The Chemistry and Photochemistry of the Insecticide Carbaryl in Aquatic Environments

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Contents

Acknowledgements i

1. Introduction 1

2-3 Experimental
   2.1 Materials 3
   2.2 Apparatus 3
   2.3 Stock Solutions 3
   2.4 Detection of Carbaryl and its Degradation Products 5
      2.4.1 Carbaryl Calibration Graph 5
      2.4.2 1-Naphthol Calibration Graph 6
      2.4.3 1-Naphtholate Calibration Graph 6
      2.4.4 Discussion of Detection Technique 6
   2.5 Solution Stability Study 7
   2.6 Study of Carbaryl and 1-Naphthol Hydrolysis 7
   2.7 Design of Photolysis Apparatus 8
   2.8 Photolysis Procedure 9
   2.9 Potassium Ferrioxate Actinometer 9
   3.0 Safety Aspects 10
   3.1 Humic Substance Extraction 10
   3.2 Quartz Fluorescent Tube Calibration 10

4. Results 11
   4.1 Solution Stability Study 11
   4.2 Carbaryl 11
      4.2.1 Carbaryl Photolysis 11
      4.2.2 Carbaryl Hydrolysis 12
   4.3 1-Naphthol 12
      4.3.1 1-Naphthol Alkaline Degradation 14
      4.3.2 1-Naphthol Photolysis 14
   4.4 1-Naphtholate 17
      4.4.1 1-Naphtholate Alkaline Degradation 17
      4.4.2 1-Naphtholate Photolysis 17

5. Conclusions 18

Appendix 1 Error Analysis 20

References 20
Acknowledgements

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1. **Introduction**

Large quantities of pesticides have been used for many years in New Zealand to control agricultural and horticultural pests. The fate of a pesticide after application as a powder or solution to the target organism depends on its chemical structure and mode of application. Once in the environment, it may degrade by any number of physical, chemical or biological mechanisms. The time scale for this degradation can span from hours to years and the degradation products may be as toxic, if not more, than the parent molecule. Thus pesticides may become "xenobiotic" pollutants and eventually have adverse effects not only in the environment but they may also bioaccumulate in the food chain and even ultimately affect humans.

One of the common degradation pathways of many chemical species in the environment is via sunlight-induced **photochemistry** either in the solid state or in solution. The aim of this project was to examine this degradation pathway for solutions of a pesticide in common usage in New Zealand. It was hoped that the results from this study would provide some indication of the relative importance of sunlight as a method of pesticide degradation. In the New Zealand environment, a significant fraction of all pesticides are ultimately washed off the target plants and organisms to form solutions in natural aquatic environments such as streams, rivers, lakes and coastal estuaries.

A recent South Island survey of pesticide usage [1] was carefully examined for a pesticide which fulfilled the following conditions:

1. Extensively used in the local area (ie. Dunedin/Silver Peaks).
2. Significant toxicity.
3. Did not contain halogens. The usual and most sensitive method of analysis of organohalogen pesticides involves GC methods with Electron Capture detection and this facility was not available in the Chemistry Department.

Two pesticides, Roundup (TM) (active ingredient, glyphosate) and Carbaryl(TM) (1-naphthyl-N-methylcarbamate) were selected. However only Carbaryl was considered after difficulties were experienced with the major assay technique for Roundup which involves a complicated and lengthy HPLC procedure.

Carbaryl (also known by its alternative trade name of Sevin) is a contact and stomach insecticide used against pests of fruit, vegetables and other crops. It is widely used throughout New Zealand as well as the Dunedin/Silver Peaks area for both horticulture and in domestic gardens. Overseas, it has also been used to control pests of oysters in shallow estuaries [13]. It is a popular insecticide in part due to its low toxicity to non-target organisms (LD$_{50}$ = 2.0 mg/kg in rabbits and 4.3 mg/dm$^3$ for rainbow trout). It has also become popular because of the general move away from the use of very persistent organochlorine pesticides such as 2,4,5 T and DDT.
Fig. 1. Previous research on carbaryl and some of its degradation products.
There have been several previous reports of the photochemistry of carbamate pesticides in organic solvents with excitation wavelengths in the 220 - 265 nm range [2-6a]. Carbaryl has been shown to photodegrade under such conditions via a Photo-Fries mechanism [25-26] to give 1-naphthol and naphthalene as the major degradation products.

