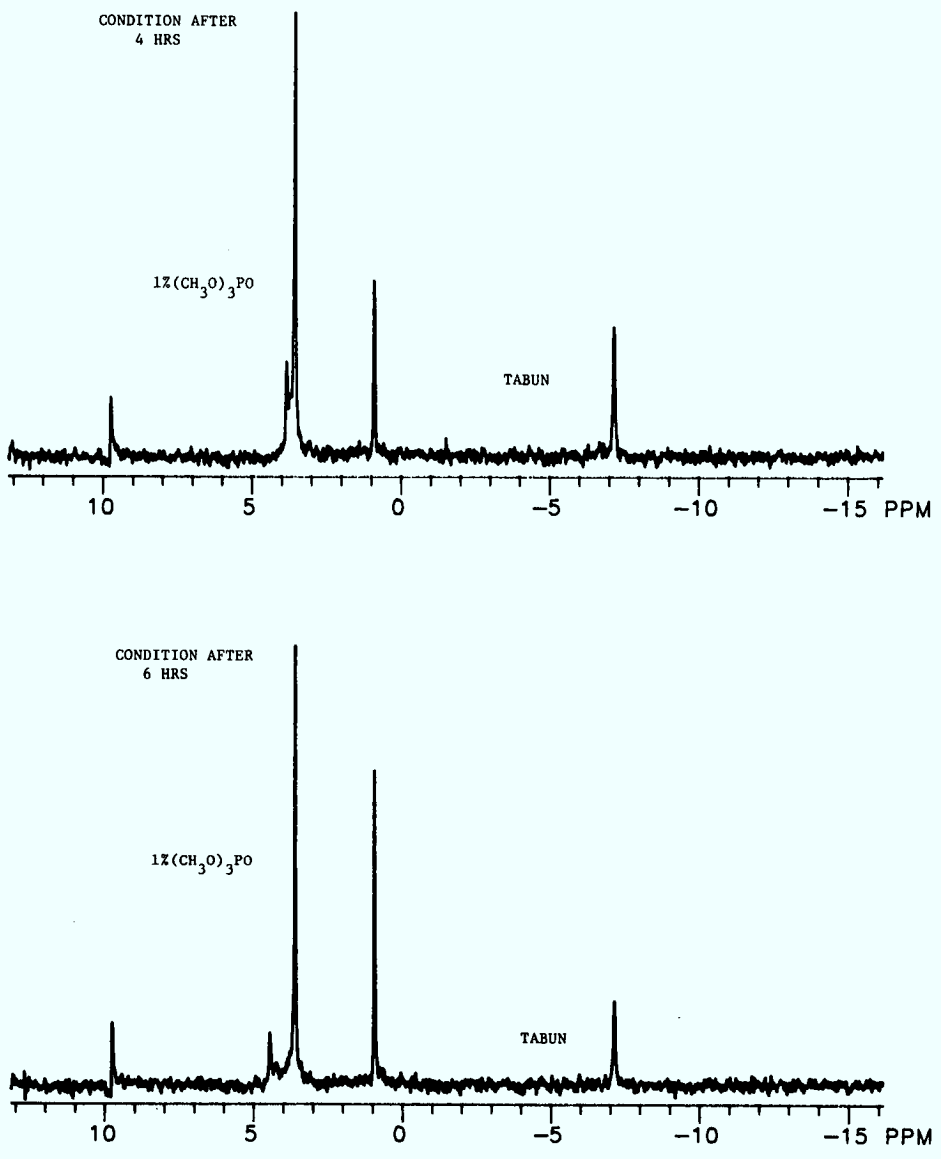
APPENDIX 2.0 (CONT'D)

FIGURE 3: The ^{31}P-NMR Spectra of Tabun at Various Time Intervals



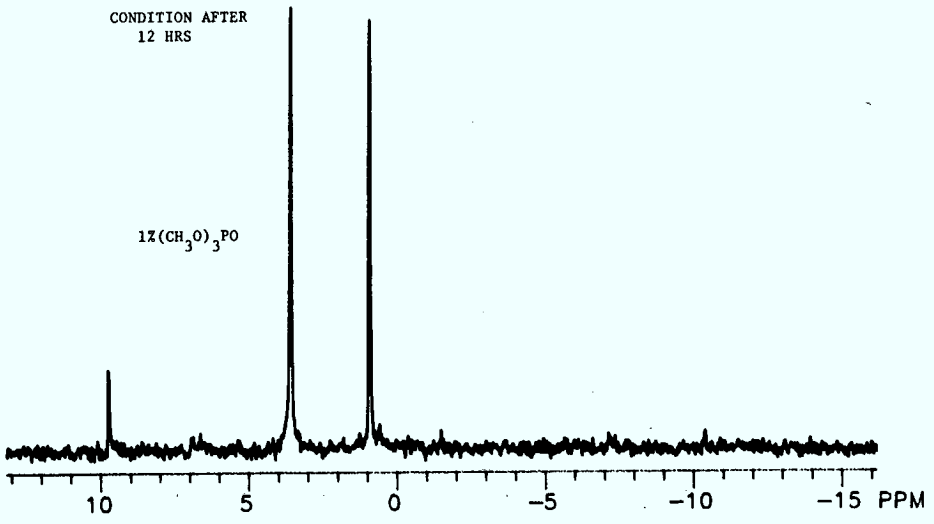
APPENDIX 2.0 (CONT'D)

