Final Report DHS HS-STEM Fellowship Program (2015)

"Investigating the Hydrolysis Reactions of a Chemical Warfare Agent Surrogate: A Systematic Study using ^{1}H, ^{13}C, ^{17}O, ^{19}F, ^{31}P, and ^{35}Cl NMR Spectroscopy"

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Introduction:

During the summer of 2015, I participated in the DHS HS-STEM fellowship at Sandia National Laboratories (SNL, NM) under the supervision of Dr. Todd M. Alam in his Nuclear Magnetic Resonance (NMR) Spectroscopy research group. While with the group, my main project involved pursing various hydrolysis reactions with Diethyl Chlorophosphate (DECP), a surrogate for the agent Sarin (GB). Specifically, I performed different hydrolysis reactions, monitored and tracked the different phosphorous containing species using phosphorous (^{31}P) NMR spectroscopy. With the data collected, I performed kinetics studies mapping the rates of DECP hydrolysis. I also used the NMR of different nuclei such as ^{1}H, ^{13}C, ^{17}O, and ^{35}Cl to help understand the complexity of the reactions that take place. Finally, my last task at SNL was to work with Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) NMR Spectroscopy optimizing conditions for ^{19}F-^{31}P filtering NMR experiments.

Background:

Sarin is a Chemical Warfare Agent (CWA) and arguably one of the most deadly. It was synthesize in 1939 by German scientists **S**chrader, **A**mbros, **R**itter, and L**in**de and named in their honor. This CWA was never initially intended for use in war, but instead as an improvement for pesticides. However, even though intentions were never for harm to mankind that has not been the case.

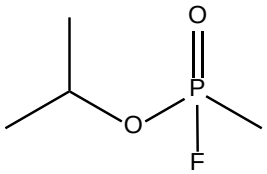


Figure 1. Structure of Sarin (GB)

Figure 2. Sarin use Timeline

As seen in Figure 2, there are multiple cases where sarin has been used for harm and most recently in 2013 in Ghouta, Syria.

When sarin enters the body, it binds irreversibly to a class of enzymes known as cholinesterases. This binding occurs by the phosphorus atom blocking the active site of the enzyme and preventing acetylcholine, a neurotransmitter from being broken down into choline and acetic acid. When this occurs, acetylcholine builds up in excess; when it is not degraded it prevents the cholinergic neuron from returning to its resting state after firing. Typically when this happens, death is almost certainly caused by asphyxiation due to the inability of muscles to allow breathing.

Motivation and Goals:

Currently, to neutralize and decontaminate CWAs, the agent is commonly diluted with large amount water. Then once a suitable concentration is reached, it is added to a field deployable batch reactor. Our goal for this project is to explore a way to neutralize and polymerize the CWA simulant *in situ* using a “wet” chemistry approach with very little dilution. It had been previously noted at SNL that the mechanisms of hydrolysis and condensation were

dramatically different between the dilute and concentrated regimes. These differences were also explored under this project.

Figure 3. Batch Reactor in Field Deployable Hydrolysis Unit

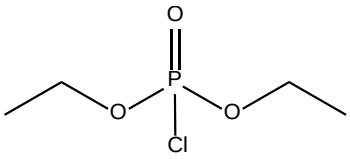


Figure 4. Structure of DECP

Experiments:

A variety of experiments were designed to simulate high concentration of DECP low concentration of decontamination reagent. In each case, 1.0mL of DECP (7 mmoles) was added to an NMR tube and an initial ^{31}P NMR spectrum was taken to assess the relative starting percentages of phosphorus containing species. Then a minimal amount of decontamination reagent was added to the tube to initiate the reaction, and a kinetic series of multiple NMR spectra were in most cases acquired overnight then followed through several weeks. Additional ^{1}H and ^{13}C NMR experiments were also performed to confirm the presence of one of the byproducts of the reaction as chloroethane (EtCl) and not ethanol (EtOH). To try and further elucidate the question of the reaction mechanism by determining the byproduct of the reaction a ^{35}Cl NMR spectroscopy experiment was set up to examine the chlorine containing species of the reaction.

To better understand the complexity of the hydrolysis reaction that occurs when water is added the ^{17}O isotope was examined using NMR spectroscopy. However, it has a natural abundance of 0.0037%. Due to its quadrupolar nature and fast relaxation time the low natural abundance was not a problem and it was possible to obtain a ^{17}O NMR spectrum of pure DECP. To attempt to track the oxygen in the hydrolysis reaction, enriched water was used ($H_2{}^{17}O$).

All experiments were performed on a Bruker Avance III 500 MHz spectrometer with 5mm broadband observe probe at 298K or 323K using standard pulse sequences.

Significant Results

1. Hydrolysis of DECP

A variety of different decontamination chemistries were explored as part of this project, and will be detailed elsewhere in a technical report (SAND Report 2015-XXXX, Kinnan, Alam, and Wilson, *in preparation*). From the ^{31}P NMR studies the half-life for DECP disappearance ($t_{1/2}$) and kinetic rate constant were evaluated. As an example, the hydrolysis of DECP with the addition of 10μL, 30μL, and 124μL of H_2O as well as 30μL and 124μL of H_2O_2 at 298K (room temperature) are summarized in Table1.

Table 1. Reaction Descriptions

Reagent	Half-life of DECP ($t_{1/2}$)	Molar Ratio (mmoles) DECP : Reagent
10μL H_2O	44450 min	1:0.085
30μL H_2O	2590 min	1:0.285
124μL H_2O	155 min	1:1
30μL H_2O_2	1343 min	1:0.0428
124μL H_2O	166 min	1:0.142