The photolysis of carbaryl in water has also been investigated using artificial light (λ>220 nm) [6-7] and sunlight (λ>290 nm) [7-8]. Again the major degradation product was identified as 1-naphthol (Fig. 1).

An alternative non-photochemical degradation pathway involving the hydrolysis of carbaryl to 1-naphthol has been shown to compete with the photolysis process. This hydrolysis is relatively rapid for aqueous carbaryl solutions at typical environmental pH values (e.g. t_{1/2} 1.3 days at pH 8) [8-11]. 1-Naphthol is also the major degradation product from photolysis and hydrolysis of carbaryl on sediment surfaces and in the soil [8,12] and it is the major metabolite of carbaryl in plants, micro-organisms and animals [14].

1-Naphthol may itself be degraded under the influence of light, pH, microorganisms and the presence of oxygen [6,9,13]. 1-Naphthol is in acid-base equilibrium with its conjugate base, 1-naphtholate (pK_a = 9.40). An appreciable amount of 1-naphtholate (6%) is present in seawater (pH 8.2). 1-Naphtholate is also light-sensitive and one of its degradation products has been identified as 2-hydroxy-1,4-naphthoquinone [11].

A variety of natural species present in aquatic environments such as humic substances, singlet oxygen, and nitrate anions are known to sensitise the breakdown of compounds previously thought to be stable in the environment [15-16]. So another aim of this project was to investigate whether these naturally occurring materials would sensitise the breakdown of aqueous solutions of carbaryl and of its degradation product, 1-naphthol.

In this study, detection methods were first developed for carbaryl, 1-naphthol and 1-naphtholate based on the fluorescence of these species. The hydrolysis of carbaryl and the degradation of 1-naphthol plus 1-naphtholate were then investigated under typical natural water pH conditions (8.2) in the absence of light and microorganisms. Photolysis equipment was developed to study the photochemical degradation of carbaryl and 1-naphthol in aqueous solution under various conditions and a comparison made with the results for degradation in the absence of light.
2. **Experimental**

2.1 **Materials**

Three sources of carbaryl were used:

1. Commercial insecticide (80% active ingredient) from Yates.
2. Technical grade (>99%).
3. Pestanal grade (>99.9%) from Riedel-de Haen.

A.R. grade 1-naphthol was purified by vacuum sublimation (m.p. 95 °C). All light sensitive compounds and solutions of these compounds were stored in the dark. A fluorescence standard was prepared by dissolving A.R. grade quinine bisulphate in Milli-Q water to give a concentration of 0.1 ppm. Buffers of pH 5.5 (sodium acetate - acetic acid, 0.2 M), pH 8.2 (boric acid, 0.1 M), and pH 13 (hydroxide - chloride, 0.2 M) were made by dissolving the A.R. grade chemicals in Milli-Q water [17]. Methanol was purified by distillation. The potassium ferrioxalate actinometer was prepared following the method outlined in ref [18]. Reagent grade sodium azide (0.1 ppm) was used as an antibacterial agent [19]. Humic acid from Aldrich was used before fulvic acid was extracted from the Taieri River [20]. All solutions were air saturated.

2.2 **Apparatus**

Absorption spectra were recorded on a Shimadzu UV-240 spectrophotometer in quartz or glass 1 cm x 1 cm cells. Fluorescence spectra were measured using a Perkin Elmer MPF-4 fluorescence spectrophotometer with a Xenon lamp using quartz 1 cm x 1 cm fluorescence cells. All spectra were taken at room temperature (20°C). Hydrolysis studies were conducted in a sealed, light proof thermostatically controlled water bath. pH measurements were recorded using a Solstat EPM-300 pH meter. Temperature was measured via a Comark 5000 digital thermometer. The photolysis apparatus consisted of a Hytek medium pressure 250 W mercury lamp, a system of lenses and a monochromator with enclosed sample compartment. More detail on this will be given later.

