ACID AND BASE CATALYSED AQUEOUS HYDROLYSIS OF THE ORGANOPHOSPHORUS PESTICIDE, DIAZINON

By
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A thesis submitted to the Department of Chemistry in conformity with the requirements for the degree of a Master of Science

Queen's University
Kingston, Ontario, Canada
April, 2001

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0-612-59396-7
ABSTRACT

This thesis details the abiotic hydrolysis of an organophosphorus pesticide, diazinon (O,O-diethyl-(2-isopropyl-6-methylpyrimidin-4-01) phosphorothioate) under controlled conditions in aqueous NaOH, HCl, and humic acid solutions. The decomposition of diazinon (1) was studied by following the appearance of products, O,O-diethyl phosphorothioic acid (2 or 2\(\cdot\)Na\(^+\)) and 2-isopropyl-6-methylpyrimidin-4-ol (3 or 3\(\cdot\)Na\(^+\)), detected spectrophotometrically.

The kinetic results were used to create a pH rate-profile for the hydrolysis of diazinon. Under alkaline conditions (0.0115 to 0.543 M NaOH) the second order rate constant was determined to be \(3.06 \times 10^{-3}\) s\(^{-1}\) at 25°C. The rate was enhanced by increasing temperature, being elevated by a factor of approximately two for every ten degree rise in temperature. The rate constants at various temperatures (25, 35 and 45°C) were used to calculate the activation energy (\(E_a\)) and the energy parameters, \(\Delta G^*\), \(\Delta H^*\), and \(\Delta S^*\). This region of the pH-rate profile was linear showing specific base catalysis by OH\(^-\).

Under acidic conditions a much more complex system was encountered. The pH rate-profile in this region was not a simple straight line, but showed first increasing rate with acid concentration (2.6 \(\times\) 10\(^{-3}\) to 0.175 M HCl), followed by a decrease in rate with acid concentration (from 0.175 M to 6.13 M HCl) and finally the rate increased again with acid concentration (from 6.13 M to 8.75 M HCl). These differences are accounted for by the many possible protonations of the substrate that can enhance reaction, and due to the decrease in the activity of water at higher acid concentrations.
A preliminary study of the effect of humic acid (at 24 ppm and 48 ppm) on the alkaline hydrolysis of diazinon was also carried out. It was found that the presence of humic acid caused a mild decrease in rate. This could be due to either the hydrophobic humic acid protecting diazinon from the hydrophilic OH⁻, or due to general base catalysis of diazinon by carboxylate groups present on the humic acid.
CLAIMS TO ORIGINALITY

The aqueous kinetics of diazinon have been studied by other researchers, however this work, to the best of the author’s knowledge, contains the following original work:

1. This is the first study on the kinetics of the aqueous hydrolysis of diazinon in the pH range of 12-14.

2. The data presented include the activation parameters for the aqueous hydrolysis of diazinon in the pH range of 12-14.

3. This is the first study on the kinetics of the hydrolysis of diazinon in very acidic solution (0.2M \text{-} 9 \text{ M HCl}).

4. This work is the first to present the activation parameters for the aqueous hydrolysis of diazinon in very acidic solution.

5. This is the first study to examine the effect of humic acid on the rate of alkaline aqueous hydrolysis of diazinon.

6. The $pK_a$ values of 2-isopropyl-6-methylpyrimidin-4-ol are reported in this work.
ACKNOWLEDGEMENTS

I am sincerely grateful for the help of my supervisors, Professors E. Buncel and G.W. vanLoon, during the experimental portion of this work, and for their input into the writing of this document. I thank them for their support, guidance and encouragement during my time at Queen’s University.

I would also like to extend thanks to Professor J.M. Dust (visiting Professor from Sir Wilfred Grenfell College, Memorial University of Newfoundland) for his suggestions regarding the interpretations of experimental results, and guidance throughout his annual visits to Queen’s.

Thanks also goes out to Mr. P. Mulligan, Dr. F. Sauriol, Mr S. Meskis, and all the other technical staff at Queen’s University for their assistance with my research. A special thanks to Mr. R. Roberts and Mr. E. Maracle in the electronics shop for keeping the spectrophotometers running. I would also like to thank the Graduate Secretary, Ms. A. Keyes, for helping with all the paper work required to obtain a Master of Science.

My appreciation goes out to my present colleagues, Abdelhamid Esbata, Adnaan Wasey, Ahmed Aman, Asri Ghani, Dalia Abdallah, David Kreller, Kristen Exall, Salma Shirin, Vimal Balakrishnan, and Xiumei Han, and to my past colleagues, Ishaq Eneji and James Wotyk. I would like to particularly thank Vim for his help in getting this project off the ground when I first arrived in Kingston, and for his helpful discussions about the interpretation of my experimental results. I thank everyone for making my time here more enjoyable.
For their love and support I would like to thank my parents, my two brothers, Stanley and Peter, and my fiancee, Steve, and his family.

Finally my appreciation goes out to the National Council for Engineering and Research and Queen’s University for financial support throughout the period of this study.
DEDICATION

To Steve

Without his support I would not be here today.
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1. INTRODUCTION

1.1. General

Pesticides are a widely used class of compounds needed to help improve yields of food and fibre crops, remove household pests, and kill disease carrying insects. Although these compounds can be beneficial, they need to be used with care as they have the ability to harm the environment by affecting non-target organisms through application to non-target areas by spray drift during application or in water run-off. It is for this reason that it is necessary to determine how these chemicals behave in the environment. It is important to know the rate of degradation of a pesticide to determine its persistence. As well, it is crucial to identify the metabolites or products formed from their degradation as these compounds can be more toxic than the parent compound, and will also have effects within the environment. The ideal pesticide would be a compound that is selective to the desired pest, has a lower persistence and produces harmless degradation by-products.

1.2. Historical development of pesticides

The first chemical pesticides were inorganic compounds, such as the fungicide Bordeaux mixture (copper sulfate/lime mixture) and the insecticides Paris Green (cupric and arsenious oxides) and calcium arsenate\(^1,2\). A few early organic pesticides were derived from natural sources, such as pyrethrum from the chrysanthemum\(^1\). Another naturally occurring insecticide is nicotine, from tobacco, which was used before the 1700's\(^1\). In the 1930's synthetic pesticides were developed, which now dominate the pesticide market.
1.3. Development of biologically active organophosphorus compounds

It was noted by the German scientist, Dr. Gerhard Schrader, that organophosphorus compounds could be used as nerve gases\(^1,2,3\). He developed several different highly toxic organophosphorus compounds before and during the Second World War; one of the first nerve gases was a compound called tabun\(^2\). Table 1.1 gives the structures and toxicity of three nerve gases, including tabun, which were developed during the Second World War.
Table 1.1. Organophosphorus nerve gases

<table>
<thead>
<tr>
<th>Common name</th>
<th>Structure</th>
<th>$LD_{50}^a$ (injected (mg kg$^{-1}$ mice))</th>
<th>adsorbed through skin (mg per man)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarin</td>
<td>$\begin{array}{c} CH_3 \ H-C-O-P-F \ CH_3 \end{array}$</td>
<td>0.33</td>
<td>2000</td>
</tr>
<tr>
<td>Tabun</td>
<td>$\begin{array}{c} CH_3CH_2O-P-CN \ N(CH_3)_2 \end{array}$</td>
<td>0.34</td>
<td>1500</td>
</tr>
<tr>
<td>Soman</td>
<td>$\begin{array}{c} CH_3 \ H_3C-C-C-O-P-F \ CH_3 \end{array}$</td>
<td>0.14</td>
<td>1250</td>
</tr>
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</table>

$^a$Data from reference 2.
Note: $LD_{50}$ is the dose which kills 50% of the test sample.

It was also discovered that these compounds had insecticidal, in addition to toxic properties. Organophosphorus pesticides operate by binding to the enzyme cholinesterase, preventing it from catalyzing the hydrolysis of the neurotransmitter acetylcholine$^4$. Figure 1.1 compares the action of acetylcholine and an organophosphorus pesticide at the active site of the enzyme. The organophosphorus pesticides bind much more strongly to the active site of the enzyme, taking hours to weeks to be removed, whereas acetylcholine is cleaved in microseconds.
One of the first organophosphorus compounds, developed during the Second World War to be used as an insecticide was tetraethyl pyrophosphate, or TEPP\(^5\); however, it was not ideal as it was rapidly hydrolyzed and had a very high mammalian toxicity\(^2\). Near the end of the Second World War, in 1944, a more suitable organophosphorus pesticide, parathion, was developed by Schrader\(^1,3\). This discovery sparked the development of thousands of other organophosphorus compounds with insecticidal properties. Today organophosphorus pesticides dominate the market; 40% of the global insecticide use in 1989 was composed of organophosphorus pesticides\(^6\). Table 1.2 gives
the structures and some physical data about TEPP, parathion, and some other organophosphorus pesticides currently under study by the author's group.
Table 1.2. Organophosphorus pesticides

<table>
<thead>
<tr>
<th>Common name</th>
<th>Structure</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Physical data</th>
</tr>
</thead>
</table>
| TEPP        | ![Structure](image) | 2             | Vp 21 mPa (20°C)<sup>c</sup>  
Bp 135-138°C (133 Pa)<sup>c</sup> |
| Parathion   | ![Structure](image) | 6<sup>d</sup> | K<sub>ow</sub> 6,800  
Solubility 11 mg/L (20°C)  
Vp 0.89 mPa (20°C)  
Bp 150°C (80 Pa)  
K<sub>H</sub> 6.2 x 10<sup>-6</sup><sup>e</sup> |
| Diazinon    | ![Structure](image) | 300-400       | K<sub>ow</sub> 6,500<sup>f</sup>  
Solubility 60 mg/L (20°C)  
Vp 12 mPa (25°C)  
Bp 83-84°C (27 mPa)  
K<sub>H</sub> 5.8 x 10<sup>-5</sup><sup>e</sup> |
| Pirimiphos-methyl | ![Structure](image) | 2050         | K<sub>ow</sub> 16,000  
Solubility 8.6 mg/L (30°C)  
Vp 2 mPa (20°C) |
| Quinalphos  | ![Structure](image) | 71           | K<sub>ow</sub> 27,400  
Solubility 17.8 mg/L (22-23°C)  
Vp 0.346 mPa (20°C)  
Bp 142°C (40 mPa, decomp) |
| Fenitrothion| ![Structure](image) | 1700         | K<sub>ow</sub> 2,700  
Solubility 21 mg/L (20°C)  
Vp 18 mPa (20°C)  
Bp 140-145°C (13 Pa) |

<sup>a</sup>All Data is from reference 7, unless noted otherwise  
<sup>b</sup>See note in Table 1.1, and data is for rat(oral) in mg kg<sup>-1</sup>  
<sup>c</sup>Data from reference 8  
<sup>d</sup>Data from reference 2  
<sup>e</sup>Data from reference 9  
<sup>f</sup>Data from reference 10  

Note: solubility is in water, K<sub>ow</sub> is the octanol water partition coefficient, Vp is the vapor pressure, Bp is the boiling point and K<sub>H</sub> is the Henry's Law coefficient.
1.4. Diazinon

1.4.1. General

In the present work one organophosphorus pesticide was studied: O,O-diethyl-O-(2-isopropyl-6-methylpyrimidin-4-ol) phosphorothioate, or by common name, diazinon (1). Some of its physical properties are listed in Table 1.2. Commercial production of diazinon began in 1952 by the Ciba-Geigy Corporation\textsuperscript{11} and the insecticidal properties of diazinon were first reported in the literature in 1953 by R. Gasser\textsuperscript{7}. The synthesis of diazinon, as detailed by Gysin and Margot\textsuperscript{12}, from J.R. Geigy S.A., and others\textsuperscript{13}, is shown in Figure 1.2.

![Figure 1.2. Synthesis of diazinon\textsuperscript{11,12}](image)

Diazinon is both an insecticide and an acaricide, which can be used on a wide array of pests. It has been used against cockroaches, fleas, lice, bedbugs, fruit flies, ants, silver fish, mites, soil nematodes, and flower thrips\textsuperscript{7,11,14}. From this list of pests it is clear diazinon can be used for many applications including farming, forestry, gardening,
veterinary medicine and household pest removal. In fact diazinon is the most widely used pesticide for lawn care. Prior to 1983 it was estimated that 1.18 million kg of diazinon were used annually in the United States alone. Less than 10 years later, in 1990, the amount of diazinon used the United States annually had nearly quadrupled, to 4.67 million kg. Diazinon was also one of the most heavily used organophosphorus pesticides by farms in Ontario in 1988. Clearly with such large inputs of this chemical it is necessary to understand its interactions in the natural environment.

1.4.2. Chemical decomposition of diazinon

1.4.2.1. Decomposition with perborate ion

Due to the potential of spills at the manufacturing plant or application site of a pesticide, methods for removal of a large dose of chemical must be available. It has been suggested that sodium perborate could be used for such a purpose. Qian et al. compared the rate of degradation of diazinon with sodium perborate (0.03 M) at 26°C in tap water (pH 9.94), lake water (pH 9.88), and deionized water (9.68). The rate of degradation of diazinon in the aqueous samples were fast, with half-lives of 16 minutes in tap water, 21.3 minutes in lake water and 27.2 minutes in deionized water. For comparison, in the absence of perborate ion, at pH 10.4 (20°C) diazinon has a half-life of 8,700 minutes.

Qian et al. state that in the sodium perborate system the active nucleophile is not the perborate ion itself, but hydroperoxide anion (HO₂⁻) which is formed by the following process:

$$\text{H}_2\text{O} + \text{H}_2\text{BO}_4^- \rightleftharpoons \text{HO}_2^- + \text{H}_3\text{BO}_3$$
However, a further study, carried out on paraoxon (4) and ethyl p-nitrophenylmethylphosphonate (5) showed that the kinetics could not be explained solely by HO$_2^-$, but that the perborate ion itself also acts as a nucleophile; thus, the decomposition is caused both by hydroperoxide anion and the perborate ion$^{18}$.

Mixtures of water and soil have also been tested by Qian et al. for the effect of perborate ion (0.03 M) on the decomposition of diazinon$^{16}$. It was found that the addition of soil, above 0.1%, to the system reduced the rate of decomposition, and at 5% soil complete decomposition was not observed ($t_{1/2} > 240$ minutes). It was determined that the reaction rate decreased due to the perborate ion reacting with soil components as well as diazinon. If the 5% soil sample was centrifuged most of the pesticide was recovered showing the rate was not decreased due to adsorption of the pesticide on the soil. When a higher concentration of perborate (0.12 M) was used in a 5% soil sample the rate was greatly increased, with a half-life of only 9.4 minutes.

Treatment of a contaminated pond (40,000 liters) with sodium perborate would only cost 142 dollars$^{16}$. Thus, perborate could be used a fast and cheap clean-up method for soil and water contaminated by diazinon.
1.4.2.2. Decomposition with sodium hypochlorite

Diazinon can end up in rivers and lakes through agricultural run-off. One study looked at diazinon concentrations in the San Joaquin River and its tributaries, in California, where several orchards are located\textsuperscript{19}. It was found that diazinon was present in the water. Especially high concentrations, up to 7 $\mu$g/L, were observed after a rainstorm. This is a high enough level to have harmful effects on the aquatic environment, being larger than the LC$_{50}$ for some aquatic life forms. The LC$_{50}$ for some aquatic life forms are given in Table 1.3.

### Table 1.3. LC$_{50}$\textsuperscript{a} Data for aquatic life exposed to diazinon

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>LC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acatia tonsa</td>
<td>Copepod (zooplankton)</td>
<td>2.57 $\mu$g/L\textsuperscript{b}</td>
</tr>
<tr>
<td>Daphnia pulex</td>
<td>Water flea (zooplankton)</td>
<td>0.9 $\mu$g/L</td>
</tr>
<tr>
<td>Ceriodaphnia dubia</td>
<td>Water flea (zooplankton)</td>
<td>0.35 $\mu$g/L\textsuperscript{c}</td>
</tr>
<tr>
<td>Menidia beryllina</td>
<td>Inland silverside (ray-finned fish)</td>
<td>4.8 $\mu$g/L</td>
</tr>
</tbody>
</table>

\textsuperscript{a} LC$_{50}$ is the lethal concentration for 50\% of the test organisms

\textsuperscript{b} Data from reference 7 unless stated otherwise

\textsuperscript{c} Data from reference 20

Diazinon has even been found in the effluent from waste water treatment plants in the Southeastern United States\textsuperscript{20}. The water was found to be toxic to water fleas (Ceriodaphnia dubia) with concentrations ranging from 0.21 to 1.31 $\mu$g/L.