FIGURE 3: (Cont'd)



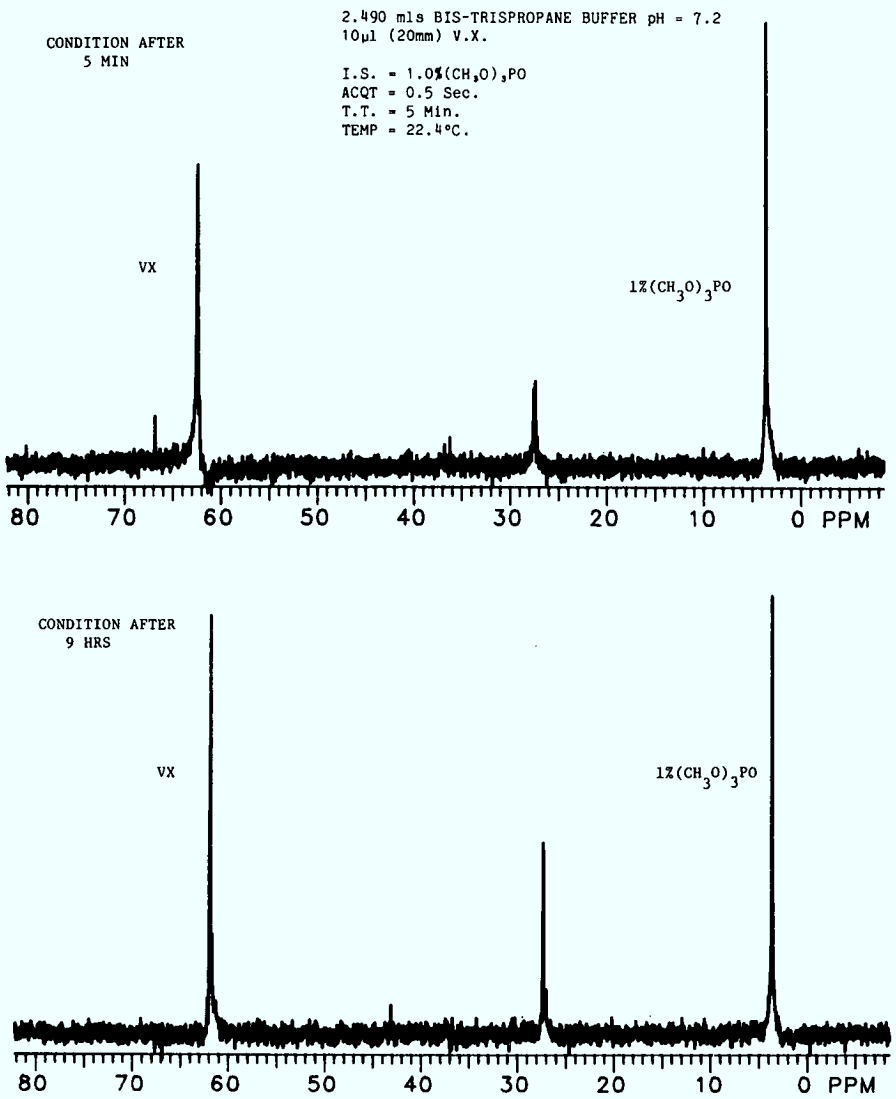
APPENDIX 2.0 (CONT'D)

FIGURE 3: (Cont'd)