2.3 **Stock Solutions**

Carbaryl and 1-naphthol have a limited solubility in water. Sanchez and Blanco (1990) [21] found water to be the best solvent for the spectrofluorimetric determination of carbaryl and 1-naphthol. Ethanol and methanol were determined to be the best co-solvents. Methanol was chosen as Wolfe et. al. [29] used it as co-solvent for their carbaryl photolysis studies. The solutes were dissolved in methanol (50 cm³) with complete dissolution taking approximately half an hour for carbaryl and being immediate for 1-naphthol. These solutions were then diluted to 1 dm³ with Milli-Q water.
Before the Pestanal grade carbaryl was available, stock solutions were made using technical grade carbaryl at a concentration of 100 ppm (100 mg in 1 dm³) with 5\% (v/v) methanol. The solubility of carbaryl is reported to be 120 mg/dm³ water [10]. After about one day a precipitate formed in the solution, and thus the stock solution was thought to be supersaturated with respect to carbaryl. Carbaryl stocks at 100 ppm were then ultrasonically treated for three hours immediately after the dilution with Milli-Q water in an attempt to aid the dissolution. However this procedure did not stop the precipitation. A 100 ppm solution of carbaryl with distilled ethanol as a co-solvent also gave rise to precipitation. Finally a 100 ppm solution of Pestanal grade carbaryl was prepared and left for a week. A precipitate did not form in this solution. Thus the precipitate observed in the other solutions must have arisen from impurities in the technical grade material or the precipitation of carbaryl was aided by impurities in this technical grade material.

In order to save materials, stock solutions of 50 ppm (50 mg in 1 dm³) Pestanal grade carbaryl were prepared with 5\% (v/v) methanol, in Milli-Q water.

Solubility problems were not encountered with 1-naphthol. Initially 100 ppm stocks were made but a concentration of 50 ppm with 5\% (v/v) methanol was adopted for convenience when this was found to be satisfactory for carbaryl.
Fig. 2. Spectral characteristics of carbaryl, 1-naphthol & 1-naphtholate.
2.4 Detection of Carbaryl and its Degradation Products

2.4.1 Carbaryl Calibration Graph

Initial studies began with carbaryl extracted from the commercial pesticide [21]. Preliminary fluorescence and absorption spectra were obtained using this extract.

The spectral characteristics of carbaryl are shown in Fig. 2. The fluorescent emission maximum intensity is measured at $\lambda_{\text{max,em}} = 330$ nm (note the difference in maximum emission wavelength compared to ref [21] probably due to a slight bathochromic shift when methanol is used as co-solvent), with excitation wavelength set at $\lambda_{\text{ex}} = 285$ nm.

The spectrofluorimeter settings were as follows:
- excitation and emission slit widths 10 nm
- scan speed 60 nm/min
- chart speed 60 mm/min
- pen 1 range 10

All measurements were made in the "energy" mode. All other parameters and the warm up procedure are as outlined in the MPF-4's handbook.

An initial calibration graph was obtained using a 100 ppm technical grade carbaryl stock. As previously mentioned, a precipitate started to form in these stock solutions after a day. Once the Pestanal grade carbaryl was obtained, a calibration graph using a 50 ppm stock was attempted. Aliquots of the stock were diluted with Milli-Q water and a linear calibration graph was obtained in the 0.01-0.06 ppm range.

A normalisation process was used to correct for variations in the intensity of the excitation wavelength due to minor lamp fluctuations. The quinine bisulphate standard (0.1 ppm) was measured ($\lambda_{\text{ex}} = 285$ nm, $\lambda_{\text{max,em}} = 282$ nm) and corrected for baseline (Milli-Q water, pH 5.6) to give its corrected fluorescent intensity (CFI [QS]). The fluorescent intensity of the various carbaryl solutions were first corrected for baseline to give their corrected fluorescent intensities (CFI [carbaryl]). This corrected fluorescent intensity value was then normalised by dividing through by the corrected fluorescent intensity of the standard to give the normalised corrected fluorescent intensity (NCFI [carbaryl]). A plot of the normalised corrected fluorescent intensity of carbaryl against carbaryl concentration is linear in the 0.01 - 0.06 ppm concentration range (Fig. 3). Regression analysis gave the following formula:

$$\text{NCFI [carbaryl]} = \text{SLOPE} \times \text{[carbaryl]} \text{ ppm} - \text{INTERCEPT}$$