As diazinon is present in lakes and rivers it has the potential to be present in drinking water that will undergo treatment to remove abiotic and biotic contaminants. One common method to purify drinking water is through chlorination, or treatment with sodium hypochlorite (NaOCl)\textsuperscript{21}. Diazinon undergoes a complex series of reactions on
treatment with sodium hypochlorite, buffered with calcium carbonate. One reaction sequence proposed by Dennis et al. is shown in Figure 1.3\textsuperscript{22}.

![Chemical reaction diagram]

**Figure 1.3.** Chlorination by-products of diazinon

Trichloroacetate ion, one of the final products shown in Figure 1.3, is itself a herbicide, which has a much lower mammalian toxicity (LD\textsubscript{50} 3200 mg/kg) than diazinon\textsuperscript{21}.

Trichloroacetate and dichloroacetate ions were also observed in a separate study\textsuperscript{22}. It was also found that if the pH became acidic (>6.8) diazoxon did not undergo further reaction and accumulated\textsuperscript{22,23}. Diazoxon is the P=O analog of diazinon, and is much more toxic
than diazinon itself. Thus, in the chlorination of waste waters containing diazinon, careful control of the pH is required to ensure complete degradation. Also for water contaminated with diazinon, chlorination may be a better treatment option than ozonation. This is due to the fact that during ozonation, diazinon is converted into diazoxon, but ozone is unable to degrade it further.  

1.4.3. Environmental fate of diazinon  

1.4.3.1. Microbial degradation  

It has been found that diazinon can be degraded by naturally occurring microorganisms. One study investigated a mixture of microorganisms found in activated sludge (Flavobacterium, Alcaligenes, Corynebacterium and Pseudomonas). In an aerobic sterilized sludge sample (pH 6.9), diazinon had a half-life of 22.2 days, whereas in an activated sludge sample, diazinon had a half-live of 15.3 days. The rate enhancement could be improved by adding a carbon source (glucose) and a nitrogen source (hydrolyzed protein), to a half-life of 10.1 days. The same trend was observed under anaerobic conditions, only the rate was slower in all three cases. It has also been determined that naturally occurring microorganisms in marsh water can enhance the rate of degradation of diazinon.

It has been found that diazinon degradation by one microorganism can be affected by other microorganisms. Arthrobacter or Streptomyces alone can not degrade the ring portion of diazinon. However, when placed together they can degrade the pyrimidinol ring of diazinon, showing a synergistic relationship.
Also, the length of time a field has been treated with diazinon can affect microbial degradation. A rice paddy field had been treated with diazinon every 20 days, however after 3.5 years the treatment failed. It was determined that over the 3.5 year period microorganisms in the soil developed the ability to degrade diazinon, mineralizing the pyrimidinol containing product to carbon dioxide, in 3 to 5 days, making the treatment ineffective. The microorganisms were found to be specific to the treated fields, as soil and water samples from diazinon free fields could not rapidly degrade diazinon. Also the microorganisms in the treated fields were specific to diazinon, as they could not degrade other organophosphorus pesticides such as, chloropyrifos (6) or parathion (see Table 1.2).

\[
\begin{align*}
\text{H}_3\text{CH}_2\text{CO} & \quad \text{O} \\
\text{Cl} & \\
\text{H}_3\text{CH}_2\text{O} & \quad \text{O} \\
\text{N} & \\
\text{Cl} & \\
\end{align*}
\]

6

1.4.3.2. Mammalian metabolism

Diazinon undergoes a complex metabolic process in the mammalian system. Figure 1.4. outlines the metabolic degradation of diazinon in the mammal. Several different cellular components are involved including liver microsomes, \( \text{O}_2/\text{NADPH}_2 \) (nicotinamide adenine dinucleotide phosphate) system, and glutathione dependent aryl transferase. Mammalian metabolism of diazinon produces both toxic and relatively nontoxic compounds. The toxic compounds include isomers of hydroxydiazinon and diazoxon (see Figure 1.4 for structures). The relatively harmless metabolites include
O,O-diethyl phosphorothioic acid, 2-isopropyl-6-methylpyrimidin-4-ol
(LD50 mice 2,700 mg/kg)17, and several oxidation products of 2-isopropyl-6-methyl-
pyrimidin-4-ol. The metabolites O,O-diethyl phosphorothioic, 2-isopropyl-6-
methylpyrimidin-4-ol and its oxidation products, and isomers of hydroxydiazinon are
excreted from the body in urine11,28,29,30.

Figure 1.4. Outline of the metabolic degradation of diazinon in the mammal10
1.4.3.3. Soil interactions

As diazinon is applied to farm fields and household lawns it is important to know how it interacts and degrades in soils. It has been found that diazinon is adsorbed more strongly by organic rather than sandy soils\textsuperscript{31,32}. Adsorption isotherm experiments (Freundlich curves) revealed a K value of 325 pmol/g for an organic soil and a K value of only 2 pmol/g for a sandy soil\textsuperscript{31}. K is the ordinate intercept of the adsorption isotherm, which indicates the extent of adsorption. In a similar study the same result was obtained showing the largest K values for soils high in organic matter compared to soils low in organic matter\textsuperscript{32}. It was also found that diazinon has a relatively high organic carbon partition coefficient (K\textsubscript{oc}) of 1,700\textsuperscript{33}. As expected diazinon has a higher leachability in sandy soils than in organic soils. When soil samples were washed 10 times with 200 mL of water, 94.6% of the diazinon was recovered from the sandy soil, whereas only 49.9% was recovered from the organic soil\textsuperscript{31}. It has also been found that for clay minerals the interlayer cation present plays an important role in diazinon adsorption. Diazinon does not bind to Ca\textsuperscript{2+} montmorillonite, but it does bind to montmorillonite with Cu\textsuperscript{2+} or Ni\textsuperscript{2+} cations due to the vacant d orbitals of the copper and nickel cations\textsuperscript{34}.

1.4.3.4. Soil degradation

Studies have also examined the rate of degradation of diazinon in soil. It was found that diazinon degrades faster in sterile organic soil (t\textsubscript{1/2} = 6.5 weeks) than in a sterile sandy soil (t\textsubscript{1/2} = 12.5 weeks)\textsuperscript{35}. The authors also found that in non-sterile soil samples, the rate was much faster in both organic soil (t\textsubscript{1/2} = 2 weeks) and in sandy soil (t\textsubscript{1/2} <1 week), due to microbial degradation. The rate of degradation of diazinon in two
types of soil from Southeastern Ontario was also investigated. The authors do not indicate if the soil was sterile; however, based on the relatively long half-lives it appears to have been sterilized. They also found diazinon to degrade quicker in organic soils ($t_{1/2} = 6$ weeks) versus mineral soils ($t_{1/2} = 15$ weeks).

1.4.3.5. Degradation in natural water and photolysis

As diazinon has been found in natural water sources, it is important to have a clear understanding of the processes which can take place in water. Several studies of the degradation of diazinon in natural waters have been undertaken. Well (pH 8.3) and ground water (pH 8.5), were studied in the dark and in sunlight, under ambient temperatures (-4 to 37°C). Diazinon was found to have a half-life of 99 days in the dark and 88 days in sunlight. However, it is not clear if this difference results from photolysis or from temperature differences due to exposure to sunlight, or to microbial degradation differences as the authors do not mention sterilization of the water samples.

The hydrolysis of diazinon was compared in river water (pH 7.3), filtered river water (pH 7.3) and sea water (pH 8.1). Diazinon was found to be more persistent in river water ($t_{1/2} = 80$ days) than in filtered river water ($t_{1/2} = 52$ days). The authors suggest that this may be due to the adsorption of diazinon on to particulate matter present in the river water. Samples of river water and sea water were also exposed to sunlight at ambient temperatures. The rate of degradation in sea water was largely unchanged (dark, 22°C, $t_{1/2} = 50$ days; sunlight, variable, $t_{1/2} = 47$ days); however, the rate in river water was significantly faster (dark, 22°C, $t_{1/2} = 80$ days; sunlight, variable, $t_{1/2} = 43$ days). The authors suggest that humic acid present in the river water, but not in sea water, may
have acted as a photosensitizer, increasing the rate through photolysis. In the presence of sunlight humic acid can produce photo active species such as singlet oxygen, peroxo radicals and superoxide anions, which are very reactive.\textsuperscript{38}

Diazinon degradation was also investigated in estuarine water (pH 7.8) under ambient temperatures.\textsuperscript{39} Diazinon was found to have a half-life of 10.6 days. This is much faster than the rates seen in sea and river water. The difference could be due to such things as different microbial populations or different weather patterns during the studies.

Diazinon photolysis in fog has been observed to produce diazoxon.\textsuperscript{40} This was determined as the concentration of the P=O compound was much lower than P=S in the morning but was much higher than P=S in the evening. Another product of diazinon photolysis is hydroxydiazinon, which was found to form when neat diazinon was subjected to UV irradiation.\textsuperscript{41} In another study aqueous samples of diazinon were irradiated with UV light.\textsuperscript{42} First 2-isopropyl-6-methyl-pyrimidin-4-ol and O,O-diethyl phosphorothioic acid were produced. The O,O-diethyl phosphorothioic acid undergoes further reaction to produce sulfate ions. It is also possible that the sulfate ions could be produced from diazinon being converted to diazoxon, as was seen in the ozonation of diazinon, followed by further degradation of diazoxon to produce 2-isopropyl-6-methyl-pyrimidin-4-ol and O,O-diethyl phosphoric acid in the presence of water and UV light.

Through the use of diazinon as a pesticide, this anthropogenic compound has been introduced into the natural environment. Studies have looked at how diazinon interacts with living organisms, soils and water. It will also be important to know by what
processes, or mechanisms, diazinon degrades to help us better predict its behavior under different conditions.

1.5. Mechanism at the phosphorus center in phosphorus triester compounds

Diazinon is a phosphorus triester, and studies on the mechanism of this class of compound have been carried out over the past few decades. The general form of the reaction is given in Equation 1.1.

\[ \text{R}_1\text{P}-\text{X} + \text{Nu}^- \rightleftharpoons \text{R}_1\text{P}-\text{Nu} + \text{X}^- \]  

\[ (1.1) \]

\( Z = \text{O} \) or \( S \)

\( \text{X} = \) leaving group

\( \text{R}_1, \text{R}_2 = \text{alkyl}, \text{O-alkyl}, \text{aryl}, \text{O-aryl}, \text{OH}, \text{H} \)

The main types of reaction mechanisms that have been identified for this reaction are bimolecular nucleophilic substitution at phosphorus (\( S_{N2} (P) \)), addition-elimination at phosphorus, and unimolecular nucleophilic substitution at phosphorus (\( S_{N1} (P) \)).

1.5.1. \( S_{N2} (P) \)

In this mechanism, the reaction occurs as a concerted process, or bond formation and breakage occur at the same time, as depicted in Equation 1.2.

\[ \text{Nu}^- + \text{R}_1\text{P}-\text{X} \rightleftharpoons \text{R}_1\text{P} - \text{Nu} + \text{X}^- \]  

\[ (1.2) \]

In this mechanism there is a trigonal bipyramidal transition state, where the nucleophile
and the leaving group have a partial negative charge.

1.5.2. Addition-elimination

During this process the formation of a trigonal bipyramidal intermediate (TBP) occurs before the departure of the leaving group, as shown in Equation 1.3.

\[
\text{Nu}^- + \text{R}_1-\text{P}-\text{X} \xrightarrow{\text{Z}} \text{Nu}-\text{P}-\text{X} \xrightarrow{\text{R}_1-\text{P}\text{-Nu}} \text{R}_1-\text{P}\text{-Nu} + \text{X}^- \quad (1.3)
\]

The pentacoordinate TBP formed is similar in structure to the pentacoordinate transition state found in the S\textsubscript{N}2 (P) mechanism. The intermediate has a negative charge on the Z atom. It has been found that the nucleophile will attack and the leaving group will depart thought the apical positions of the TBP, resulting in the inversion of the configuration\textsuperscript{3}. However, the lack of inversion of configuration can not rule out this mechanism as the intermediate can undergo isomerization by one of two processes (Berry pseudorotation or turnstile rotation)\textsuperscript{2,46,47}.

1.5.3. S\textsubscript{N}1 (P)

In the S\textsubscript{N}1 (P) process, sometimes referred to as an elimination-addition process, the rate determining step is the dissociation of the leaving group, followed by a fast addition of the nucleophile to the metaphosphate species\textsuperscript{2}. This mechanism is outlined in Equation 1.4.

\[
\text{Nu}^- + \text{R}_1\text{O}-\text{P}-\text{X} \xrightarrow{\text{Z}} \text{Nu}+\text{R}_1\text{O}\text{P}^+ \xrightarrow{\text{Z}} \text{R}_1\text{O}+\text{P}-\text{Nu} + \text{X}^- \quad (1.4)
\]
This mechanism is only possible if there is an electron donating group, such as oxygen, next to the phosphorus to help stabilize the intermediate. This type of mechanism has also been seen in compounds in which phosphorus has a high degree of steric hindrance. The three different mechanisms are represented in a More O’Ferrall-Jencks reaction coordinate diagram in Figure 1.5.

\[
\begin{align*}
\text{SN1 (P)} & : \text{Nu}^– + R_1O\text{P}^+ + X^- \\
\text{SN2 (P)} & : R_1O\text{P}^-\text{Nu} + X^- \\
\text{Addition-elimination} & : \text{Nu}^– + R_1O\text{P}^-X \quad \text{Nu}^–\text{P}^-X
\end{align*}
\]

**Figure 1.5.** More O’Ferrall-Jencks diagram representing the relationship between the three mechanisms of nucleophilic substitution at phosphorus.

This diagram shows that any individual reaction mechanism may not be purely one type or the other, but maybe lie somewhere in between. For example, if the departure of the leaving group occurs just slightly before bond formation, the transition state will start to resemble the SN1 (P) process, rather than an SN2 (P).
1.6. Mechanism at carbon centers in phosphorus triester compounds

Beyond attack at phosphorus, reaction can also take place at the aliphatic carbon of O-alkyl groups attached to phosphorus, via a $S_{N}2$ (C) mechanism. Attack can also occur at the aromatic ring of O-aryl groups attached to phosphorus by way of an aromatic nucleophilic substitution mechanism ($S_{N}A r$).

1.6.1. $S_{N}2$ (C)

This reaction occurs by the nucleophile attacking the carbon of an O-alkyl group as detailed in Equation 1.5.

\[
\begin{align*}
R_1\text{—}P\text{—}O\text{—}CH_2R & \quad \text{Nu}^- \\
& \quad \rightarrow \\
R_1\text{—}P\text{—}O^- + \text{Nu—CH}_2R
\end{align*}
\]

This process will only be significant under neutral conditions as hydroxide ion favors attack at phosphorus over saturated carbon\textsuperscript{49}.

1.6.2. $S_{N}A r$

In general $S_{N}A r$ reactions occur when the ring is activated by the presence of electron withdrawing groups (EWG), such as nitro groups\textsuperscript{50,51}. This reaction follows an addition elimination mechanism. The first step is addition of the nucleophile to yield a cyclohexadienyl anion, or Meisenheimer type complex\textsuperscript{52}. The intermediate is no longer aromatic, possessing an sp\textsuperscript{3} hybridized carbon. The final step is the loss of the leaving group to give the substituted product. The intermediate is not observed unless the ring is highly electron-deficient, otherwise the intermediate is designated as a model for the transition state. The reaction proceeds as shown in Equation 1.6.
An aromatic ring which does not contain electron-withdrawing substituents is unlikely to follow this mechanism due to repulsions between the π ring electrons and the attacking nucleophile.

There are many possible reactions that can occur with phosphorus triester compounds; these will be further described and related to diazinon degradation in the discussion.

1.7. Purpose of this study

In this laboratory, several organophosphorus pesticides have been studied. The effect of metal ethoxides and surfactants on the degradation of fenitrothion have been investigated. Also, research on the nucleophilic substitution of fenitrothion with oxygen nucleophiles was carried out. In addition, the behavior of pirimiphos-methyl in soil-
water mixtures has been explored\textsuperscript{54}. Presently, the study of diazinon, which is similar in structure to pirimiphos-methyl, can broaden the understanding of the behavior of organophosphorus pesticides.

In this study the main goal was to develop a pH rate-profile for the aqueous hydrolysis of diazinon. This profile could be used to help determine the rate of hydrolysis of diazinon under different pH conditions, as well as reveal information about the mechanisms taking place. Although the environmental region (pH 5-8) was not studied here, the reaction has been studied by other workers under similar conditions\textsuperscript{36}, and this data was used in the pH profile. The plot extends beyond the environmental region to relatively basic systems (pH 12-14) and into highly acidic conditions (8.75 M HCl). This information can be used to help define further the behavior of this pesticide.