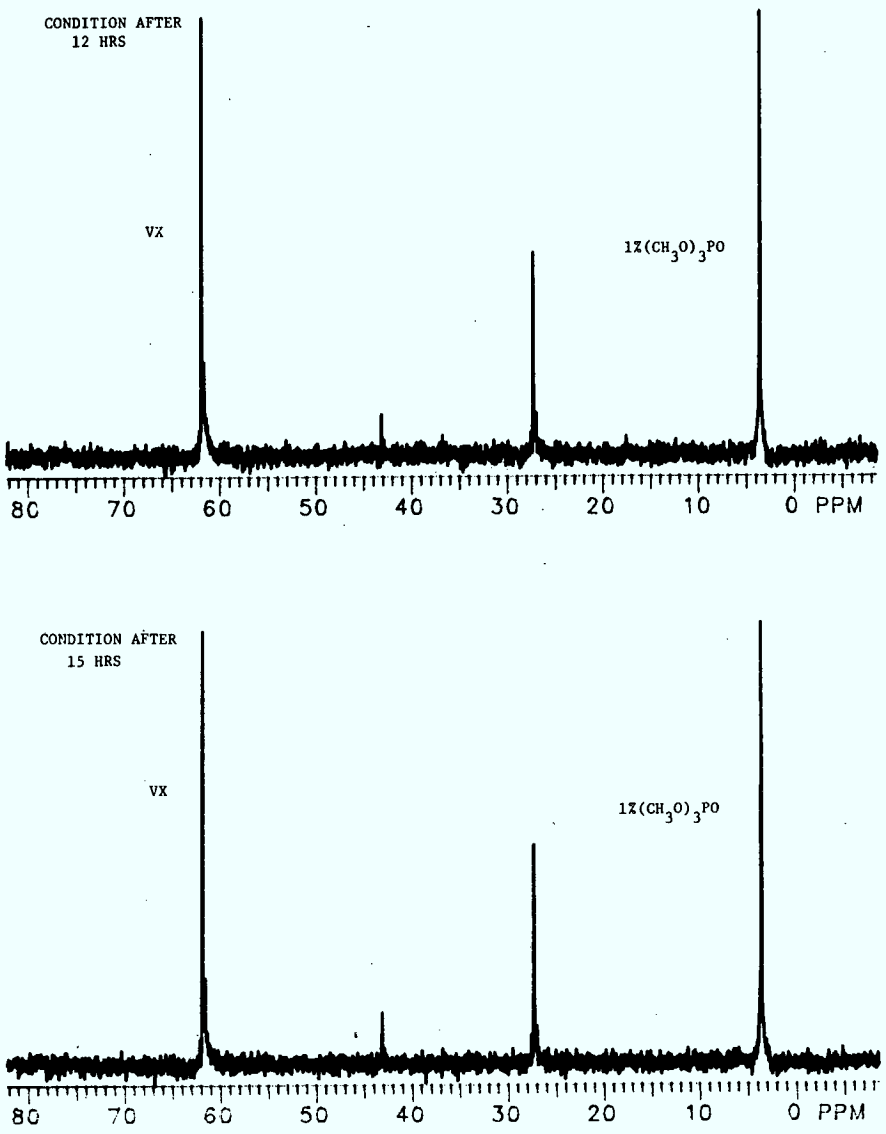
APPENDIX 2.0 (CONT'D)

FIGURE 4: The ^{31}P-NMR Spectra of VX at Various Time Intervals



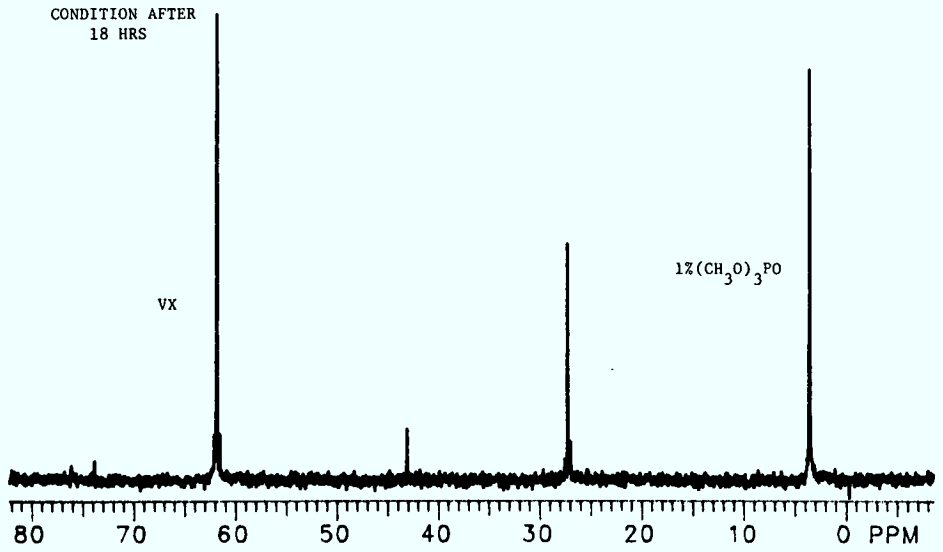
APPENDIX 2.0 (CONT'D)

FIGURE 4: (Cont'd)



APPENDIX 2.0 (CONT'D)

FIGURE 4: (Cont'd)



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4. AUTHORS (Last name, first name, middle initial)

SHILOFF, J. Deborah, LACROIX, Benoit V. and BOVENKAMP, John W.

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(U) The use of ^{31}P-NMR spectrometry as a technique for monitoring the hydrolysis of sarin, soman, tabun and VX in water, in Hoskin buffer, and in Hoskin buffer in the presence of five commercially available enzymes was investigated. Calculated values for half-lives were demonstrated to be in line with experimentally observed times to 100% hydrolysis as well as with values from the literature. This investigation showed that ^{31}P-NMR spectrometry can be used as a semiquantitative technique to follow the hydrolysis hence, the decontamination, of phosphorus-containing chemical warfare agents. (U)

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