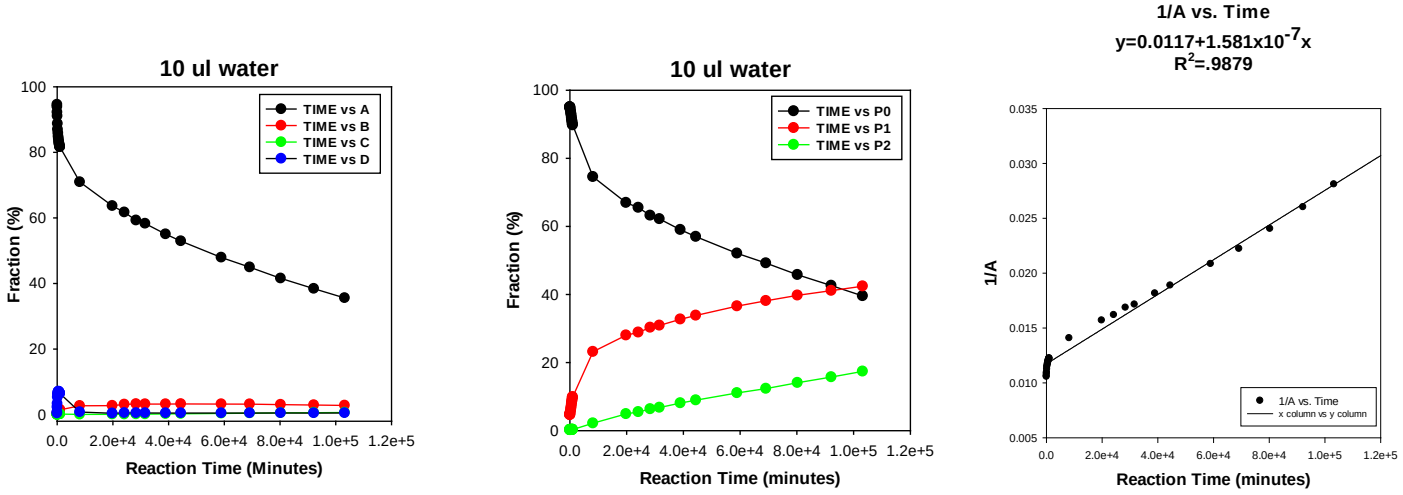


Figure 5. 10μL H_2O, %P vs. Time and Kinetics

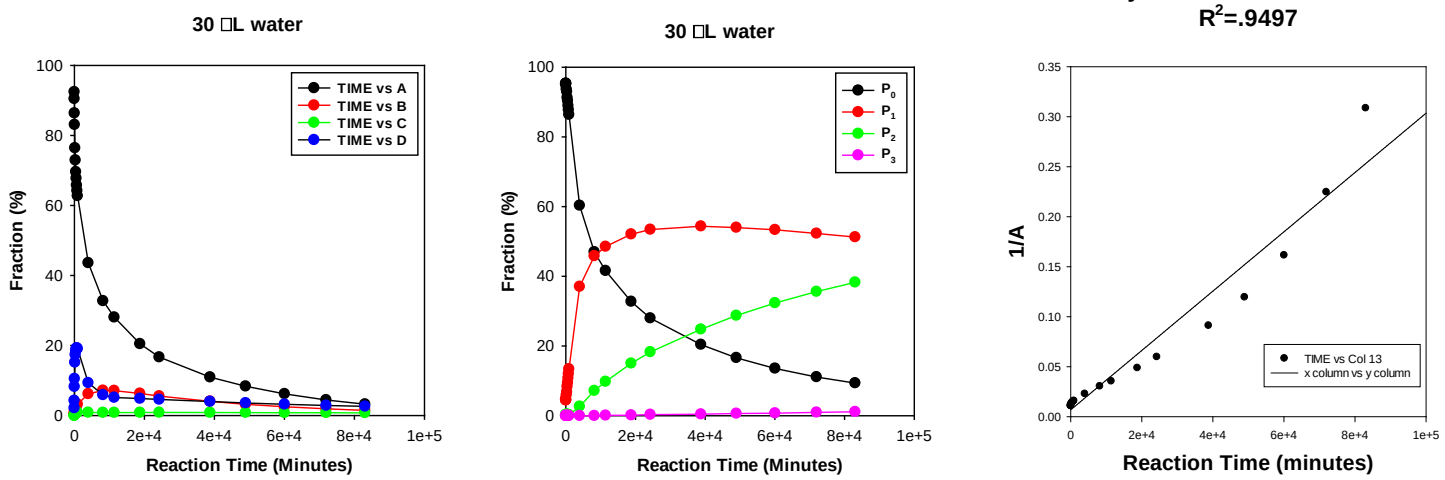


Figure 6. 30μL H_2O, %P vs. Time and Kinetics

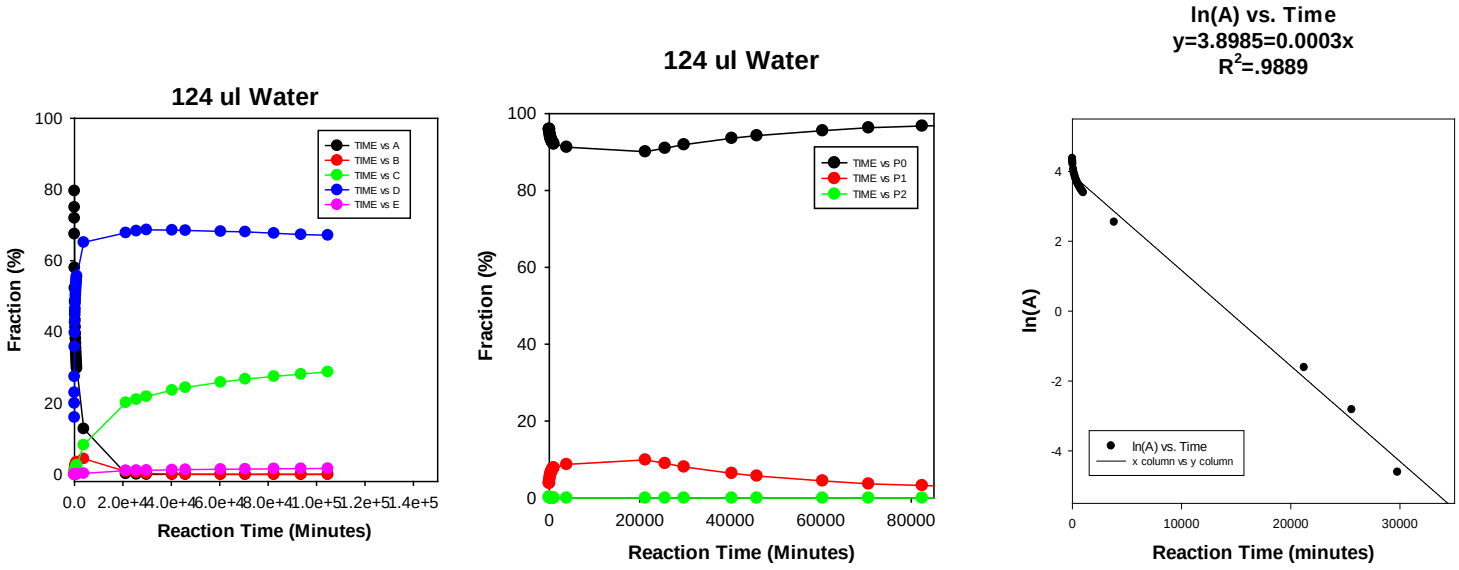


Figure 7. 124μL H_2O %P vs. Time and Kinetics

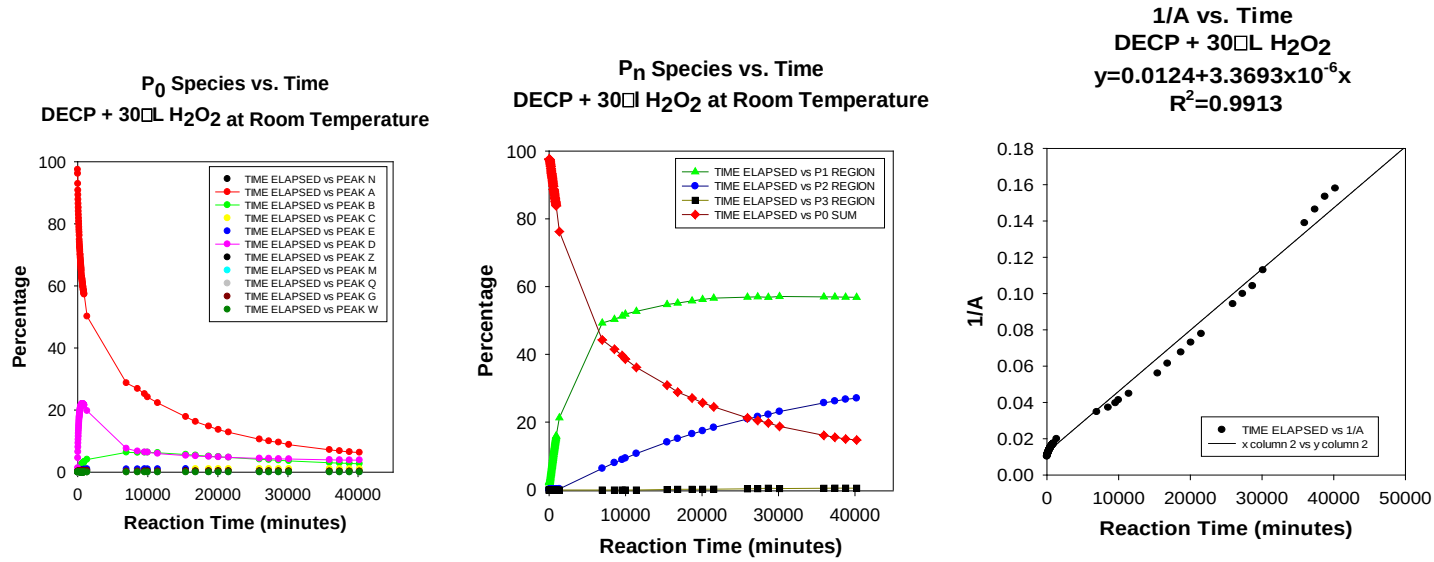


Figure 8. 30μL H_2O_2 %P vs. Time and Kinetics

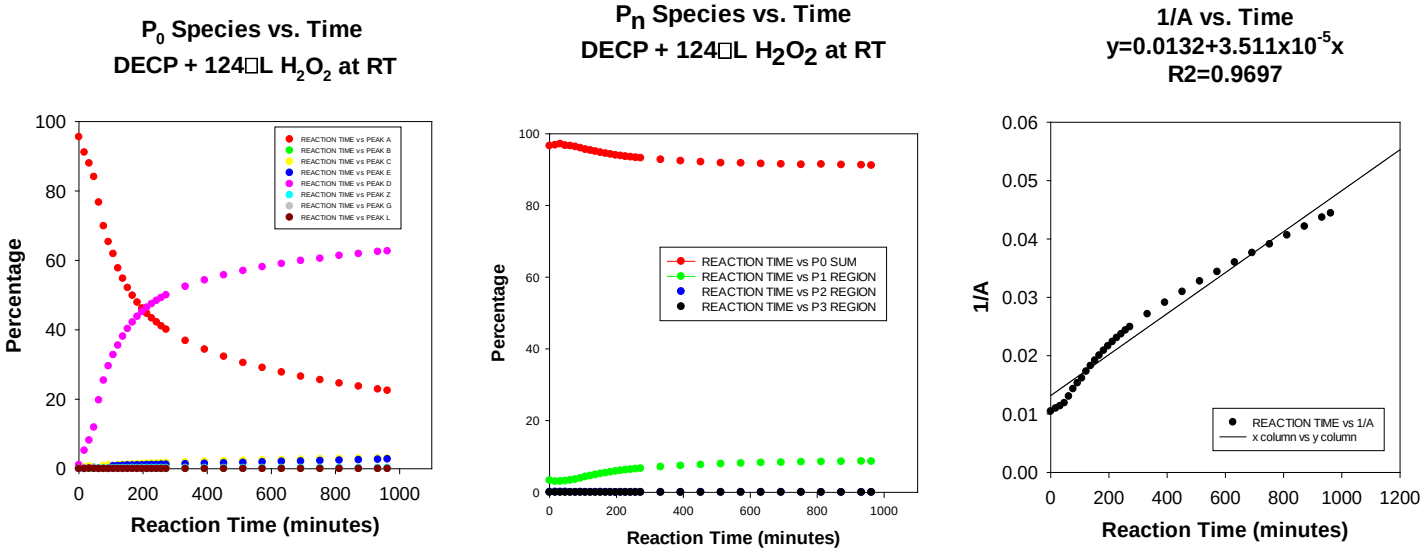


Figure 9. 124μL H_2O_2 %P vs. Time and Kinetics

From the data collected, it is obvious that at room temperature the reactivity of DECP with H_2O and H_2O_2 were very similar in terms of products formed. However, the major difference is with the addition of 30μL of H_2O_2 the reaction proceeds at a much faster rate than H_2O. The half-life is approximately 1000 minutes faster than that of 30μL H_2O. Another very apparent difference is that rate order model between 124μL of H_2O and H_2O_2. The reaction with

water appears to behave in a first order fashion with respect to DECP; on the other hand, when peroxide is used instead, the reaction appears to behave in a second order manner. The half-life of DECP is very similar between the two and their product build up also suggest the major product components are the same. More studies may need to be done to investigate the difference in the first and second order rates.