The second part of the present study was to determine what effect a naturally occurring substance, humic acid, would have on the hydrolysis of diazinon. The rate in aqueous alkaline solution will be compared to aqueous alkaline solutions containing humic acid. This will further our understanding of organophosphorus pesticide interactions in the environment. This information can help determine whether the pesticide will be stabilized in soil or water of high humic acid content, or if the hydrolysis will be catalyzed, reducing the persistence of the pesticide.
2. EXPERIMENTAL

2.1. Purification of materials

2.1.1. General

Diazinon, O,O-diethyl chlorothiophosphate, acetone, chloroform, chloroform-d, deuterium oxide, 1,4-dioxane-d_8, ethanol, concentrated HCl, hexanes, humic acid, anhydrous MgSO_4, NaCl, and NaOH were obtained from commercial sources of the highest purity possible and used without further treatment. Other materials were treated as specified in detail below.

2.1.2. 1,4-dioxane

The purification of 1,4-dioxane was a two-step process. First, reagent grade 1,4-dioxane was refluxed over stannous chloride for at least 6 hours followed by distillation to remove peroxides. Secondly, to remove residual water, the 1,4-dioxane was refluxed over sodium metal for at least 6 hours followed by distillation. The 1,4-dioxane was collected under a stream of nitrogen and stored in the freezer under nitrogen to prevent decomposition.

2.1.3. 2-Isopropyl-6-methylpyrimidin-4-ol(3)

Solid compound 3 obtained from Aldrich (99%) was found to contain a fine black impurity. The impurity was removed by dissolution in hot acetone followed by hot gravity filtration. Once cooled, fine white needles of pure 3 were produced. The melting point determined on a Mel-temp melting point apparatus was found to be 172-173.5°C (lit. 172-175°C).
2.1.4. Water

Distilled water was deionized by passing through a column containing an ion exchange resin. The water was then boiled in a three-neck round bottom flask. While cooling, nitrogen was passed through the water to prevent dissolution of oxygen and carbon dioxide. The water was then stored under nitrogen. This water was used to make up all aqueous stock solutions used in the kinetic studies.

2.2. Preparation of stock solutions

2.2.1 General

All stock solutions were made up in volumetric flasks and sealed with rubber septa and Parafilm®. As well, all solutions were stored in the refrigerator under nitrogen.

2.2.2. Sodium Hydroxide

NaOH pellets were dissolved in water and the solution was standardized by titration against dry potassium hydrogen phthalate (KHP) using phenolphthalein as an indicator.

2.2.3. Hydrochloric acid

Concentrated HCl was diluted with water to the desired concentration and the resulting solution was standardized against previously standardized NaOH using phenolphthalein as indicator.

2.2.4. Sodium Chloride

Weighted amounts of NaCl were dissolved in water to give the desired concentration. These solutions were used to keep a constant ionic strength in kinetic studies, in order to determine the effect of ionic strength on the rate of hydrolysis in
aqueous NaOH.

2.2.5. Humic Acid

A large stock solution (250 mL) of 0.509 M sodium hydroxide was made and standardized as above. Ten 25 mL volumes of solution were made from this stock solution, with compositions given in Table 2.1.

<table>
<thead>
<tr>
<th>Stock solution number</th>
<th>0.509 M NaOH (mL)</th>
<th>Water (mL)</th>
<th>Humic acid (ppm)</th>
<th>[NaOH] (x 10^{-2} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.0</td>
<td>0</td>
<td>0</td>
<td>50.9</td>
</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>0</td>
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</tr>
<tr>
<td>10</td>
<td>0.50</td>
<td>24.5</td>
<td>112</td>
<td>0.943</td>
</tr>
</tbody>
</table>

To determine the effect of the humic acid on the [OH^-], stock solution 10 was titrated against KHP using phenolphthalein as indicator, and found to be 9.43 x 10^{-3} M. This was used to correct [OH^-] in all other stock solutions containing humic acid. In the kinetic experiments, different amounts of humic acid-containing and
non humic acid-containing stock solutions were mixed to produce varying humic acid concentrations. The cuvettes were filled as outlined in Table 2.2.

**Table 2.2.** Composition of cuvettes used in determination of effect of humic acid on alkaline hydrolysis of diazinon

<table>
<thead>
<tr>
<th>Cuvette</th>
<th>NaOH stock solutions</th>
<th>[humic acid] (ppm)</th>
<th>[NaOH] (x 10^2 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.50 mL of 1</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2.00 mL of 1</td>
<td>0.50 mL of 2</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>1.50 mL of 1</td>
<td>1.00 mL of 2</td>
<td>48</td>
</tr>
<tr>
<td>D</td>
<td>2.50 mL of 3</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>2.00 mL of 3</td>
<td>0.50 mL of 4</td>
<td>25</td>
</tr>
<tr>
<td>F</td>
<td>1.50 mL of 3</td>
<td>1.00 mL of 4</td>
<td>50</td>
</tr>
<tr>
<td>G</td>
<td>2.50 mL of 5</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>2.00 mL of 5</td>
<td>0.50 mL of 6</td>
<td>24</td>
</tr>
<tr>
<td>I</td>
<td>1.50 mL of 5</td>
<td>1.00 mL of 6</td>
<td>48</td>
</tr>
<tr>
<td>J</td>
<td>2.50 mL of 7</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>K</td>
<td>2.00 mL of 7</td>
<td>0.50 mL of 8</td>
<td>24</td>
</tr>
<tr>
<td>L</td>
<td>1.50 mL of 7</td>
<td>1.00 mL of 8</td>
<td>48</td>
</tr>
<tr>
<td>M</td>
<td>2.50 mL of 9</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>2.00 mL of 9</td>
<td>0.50 mL of 10</td>
<td>22</td>
</tr>
<tr>
<td>O</td>
<td>1.50 mL of 9</td>
<td>1.00 mL of 10</td>
<td>45</td>
</tr>
</tbody>
</table>

Note: Numbers refer to solutions in Table 2.1.

**2.2.6. Diazinon**

The purity of diazinon obtained from Chem Services (99.5%) was checked by gas chromatography using an electron-impact (EI) mass spectrometry detector (GC/MS) and
NMR. Two peaks were seen in the chromatogram: one with a retention time of ~3 minutes due to chloroform (solvent) and a second at 26.6 minutes due to diazinon. The MS, parent peak of 304 m/z, and fragmentation pattern were consistent with diazinon. The NMR spectra ($^1$H, $^{13}$C and $^{31}$P) were consistent with diazinon (see appendix B for spectra and interpretation).

A 15 μL aliquot of diazinon was dissolved in 5 mL of dry 1,4-dioxane resulting in a 1.10 x 10$^{-5}$ M solution of diazinon. In all kinetic studies 20 μL of this solution was added to the quartz cuvettes. The total volume in the cuvette including diazinon, acid, base, and/or sodium chloride was 2.52 mL, producing a final diazinon concentration of 8.73 x 10$^{-5}$ M in every kinetic run.

2.2.7. Product reference compounds

A 0.0761 g portion of 3 was dissolved in 50 mL of dry 1,4-dioxane to produce a 1.00 x 10$^{-2}$ M solution. As well, a 0.1367 g portion of 2 was dissolved in 50 mL of dry 1,4-dioxane to give a 1.61 x 10$^{-3}$ M solution.

2.3. Determination of pK$_a$ values of 2-isopropyl-6-methylpyrimidin-4-ol

A titration was used to determine two of the pK$_a$ values of 3. A solution of 3 (3.02 x 10$^{-2}$ M) in NaOH (0.108 M), was titrated with HCl (1.25 M) and the pH was determined using a standard pH meter equipped with an Ag/AgCl electrode. The pH meter was calibrated using four buffers (2.00, 4.00, 7.00 and 10.00). The acid was added to 100 mL of basic solution containing 3 in 0.02 mL increments from a 10 mL class A burette. As well spectrophotometric scans were carried out using 2 mL of a 1.31 x 10$^{-4}$ M solution of 3 in 1.09 x 10$^{-2}$ M HCl. To this, increments of NaOH solutions
(2.85 M, 0.285 M, 0.0285 M, 2.85 \times 10^{-3} \text{ M} \text{ or } 2.85 \times 10^{-4} \text{ M}) \text{ were added (5 or } 1.5 \mu \text{L at a time) and a specturm recorded after each addition. Further analysis of this compound included an infrared (IR) spectrum taken in a sodium bromide disk on a Bomem MB-Series Infrared instrument (see appendix B for spectrum).

2.4. Kinetic studies of the hydrolysis of diazinon

2.4.1. General

Kinetics were following by ultra violet-visible (UV-VIS) spectrophotometry using either a Varian CARY3, Hewlett-Packard 8452A, or Perkin-Elmer \lambda-20 spectrophotometer. Temperature was maintained with a Peltier device in the case of the CARY3; whereas the 8452A and \lambda-20 instruments used a circulating water bath for temperature control. All kinetics were carried out under pseudo-first order conditions, ensuring that the concentration of base or acid was at least 28 times greater than the initial concentration of diazinon. Sodium hydroxide solutions were added to the cuvettes using gas-tight syringes while HCl solutions were added using two class A pipettes (2 mL and 0.5 mL). The cuvettes were then placed in the spectrophotometer cell holder for at least 30 min to allow for temperature equilibration. Then 20 \mu L of the substrate solution was added using a 25 \mu L gas tight syringe and the run started. Cuvettes were cleaned using the following sequence of solvents; soapy water, distilled water, and acetone.

2.4.2. Kinetic Methods

Repetitive scanning of the hydrolysis of diazinon was carried out under acidic and basic conditions between 200 nm and 500 nm, to monitor the appearance of products, disappearance of substrate and to observe the isosbestic behaviour of the reaction.
Isosbestic behaviour provides information about the reaction; for example, the presence of isosbestic points shows that there are no long-lived intermediates.

The appearance of products was monitored at 229 nm for the basic system, which corresponds to the maximum absorption ($\lambda_{\text{max}}$) under basic conditions. In the case of the acidic system, $\lambda_{\text{max}}$ was at or near 230 nm, being shifted slightly with increasing acid concentration to 232 nm. The $\lambda_{\text{max}}$ was determined from the repetitive scanning experiments (see Section 3.1.3). Beer's law was used to calculate the molar absorptivity at 229 nm ($e_{229\text{nm}}$) under alkaline conditions and at 230 nm ($e_{230\text{nm}}$) under acidic conditions of the products, 3 and 2, to determine the expected infinity absorption value ($A_{\infty}$) under both experimental conditions.

The hydrolysis reactions in NaOH and HCl were followed for 3 half-lives ($3t_{1/2}$) and the $A_{\infty}$ taken at 10 $t_{1/2}$. Pseudo-first order rate constants ($k_{\text{obs}}$) were calculated from a plot of $3+\log(A_{\infty}-A_t)$ versus time, where $A_t$ is the absorbance at time $t$. As well, the slope of the plot of $k_{\text{obs}}$ versus [NaOH] yielded the second order rate constant, $k_2$.

The effect of ionic strength on the rate of alkaline hydrolysis was investigated by examining the rate over an ionic strength range of 0.109 to 0.446 M, raised in 0.05 intervals. It was found that the rate was not affected as long as the ionic strength was ≤0.347 M. As only one solution in the alkaline region went over this ionic strength range (0.543 M NaOH) no attempt was made to maintain a constant ionic strength.

2.5. Syntheses

2.5.1. General

Product analysis of synthesised compounds involved $^1\text{H}$, $^{13}\text{C}$ and $^{31}\text{P}$ nuclear
magnetic resonance (NMR) and GC/MS. The NMR spectra were run on a Bruker 300 MHz Avance spectrometer using CDCl₃ as solvent, unless otherwise stated. The GC/MS analyses were carried out on a Fisons 8000 Series GC/MS instrument. All spectra can be found in Appendix B.

2.5.2. O,O-Diethyl phosphorothioic acid(2) and triethyl thiophosphate(7)

Compounds 2 and 7 were prepared by a modification of Mastin, Norman and Weilmuenster's method² as outlined in Scheme 1.

\[
\text{NaOH} + \text{CH}_3\text{CH}_2\text{OH} \xrightarrow{\text{CH}_3\text{CH}_2\text{O}} \text{CH}_3\text{CH}_2\text{ONa} + \text{H}_2\text{O}
\]

\[
\text{CH}_3\text{CH}_2\text{O}^{\|}\text{P}^{\text{Cl}} + \text{NaOH} \xrightarrow{\text{EtOH}} \text{CH}_3\text{CH}_2\text{O}^{\|}\text{P}^{\text{ONa}} + \text{CH}_3\text{CH}_2\text{O}^{\|}\text{P}^{\text{OCH}_2\text{CH}_3} \\
+ \text{NaOCH}_2\text{CH}_3 \\
(2-\text{Na})
\]

\[
(6)
\]

\[
+\text{NaCl}
\]

Scheme 2.1. Preparation of 2-'Na⁺ and 7

Diethyl chlorothiophosphate (MM=188.61 g/mol, d=1.2 g/mL, 15.0 mL or 18.0 g, 0.0954 mol, Aldrich) was added dropwise to a stirred solution of NaOH (MM=40 g/mol, 7.50 g, 0.1875 mol, Aldrich) in 150 mL of absolute ethanol. Once all the diethyl chlorothiophosphate was added, the resulting solution was stirred for 3 hours. Next the solution was chilled in an ice bath and filtered to remove NaCl formed during the reaction. The filtrate was concentrated by rotary evaporation and the resulting slurry was filtered to remove residual NaOH and the sodium salt of 2 (2-'Na⁺) while the filtrate contained 7. The filtrate was dried with anhydrous MgSO₄ and after filtration the ethanol was removed by rotary evaporation. The resulting oil was further purified by flash
column chromatography using silica gel as the stationary phase and a mixed solvent of 80% hexanes/20% chloroform as eluent. Solvent was removed from the solution of 7 first by rotary evaporation followed by drying under vacuum overnight. This procedure gave a final yield of 52% (9.88 g, 0.0498 mol) of 7, and a final crude yield of 54% (11.62 g, 0.0605 mol) of \( \text{2 Na}^+ \).

A 2 g portion of \( \text{2 Na}^+ \) was acidified using HCl (~10 mL of 6 M, until pH indicator paper acidic) and then extracted with 3 x 10 mL portions of chloroform to extract 2. The organic layer was dried using anhydrous MgSO\(_4\) and, after filtration, the chloroform was removed by rotary evaporation followed by drying under vacuum overnight. This procedure gave a final yield of 16.4% (0.324 g, 1.90 x 10\(^{-3}\) mol) of 2.

The purity and identity of 7 was determined by GC/MS and NMR. There were two peaks in the chromatogram, one with a retention time of ~3 minutes due to chloroform and a second at 12.7 minutes due to 7. The MS, parent peak at 198 m/z, was consistent with the proposed compound's identity. The NMR spectra were assigned as follows (numbering as in the diagram below):

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{O} & \xrightarrow{S} \text{P}-\text{OCH}_2\text{CH}_3 \\
\text{CH}_3\text{CH}_2\text{O} & \xrightarrow{a} b
\end{align*}
\]

\(^1\text{H}: 1.25 \text{ ppm} (t, J_{\text{H}-\text{H}}=7.1 \text{ Hz, 9H, a}) 4.07 \text{ ppm} (d \text{ of } q, J_{\text{H}-\text{H}}=7.1 \text{ Hz, } J_{\text{H}-\text{p}}=9.6 \text{ Hz, 6H, b}).
\]

\(^13\text{C}: 16.3 \text{ ppm} (d, J_{\text{C}-\text{p}}=7.5 \text{ Hz, b}) 64.5 \text{ ppm} (d, J_{\text{C}-\text{p}}=5.5 \text{ Hz, a}).
\]

\(^{31}\text{P}: 68.6 \text{ ppm}\)
Where $t =$ triplet, $d =$ doublet, and $q =$ quartet. For a singlet no letter will be shown. In brackets the multiplicity is given followed by the coupling constant and the number of equivalent hydrogens (or carbons) represented in that peak, and finally the letter identifying the atom from the structure presented.

The purity and identity of 2 were determined by MS and NMR. The MS parent peak at 170 m/z, was consistent with the proposed compound's identity. The NMR spectra were assigned as follows (numbering as in the diagram below):

\[ \text{CH}_3\text{CH}_2\text{O} \begin{array}{c} S \\ \| \\ \text{CH}_3\text{CH}_2\text{O} \end{array} \text{P—OH} \]

$^1$H: 1.33 ppm ($t$, $J_{\text{Ha-Hb}} = 7.1$ Hz, 6H, a) 4.20 ppm (d of q, $J_{\text{Hb-Ha}} = 7.0$ Hz, $J_{\text{Hb-P}} = 9.3$ Hz, 4H, b) 7.59 ppm (c).