The slope of the graph was $82 \pm 5$ and the co-efficient of regression, $r^2$ is 0.999. Monitoring this calibration graph for six days showed it to be stable. Repetition of the calibration procedure gave slopes of $79 \pm 5$ ($r^2 = 0.999$) and $75 \pm 5$ ($r^2 = 0.999$).
Fig. 3. Carbaryl calibration graph pH 5.5.
During the course of this research it became clear that the pH of all solutions needed to be controlled more carefully and it was necessary to determine another calibration graph at pH 5.5. The absorption spectra of the pH 5.5 buffer showed that it does not absorb in the region of interest (285 - 295 nm) and thus use of this buffer will not affect the fluorescent signal of carbaryl or 1-naphthol. The same situation applies to the pH 8.2 and pH 13 buffers. Regression analysis of this calibration graph gave:

\[
\text{NCFI [carbaryl]} = (77 \pm 4) \times [\text{carbaryl}] \text{ ppm} - 0.11 \quad r^2 = 0.999
\]

The concentration of carbaryl in any solution is given by:

\[
[\text{carbaryl}] \text{ ppm} = \frac{(\text{NCFI [carbaryl]} - 0.11)/77 \pm 4}{\text{ppm}}
\]

This is accurate to approximately 10% (a partial error analysis is given in Appendix 1). It should be noted that below 0.03 ppm, the carbaryl peak exists as a shoulder on the Raman peak of water (\(\lambda_{\text{max,em}} = 313 \text{ nm at } \lambda_{\text{ex}} = 285 \text{ nm}\)). A detection limit lower than 0.01 ppm could be reached if the Raman peak could be subtracted from the spectrum. This could best be done using computer software if data logging facilities were available.

### 2.4.2 1-Naphthol Calibration Graph

This was obtained following the same procedure and instrumental settings as for carbaryl. The spectral characteristics of 1-naphthol are shown in Fig. 2. An excitation wavelength of \(\lambda_{\text{ex}} = 295 \text{ nm}\) was used and the maximum fluorescent intensity measured at \(\lambda_{\text{max,em}} = 460 \text{ nm}\). The quinine bisulphate standard was measured at \(\lambda_{\text{ex}} = 295 \text{ nm}\), its maximum emission being at \(\lambda_{\text{max,em}} = 440 \text{ nm}\). Aliquots of a 50 ppm stock solution of the purified 1-naphthol were diluted with the pH 5.5 buffer. A linear graph of normalised corrected fluorescent intensity against concentration was obtained in the 0.006 - 0.2 ppm range. Regression analysis gave:

\[
[\text{1-naphthol}] \text{ ppm} = \frac{\text{NCFI [1-naphthol]} - 0.0064)/12.6 \pm 0.4}{\text{ppm}} \quad r^2 = 0.999
\]

### 2.4.3 1-Naphtholate Calibration Graph

Again this was measured following the same procedure and instrumental settings as for carbaryl. The spectral characteristics of 1-naphtholate are shown in Fig. 2. An excitation wavelength of \(\lambda_{\text{ex}} = 330 \text{ nm}\) was used and the maximum fluorescent intensity measured at \(\lambda_{\text{max,em}} = 460 \text{ nm}\). The quinine bisulphate standard was again measured at \(\lambda_{\text{ex}} = 295 \text{ nm}\) as the 0.1 ppm solution went off the scale of the instrument at \(\lambda_{\text{ex}} = 330 \text{ nm}\). A pH 13 hydroxide-chloride buffer was used to dilute aliquots of a 50 ppm 1-naphthol-stock. At this pH, given a \(\text{pK}_a\) of 9.40 for 1-naphthol, 0.025% 1-naphthol will be present. Solutions to be measured were left for approximately 30 minutes to ensure almost complete conversion to 1-naphtholate. A linear graph of the normalised corrected fluorescent intensity against concentration was linear in the range 0.001 - 0.05 ppm. Regression analysis gave:

\[
[\text{1-naphtholate}] \text{ ppm} = \frac{\text{NCFI [1-naphtholate]} + 0.0088)/51 \pm 2}{\text{ppm}} \quad r^2 = 0.999
\]
2.4.4 Discussion of Detection Technique

Carbaryl has been detected using many techniques including gas chromatography, derivatisation methods [9,22-23] and HPLC with fluorescent detection [7,24]. Spectrophotometric techniques have been developed that involve the conversion of carbaryl to 1-naphthol by alkaline hydrolysis [8]. This involves measuring the 1-naphthol initially present and then adding hydroxide and measuring the combined carbaryl and 1-naphthol. The amount of carbaryl is then calculated by the difference. The World Health Organisation method involves colorimetric determination of carbaryl in the 1-naphthol form. Fluorescence techniques have been used [21] to quantitatively measure carbaryl in neutral media and as 1-naphtholate in basic media.