2. The Spiking Experiment and Results

To confirm the presence of EtCl produced during the reaction, a spiking experiment was set up so that DECP was allowed to react for approximately 4 hours at 323K with 30μL H_2O and then spiked with 50 μL of 200 proof EtOH. A ^{13}C spectrum and ^{35}Cl spectrum were obtained. The carbon spectrum clearly shows a distinction between two different species in the mixture. To further confirm this, the chlorine spectrum shows a large peak that is assumed to be EtCl, since DECP does not give a ^{35}Cl NMR signal likely because the molecule is too large.

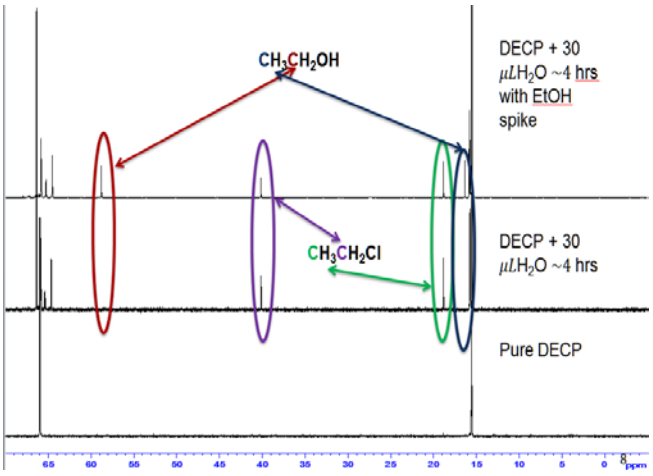


Figure 10. ^{13}CEtOH Spiking Experiment

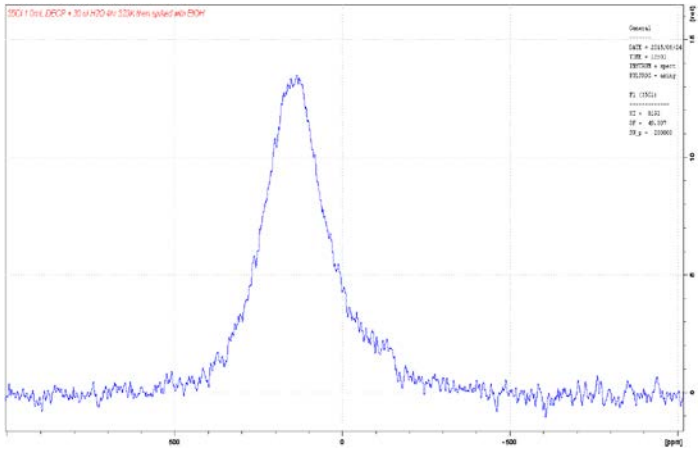


Figure 11. ^{35}Cl NMR of Reaction Progress

3. ^{17}O NMR Spectroscopy

Unfortunately the use of ^{17}O NMR was not as helpful as hoped. Figure 12 show the ^{17}O spectrum of neat (unreacted) DECP the doublet corresponds to the phosphorus oxygen double bond (P=O) and the broad singlet corresponds to phosphorus oxygen ester bond (P-OEt). As seen in Figure 13, there are two new broad singlets that could correspond to new ester bonds (P-OX). It should be noted that all of the enriched water has been used up as it appears as a singlet at δ=0.00 ppm and was visible in the very initial stages of the reaction. Ideally, the dimer and trimer ester linkage bonds would have been present in the spectrum; however, they are not visible.

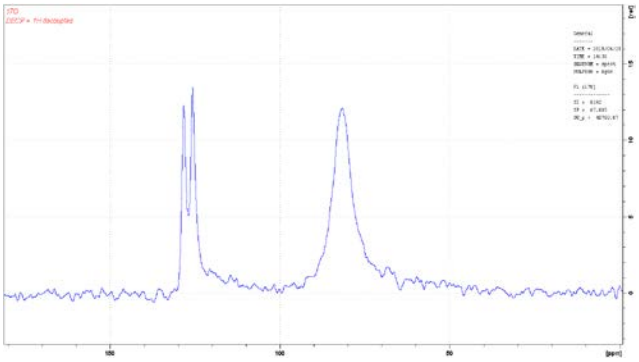


Figure 12. ^{17}O NMR of Unreacted DECP

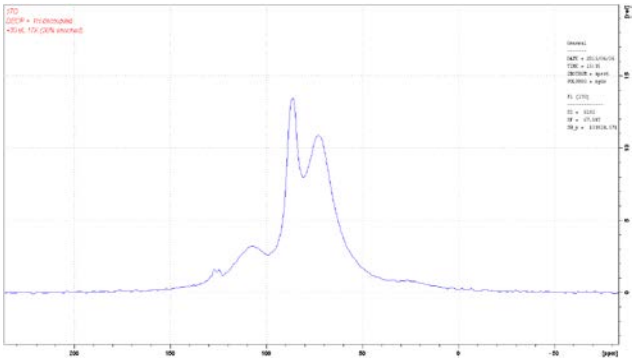


Figure 13. ^{17}O NMR of Reaction Progress

4. ^{19}F-^{31}P INEPT Optimization

A side project pursed was to optimize the INEPT pulse program for ^{19}F-^{31}P containing compounds. To do this, 1-Butyl-1-methylpyrrolidinium hexafluorophosphate in CD_3CN was used. Using different composite pulse sequences and varying different parameters for optimization, in each case, the normalized signal intensity was plotted as a function of the varied parameters (i.e. set *J* value, offset frequency of the observed channel, and offset frequency of the non-observed channel). It was observed that adding a composite pulse to the observed channel

did not have a significant effect on the signal strength; however on the non-observed channel signal was greatly affected. There is still more work to be done on this area, especially if a refocused delay could be harnessed to use with this technique.

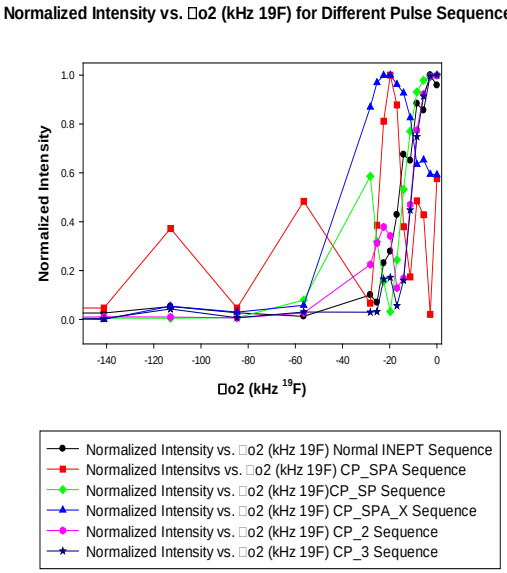


Figure 14. INEPT Study of Offset Frequency

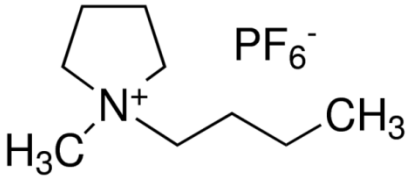


Figure 15. 1-Butyl-1-methylpyrrolidinium hexafluorophosphate

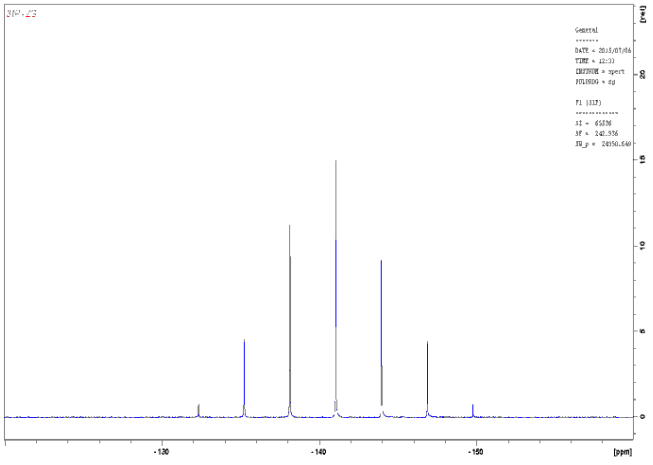


Figure 16. ^{31}P NMR of 1-Butyl-1-methylpyrrolidinium hexafluorophosphate

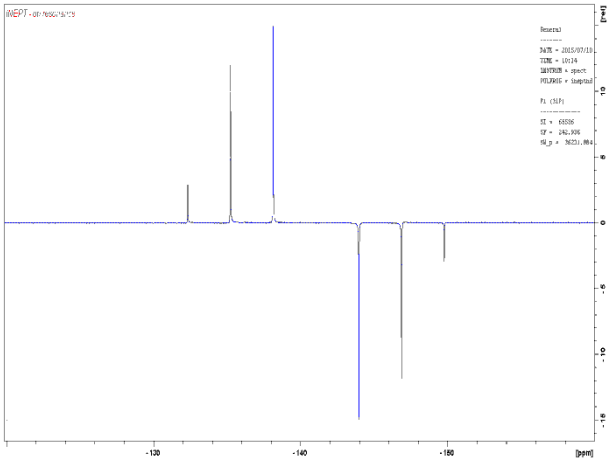


Figure 17. ^{31}P INEPT NMR of 1-Butyl-1-methylpyrrolidinium hexafluorophosphate

There are clear distinct differences in the spectrum produced from the normal pulse program used for ^{31}P NMR and the INEPT pulse sequence. Most notably is how the INEPT transforms a septet into a non-traditional septet where the central peak is null.