$^{13}$C: 16.2 ppm (d, $J_{\text{C-Ha}} = 7.7$ Hz. a) 64.8 ppm (d, $J_{\text{C-Hb}} = 5.5$ Hz, b).

$^{31}$P: 58.4 ppm

$^{31}$P NMR was also run of 2·Na$^+$ in D$_2$O. The NMR peak was assigned as follows (numbering as in the diagram below):

\[ \text{CH}_3\text{CH}_2\text{O} \begin{array}{c} S \\ \| \\ \text{CH}_3\text{CH}_2\text{O} \end{array} \text{P—ONa} \]

$^1$H: 1.15 ppm ($t$, $J_{\text{Ha-Hb}} = 7.1$ Hz, 6H, a) 3.86 ppm (d of q, $J_{\text{Hb-Ha}} = 7.1$ Hz, $J_{\text{Hb-P}} = 0.8$ Hz, 4H, b).
\( ^{13}\text{C} \): 15.8 ppm (d, \( J_{\text{C},\text{P}} = 7.5 \text{ Hz} \), a) 63.0 ppm (d, \( J_{\text{C},\text{P}} = 5.9 \text{ Hz} \), b).

\( ^{31}\text{P} \): 55.9 ppm

2.6. Product analysis by \( ^{31}\text{P} \) NMR of the hydrolysis of diazinon

The products of the hydrolysis of diazinon were studied by \( ^{31}\text{P} \) NMR to determine if, as expected, only one phosphorus-containing product was produced. Three different conditions were used:

1) Basic: aqueous solution containing 0.409 M \( \text{NaOH} \), 18\% (v/v) 1,4-dioxane-\( \text{d}_8 \) and saturated with diazinon,

2) Mildly acidic: aqueous solution containing \( 2.26 \times 10^{-3} \text{ M HCl} \), 18\% (v/v) 1,4-dioxane-\( \text{d}_8 \) and saturated with diazinon,

3) Strongly acidic: aqueous solution containing 2.17 M \( \text{HCl} \), 9.6\% (v/v) 1,4-dioxane-\( \text{d}_8 \) and 0.015 M diazinon.

To the basic and mildly acidic solutions more 1,4-dioxane-\( \text{d}_8 \) was added (+ 0.1 mL) due to reduced solubility of diazinon. For the same reason, saturated solutions of diazinon were used under basic and mildly acidic conditions. Standards were run of diazinon and the expected product 2.
3. RESULTS

3.1. Reaction in basic media

3.1.1. NMR identification of phosphorus-containing product

The base catalysed aqueous hydrolysis of diazinon, according to Scheme 3.1, was studied by $^{31}\text{P}$ NMR to determine if, as expected, O,O-diethyl phosphorothioic acid (2) was the only phosphorus-containing product formed (as described in Section 2.6).

![Scheme 3.1. Outline of the base catalysed aqueous hydrolysis of diazinon](image)

The $^{31}\text{P}$ NMR confirmed the formation of 2 as the sole phosphorus-containing product formed during the alkaline hydrolysis of diazinon. The $^{31}\text{P}$ NMR spectrum taken 24 hours after the start of reaction is shown in Figure 3.1. This experiment also verified that diazinon does not undergo isomerization to the P=O form under alkaline conditions. This result also rules out any $S_N 2$ (C), which would have produced a different phosphorus-containing product as seen in Scheme 3.2. This type of process is typically seen in...
phosphorus pesticides near neutral pH and was also observed as a competitive pathway under ethanolysis in fenitrothion in this group.$^{58}$

\[
\text{Scheme 3.2. Products from } S_{N}2 \text{ (C) process}
\]

\[
\begin{align*}
\text{Products from } S_{N}2 \text{ (C) process} \\
\end{align*}
\]

\[
\text{Figure 3.1. } ^{31}\text{P NMR of reaction of diazinon under basic conditions, after 24 hours}
\]
3.1.2. UV/VIS Spectrophotometric analysis of reaction products in basic media

Both products, 2-isopropyl-6-methylpyrimidin-4-ol (3) and O,O-diethyl phosphorothioic acid (2) were studied by UV/VIS spectrophotometry by determining their molar absorptivity (€) under alkaline conditions. First, repetitive scans of the reaction were carried out to determine a wavelength which undergoes maximum change during the reaction. This was determined to be 229 nm. The repetitive scans are shown in Figures 3.2, 3.3, 3.4, 3.5 and 3.6. Next, Beer-Lambert analysis of the two products was used to determine the theoretical absorbance at infinite time (A∞) at 229 nm. From the Beer-Lambert analysis it was found that the wavelength of maximum absorbance (λmax) of 3 is 229 nm with €229 being 9.53 x 10³ cm⁻¹ M⁻¹. The λmax for 2 was found to be 219 nm, thus, at 229 nm we are on a shoulder of the spectrum for this product. The €229 of 2 was found to be 1.49 x 10³ cm⁻¹ M⁻¹. From this the theoretical A∞ was calculated to be 0.91. In all kinetic runs the A∞ used is within 5% of this value, except for the repetitive scans which show absorbances about 0.1 units higher. As kinetic and Beer-Lambert data were collected on the CARY 3 and the repetitive scans were taken using the HP 2845A it is believed this discrepancy may have to do with instrumental differences, including the fact that the baseline of the repetitive scans was ~0.05 absorbance units.

3.1.3. Kinetic studies of the base catalysed aqueous hydrolysis of diazinon

The base catalysed aqueous hydrolysis of diazinon, according to Scheme 3.1, previously shown, was studied spectrophotometrically, by following the formation of Products, 3 and 2. The reaction was followed at three temperatures, 25, 35 and 45°C. In all cases, the concentration of NaOH was present in at least a 100-fold excess over
diazinon (8.73 x 10^{-5} M) to ensure that pseudo-first order conditions were met. The reaction was followed to ten-half lives (ca. 99.9% complete reaction) and the rate constants determined using information from the first three half-lives. The determination of the pseudo-first order rate constant is shown for a representative run in Appendix A. Data from these kinetic experiments are presented in Tables 3.2, 3.3 and 3.4. From the repetitive scanning plots (Figures 3.2 - 3.6) it can be seen that, in all cases studied here, there are clean isosbestic points. The dependence of rate on the concentration of NaOH under varying temperatures is shown in Figure 3.7. The slope of this plot gave the second order rate constant, k_2. From Figure 3.7 it is clear that there is a positive linear relationship between k_{obs} and [NaOH]. It can also be seen, as expected, that as the temperature increases so does the rate of reaction.

Table 3.1. Pseudo-first order rate constants (k_{obs}) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying [NaOH] at 25°C.

<table>
<thead>
<tr>
<th>[NaOH]_0 (M)</th>
<th>Calculated pH</th>
<th>k_{obs} (x 10^{-4} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0115</td>
<td>12.06</td>
<td>0.373 ± 0.009</td>
</tr>
<tr>
<td>0.0228</td>
<td>12.36</td>
<td>0.932 ± 0.001</td>
</tr>
<tr>
<td>0.109</td>
<td>13.04</td>
<td>3.50 ± 0.025</td>
</tr>
<tr>
<td>0.326</td>
<td>13.51</td>
<td>10.3 ± 0.150</td>
</tr>
<tr>
<td>0.543</td>
<td>13.73</td>
<td>16.7 ± 0.100</td>
</tr>
</tbody>
</table>

k_2 = 3.06 x 10^{-3} M^{-1} s^{-1}

^aThe error limits in the k_{obs} value were taken as the average deviation following the method of Balakrishnan. In this case the two rate constants were 3.64 x 10^{-5} s^{-1} and 3.82 x 10^{-5} s^{-1} with a mean of 3.73 x 10^{-5} s^{-1}. The difference from the mean is ±0.09 x 10^{-4} s^{-1}. Thus, the rate is reported as (3.73±0.09) x 10^{-5} s^{-1}. In cases where three experimentally determined values of k_{obs} were employed to determine the mean, the error limit used was the standard deviation about that mean.
**Table 3.2.** Pseudo-first order rate constants ($k_{obs}$) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying [NaOH] at 35°C.

<table>
<thead>
<tr>
<th>[NaOH]₀ (M)</th>
<th>Calculated pH</th>
<th>$k_{obs}$ (x 10^{-4} s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0115</td>
<td>12.06</td>
<td>0.788 ± 0.007⁺</td>
</tr>
<tr>
<td>0.0228</td>
<td>12.36</td>
<td>1.94 ± 0.000</td>
</tr>
<tr>
<td>0.109</td>
<td>13.04</td>
<td>7.48 ± 0.046</td>
</tr>
<tr>
<td>0.326</td>
<td>13.51</td>
<td>21.1 ± 0.150</td>
</tr>
<tr>
<td>0.543</td>
<td>13.73</td>
<td>33.0 ± 0.250</td>
</tr>
</tbody>
</table>

$k_2 = 6.06 \times 10^{-3}$ M⁻¹ s⁻¹

⁺ See footnote in Table 3.1

**Table 3.3.** Pseudo-first order rate constants ($k_{obs}$) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying [NaOH] at 45°C.

<table>
<thead>
<tr>
<th>[NaOH]₀ (M)</th>
<th>Calculated pH</th>
<th>$k_{obs}$ (x 10^{-3} s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0115</td>
<td>12.06</td>
<td>0.157 ± 0.001⁺</td>
</tr>
<tr>
<td>0.0228</td>
<td>12.36</td>
<td>0.409 ± 0.013</td>
</tr>
<tr>
<td>0.109</td>
<td>13.04</td>
<td>1.59 ± 0.026</td>
</tr>
<tr>
<td>0.326</td>
<td>13.51</td>
<td>4.45 ± 0.045</td>
</tr>
<tr>
<td>0.543</td>
<td>13.73</td>
<td>7.01 ± 0.214</td>
</tr>
</tbody>
</table>

$k_2 = 1.29 \times 10^{-2}$ M⁻¹ s⁻¹

⁺ See footnote in Table 3.1
**Figure 3.2.** Repetitive scans of the base catalysed (0.0115M NaOH) aqueous hydrolysis of diazinon (scans taken at intervals of 1800 seconds).

**Figure 3.3.** Repetitive scans of the base catalysed (0.0228 M NaOH) aqueous hydrolysis of diazinon (scans taken at intervals of 750 seconds).
Figure 3.4. Repetitive scans of the base catalysed (0.109 M NaOH) aqueous hydrolysis of diazinon (scans taken at intervals of 200 seconds).

Figure 3.5. Repetitive scans of the base catalysed (0.326 M NaOH) aqueous hydrolysis of diazinon (scans taken at intervals of 70 seconds).
Figure 3.6. Repetitive scans of the base catalysed (0.543 M NaOH) aqueous hydrolysis of diazinon (scans taken at intervals of 40 seconds).
Figure 3.7. The dependance of $k_{obs}$ on $[\text{NaOH}]_0$ for the base catalysed aqueous hydrolysis of diazinon at three temperatures. The data can be found in Tables 3.1, 3.2 and 3.3.
3.1.4. Activation parameters

The hydrolysis reaction carried out in the presence of NaOH was studied at three temperatures: 25, 35 and 45°C. The second order rate constants ($k_2$) from these three temperatures were used to determine the activation parameters of the reaction. An Arrhenius plot, $\log k_2$ versus $1/T$, was used to determine the activation energy and the log of the frequency factor ($\log A$). This plot is presented in Figure 3.8. The activation energy was found from the slope and $\log A$ as the y-intercept, as shown in Equation 3.3.

$$\log k_2 = \log A - \frac{E_a}{2.303 RT} \tag{3.3}$$

Next, an Eyring plot, $\ln (k_2 (or \ k_{obs})/T)$ versus $1/T$, was used to determine the enthalpy ($\Delta H^*$) and entropy ($\Delta S^*$) values. This plot is shown in Figure 3.9. $\Delta H^*$ and $\Delta S^*$ values were calculated from this plot using the relationships given in Equations 3.4 and 3.5, respectively, where $R$ is the gas constant, $k$ is Boltzmann’s constant and $h$ is Planck’s constant.

$$slope = \frac{-\Delta H^*}{R} \tag{3.4}$$

$$intercept = \ln\left(\frac{k}{h}\right) + \frac{\Delta S^*}{R} \tag{3.5}$$

The Gibb’s free energy ($\Delta G^*$) term was calculated from $\Delta H^*$ and $\Delta S^*$, at 25°C, using the relationship in Equation 3.6.

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{3.6}$$
The activation parameter results, with standard errors, are as follows:

\[ E_a = (56.6 \pm 2.7) \text{ kJ mol}^{-1} ((13.5 \pm 0.7) \text{ kcal mol}^{-1}) \]

\[ \log A = 7.40 \pm 0.5 \]

\[ \Delta H^* = (54.1 \pm 2.7) \text{ kJ mol}^{-1} ((12.9 \pm 0.6) \text{ kcal mol}^{-1}) \]

\[ \Delta S^* = (-112 \pm 11) \text{ J mol}^{-1} \text{ K}^{-1} ((-26.8 \pm 2.7) \text{ cal mol}^{-1} \text{ K}^{-1}) \]

\[ \Delta G^* = (87.5 \pm 13) \text{ kJ mol}^{-1} ((20.9 \pm 3.2) \text{ kcal mol}^{-1}) . \]
Figure 3.8. Arrhenius plot for base catalysed aqueous hydrolysis of diazinon.
Figure 3.9. Erying plot of base catalysed aqueous hydrolysis of diazinon.
3.1.5. Effect of ionic strength on rate of reaction

The effect of ionic strength on the rate of alkaline hydrolysis of the organophosphorus compounds O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate (or fenitrothion) and 4-nitrophenyl diphenyl-phosphate ester, has been investigated by Omakor and Ba-Saif et al., respectively. Omakor found that the rate of hydrolysis was not affected over an ionic strength range of 0.04 to 0.107 M. Ba-Saif's group have covered a higher range of ionic strength, 0.1M to 0.5 M, raised in 0.1M intervals. They found that the rate was unaffected as long as the ionic strength was ≤ 0.3 M, but at higher values than 0.3 M the rate decreased.

In the present study, the effect of ionic strength on the rate of alkaline hydrolysis was studied from 0.109 to 0.446 M, raised in 0.05M intervals. The results from this test are shown in Table 3.4. The deviation of each run with higher ionic strength from the control run (0.109 M NaOH) was calculated. It was found that the rate was not significantly affected (i.e. <5% deviation) as long as the ionic strength was ≤0.347 M. Similar to Ba-Saif's group, the rate decreased when the ionic strength was higher than 0.347 M. As only one base concentration used in this study had an ionic strength greater than this limit (0.543 M) no attempt was made to maintain a constant ionic strength in subsequent kinetic studies.
Table 3.4. Pseudo-first order rate constants ($k_{\text{obs}}$) for the hydrolysis of diazinon (8.73 x $10^{-5}$ M) in the presence of aqueous NaOH (0.109 M) at 25°C under varying ionic strength ($\mu$).

<table>
<thead>
<tr>
<th>[NaCl] (M)</th>
<th>$\mu$ (M)</th>
<th>$k_{\text{obs}}$ (x 10$^{-4}$ s$^{-1}$)</th>
<th>% deviation from control run</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>0.109$^a$</td>
<td>3.48</td>
<td>---</td>
</tr>
<tr>
<td>0.0397</td>
<td>0.149</td>
<td>3.46</td>
<td>0.6$^b$</td>
</tr>
<tr>
<td>0.0992</td>
<td>0.208</td>
<td>3.39</td>
<td>2.6</td>
</tr>
<tr>
<td>0.139</td>
<td>0.248</td>
<td>3.36</td>
<td>3.5</td>
</tr>
<tr>
<td>0.198</td>
<td>0.307</td>
<td>3.34</td>
<td>4.1</td>
</tr>
<tr>
<td>0.238</td>
<td>0.347</td>
<td>3.32</td>
<td>4.7</td>
</tr>
<tr>
<td>0.298</td>
<td>0.407</td>
<td>3.23</td>
<td>7.4</td>
</tr>
<tr>
<td>0.337</td>
<td>0.446</td>
<td>3.25</td>
<td>6.8</td>
</tr>
</tbody>
</table>

$^a$The ionic strength was calculated using the following equation:

$$\mu = \frac{1}{2} \sum_{i} C_i Z_i^2$$

$^b$The percent deviation was calculated as the difference between the two values divided by the mean of the two values multiplied by 100. In this case:

$$\frac{3.48-3.46}{3.47} \times 100 = 0.6\%$$

3.2. Reaction in acidic media

3.2.1. $pK_a$ of products

The $pK_a$ of the products can be used as an aid in deciphering the reaction mechanism, by helping to determine the ability of the product as a leaving group and to determine if the substrate will be protonated under reaction conditions. The $pK_a$ of O,O-diethyl phosphorothioic acid (2) was reported in the literature as 1.83$^{60}$. The $pK_a$ of 2-isopropyl-6-methylpyrimidin-4-ol (3) was estimated by a pH titration as detailed in
Section 2.3. The titration curve from this experiment is presented in Figure 3.10. From this titration curve two $pK_a$ values were estimated as ~9.3 and ~2.7. The first value, $pK_{a2}$, is consistent with other reported pyrimidin-4-ols (see figure 3.11), as the addition of alkyl groups causes base strengthening. The second value, $pK_{a1}$, is also consistent with other reported compounds as seen in Figure 3.11.
Figure 3.11. pKₐ values for a series of pyrimidin-4-ols

The spectrophotometric data showed the various spectra associated with each species of pyrimidinol; cationic, neutral and anionic (see discussion for full details). The overlaid spectra are shown in Figure 3.12. There are two regions of the spectra which show significant change, at 230 nm and a 260 nm. These wavelengths were used to produce pH versus absorbance plots and are shown in Figure 3.13, which places the data from both wavelengths on the one graph so that their similarity can be clearly seen. Both show a change in absorbance at ~pH 2 and ~pH 9. These results show rough pKₐ values of ~2.5 and ~9.7, which agree with the titration.
Figure 3.12. Scans of spectroscopic titration of 3

Figure 3.13. Comparison of titration curves at 230 and 260 nm
3.2.2. NMR identification of phosphorus-containing product

Acid catalysed aqueous hydrolysis of diazinon, according to Scheme 3.3, was studied by \(^{31}\)P NMR under two different acid concentrations to determine if, as expected, O,O-diethylphosphorothioic acid (2) was the only phosphorus-containing product formed (as described in Section 2.6). \(^{31}\)P NMR confirmed the formation of 2 as the sole phosphorus-containing product formed during the acid hydrolysis of diazinon, under both concentrations. The \(^{31}\)P NMR spectra taken 24 hours after the start of reaction are shown in Figure 3.14, and 3.15. This experiment also verified that diazinon does not undergo isomerization to the P=O form under acidic conditions. This result also rules out any other reaction taking place besides the expected process, as no other phosphorus containing products were seen in the \(^{31}\)P NMR spectra.
Figure 3.14. $^{31}$P NMR of reaction of diazinon under mildly acidic conditions, after 24 hours

Figure 3.15. $^{31}$P NMR of reaction of diazinon under strongly acidic conditions, after 24 hours
Scheme 3.3. Acid catalysed aqueous hydrolysis of diazinon

3.2.3. UV/VIS Spectrophotometric analysis of reaction products in acidic media

Both products, 2-isopropyl-6-methylpyrimidin-4-ol (3) and diethyl phosphorothioic acid (2) were studied using UV/VIS spectrophotometry to determine their molar absorptivity (ε) under acidic conditions. First, repetitive scans of the reaction were carried out to determine a wavelength which undergoes maximum change during the reaction. This was found to vary between 230 and 232 nm, increasing as acid concentration increases. The repetitive scans are shown in Figures 3.16, 3.17, 3.18, 3.19, and 3.20. Next, Beer-Lambert analysis of the two products was used to determine the
theoretical absorbance at infinite time ($A_\infty$) at 230 nm. The Beer-Lambert analysis was carried out at the five different acid concentrations; it was found that the wavelength of maximum absorbance ($\lambda_{\text{max}}$) of 3 was 230 nm in 0.175 and 1.05 M HCl, 232 nm in 2.98 and 6.13 M HCl, and 234 nm in 8.75 M HCl. The $\varepsilon_{230}$ values at each acid concentration are reported in Table 3.5. The same was process was preformed using 2, the $\lambda_{\text{max}}$ in 0.175 M HCl was 208 nm, in 1.05 M HCl it was 210 nm, and in 2.98, 6.13 and 8.75 M HCl the $\lambda_{\text{max}}$ was 212 nm. In all cases 230 nm is on a shoulder of the absorbance peak for this product. The $\varepsilon_{230}$ of 2 are reported in Table 3.5. From the $\varepsilon_{230}$ of compounds 3 and 2, the theoretical $A_\infty$ at each acid concentration were calculated, and are presented in Table 3.5. In all kinetic runs the $A_\infty$ used is within 5% of this value, except for runs using 0.175 M and 8.75 M HCl in which the actual $A_\infty$ was higher. However, in all cases the actual $A_\infty$ values from repetitive runs agree well (<5% deviation).

**Table 3.5.** Molar absorptivity values for 3 and 2 under varying acid concentration

<table>
<thead>
<tr>
<th>[HCl] (M)</th>
<th>$\varepsilon_{230}$ of 3 (x 10$^3$ cm$^{-1}$ M$^{-1}$)</th>
<th>$\varepsilon_{230}$ of 2 (x 10$^3$ cm$^{-1}$ M$^{-1}$)</th>
<th>Theoretical $A_\infty$</th>
<th>Actual $A_\infty$</th>
<th>% Deviation$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.175</td>
<td>10.2</td>
<td>1.46</td>
<td>1.02</td>
<td>1.19</td>
<td>15</td>
</tr>
<tr>
<td>1.05</td>
<td>10.2</td>
<td>1.24</td>
<td>1.00</td>
<td>1.01</td>
<td>1.0</td>
</tr>
<tr>
<td>2.98</td>
<td>10.1</td>
<td>1.02</td>
<td>0.97</td>
<td>0.95</td>
<td>2.0</td>
</tr>
<tr>
<td>6.13</td>
<td>9.38</td>
<td>0.654</td>
<td>0.88</td>
<td>0.91</td>
<td>3.4</td>
</tr>
<tr>
<td>8.75</td>
<td>9.10</td>
<td>0.261</td>
<td>0.82</td>
<td>0.88</td>
<td>7.1</td>
</tr>
</tbody>
</table>

$^a$See Table 3.4, footnote b.
3.2.4. Kinetic studies of the acid catalysed aqueous hydrolysis of diazinon

The acid catalysed aqueous hydrolysis of diazinon, according to Scheme 3.3, previously shown, was studied spectrophotometrically, by following the formation of products 3 and 2. The reaction was followed at three temperatures, 25, 35 and 45°C (but only at 25°C for runs using 2.60 x 10⁻³ M acid). In all cases the concentration of hydrochloric acid was present in at least a 28-fold excess over diazinon (8.73 x 10⁻⁵ M) to ensure that pseudo-first order conditions were met. The reaction was followed to ten half lives (ca. 99.9% complete reaction) and the rate constants determined using data from the first three half lives. The determination of the pseudo-first order rate constant was the same as for base catalysed aqueous hydrolysis, for which a representative run is shown in Appendix A. From the repetitive scans of the reaction at the five highest acid concentrations it can be seen that there are no clean isosbestic points, albeit scans at 0.175 M and 8.75 M are much cleaner than the other three concentrations. Another feature these plots reveal is that at 2.98 M and 6.13 M the absorbance decreases for the first few points rather than increasing. When the kinetic data were treated at these concentrations the initial decrease was omitted when determining the rate constant. Data from these kinetic experiments are presented in Tables 3.6, 3.7, 3.8 and 3.9.

The dependance of the rate on the concentration of hydrochloric acid under varying temperatures is shown in Figure 3.21. From Figure 3.21 several important points may be noted. First, as expected, at all acid concentrations as the temperature increases, so does the rate of reaction. Secondly, unlike NaOH, there is no linear relationship between rate and acid concentration. In acid, the rate increases with acid concentration.
until it reaches an apparent maximum at ~0.2 M HCl, then the rate decreases until ~6 M HCl. Finally, there is an increase in rate seen from 6-9 M HCl.

Table 3.6. Pseudo-first order rate constants (k_{obs}) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying concentration of hydrochloric acid at 25°C.

<table>
<thead>
<tr>
<th>[HCl]_o (M)</th>
<th>H_o^a</th>
<th>k_{obs} ( x 10^{-5} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.175</td>
<td>0.0543</td>
<td>13.2 ± 0.050^b</td>
</tr>
<tr>
<td>1.05</td>
<td>-0.282</td>
<td>11.0 ± 0.250</td>
</tr>
<tr>
<td>2.98</td>
<td>-1.02</td>
<td>3.06 ± 0.176</td>
</tr>
<tr>
<td>6.13</td>
<td>-2.23</td>
<td>1.52 ± 0.010</td>
</tr>
<tr>
<td>8.75</td>
<td>-3.24</td>
<td>4.17 ± 0.000</td>
</tr>
</tbody>
</table>

^a values for H_o taken from C.H. Rochester^63
^b see footnote in Table 3.1

Table 3.7. Pseudo-first order rate constants (k_{obs}) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying concentration of hydrochloric acid at 35°C.

<table>
<thead>
<tr>
<th>[HCl]_o (M)</th>
<th>H_o^a</th>
<th>k_{obs} ( x 10^{-4} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.175</td>
<td>0.0543</td>
<td>3.16 ± 0.025^b</td>
</tr>
<tr>
<td>1.05</td>
<td>-0.282</td>
<td>2.35 ± 0.055</td>
</tr>
<tr>
<td>2.98</td>
<td>-1.02</td>
<td>0.809 ± 0.0009</td>
</tr>
<tr>
<td>6.13</td>
<td>-2.23</td>
<td>0.416 ± 0.001</td>
</tr>
<tr>
<td>8.75</td>
<td>-3.24</td>
<td>1.08 ± 0.070</td>
</tr>
</tbody>
</table>

^a see footnote in Table 3.6
^b see footnote in Table 3.1
Table 3.8. Pseudo-first order rate constants ($k_{\text{obs}}$) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of varying concentration of hydrochloric acid at 45°C.

<table>
<thead>
<tr>
<th>[HCl]₀ (M)</th>
<th>$H'_0$</th>
<th>$k_{\text{obs}}$ (x 10^{-4} s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.175</td>
<td>0.0543</td>
<td>7.55 ± 0.125b</td>
</tr>
<tr>
<td>1.05</td>
<td>-0.282</td>
<td>4.85 ± 0.080</td>
</tr>
<tr>
<td>2.98</td>
<td>-1.02</td>
<td>2.09 ± 0.045</td>
</tr>
<tr>
<td>6.13</td>
<td>-2.23</td>
<td>1.06 ± 0.035</td>
</tr>
<tr>
<td>8.75</td>
<td>-3.24</td>
<td>2.88 ± 0.050</td>
</tr>
</tbody>
</table>

* see footnote in Table 3.6
b see footnote in Table 3.1

Table 3.9. Pseudo-first order rate constants ($k_{\text{obs}}$) for the hydrolysis of diazinon (8.73 x 10^{-5} M) in the presence of 2.60 x 10^{-3} M HCl at 25°C.

<table>
<thead>
<tr>
<th>Trial</th>
<th>$k_{\text{obs}}$ (x 10^{-5} s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.97</td>
</tr>
<tr>
<td>2</td>
<td>6.24</td>
</tr>
<tr>
<td>3</td>
<td>6.01</td>
</tr>
</tbody>
</table>

$k_{\text{obs}} = (6.07 ± 0.15)^a x 10^{-5}$ s⁻¹

*Error limit is the standard deviation of three trials.
**Figure 3.16.** Repetitive scans of the acid catalysed (0.175 M HCl) aqueous hydrolysis of diazinon (scans taken every 10 min for first 100 min then every 130 min).

**Figure 3.17.** Repetitive scans of the acid catalysed (1.05 M HCl) aqueous hydrolysis of diazinon (scans taken every 10 min for first 100 min then every 130 min)
**Figure 3.18.** Repetitive scans of the acid catalysed (2.98 M HCl) aqueous hydrolysis of diazinon (scans taken every 10 min for first 100 min then every 130 min).

**Figure 3.19.** Repetitive scans of the acid catalysed (6.13 M HCl) aqueous hydrolysis of diazinon (scans taken every 10 min for first 100 min then every 130 min).
Figure 3.20. Repetitive scans of the acid catalysed (8.75 M HCl) aqueous hydrolysis of diazinon (scans taken every 10 min for first 100 min then every 130 min).
Figure 3.21. The dependance of $k_{obs}$ on [HCl], for the acid catalysed aqueous hydrolysis of diazinon at three temperatures. The data can be found in tables 3.6, 3.7 and 3.8.
3.2.5. Activation parameters

The hydrolysis reaction carried out in the presence of HCl was studied at three temperatures: 25, 35 and 45°C. As there was no linear relationship between $k_{\text{obs}}$ and [HCl], second order rate constants ($k_2$) from these three temperatures could not be used to determine the activation parameters of the reaction, as was done for NaOH. Instead $k_{\text{obs}}$ values from each acid concentration were used. The analysis was the same as for NaOH, except in all equations used $k_{\text{obs}}$ values were used in place of $k_2$ values. An Arrhenius plot and an Eyring plot were used to calculate $E_a$, log A, $\Delta H^*$ and $\Delta S^*$ values (see Section 3.1.4). These plots are shown in Figures 3.22 and 3.23. The $\Delta G^*$ value was calculated using the relationship seen in Equation 3.6. The activation parameter results, with standard error, are presented in Table 3.10.

**Table 3.10. Summary of activation parameters for the hydrolysis of diazinon in the presence of HCl.**

<table>
<thead>
<tr>
<th>[HCl] (M)</th>
<th>0.175</th>
<th>1.05</th>
<th>2.98</th>
<th>6.13</th>
<th>8.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kJ mol$^{-1}$</td>
<td>68.7 ± 1.2</td>
<td>58.5 ± 0.3</td>
<td>75.7 ± 0.9</td>
<td>76.5 ± 0.2</td>
<td>76.1 ± 2.1</td>
</tr>
<tr>
<td>kcal mol$^{-1}$</td>
<td>16.4 ± 0.3</td>
<td>14.0 ± 0.1</td>
<td>18.1 ± 0.2</td>
<td>18.3 ± 0.1</td>
<td>18.2 ± 0.5</td>
</tr>
<tr>
<td>$\Delta H^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kJ mol$^{-1}$</td>
<td>66.1 ± 1.2</td>
<td>55.9 ± 0.3</td>
<td>73.1 ± 0.9</td>
<td>74.0 ± 0.2</td>
<td>73.5 ± 2.1</td>
</tr>
<tr>
<td>kcal mol$^{-1}$</td>
<td>15.8 ± 0.3</td>
<td>13.4 ± 0.1</td>
<td>17.5 ± 0.2</td>
<td>17.7 ± 0.1</td>
<td>17.6 ± 0.5</td>
</tr>
<tr>
<td>$\Delta S^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J mol$^{-1}$ K$^{-1}$</td>
<td>-97.8 ± 3.9</td>
<td>-133 ± 1.9</td>
<td>-86.1 ± 2.2</td>
<td>-88.6 ± 0.6</td>
<td>-82.0 ± 4.8</td>
</tr>
<tr>
<td>cal mol$^{-1}$ K$^{-1}$</td>
<td>-23.4 ± 0.9</td>
<td>-31.8 ± 0.5</td>
<td>-20.6 ± 0.5</td>
<td>-21.2 ± 0.1</td>
<td>-19.6 ± 1.1</td>
</tr>
<tr>
<td>$\Delta G^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kJ mol$^{-1}$</td>
<td>95.2 ± 5.5</td>
<td>95.5 ± 1.9</td>
<td>98.8 ± 3.7</td>
<td>100 ± 0.9</td>
<td>97.9 ± 8.5</td>
</tr>
<tr>
<td>kcal mol$^{-1}$</td>
<td>22.8 ± 1.3</td>
<td>22.8 ± 0.5</td>
<td>23.6 ± 0.9</td>
<td>23.9 ± 0.2</td>
<td>23.4 ± 2.0</td>
</tr>
<tr>
<td>log A</td>
<td>8.15 ± 0.2</td>
<td>6.28 ± 0.1</td>
<td>8.75 ± 0.2</td>
<td>8.59 ± 0.1</td>
<td>8.95 ± 0.4</td>
</tr>
</tbody>
</table>
Figure 3.22. Arrhenius plot for acid catalysed aqueous hydrolysis of diazinon.
Figure 3.23. Erying plot of acid catalysed aqueous hydrolysis of diazinon.
3.3. Effect of humic acid on alkaline aqueous hydrolysis of diazinon

The effect of an environmental constituent, humic acid, on the aqueous hydrolysis of diazinon was determined under alkaline conditions. The reaction was studied spectrophotometrically, by following the formation of products, 2-isopropyl-6-methylpyrimidin-4-ol and diethyl phosphorothioic acid (as described in Section 2.3.2). In all cases the [NaOH] was present in at least a 100-fold excess over diazinon (8.73 x 10^{-5} M) to ensure that pseudo-first order contains were met. The reaction followed is outlined in Scheme 3.1. The reaction was followed to ten half lives (ca. 99.9% complete reaction) and the rate constants determined using data from the first three half lives. The determination of the pseudo-first order rate constant was the same as for base catalysed aqueous hydrolysis, for which a representative run is shown in Appendix A. Data from these kinetic experiments are presented in Table 3.11. The dependence of the rate on the [NaOH] in the presence of varying amounts of humic acid (0, 24 and 48 ppm) is shown in Figure 3.24. From Figure 3.24 the rate increases linearly, with [NaOH] even in the presence of humic acid. However, the rate decreases with increasing humic acid concentration, a 10% rate decrease from 0 ppm to 24 ppm humic acid and a 16% rate decrease from 0 ppm to 48 ppm.
Table 3.11. Pseudo-first order rate constants (k_{obs}) for the base catalyzed hydrolysis of diazinon (8.73 \times 10^{-3} \text{ M}) in the presence of humic acid at 25^\circ C.