Conclusions and Concluding Comments:

Throughout my summers work I've run many reactions with DECP that have revealed a wide range of kinetic behavior. Due to the similarity in structure to DECP and GB, it is quite possible that some of these reactions and their kinetic analysis could be used for future decontamination effects of CWAs or precursor materials.

Further work with ^{19}F-^{31}P INEPT pulse sequences could lead to a way to detect and monitor fluorophosphates decontamination in fast effective means. However, there are some limitations that must be considered or circumvented such as large ^{19}F-^{31}P *J*-couplings and each nuclei have a wide chemical shift range. On the other hand, preliminary studies performed show that there are some types of composite pulses that can be added to the non-observed channel that will not significantly diminish signal over a wider offset range, which is promising considering ^{19}F has approximately a 1000ppm range.

Impact on my Future Scientific Endeavors

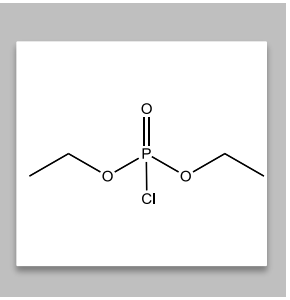
While my time at SNL has been short, it has been an amazing and rewarding experience. This opportunity has given me the chance to take part in some very unique and exciting research. Never before in my academic career have I performed as many NMR spectroscopy experiments as I have this summer. Doing this work this summer has only invigorated my desire to continue purse research in this area in the future when I attend graduate school.

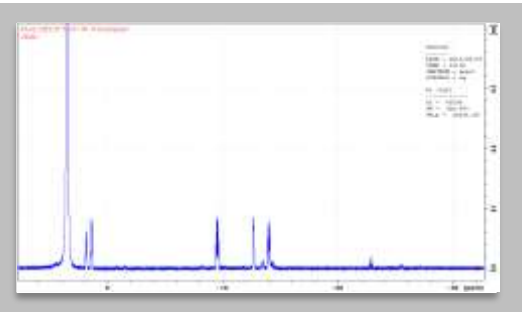
Presentations have been a consistent part of my experience here, since at our weekly group meetings everyone gave updates on the progress (or setbacks) of their current project. As well as updates to a project Dr. Alam, often would ask the group members individually to explore a specific area of NMR spectroscopy and create a small presentation and explain it to the group.

Aside from work, there have been plenty of extracurricular opportunities of things to do. For instance, during my stay, I have had the pleasure to work with several other interns from the Naval Academy and the Air Force Academy and become good friends with. We have had adventures to many different locations such as White Sands, Santa Fe, the Albuquerque Zoo, Spence Hot Springs, and also hot air ballooning over the city. Also, I got the opportunity to go boating at Elephant Butte. There have been countless opportunities provided to me to make my stay in Albuquerque feel more like home.

Acknowledgements:

Dr. Todd M. Alam and the NMR group
Dr. Mark Kinnan
Sandia National Laboratories
Department of Homeland Security
This research was supported in part by an appointment with the HS-STEM Summer Internship Program sponsored by the U.S. Department of Homeland Security (DHS) Science & Technology (S&T) Directorate Office of University Programs. This program is administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and DHS. ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract number DE-AC05-06OR23100.





Sandia National Laboratories

Exceptional service in the national interest

Investigating the Hydrolysis Reactions of CWA Simulants using NMR Spectroscopy on Multiple Nuclei

A systematic study that tracks ^{31}P containing species in a reaction of a Sarin surrogate

Brendan W. Wilson

DHS-STEM Fellow

July 23, 2015

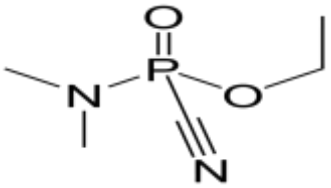
C. Eugene Bennett Department of Chemistry

This research was supported in part by an appointment with the HS-STEM Summer Internship Program sponsored by the U.S. Department of Homeland Security (DHS) Science & Technology (S&T) Directorate Office of University Programs. This program is administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and DHS. ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract number DE-AC05-06OR23100."

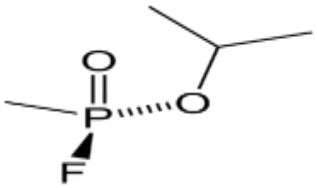
Background

- Nerve agents were developed by German Scientists in the 1940's.
- Dr. Gerhard Schrader a German scientist first synthesized tabun (GA). Further research lead to the development of sarin (GB), soman (GD), and cyclosarin (GF).
- These Chemical Warfare Agents (CWAs) were mass produced by the Germans by 1945.
- The US designated these types of agents as "G-agents".

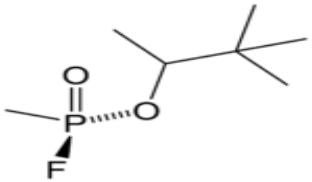
Different types of "G-agents":



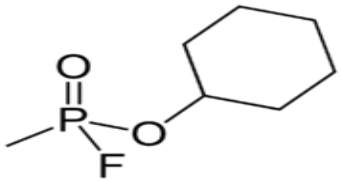
GA – Tabun (1936)



GB – Sarin (1939)



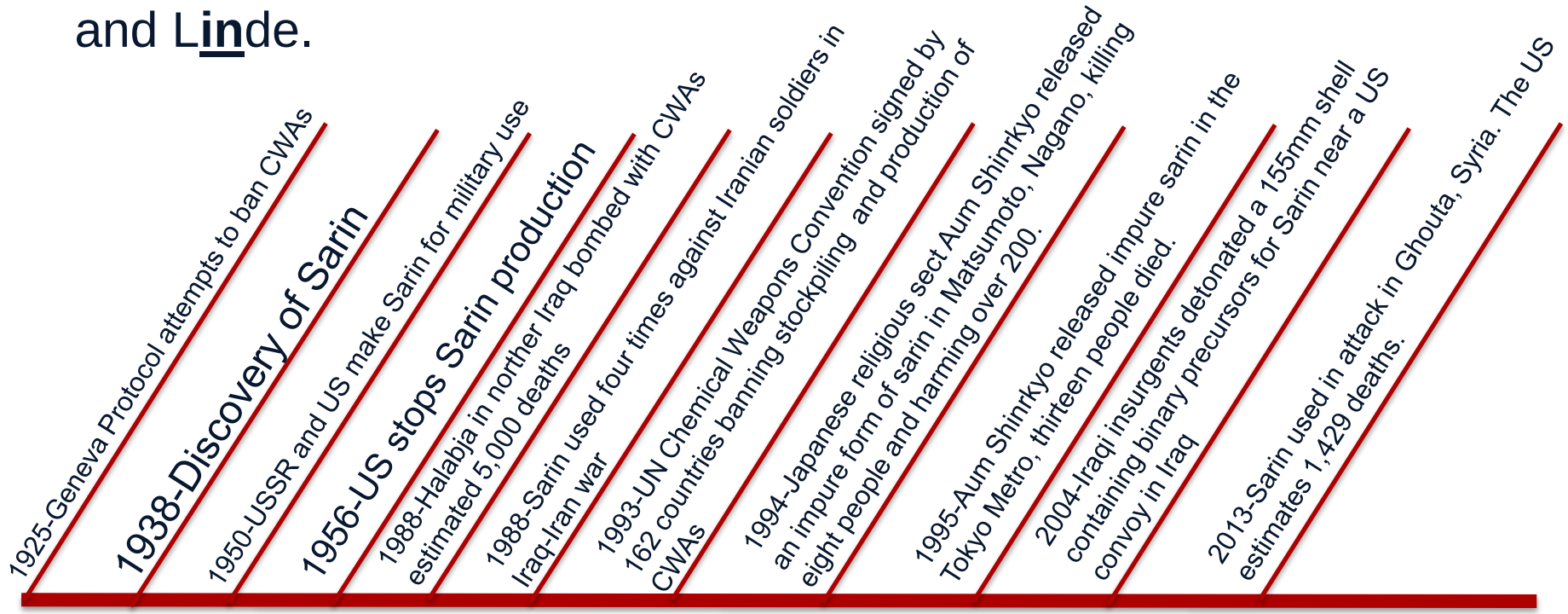
GD – Soman (1944)



GF – Cyclosarin (1949)

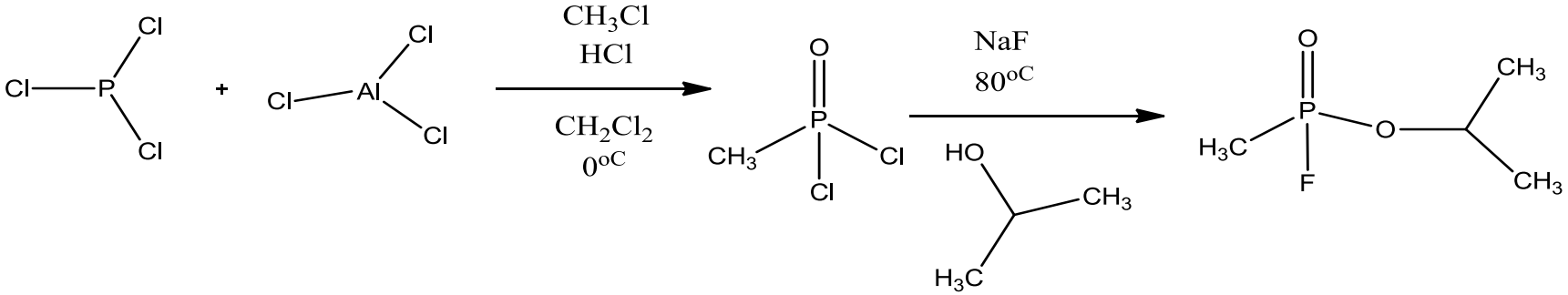
Sarin Background and Timeline

- Originally intended to be used as a pesticides.
- Most toxic of the four “G agents”.
- Sarin named in honor of researchers: **S**chrader, **A**mbros, **R**itter, and L**in**de.