<table>
<thead>
<tr>
<th>[NaOH]_o (M)</th>
<th>[Humic acid] (ppm)</th>
<th>k_{obs} (x 10^{-4} \text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.505</td>
<td>0</td>
<td>1.552</td>
</tr>
<tr>
<td>0.322</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>0.101</td>
<td>0</td>
<td>3.23</td>
</tr>
<tr>
<td>0.0303</td>
<td>0</td>
<td>0.922</td>
</tr>
<tr>
<td>0.0101</td>
<td>0</td>
<td>0.350</td>
</tr>
</tbody>
</table>

k_2 = 3.01 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}

<table>
<thead>
<tr>
<th>[NaOH]_o (M)</th>
<th>[Humic acid] (ppm)</th>
<th>k_{obs} (x 10^{-4} \text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.504</td>
<td>24</td>
<td>1.38</td>
</tr>
<tr>
<td>0.321</td>
<td>25</td>
<td>8.87</td>
</tr>
<tr>
<td>0.101</td>
<td>24</td>
<td>3.04</td>
</tr>
<tr>
<td>0.0300</td>
<td>24</td>
<td>0.876</td>
</tr>
<tr>
<td>9.92 \times 10^{-3}</td>
<td>22</td>
<td>0.320</td>
</tr>
</tbody>
</table>

k_2 = 2.72 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}

<table>
<thead>
<tr>
<th>[NaOH]_o (M)</th>
<th>[Humic acid] (ppm)</th>
<th>k_{obs} (x 10^{-4} \text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.503</td>
<td>48</td>
<td>1.28</td>
</tr>
<tr>
<td>0.320</td>
<td>50</td>
<td>8.43</td>
</tr>
<tr>
<td>0.100</td>
<td>48</td>
<td>2.67</td>
</tr>
<tr>
<td>0.0298</td>
<td>48</td>
<td>0.712</td>
</tr>
<tr>
<td>9.81 \times 10^{-3}</td>
<td>45</td>
<td>0.306</td>
</tr>
</tbody>
</table>

k_2 = 2.55 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}
Figure 3.24. The dependance of $k_{obs}$ on $[\text{NaOH}]_0$ for the base catalysed aqueous hydrolysis of diazinon in the presence of varying amounts of humic acid. The data can be found in table 3.11.
4. DISCUSSION

4.1. Analysis of reaction product 2-isopropyl-6-methylpyrimidin-4-ol

The UV/VIS behavior of 2-isopropyl-6-methylpyrimidin-4-ol (3), a product of diazinon hydrolysis, was studied under alkaline, neutral and acidic conditions. The spectra compare well with other data on similar compounds, such as 6-methylpyrimidin-4-ol and pyrimidin-4-ol. Table 4.1 details their similarities.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pyrimidin-4-ol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>6-methyl pyrimidin-4-ol&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK&lt;sub&gt;a1&lt;/sub&gt;</td>
<td>8.59</td>
<td>9.00</td>
<td>9.3</td>
</tr>
<tr>
<td>pK&lt;sub&gt;a2&lt;/sub&gt;</td>
<td>1.85</td>
<td>2.15</td>
<td>2.7</td>
</tr>
<tr>
<td>λ (ε)&lt;sup&gt;c&lt;/sup&gt; anion</td>
<td>229 (11,750)</td>
<td>230 (10,720)</td>
<td>229 (9,530)</td>
</tr>
<tr>
<td>λ (ε)&lt;sup&gt;c&lt;/sup&gt; cation</td>
<td>225(10,000)</td>
<td>229 (10,470)</td>
<td>230 (10,100)</td>
</tr>
<tr>
<td>λ(ε)&lt;sup&gt;c&lt;/sup&gt; neutral</td>
<td>225(6,760)</td>
<td>228 (7,244)</td>
<td>230 (7,113)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from reference 61  
<sup>b</sup>Data from reference 62  
<sup>c</sup>λ is in nm and ε is in cm L mol<sup>-1</sup>

The λ reported in Table 4.1. is characteristic of an aromatic system, resulting from an electronic transition of an electron from the highest energy occupied π orbital to the lowest energy empty π orbital, or a π −π* transition, as seen in benzene (at 250nm)<sup>64</sup>. The spectra also show a weaker peak at ~260 nm which has been ascribed to the electronic transition of a non-bonding lone-pair of electrons on the nitrogen to an empty π orbital of the aromatic system, or a n−π* transition<sup>64</sup>.

Pyrimidinol compounds do not exist solely as phenols and phenoxides in solution,
but, instead undergo a tautomeric equilibrium between carbonyl and enol forms. The various possible forms (and resonance structures) of pyrimidin-4-ol compounds are shown in Figure 4.1.

![Diagram of tautomeric equilibrium between carbonyl and enol forms of pyrimidin-4-ol compounds.](image)

**Figure 4.1.** Possible species of pyrimidin-4-ol based on pKₐ and tautomeric equilibria

The dominant structure present in solution can be determined through UV/VIS spectrophotometry. The way in which it is determined is through comparing the spectrum
of the hydroxy containing pyrimidinol to the UV/VIS spectra of a similar compound in which the equilibrium position is "locked" into one species. For example, to determine if the dominant form of a pyrimidin-4-ol is the phenoxide or carbonyl form, in alkaline solution, one would compare the spectrum of the pyrimidin-4-ol to that of its methoxy derivative. If the two spectra are similar then the dominant form is the phenoxide form; however, if they do not compare well, then the dominant form must be the carbonyl. This type of study has been performed on pyrimidin-4-ol\textsuperscript{61,64}, pyrimidin-2-ol\textsuperscript{61}, 6-methylpyrimidin-4-ol\textsuperscript{62} and 4,6-dimethylpyrimidin-2-ol\textsuperscript{62}. To determine the dominant form in alkaline and neutral solution the pyrimidinol spectra, under both conditions, were compared to their methoxy counterparts, revealing that the carbonyl form is preferred. To determine the dominant form under acidic conditions the spectrum of the pyrimidinol under study must be compared to its N-methylated and methoxy derivatives. If the spectrum compares well to that of the N-methylated derivative, and not the methoxy derivative, then it is the nitrogen which becomes protonated under acidic conditions, leaving the oxygen in its carbonyl form. This type of study has revealed that 4,6-dimethyl pyrimidin-2-ol\textsuperscript{62} and pyrimidin-4-ol\textsuperscript{64} are protonated via the nitrogen with the carbonyl form of the oxygen remaining under acidic conditions. Figure 4.2 shows the dominant pyrimidinol species under alkaline, neutral and acidic conditions. In the spectrophotometric scans (see Results section for spectra) an increase was seen at pH~3.2, this is believed to be due to the change of the dominant species from the acidic form to the neutral form. The second change, seen from pH 3.5 to pH 9.5, is due to the dominance of the basic form of \textit{3}. 
Figure 4.2. Dominant species of pyrimidin-4-ol

Infrared spectrometry (IR) has also been used to determine which species dominates pyrimidinol compounds. The IR of 4-methoxy- and 2-methoxypyrimidines do not show a carbonyl peak, however, the IR of pyrimidin-4-ol and pyrimidin-2-ol show a peak between 1600-1700 cm\(^{-1}\), characteristic of a carbonyl absorption\(^6\). An IR spectrum was obtained for the pyrimidin-4-ol used in this study, which also showed a characteristic carbonyl peak (1697 cm\(^{-1}\)).

It has been established that pyrimidin-4-ol compounds exist primarily as carbonyl and not phenolic compounds in solution. The system presented here is not simple as there exists equilibrium based on protonation (pK\(_a\)) as well as tautomeric equilibria. For
further detail on such systems the readers are directed to reference 65. Even though 3 will predominate as a carbonyl, for simplicity 3 will be shown as a phenolic compound in the remainder of this thesis.

4.2. Possible reaction pathways for the aqueous hydrolysis of diazinon

There are three possible pathways that the aqueous hydrolysis of diazinon could conceivably follow. These are a nucleophilic substitution at phosphorus (SN2(P)), a nucleophilic substitution at an aliphatic carbon (SN2(C)) or a nucleophilic substitution at the aromatic carbon (SNAr). The three possibilities are illustrated in scheme 4.1.

Scheme 4.1. Possible reaction pathways for the aqueous hydrolysis of diazinon

In this work the aqueous hydrolysis of diazinon was studied under basic and acidic conditions. The mechanism followed is not the same under these two different conditions. Further sections analyse basic and acidic conditions separately.
4.2.1. pH-rate profile for the aqueous hydrolysis of diazinon

The results of this work in acidic and basic media, along with data from literature, presented in Table 4.2, have been used to produce a pH rate profile for the aqueous hydrolysis of diazinon.

<table>
<thead>
<tr>
<th>pH</th>
<th>$k_{obs} \times 10^{-7} \text{s}^{-1}$</th>
<th>$t_{1/2}$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>25.5</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>5.73</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>1.47</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>1.15</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>1.49</td>
<td>54</td>
</tr>
</tbody>
</table>

$^a$Data from reference 36

The rate profile can be seen in Figure 4.3. The rate profile reported here agrees with the shape of the rate profile developed over the range of pH 2 to 9 by Ku et al. The rate profile reveals that, depending on pH, different mechanisms are at work. There are several distinct areas to the pH rate profile; the basic region (pH 12-14), the neutral or environmental region (pH 5-8), and the acidic region (pH 3 to $H_o$ -3). The acidic region itself shows three different mechanistic regimes, an increase from pH 2.6 to $H_o$ 0.05 (0.175 M HCl), a decrease to $H_o$ -2.2 (6.13 M HCl) and an increase to $H_o$ -3.2 (8.75 M HCl). The Hammett acidity function, $H_o$, was used in the strong acid region of the pH-rate profile as it relates to the acidity of these solutions better than pH. This is because in very acidic solutions the acidity is no longer related to the $[H_3O^+]$, but rather to...
the solution's ability to donate protons\textsuperscript{66}. There are other acidity functions, such as $X$, the Cox and Yates excess acidity, which have come into use in recent years\textsuperscript{66}. Different mechanistic characteristics revealed by the pH-rate profile will be further analysed in the following sections on basic, acidic and neutral hydrolysis.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure43}
\caption{pH rate-profile of the aqueous hydrolysis of diazinon (Literature data from reference 36)}
\end{figure}

4.2.2. Basic conditions

In this study the aqueous hydrolysis of diazinon was studied at 5 different
concentrations of sodium hydroxide ranging from 0.0115 M to 0.543 M (pH 12.1 to 13.7). The reaction was followed by UV/VIS spectrophotometry and any phosphorus-containing products were determined by $^{31}$P NMR. Under basic conditions only one phosphorus containing product was observed, which corresponded to a standard of O,O-diethyl thiophosphate. Thus, an $S_N2$ reaction at the aliphatic carbon was not observed, as no de-ethylated diazinon was seen in the $^{31}$P NMR spectrum, which would have been located upfield relative to diazinon.

4.2.2.1. Position of bond cleavage

C-O bond fission has been reported by Greenhalgh et al.$^{67}$ in an organophosphorus compound, fenitrothion (8). But, this was only seen at a pH $\leq$ 7.5, and only P-O bond fission was seen at a pH greater than 9. Buncel and co-workers have found that reaction was solely by attack at phosphorous for the reaction of p-nitrophenyl diphenylphosphinate (9) with alkali-metal ethoxides.$^{50,68,69,70,71,72}$ However, in a recent study Buncel and co-workers found that 8 undergoes all three possible mechanisms, $S_N2$ (P), $S_N2$ (C) and $S_NAr$ (minor pathway $\leq$ 7%) when reacted with alkali-metal ethoxides.$^{58}$

Several studies have been undertaken using 18-oxygen to determine the position of bond cleavage in the alkaline hydrolysis of organophosphorus compounds. Blumenthal and Herbert carried out oxygen-18 studies on the hydrolysis of trimethyl orthophosphate (10)$^{73}$. They found that under alkaline conditions bond cleavage was exclusively P-O. With a similar compound, triphenylphosphate (11), the same result was found.$^{74}$ Cook et al.$^{75}$ also found that this was the case, through oxygen-18 experiments, for several phosphinates, such as O-ethyldiphenylphosphinate (12) and O-isopropyl
diphenylphosphinate (13). In another 18-oxygen study, it was found that O-methyl
diisopropylphosphinate (14) underwent alkaline hydrolysis with 25% C-O bond cleavage
and the remaining 75% being P-O bond cleavage\textsuperscript{76}.

![Chemical structures](image)

Although the majority of literature studies find alkaline hydrolysis is attained
through a $S_{N2}(P)$ process, there have been reports of the reaction following a $S_{N1}(P)$
mechanism. Haake and Ossip found that strongly hindered phosphinyl chlorides, such as
di-t-butylphosphinyl chloride (15), undergo a $S_{N1}(P)$ process\textsuperscript{77}. They note that the
$S_{N2}(P)$ process is highly favoured, as the slightly less hindered diisopropylphosphinyl
chloride (16) does not follow the $S_{N1}(P)$ process, but undergoes a $S_{N2}(P)$ process. It has
also been found that with sterically hindered phosphate triesters, such as tri-t-butyl
phosphate (17) the mechanism of alkaline hydrolysis follows an $S_{N1}(C)$ process, with a
carbocation as an intermediate\textsuperscript{78}. 
As mentioned in the introduction, there has been much debate over whether the mechanism, with attack at phosphorous, involves direct displacement, resulting in a trigonal bipyramidal transition state, or if the mechanism involves a true pentacoordinate, trigonal bipyramidal intermediate. There has been evidence for the existence of a pentacoordinate intermediate. Cook and Rahhal-Arabi found that the rate of alkaline hydrolysis of a series of substituted aryl diphenylphosphinothioates (18) correlated with Hammett’s σ values and not Hammett’s σ⁻ values, supporting the existence of a pentacoordinate intermediate, and thus an addition-elimination type reaction. Buncel and co-workers have found that the reaction of substituted aryl diphenylphosphinates (19) with phenoxides and ethoxides do correlate with σ and σ⁰, also supporting the presence of a pentacoordinate intermediate. The main reasoning used against the existence of the pentacoordinate intermediate is that when 18-oxygen studies are carried out there should be an 18-oxygen exchange, allowing for 2 atoms of 18-oxygen to be incorporated into some of the final product as illustrated in Scheme 4.2.
\[
\begin{align*}
R_X^\| P-LG + ^{18}OH^- & \rightleftharpoons H^{18}O^\| P-LG \\
& \rightarrow R_X^\| P-^{18}OH^- + LG^- \\
\end{align*}
\]

Where \( X = S \) or \( O \)

\[
\begin{align*}
^{18}O \quad R_X^\| P-LG + XH^- & \rightleftharpoons XH^\| P-LG \\
& \rightleftharpoons ^{18}O \quad R_X^\| P-LG \\
\end{align*}
\]

\( LG = \text{leaving group} \)

\[
\begin{align*}
^{18}O^- \quad H^{18}O^\| P-LG & \rightarrow R_X^\| P-^{18}OH^- + LG^- \\
\end{align*}
\]

**Scheme 4.2.** \(^{18}O\) exchange during alkaline hydrolysis of phosphorus triesters

It has been determined for both 10 and 11 that no 18-oxygen exchange takes place, going against the existence of the pentacoordinate intermediate\(^7\). However, this fact alone does not rule out a pentacoordinate intermediate as it could be a very short lived species, decomposing rapidly, not allowing enough time for the 18-oxygen exchange.

![Chemical structures](image)
4.2.2.2. Mechanism of alkaline hydrolysis of diazinon

The products formed through the alkaline hydrolysis of diazinon could be produced from either the $S_{N2}$ (P) or the $S_{N}A r$ pathway. Although no experiments were carried out in this work to further differentiate the two pathways (i.e. $^{18}$O studies), the literature points toward the $S_{N2}$ (P) pathway. In the reported $^{18}$-oxygen experiments that follow the alkaline aqueous hydrolysis of phosphorus triesters (see section 4.2.2.1) predominately P-O bond fission is observed. It is clear from the kinetic experiments carried out in the present work that the alkaline hydrolysis does not follow an $S_{N1}$ (P) process, as the rate is dependent on the concentration of the nucleophile, OH-, as demonstrated in the second order rate plot that was presented in Figure 3.7. Thus, it is fair to conclude that the most likely pathway is either the $S_{N2}$ (P) pathway, with a trigonal bipyramidal intermediate, or an addition-elimination process involving a true pentacoordinate intermediate. From the $^{31}$P NMR studies there was no oxygen exchange occurring, as no P=O compounds were detected in the spectra, which may suggest the reaction does not proceed through a pentacoordinate intermediate. However, as mentioned previously, this must be considered with some caution as it does not exclude the pentacoordinate intermediate (i.e. short lived species). Also, if the concentrations of any P=O compounds were small, they may have been below the limit of detection by NMR. In either case the reaction can be illustrated as in Scheme 4.3.
4.2.3. Acidic conditions

The aqueous hydrolysis of diazinon was studied over a range of six different acid concentrations. The products were studied by $^{31}$P NMR at two different acid concentrations. Both revealed only one phosphorus-containing product, O,O-diethylphosphothioic acid. This shows that there was no conversion to a P=O compound under the conditions studied, and furthermore that there was no $S_N2$ (C) reaction taking place. The product could have been formed from either the $S_N2$ (P) or $S_NAr$ process. In the literature there have been reports of all three processes occurring with various phosphorus esters which are discussed in the following sections.

4.2.3.1. Cleavage of the C-O bond

Several organophosphorus esters have been reported to undergo an $S_N2$ (C) type process. For example, dimethyl phosphate anion (20) was found to undergo 78% O-C bond cleavage in 0.0645 M HCl$^{81}$. However, in 5 M HClO$_4$, the hydrolysis reaction was...
found to occur with 86% O-C bond cleavage. The change in the proportion of O-C bond cleavage was explained by the form of the substrate in each medium. In 0.0645 M HCl the phosphorus ester was present as a neutral species, whereas in 5 M HClO₄ the substrate was protonated, and possessed a positive charge. The acid hydrolysis of 10 has also been investigated using ¹⁸O experiments. It was found that in 1 M HCl there was predominately C-O bond cleavage (70%)[73]. In a separate experiment, it was found that 10 in 0.1 M or 1.0 M HClO₄ solutions undergoes 100% cleavage of the C-O bond[74]. However, with this compound no acid catalysis was seen going from neutral water to 3 M HClO₄. The hydrolysis of methyl methylarylphosphinates (21) in 1.0 M HClO₄ has been found to be predominately by C-O cleavage (90%)[82]. Mhala and Kiledar have studied the hydrolysis of tri-p-iodo benzyl phosphate (22) in the range of 0.5 M to 4.5 M HCl[83]. They saw a rapid increase in rate with increasing acid concentration, and proposed C-O bond cleavage. Correlation with Bunnett parameters indicate a unimolecular reaction (w = 0.83). Furthermore, log₁₀ kₘₐₗₐₜₐ correlated with -Hₒ and not log₁₀ Cₙ⁺, lending more support to a unimolecular reaction. The authors proposed that protonation of the oxygen (ether linkage) attached to phosphorus was followed by O-C bond cleavage to produce a carbocation. The final step is the attack of water on the carbocation. This reaction is best termed an A₁ process, using ester hydrolysis terminology[66]. Using ¹⁸O experiments the hydrolysis of triethylphosphate (23) in acidic media showed complete C-O bond cleavage in 1.02 M HClO₄[84]. It was suggested that the P=O oxygen becomes protonated, followed by the attack of water on carbon to produce ethanol and diethyl phosphate, following an Aₐₓₐₕ₋₂ (acid catalysed, alkyl-oxygen cleavage, bimolecular) mechanism.
Kirby and Younas have found solely P-O bond cleavage in the hydrolysis of bis-2,4-dinitrodiphenyl phosphate anion (24)\textsuperscript{85,86} and 2,4-dinitrophenyl methyl phosphate anion (25)\textsuperscript{85}. Another \textsuperscript{18}O study using 9 revealed only P-O bond cleavage for hydrolysis in the presence of HClO\textsubscript{4}\textsuperscript{87}. The results showed that the rate did not increase in a simple manner with increasing acid concentration, but instead a plot of rate versus acid concentration was ‘bell-shaped’ showing acid catalysis at lower acid concentration and acid inhibition at higher acid concentration. This type of plot is typical of an A-2 process\textsuperscript{88}, which the authors suggest. The authors do not believe this inhibition to be a result of a decrease in the activity of water, acting solely as a nucleophile, at higher acid concentration, but instead attribute it to the need of water to remove a proton from an attacking water molecule (see Scheme 4.8) or for hydration of the leaving group. In a study on p-nitrophenyl diphenyl phosphate (26), bell shaped curves are found for hydrolysis in several acids, HClO\textsubscript{4}, H\textsubscript{2}SO\textsubscript{4}, and HCl\textsuperscript{89}. The authors note that the maxima
appear above the pKₐ of the substrate, indicating the maxima are not due to complete protonation of the substrate. They believe the rate decrease is due to the fact that the transition state of the reaction needs to be hydrated; thus in higher acid concentration there is relatively less water, destabilizing the transition state, so the rate decrease is not simply due to a decrease in nucleophile. Cook and Metni have studied the acid catalysed hydrolysis of O-methyl dimethylthiophosphinate (27)³⁸,⁹⁰. They also report a bell-shaped curve for a plot of rate versus acid concentration, and propose an A-2 mechanism. They find that when the compound is reacted with strong acid (25-85% D₂SO₄), both P=S and P=O product are obtained. This result suggests that the mechanism followed is attack at phosphorus involving a pentacoordinate intermediate, which would account for the S-O exchange (see Scheme 4.2). The exchange of oxygen with solvent was also seen in labelled diphenyl methyl phosphonate (28)³¹. The authors also put this forth as evidence for the existence of a pentacoordinate intermediate.
4.2.3.3. Mechanism of acidic hydrolysis of diazinon

As seen, hydrolysis of organophosphorus esters in acid medium occurs via a variety of processes. The type of mechanism followed depends on many factors, even showing different mechanisms at different acid concentrations. Thus, at the present it is not totally clear which mechanism is at work in the acid catalysed hydrolysis of diazinon. However, one can speculate about the mechanism taking place through comparison with literature results, analysis of the rate profile, and consideration of the pKₐ values of the products. The structures of the products indicating pKₐ's are shown in figure 4.4.

\[
\begin{align*}
\text{pK}_a &\approx 2.7 & \text{pK}_a &\approx 6^a \\
\text{pK}_a &\approx 9.3 & \text{pK}_a &= 1.83^b \\
\text{pK}_a &\approx 2.4^c & \text{pK}_a &\approx 2.6^d
\end{align*}
\]

Figure 4.4. pKₐ values for diazinon and the products of diazinon hydrolysis

Note: When not referenced, the values are from this work.

\(^a\)Based on the pKₐ of pyrimidin-4-ol \(^b\)From reference 60

\(^c\)From reference 42 \(^d\)From reference 92

The rate of reaction in aqueous acid increases over the range of 0.003 M to 0.2 M HCl, then the rate decreases to a minimum at ~6 M HCl. The rate then increases from 6 M to 9 M HCl. From 0.003 M to 6 M the plot is 'bell shaped' as was seen with other
organophosphorus compounds, previously mentioned, such as p-nitrophenyl diphenyl phosphate. As previously noted there is not a clear-cut reason for this type of plot, though literature suggests an A-2 mechanism which involves water in the rate determining step. The pK\text{a} of the first ring nitrogen is \(-2.6\), which means that at the low end of the acid range (pH = 2.6, 0.003 M HCl) the substrate should be appreciably protonated (\(-50\%\)) on one nitrogen. At the rate maximum (\(-0.175\) M HCl) the nitrogen will be fully protonated. The initial increase could be due to increased concentration of protonated substrate which will enhance the rate of reaction, by activating the molecule towards attack by the presence of a positive charge. The enhancement due to protonation will only be seen until complete protonation. The rate decrease beyond complete protonation could be due to the reduced activity of the nucleophile, water, in the higher acid concentrations, which is no longer counter balanced by increased protonation. This would suggest an A-2 mechanism in which the rate depends on the relative amount of water available for reaction. Some uncertainty remains about this proposed mechanism as reaction could occur at phosphorus, as presented in Scheme 4.4, or by reaction at the aromatic carbon as presented in Scheme 4.5.
Scheme 4.4. A-2 mechanism for acid catalysed aqueous hydrolysis of diazinon with attack at phosphorus ($S_N^2 (P)$).
Scheme 4.5. A-2 mechanism for the acid catalysed aqueous hydrolysis of diazinon with attack at aromatic carbon (S$_{N}$Ar)
Either the $S_N2$ (P) or the $S_NAr$ mechanism could be envisioned as occurring. As mentioned earlier, the acid catalysed hydrolysis of 26 revealed P-O bond cleavage\textsuperscript{89}. With one electron withdrawing group on the aromatic ring, which enhances the ring toward reaction, bond cleavage still took place at phosphorus. As well, Kirby and Yonas observed solely P-O bond cleavage in the hydrolysis of 24 and 25\textsuperscript{85}. Here, even with two electron withdrawing groups to enhance an $S_NAr$ pathway, the reaction proceeded via the $S_N2$ (P) mechanism. Thus, in the present case, with only one nitrogen protonated to enhance the $S_NAr$ pathway, literature results point toward the $S_N2$ (P) mechanism. However, care should be used as hydrolysis was seen to proceed predominately via the $S_NAr$ pathway with 21 which did not contain electron withdrawing substituents on the ring\textsuperscript{82}. Thus, the mechanism could conceivably still be occurring via the $S_NAr$ pathway, although it does appear to be less likely.

The next feature of the rate profile in acid medium is a minimum in rate at ~6 M HCl followed by an increase from 6 M to 9 M HCl. The rate increase indicates that a mechanistic change is taking place in the system. There are many possible processes which could be taking place. For example additional sites for protonation are possible, such as at the sulfur attached to phosphorus. This could enhance the rate by making the phosphorus centre more positive, and hence a better site of attack for the H$_2$O nucleophile, as shown in Scheme 4.6. If this protonation occurs appreciably in very acidic solution (9 M) the improved nucleophilicity of the phosphorus centre could be responsible for the rate increase seen at 9 M HCl.
Scheme 4.6. Mechanism for the acid catalysed aqueous hydrolysis of diazinon for attack at phosphorus with protonation at sulfur.

Even though it seems most plausible that, with protonation at sulfur, attack by $\text{H}_2\text{O}$ will be at phosphorus, it is possible that attack could occur at carbon. Lyznicki et al. found $\text{O-C}$ cleavage of triethyl phosphate and proposed protonation of the $\text{P=O}$ oxygen\textsuperscript{84}. Thus, even with the apparently more favourable positive phosphorus centre (due to protonation of $\text{P=O}$), attack could still be at carbon. However, care must be taken as in our study
attack at carbon would be an $S_{N}Ar$ reaction whereas for Lyznicki and co-workers attack at carbon is a $S_{N}2(C)$ process. One possible reason why attack was seen at carbon rather than at phosphorus in Lyznicki and co-worker's case is that $H_2O$ prefers attack at aliphatic carbon sites over phosphorus sites, resulting in the $S_{N}2(C)$ mechanism dominating. This is evident when comparing the reactivity of water to hydroxide. For attack at a saturated carbon, $OH^-$ is a better nucleophile than $H_2O$ by a factor of 10,000; however, for reactivity at phosphorus, the factor is much greater, $10^8$, showing that water prefers saturated carbon over phosphorus$^{74,93}$.

There is still another possible protonation, that of the phenoxy oxygen, that could be behind the rate increase from 6 M to 9 M HCl. This would lead to enhancement of either the $S_{N}Ar$ or the $S_{N}2(P)$ mechanism as shown in Scheme 4.7, since protonation at this position makes the leaving group better in either case.

![Scheme 4.7. Protonation of oxygen causing enhancement of either an $S_{N}2(P)$ or $S_{N}Ar$ process.](image)

A final possible protonation would be the second ring nitrogen. This would further enhance the effect of the first ring protonation, which could also explain the rate increase. The rate increase could also be due to a mechanism change. For example, with only one ring nitrogen protonated, the ring may not be activated enough for the reaction
to follow an $S_N$Ar mechanism. However, with two nitrogens protonated, the ring may now be sufficiently activated to cause reaction to occur by the $S_N$Ar pathway. A switch to an $S_N$Ar mechanism may also be viable as pyrimidine rings are in general more susceptible to aromatic attack versus benzene as the nitrogens, even without protonation, can act as electron withdrawing groups$^{64}$. One must also consider the possibility that the reaction may not be purely one or the other, but rather that the ratio of $S_N2$ (P) to $S_N$Ar changes with varying HCl concentration.

4.2.3. Neutral conditions

From the pH rate profile of diazinon it can be seen that the rate of hydrolysis is slowest under environmental conditions (pH 5-8). This behaviour is also seen in many other organophosphorus compounds. Compound 10, even at 100°C, has a half-life of 5.3 hours$^{64}$. Compound 22 is even more persistent with a half life of 21.2 hours, at 100°C$^{94}$. At a more environmentally normal temperature, 37°C, diethyl 4-nitrophenyl-phosphorothioate (29) has a very long half-life of 1.9 years$^{95}$. It has been determined that trialkyl phosphates, such as 10 and 22, normally follow the $S_N2$ (C) mechanism under neutral conditions$^{64}$; thus, as expected $^{18}$O experiments of the neutral hydrolysis of 10 have shown predominately C-O cleavage (70%, 100%)$^{78,79}$. P-O cleavage was observed for 2,4-dinitrophenyloxy-2-oxo-1,3,2-dioxophosphorinane (30) in a 0.05 M acetate buffer (pH 4)$^{96}$. The authors propose that under these conditions 40% reaction is due to water, so that in pure water the reaction should also reveal P-O bond cleavage. It was found that the hydrolysis of 30 under neutral conditions, follows the $S_N2$ (P) mechanism, with general base catalysis by a second molecule of H$_2$O as seen in Scheme 4.8.
Studies have been carried out on the rate of hydrolysis of organophosphorus compounds in the natural environment. One study followed the hydrolysis of several organophosphorus pesticides, including diazinon, in river and well water (pH 8.5 and 8.3, respectively)\textsuperscript{15}. It was found that diazinon had a half-life of 14 days at 21°C in these natural waters. Another study examined the rate of hydrolysis of organophosphorus pesticides in distilled deionized water (pH 6.1), sea water (pH 8.1) and river water (pH 7.3)\textsuperscript{37}. The half-lives of diazinon in these waters at 22°C were found to be 69 days in distilled deionized water, 50 days in sea water and 80 days in river water. The results from these two studies do not seem consistent. The first study may be more accurate as they performed four replicates of each experiment whereas in the second study only a single experiment was done. However, comparing the results is complicated as neither study ensured that the natural water was sterile, thus the differences could be due to biotic
degradation taking place. These results can be compared to those used in the pH rate profile performed in sterile phosphate buffer solutions by Chapman and Cole\textsuperscript{36}. These authors found that the half-life of diazinon at pH 7.0 was 70 days and at pH 8.0 was 54 days at 25±3°C. It is interesting to note that when diazinon is hydrolysed in water (i.e. not a buffer) the reaction is auto catalytic as acid is produced during reaction\textsuperscript{60}. The results of Chapman and Cole agree fairly well with the results of the second study, comparing results from similar pH. Perhaps the river and well water used in the first study contained microorganisms adept at diazinon degradation, or some organic or inorganic compound which can catalyse the rate of hydrolysis. Several dissolved and soil materials have been found to catalyse the hydrolysis of organophosphorus compounds. For example iron oxide\textsuperscript{97}, cobalt\textsuperscript{98} and copper\textsuperscript{99,100,101} have all been found to catalyse the hydrolysis of organophosphorus compounds. The effect of copper on diazinon has been investigated. At pH 4.9 after 48 hours 81% of the initial amount of diazinon in solution remained, however, in the presence of copper (II) sulphate only 6% of the initial amount of diazinon remained\textsuperscript{100}. The catalytic effect of Cu (II) has been explained by the coordination of the pesticide and water to the metal as shown below\textsuperscript{101}.

\begin{center}
\includegraphics{diagram.png}
\end{center}

Perhaps the river and well water contained iron oxide, cobalt or copper or other catalysts,
which would explain the much faster rate of degradation seen in that study.