Motivation and Sarin

- Chemical Warfare Agents (CWAs) are opportunities for terror attacks.
- Sarin is a deadly CWA with LD_{50}'s (lethal dose to kill 50% of the population) on the order of $5 - 20\,\frac{\mu g}{kg}$ by absorbtion[1] for various cases, its vapors are deadly.
- Sarin cause irreversible inhibition to a class of enzymes known as cholinesterases.
- It is not very stable and vaporizes easily. Typically is only found pure for a few weeks to a few months at max.
- Most synthetic routes are few steps and available online free of charge and are only a two step synthesis[2].

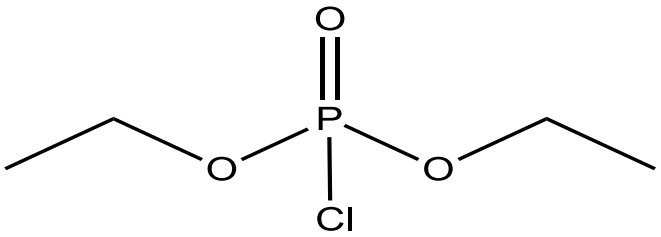


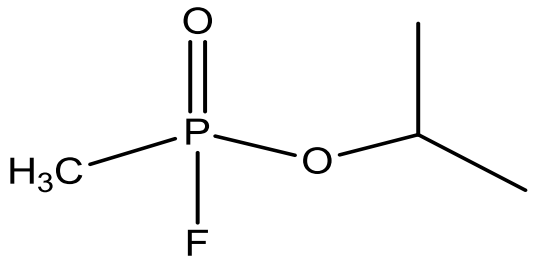
Cape Ray

- Housed 1,038.5 tons of CWAs and precursors that Syria declared.
- Ship contained two field hydrolysis units.
  - Must dilute the CWA to decontaminate with reactor.
- Mission took place in the Mediterranean Sea.
- Endeavor Started on July 3, 2014.
- August 11, 2014 marked 75% decontamination.
- August 18, 2014 the neutralization process was finished.

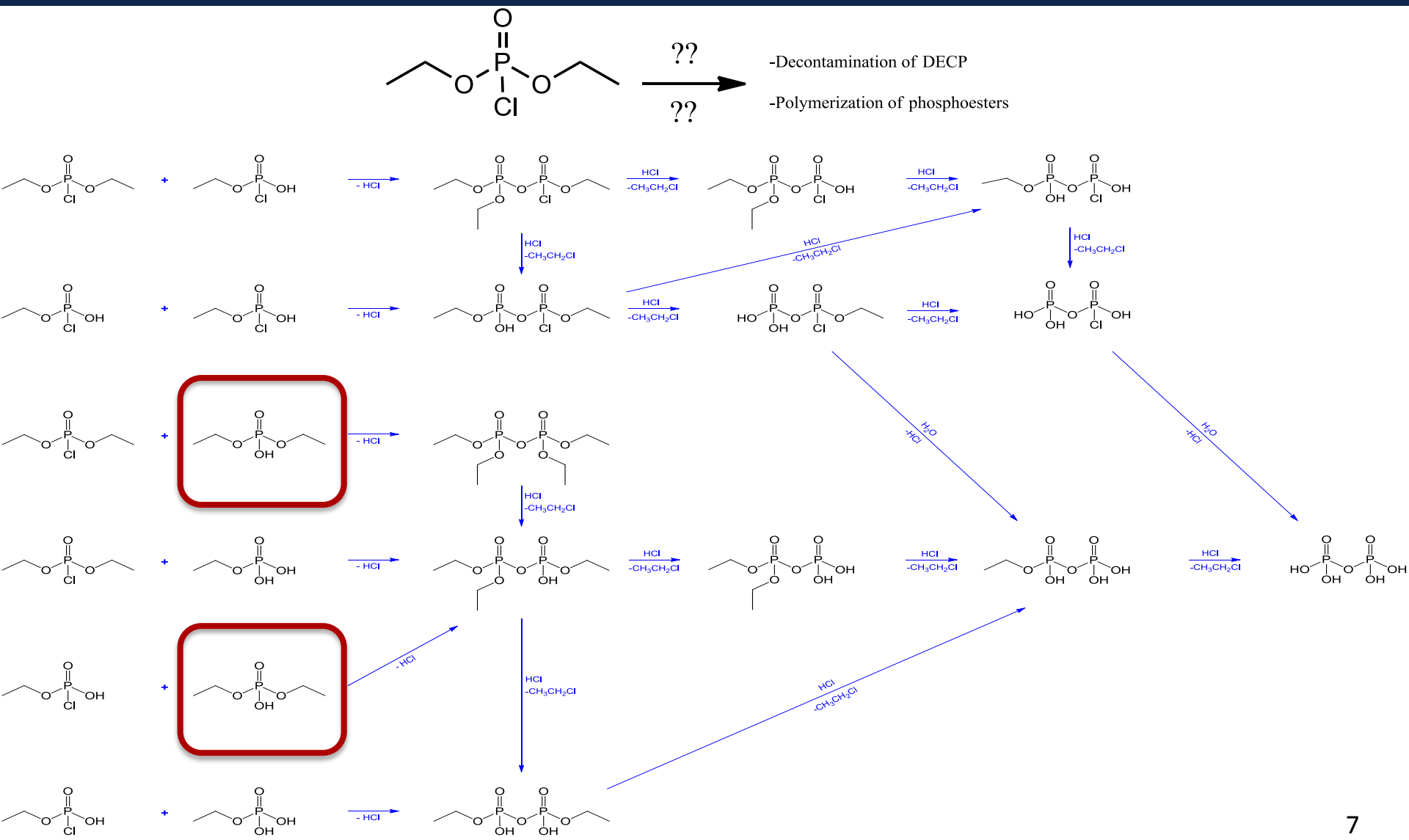
Precursors and DECP

- DECP-diethyl chlorophosphate is similar in structure to sarin, and other precursors but much more stable.
- This is a safer compound with similar reactivity due to the phosphorus-halogen bond, and phosphoester nature of the molecule.
- With this compound we can simulate situations that could be encountered in the field. Specifically, high concentration of CWAs and low concentration decontamination reagent.
  - Hydrolysis reactions of DECP are completely different depending on concentration.
  - At low concentration, there is primarily one product formed.
  - At high concentration, there are many products formed and an increase in the complexity of the reaction.