4.3. Effect of humic acid on the alkaline hydrolysis of diazinon

As previously mentioned, inorganic substances, like metals, are capable of accelerating the rate of hydrolysis. There are also organic material found in soil and water, such as lignin, humin, and fulvic and humic acids that can affect the hydrolysis of pesticides. Other studies of pesticides with various natural organic compounds have been undertaken. Studies have found that the rate of degradation of atrazine (31) is enhanced by organic material such as fulvic and humic acid. In a study on the 1-octyl ester of (2,4-dichlorophenoxy)acetic acid (32) it was found that the rate of alkaline hydrolysis decreased in the presence of humic acid. In this investigation the effect of humic acid on the rate of hydrolysis under basic conditions was studied. The plot shown in Figure 3.22 compares the rate of hydrolysis with no humic acid to the rate in the presence of 24 ppm and 48 ppm humic acid. From this plot it is clear that the presence of humic acid inhibits the hydrolysis of diazinon to a small extent. Humic acids are large macromolecules of varying composition formed from the decomposition of plant and microbial material, with a generic structure as shown below.
From this structure it is clear that humic acids possess hydrophobic areas with which hydrophobic organic compounds could become associated; for diazinon, the possibility of associating with organic matter is reflected in the log value of the octanol water partition coefficient (log $K_{ow}$ = 3.81)$^{106}$. Based on this, a large portion of the diazinon should become associated with the dissolved humic material. The reason for the rate decline is not yet known, it could simply be caused by the humic acid protecting the diazinon from the hydrophilic hydroxide nucleophile. The humic acid has carboxylate groups. Under the conditions of the experiment (alkaline) these groups will be in their ionized form. Alternatively, it is possible that while associated with the humic acid diazinon undergoes general base catalysis by attack of the carboxylate groups of the humic acid. Carboxylate ion is not as strong a nucleophile as hydroxide; thus the rate decreases.
4.4.1. Comparison of $^{31}$P NMR of phosphorus compounds

The chemical shift ($\delta$) of a phosphorus peak in a $^{31}$P NMR is affected by the environment surrounding the phosphorus atom. The $\delta$ value of an atom is affected by the electron density in its environment. If a phosphorus atom has a greater electron density around it, it is said to be shielded and the $\delta$ value will be further upfield. Conversely, if the electron density around the phosphorus atom is reduced, a decrease in shielding, or deshielding, the $\delta$ value will be further downfield. In this work $^{31}$P NMR spectra were obtained for several phosphorus compounds. The chemical shifts of these phosphorus compounds show a variety of $\delta$ values. The $^{31}$P NMR data of the compounds studied in this work as well as compounds studied previously in our laboratory are presented in Table 4.3.
Table 4.3. NMR data from phosphorus containing compounds (solvent CDCl₃)

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>$^{31}$P NMR $\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CH₃CH₂O−P−OCH₂CH₃</td>
<td>68.6</td>
</tr>
<tr>
<td></td>
<td>OCH₂CH₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH₃CH₂O−P−OH</td>
<td>58.4</td>
</tr>
<tr>
<td></td>
<td>OCH₂CH₃</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>CH₃CH₂O−P−O−</td>
<td>61.4</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OCH₂CH₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pirimiphos-methyl</td>
<td>65.2$^{54}$</td>
</tr>
</tbody>
</table>

According to Gorenstein$^{107}$ the $\delta$ value of a phosphorus compound can be affected by many factors, such as σ-bond angles, conformation, resonance stabilization, electronegativity of attached ligands and π-bond overlap. Gorenstein has also found that to clearly see trends in $^{31}$P NMR it is best to compare structurally similar phosphorus compounds. This is why the compounds presented in Table 4.3 have been broken into
two groups, A and B. Of the two compounds in group A, one might be surprised that the compound with the stronger electron withdrawing group (-OH) is further upfield relative to the compound with the weaker electron withdrawing group (-OCH₂CH₃). In ¹H and ¹³C NMR the normal trend is that the electron withdrawing group will remove electron density from around the atom, resulting in deshielding, and thus a downfield shift. It has been found for several series’ of phosphorus compounds, including substituted phenylphosphonic difluorides (33)¹⁰⁸, phenylphosphonic acids (34)¹⁰⁹, α-diethylphosphonocinnamates (35)¹¹⁰, ethyl α-diethylphosphonocinnamates (36)¹¹⁰ and arylphosphonic dichlorides (37)¹¹¹ that there is an inverse chemical shift trend, as also seen in the two compounds in group A in this work. One possible reason for this shielding effect caused by an electron withdrawing group is that the electron density around the phosphorus atom is decreased, which results in an increased favouring of the resonance form B¹¹², seen in Figure 4.5.

![Figure 4.5. Resonance forms of P=S triesters](image)

In other words, the more electron withdrawing substituent makes π bonding between the 3d orbital of phosphorus and 3p orbital of sulfur more favourable, which results in a greater electron density around the phosphorus atom, causing the observed upfield chemical shift. This trend can also be seen when we compare the δ values of 2 in basic
and acidic media. Under basic conditions the compound will be ionized (-O\(^-\)), and the \(\delta\) value should be further upfield than under acidic conditions in which the compound is protonated (-OH). This is seen as under acidic conditions the \(\delta\) value is 62.0 ppm, whereas under basic conditions it is 55.9 ppm.

Another trend is revealed through the compounds in group B. Here with more electron withdrawing groups on the aromatic ring (nitrogens) the \(\delta\) value is further downfield. This effect has been seen in substituted phenyl dimethylphosphinates(38)\(^{112}\), phenyl methylphenylphosphinates(39)\(^{112}\), and phenyl diphenylphosphinates(40)\(^{112, 113}\). This trend is also explained by the stabilization of one resonance form over another\(^{112, 113}\). The two resonance forms are shown in Figure 4.6.

![Resonance forms of P-O-O(phenox)triesters](image)

**Figure 4.6.** Resonance forms of P-O-O(phenox)triesters

By examining Figure 4.6, one can see that electron withdrawing groups on the ring will remove electron density from the oxygen, destabilizing resonance form B, favouring A. In resonance form A, the phosphorus atom is more deshielded and will appear further downfield. The aromatic ring of diazinon contains 2 nitrogens, whereas the aromatic ring of pirimiphos-methyl contains three. Thus, resonance form A is favoured more heavily
in pirimiphos-methyl than in diazinon, resulting in diazinon appearing further upfield in the $^{31}\text{P}$ NMR.

![Chemical structures](image-url)
5. CONCLUSIONS AND FUTURE WORK

5.1. Conclusions

5.1.1. General

The rate of the aqueous hydrolysis of diazinon has been studied under alkaline and aqueous conditions. These data, along with literature results, were used to produce a pH rate-profile for the reaction. This plot showed that the degradation of diazinon is highly dependant on pH, with rate being the slowest under environmental conditions (pH 5-8).

5.1.2. Alkaline conditions

Under alkaline conditions the second order rate constant was found to be $3.06 \times 10^{-3}$ M$^{-1}$s$^{-1}$ at 25°C. $^{31}$P NMR revealed that the reaction produced O,O-diethyl-phosphorotheic acid. This region of the pH-rate profile was a straight line showing specific base catalysis. The reaction mechanism is thought to be a $S_{N}2$ (P) process, and the overall process can be represented as seen in Equation 5.1.

\[
\begin{align*}
\text{N} & - \text{O} - \text{S} - \text{OCH}_2\text{CH}_3 + \cdot \text{OH} & \rightarrow & \text{N} & - \text{O} - \cdot + \cdot \text{O} - \text{S} - \text{OCH}_2\text{CH}_3 \\
\end{align*}
\]

(5.1)

5.1.3. Acidic conditions

The hydrolysis of diazinon under acidic conditions revealed a more complex system than under alkaline conditions. This region of the pH rate-profile showed three distinct behaviors, which are due to the many different possible protonations of diazinon. Based on comparison to reports in literature, the most likely mechanism in the region of
2.60 \times 10^{-3} \text{ to } 0.175 \text{ M HCl} is an \text{S}_2(P) \text{ process which is enhanced by the protonation of one ring nitrogen of diazinon. The decrease seen in the rate-profile above } \sim 2 \text{ M HCl} \text{ is plausibly due to the decrease in the activity of water with increasing acid concentration. The final increase in rate seen from 6.13 \text{ M to } 8.75 \text{ M HCl} \text{ could be due to other possible protonations (e.g. second ring nitrogen, sulfur, or phenoxy oxygen) which would heighten the rate of reaction. This increase may result with a change in mechanism from an \text{S}_2(P) \text{ to an } \text{S}_N\text{Ar process, especially if the second ring nitrogen is protonated as the ring will be more electron deficient.}}}

\text{31P NMR revealed that the same phosphorus containing product that is formed under alkaline conditions is also formed under acidic conditions. The overall process under acidic conditions can be depicted as in Equation 5.2.}

\begin{align*}
\text{HNO}_2 \text{OH} + \text{H}_2\text{O} & \xrightarrow{\text{H}^+} \text{HNO}_2\text{OH} + \text{H}_2\text{O} \\
\text{N} & \text{N} \\
\text{N} & \text{N} \\
\text{OH} & \text{OH} \\
\text{P} & \text{P} \\
\text{OCH}_2\text{CH}_3 & \text{OCH}_2\text{CH}_3 \\
\end{align*}

\text{(5.2)}

\textbf{1.5.3. Humic acid}

\text{It was also determined that the alkaline hydrolysis of diazinon is inhibited in the presence of humic acid. This may be due simply to the humic acid acting to protect the pesticide from the attack of the hydrophilic nucleophile. Alternatively, the inhibition could be caused by general base catalysis taking place by way of the carboxylate groups of the humic acid.}
5.2. Future work

5.2.1. Aqueous hydrolysis

To unambiguously determine the mechanisms taking place under alkaline, neutral and acidic conditions it would be useful to carry out $^{18}$O studies. As the reaction products for attack at carbon or phosphorus are the same, labeled oxygen would enable us to tell them apart. As well the pH rate-profile should be developed further. First the regions of pH 2-4 and 9-11 need to determined using buffer systems (e.g. acetate, phosphate, and carbonate). Also it would be of interest to extend further into the acid region to discover whether the rate continues to increase above 8.75 M HCl.

5.2.2. Environmental studies

As diazinon is used in the natural environment it would be of use to determine the effect of other natural molecules on the rate of hydrolysis. These might include such things as fulvic acid, clay minerals, lignin and metal ions and oxides. Of key interest would be their effect, as well as humic acids effect, on the rate in the pH range of 5-8, the environmental region.

5.2.3. Remediation

As diazinon is a toxic chemical, it is important that there be cheap and effective ways to remediate contaminated soils and water. It has been found the cyclodextrins can affect the rate of the hydrolysis of organophosphorus pesticides. It would be of benefit to see what effect these molecules have on the hydrolysis of diazinon and to perhaps develop new remediation technologies in the form of modified cyclodextrins.
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APPENDIX A: Determination of $k_{\text{obs}}$ for the alkaline hydrolysis of diazinon
APPENDIX A: Determination of $k_{obs}$ for the 
alkaline hydrolysis of diazinon

In this section the determination of $k_{obs}$ from a representative kinetic run is detailed. The reaction is diazinon ($8.73 \times 10^{-5}$ M) in a 0.543 M aqueous solution of NaOH, at 25°C. The reaction was monitored spectrophotometrically at 229 nm, observing the appearance of products. Table A.1 gives the kinetic data for three half-lives (absorbance at given time) used to create Figure A.1, a plot of absorbance versus time.

The value of $A_\infty$ was taken as the maximum, constant, absorbance value reached on Figure A.1. The fact that the absorbance remains essentially constant shows that the reaction has reached completion as no more product is being produced.

Next a plot of $[3 + \log (A_\infty - A_o)]$ versus time, was produced, and is shown in Figure A.2. The factor of three was added for convenience so that the line would have positive y-values, making it conceptually easier to interpret. The slope of this line, under pseudo-first order conditions, is equal to $-(\log_{10} e) k_{obs}$. From the initial observed rate constant a value of $t_{1/2}$ was calculated. The plot of $[3 + \log (A_\infty - A_o)]$ versus time was then redrawn to contain only three half-lives of data based on the $t_{1/2}$, and the rate constant re-calculated. This process was interactively repeated until a constant value for $k_{obs}$ was obtained. The pseudo-first order rate constant for the hydrolysis of diazinon in 0.543 M NaOH, as determined from the slope of the line in Figure A.2, is $1.68 \times 10^{-3}$ s$^{-1}$. 
Table A.1. Data spanning three half-lives for the hydrolysis of diazinon in 0.543 M NaOH, at 25°C. This data is plotted in Figure A.1. and A.2. The absorbance was measured at 229 nm with $A_{\infty} = 0.896$.

<table>
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<tr>
<th>Time (s)</th>
<th>Absorbance</th>
<th>$3 + \log (A_\infty - A)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.65</td>
<td>0.338</td>
<td>2.75</td>
</tr>
<tr>
<td>61.45</td>
<td>0.390</td>
<td>2.76</td>
</tr>
<tr>
<td>121.35</td>
<td>0.439</td>
<td>2.66</td>
</tr>
<tr>
<td>181.65</td>
<td>0.477</td>
<td>2.62</td>
</tr>
<tr>
<td>241.55</td>
<td>0.521</td>
<td>2.57</td>
</tr>
<tr>
<td>301.35</td>
<td>0.561</td>
<td>2.52</td>
</tr>
<tr>
<td>361.55</td>
<td>0.591</td>
<td>2.48</td>
</tr>
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<td>421.45</td>
<td>0.618</td>
<td>2.44</td>
</tr>
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<td>481.35</td>
<td>0.645</td>
<td>2.40</td>
</tr>
<tr>
<td>541.45</td>
<td>0.671</td>
<td>2.35</td>
</tr>
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<td>601.35</td>
<td>0.691</td>
<td>2.31</td>
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<td>661.35</td>
<td>0.711</td>
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<td>721.25</td>
<td>0.732</td>
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<td>901.25</td>
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<td>961.65</td>
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<td>1141.65</td>
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</tr>
<tr>
<td>1201.45</td>
<td>0.820</td>
<td>1.88</td>
</tr>
<tr>
<td>1291.45</td>
<td>0.830</td>
<td>1.82</td>
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Figure A.1. A representative kinetic run, showing the change in absorbance at 229 nm over time. This plot depicts the reaction of diazinon with 0.543 M NaOH at 25°C.
Figure A.2. Plot to determine $k_{\text{obs}}$ for the reaction of diazinon with 0.543 M NaOH at 25°C. The data for this plot are found in Table A.1.
APPENDIX B: Spectra used in compound identification and characterization
Figure B.1. GC chromatogram of diazinon (solvent is chloroform)
Figure B.2. MS spectrum of diazinon
Figure B.3. $^1$H NMR spectrum of diazinon (in CDCl$_3$)
Figure B.4. $^{13}$C NMR spectrum (J-modulated) of diazinon (in CDCl$_3$)
Figure B.5. $^3$P NMR spectrum of diazinon (in CDCl$_3$)
Figure B.7. GC chromatogram of 7 (solvent is chloroform)
Figure B.9. $^1$H NMR spectrum of 7 (in CDCl$_3$)
Figure B.11. $^{31}$P NMR spectrum of 7 (in CDCl$_3$)

$\text{CH}_3\text{CH}_2\text{O}\_\text{S}$
$\text{CH}_2\text{CH}_2\text{O}$

$\text{CH}_3\text{CH}_2\text{O} - \text{OCH}_2\text{CH}_3$
Figure B.12. MS spectrum of 2
Figure B.13. $^1$H NMR spectrum of 2 (in CDCl$_3$)
Figure B.14. $^{13}$C NMR spectrum (J-modulated) of 2 (in CDCl$_3$)
Figure B.15. $^{31}$P NMR spectrum of 2 (in CDCl$_3$)
Figure B.16. $^1$H NMR spectrum of 2 $^\cdot$Na$^+$ (in $D_2$O)
Figure B.17. $^{13}$C NMR spectrum (J-modulated) of 2·Na$^+$ (in D$_2$O)
Figure B.18. $^{31}$P NMR spectrum of $2\cdot$Na$^+$ (in D$_2$O)