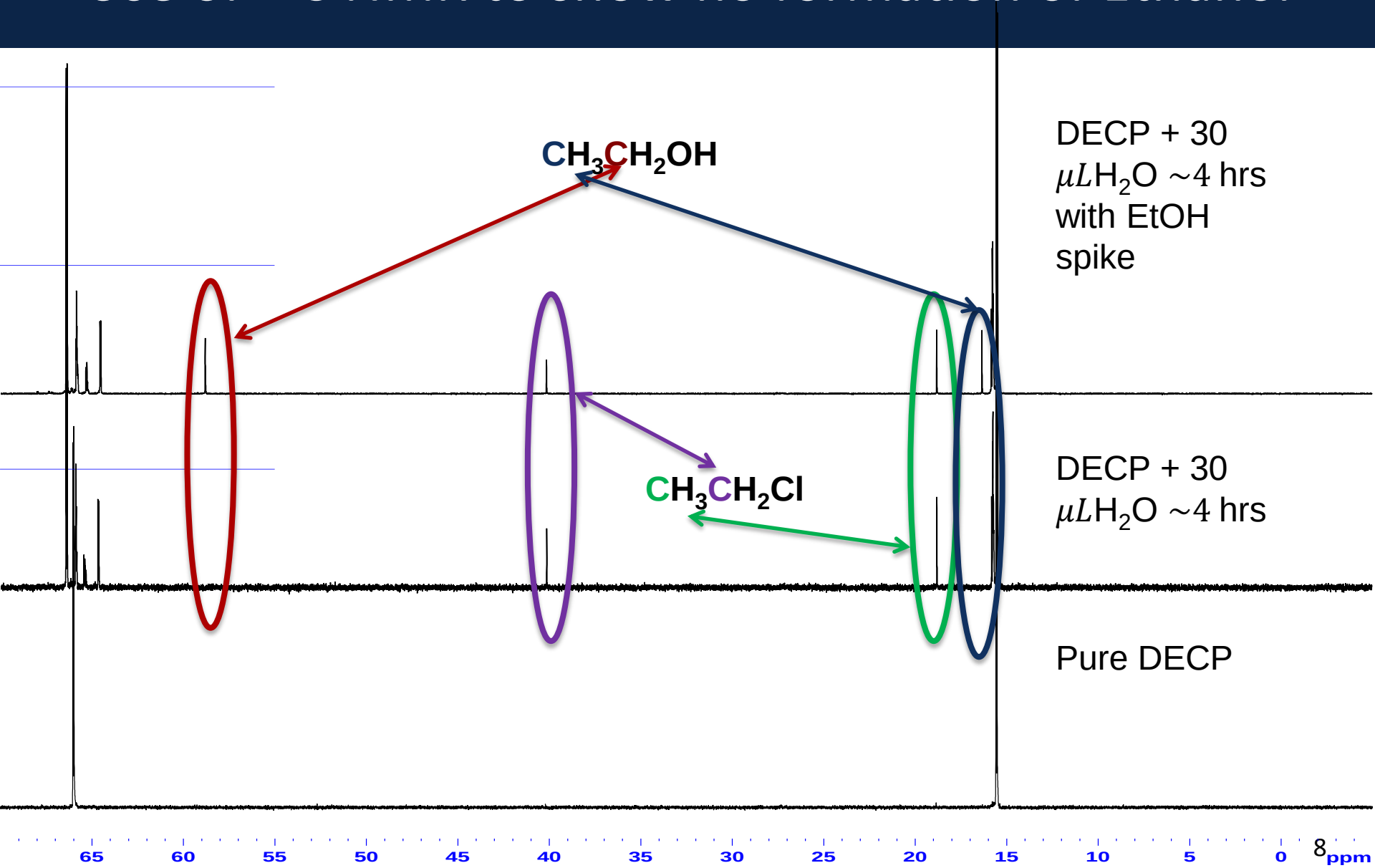




Complexity of the Reactions

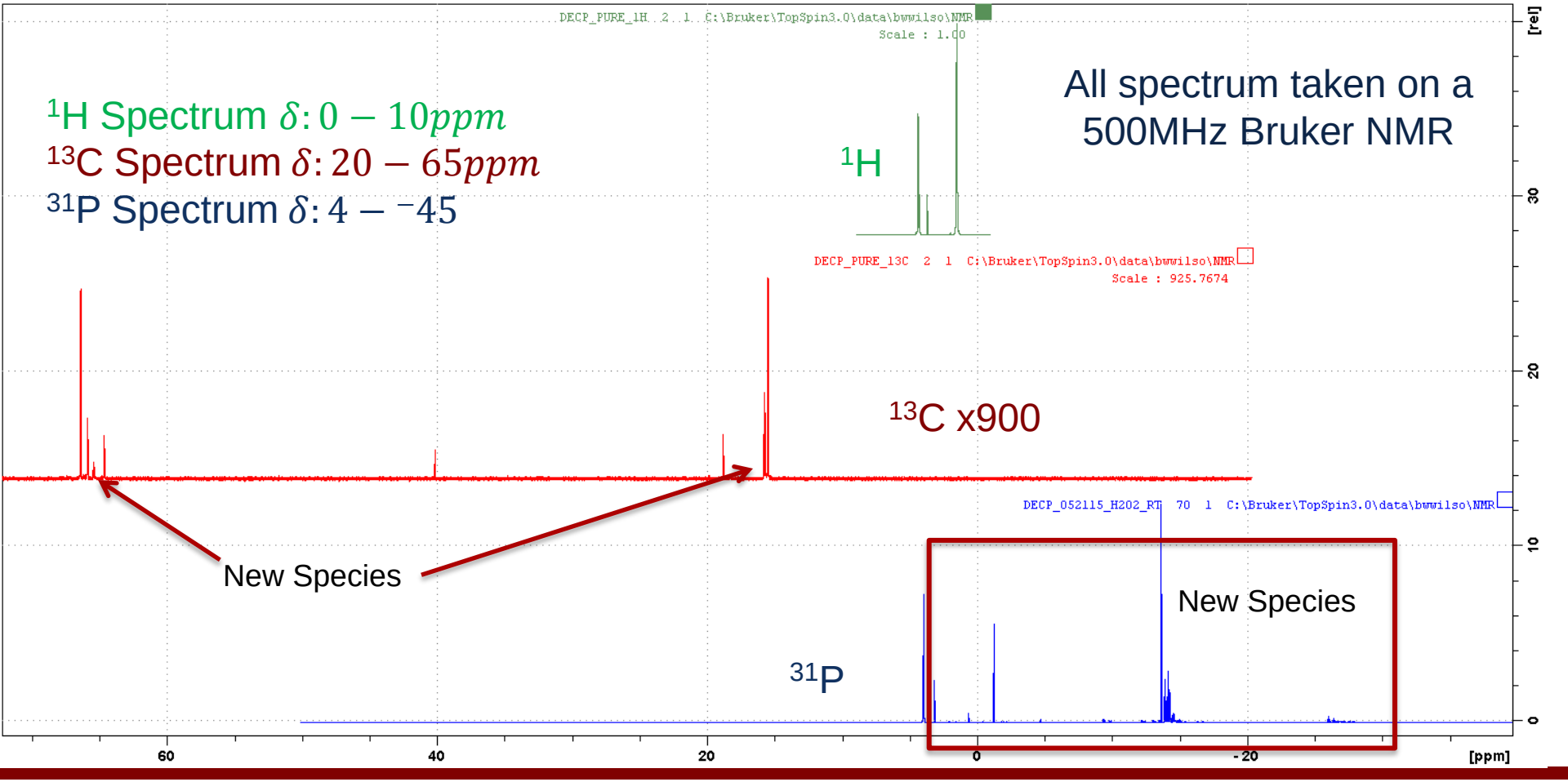


Use of ^{13}C NMR to show no formation of Ethanol



Why ^{31}P NMR spectroscopy and not ^{1}H or ^{13}C?

- The best answer: more distinction in chemical shift between different species and ^{31}P is 100% natural abundance as opposed to ^{13}C being 1.1%.



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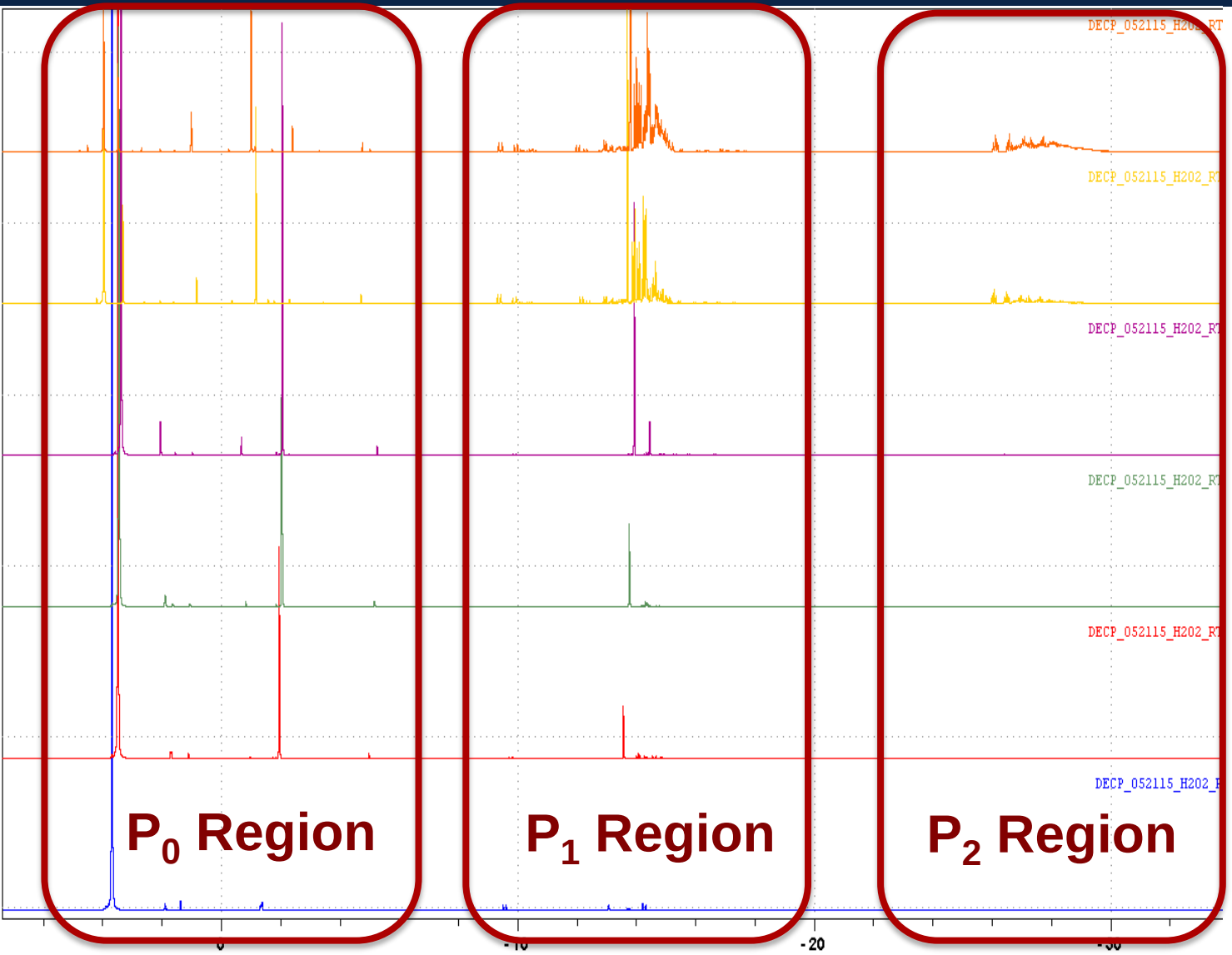
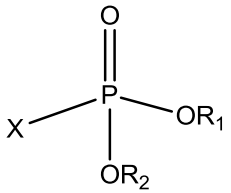
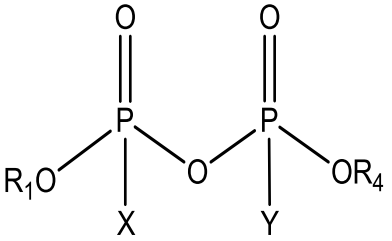


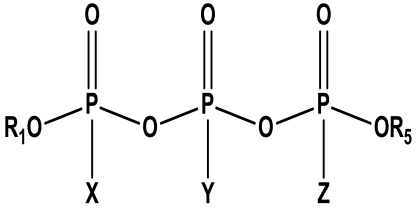
P_0 Species



P_1 Species



P_2 Species



X=Cl or OR_2, Y=Cl or OR_3, Z=Cl or OR_4

Reactions with DECP

Reaction	Conditions
10μL H_2O	323K
30μL H_2O_2 (30%)	Room Temperature
30μL 1N HCl	Room Temperature
30μL 1N NaOH	Room Temperature
10μL H_2O_2 (30%)	323K
10μL H_2O_2 (30%)	Room Temperature adding 10μL every 12 hours
10μL H_2O_2 (30%)	323K adding 10μL every 12 hours
10μL H_2O	Room Temperature adding 10μL every 12 hours
10μL H_2O	323K adding 10μL every 12 hours
30μL 3N NaOH	Room Temperature
30μL 3N HCl	Room Temperature
124μL H_2O_2 (30%)	Room Temperature
124μL H_2O	Room Temperature

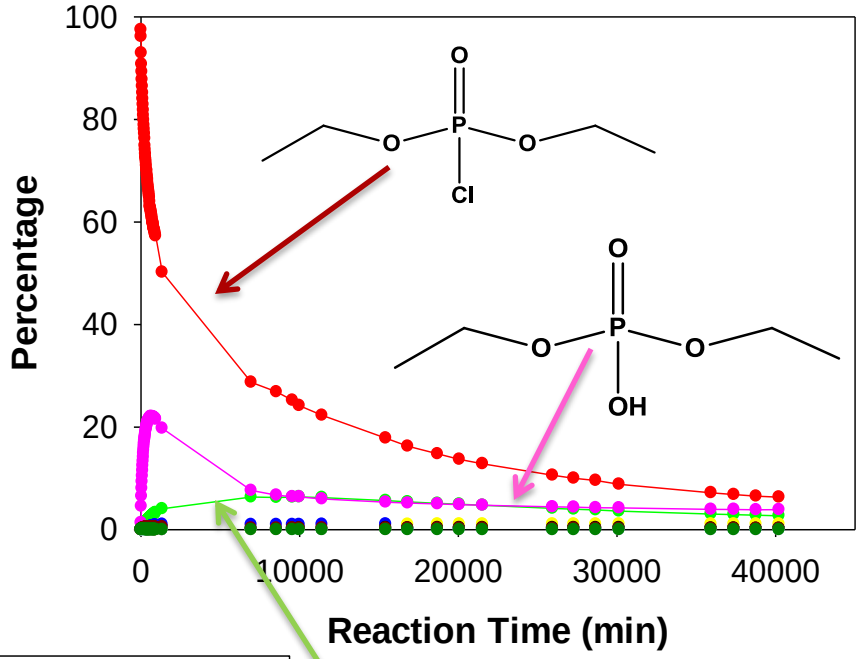
Other reaction with various reagents were pursed, but can not be discussed at this time.

All reactions employ 1.0mL of DECP

30μL H_2O_2 (30%) at RT

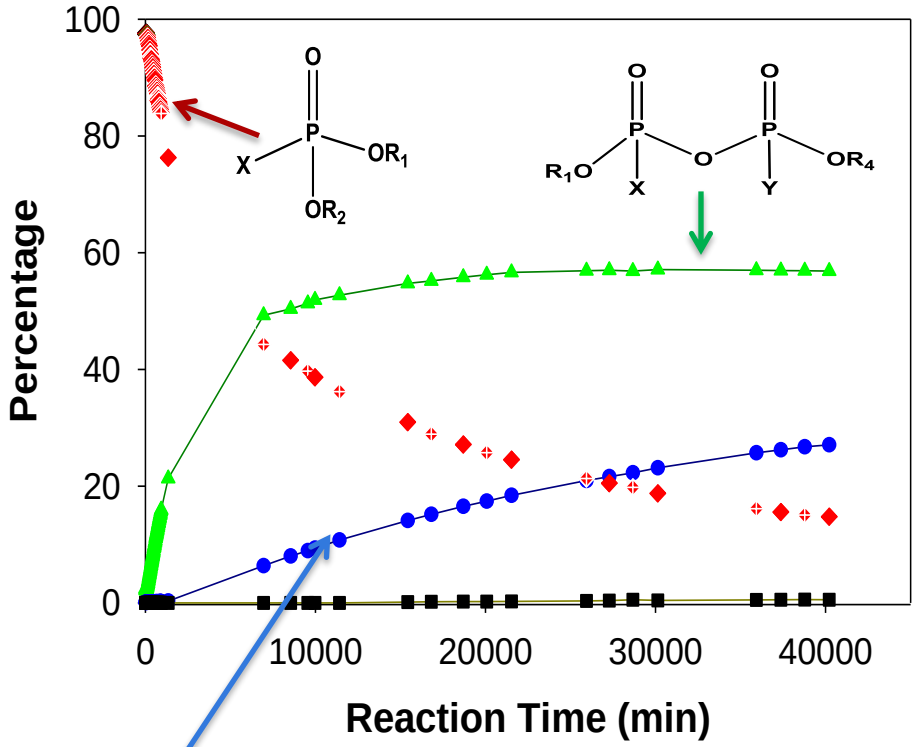
P_0 Species vs. Time

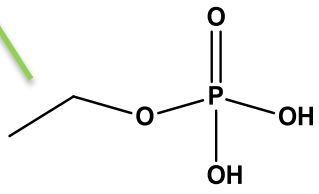
DECP + 30µL H_2O_2 at Room Temperature

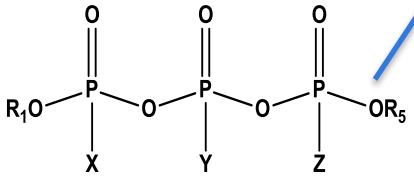


P_n Species vs. Time

DECP + 30µl H_2O_2 at Room Temperature







X=Cl or OR_2

Y=Cl or OR_3

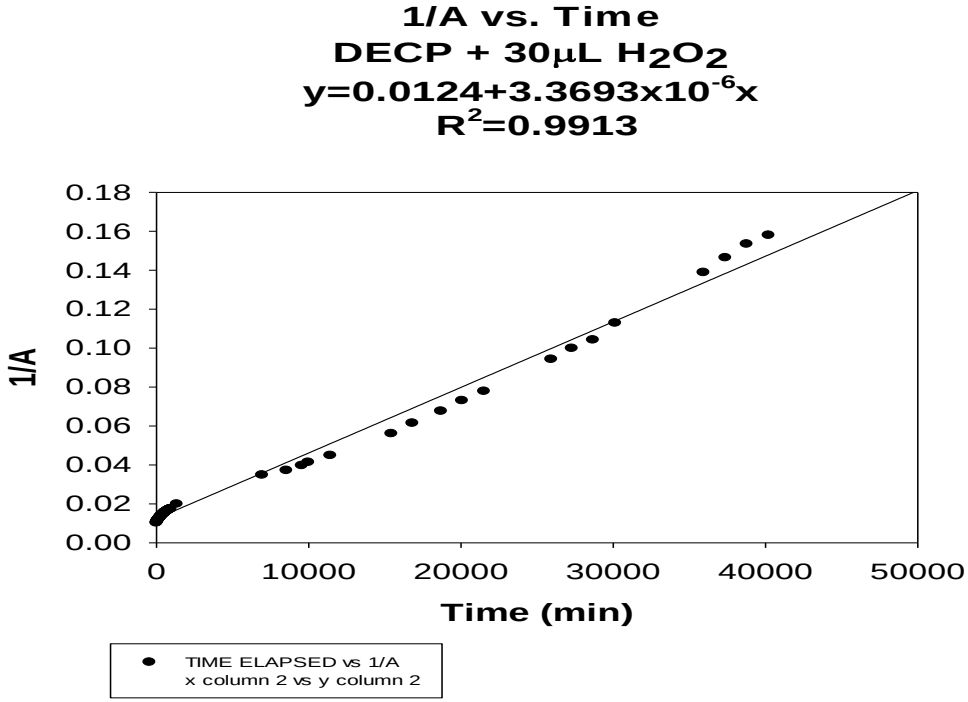
Z=Cl or OR_4

- TIME ELAPSED vs PEAK N
- TIME ELAPSED vs PEAK A
- TIME ELAPSED vs PEAK B
- TIME ELAPSED vs PEAK C
- TIME ELAPSED vs PEAK E
- TIME ELAPSED vs PEAK D
- TIME ELAPSED vs PEAK Z
- TIME ELAPSED vs PEAK M
- TIME ELAPSED vs PEAK Q
- TIME ELAPSED vs PEAK G
- TIME ELAPSED vs PEAK W

- TIME ELAPSED vs P1 REGION
- TIME ELAPSED vs P2 REGION
- TIME ELAPSED vs P3 REGION
- TIME ELAPSED vs P0 SUM

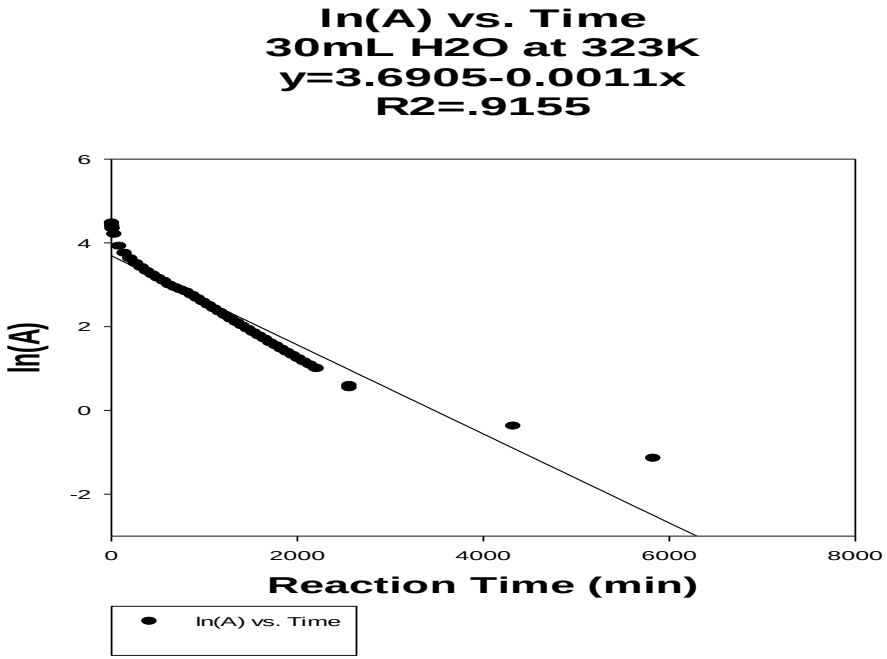
30μL H_2O_2 (30%) at RT Reaction "Kinetics"

- Appears to be 2nd with respect to DECP and H_2O_2.
- Estimated $t_{1/2} = 1343\ min.$
- Estimated $k = 1.6847 \times 10^{-6} \frac{1}{\% \cdot min}$.



30μL H_2O at 323K Reaction "Kinetics"

- Appears to be 1st order with respect to DECP.
- Estimated $t_{1/2} = 85\ min$.
- Estimated $k = 0.0011 \frac{1}{min}$.



Stoichiometric Equivalents

- 1.0mL DECP $\approx 4.16 \times 10^{21}\ molecules \approx 7$ mmoles.
- 10μL $\approx 3.34 \times 10^{20}\ molecules \approx\ .6$ mmoles.
  - Roughly 12:1 DECP:H_2O (molecules).
- 30μL $H_2O \approx 1.0025 \times 10^{21}\ molecules \approx 2$ mmoles.
  - Roughly 4:1 DECP: H_2O (molecules).
- 124μL $H_2O \approx 4.14 \times 10^{21} molecules \approx 7$ mmoles.
  - Roughly 1:1 DECP:H_2O (molecules)
- 30μL $H_2O_2 \approx 1.76 \times 10^{20}\ molecules \approx\ .3$ mmoles.
  - Roughly 23:1 DECP:H_2O_2 (molecules).
- 124μL $H_2O_2 \approx 7.31 \times 10^{20} molecules \approx 1.2\ mmoles$.
  - Roughly 6:1 DECP:H_2O_2 (molecules)

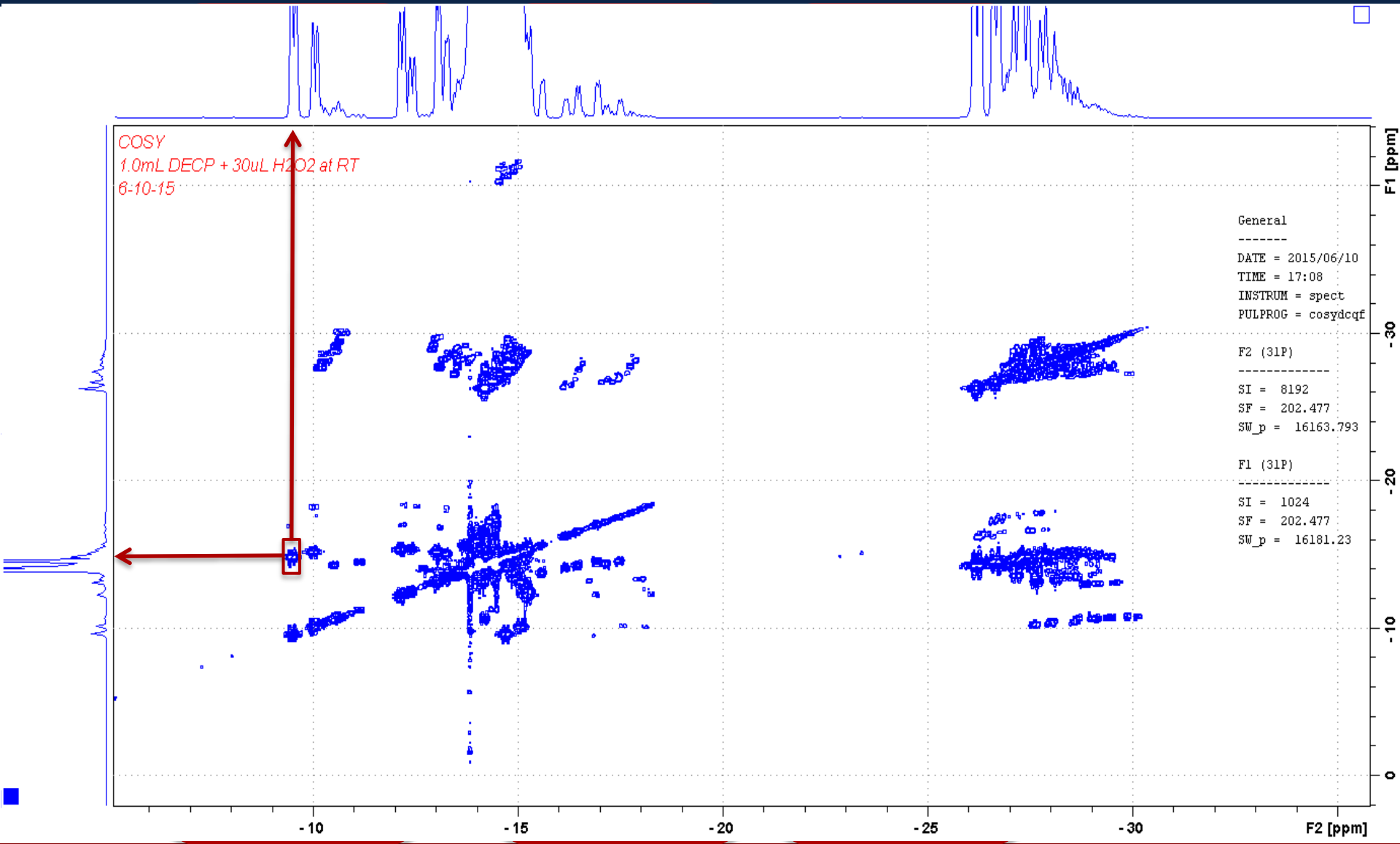
Summary of "Kinetics"

Reaction Conditions	Estimated Half-Life	Estimated $t_{1/4}$ of P1 Generation	Estimated Rate Constant	Rate Order Model
1.0 mL DECP + 10μL H_2O @ 323K	4390 min	976 min	$1.5786 \times 10^{-4} \frac{1}{min}$	1st
1.0 mL DECP + 30μL H_2O @ 323K	85 min	990 min	$0.0011 \frac{1}{min}$	1st
1.0 mL DECP + 30µL H_2O_2 @ RT	1343 min	1000 min	$1.42132 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0 mL DECP + 30μL 1N HCl @ RT	3162 min	2684 min	$1.0938 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0 mL DECP + 30μL 1N NaOH @ RT	2642 min	2147 min	$1.3582 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0 mL DECP + 30μL 3N HCl @ RT	2570 min	2075 min	$1.4915 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0 mL DECP + 30 μL 3N NaOH @ RT	2129 min	1600 min	$1.3159 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0 mL DECP + 3N HCl @ RT	2570 min	1971 min	$1.0041 \times 10^{-6} \frac{1}{\% \cdot min}$	2nd
1.0mL DECP + 124μL H_2O_2 @ RT	166 min	Not Reached	$1.7588 \times 10^{-5} \frac{1}{\% \cdot min}$	2nd
1.0mL DECP + 124μL H_2O @ RT	153 min	Not Reached	$0.0003 \frac{1}{min}$	1st

Other reaction with various reagents were pursed, but can not be discussed at this time.

*Half-life's and rate constants were found using the interpolation function in SigmaPlot.

2D NMR Spectroscopy ^{31}P-^{31}P COSY



Advantages and Use of ^{31}P-^{31}P COSY

- Both axes correspond to ^{31}P Spectrum (homonuclear correlation).
- A cross-peak indicates a correlation (communication between nuclei).
- The coupling values are specific to each molecule. Allows for more exact measure of the coupling constants.
- In the P_1 and P_2 regions it shows which phosphorous compounds are corrlated by ^{31}P-^{31}P *J*-coupling; each compounds coupling is unique.

Conclusions

- The fastest reactions involved a peroxide species.
- The reaction mechanism and the complexity of the reaction is dependent on the initial concentration of DECP
- Using ^{13}C NMR it was possible to confirm the presence of EtCl and not EtOH as a byproduct of the hydrolysis reaction.
- ^{31}P-^{31}P COSY allows examination of which ^{31}P containing species are correlated.

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- Department of Homeland Security

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