The detection technique developed here allows the consecutive quantitative determination of carbaryl, 1-naphthol and 1-naphtholate, all from the same sample, simply by changing the excitation wavelength.

Before any of the three species were measured, the standard quinine bisulphate and a Milli-Q water blank were run first. Then the blank appropriate to the sample to be analysed was measured at the excitation emission wavelengths used for the sample. Finally the sample itself was analysed.

When applied to photolysis studies this allows the detection of all three species in the photolysis reaction vessel itself. Thus the need to take subsamples of the reacting solution for processing and subsequent measurement is eliminated and this saves considerable time.

2.5 Solution Stability Study

Solutions of carbaryl and 1-naphthol at various concentrations in pH 5.5 buffer were measured over 7-9 days to assess the stability of these species at this pH. It was after this study that the instrumental variation of the spectrofluorimeter was noticed and the decision was made to normalise all data to the quinine bisulphate standard.

2.6 Study of Carbaryl Hydrolysis and 1-Naphthol Degradation

Carbaryl solutions of various concentrations and 0.2 ppm solutions of 1-naphthol, both in pH 8.2 buffer, were kept at constant temperature (20 ± 0.5°C) and in the dark via a light sealed thermostated waterbath. Sodium azide was added at a concentration of 0.1 ppm to some solutions to eliminate any bacteria present. The solutions were measured at time intervals of six hours to one day.
Fig. 4. Photolysis apparatus.

Fig. 5. Positions of the iris and the two quartz lenses on the optical bench.
2.7 Design of Photolysis Apparatus

The photolysis equipment developed as part of this project is shown in Fig. 4. The emitted radiation is firstly contained in an aluminium tube (approximate length 15 cm and diameter 5 cm) and then passed through an iris of diameter 3 cm. It is then focused by two quartz lenses onto the entrance slit of the monochromator (obtained from an atomic absorption spectrometer).

The system of lenses is shown in Fig. 5. A lot of time was spent in the design of this system, especially in adjusting the positions of the iris and the two lenses in order to get the maximum light intensity possible entering the monochromator. The distance from the collecting lens in the monochromator (Fig. 5) to the first slit was 11 cm. Thus it was desirable to focus the light beam to a point 11 cm in front of the collecting lens.

In sunlight, wavelengths less than 290 nm are absent due to their absorption by the ozone layer. The most intense emission line from the mercury lamp nearest to this threshold value is centred at 313 nm (Fig. 6): Thus all quantitative photolysis studies were conducted at 313 nm. The more intense emission line at 365 nm was not used as neither carbaryl or 1-naphthol absorb appreciably at this wavelength. Also some of the previous reports of carbaryl photolysis had used the specific wavelength of 313 nm.

Initially the light intensity at 313 nm was monitored using a plate covered in paint that fluoresces when ultraviolet light is incident upon it. A new photocell detection system was then developed which comprised of a photocell housed in the old monochromator photocell detector casing. This system was then placed into the monochromator cavity that the previous photocell occupied. The new photocell was connected to a variable range voltmeter to monitor the relative incident light intensity. This system proved to be very successful for monitoring the light intensity and hence maximising the intensity of light entering the monochromator. The emission band from the lamp is approximately 2 nm wide and is centred at 313.5 nm.

A replica of the old photocell casing was manufactured. This was designed to hold a standard 1 cm x 1 cm quartz fluorescent cell in the path of the light beam in the exact position of the photocell detector. This cell holder could be positioned in the monochromator cavity and held exactly in place by two positioning pins. The cell holder was painted matt black to prevent light that passed through the sample cell from reflecting back into the sample cell. A slot in the top allowed the sample cell to be placed in the cell holder. This was then light sealed with a rubber bung. There is provision for a hole to be drilled in this rubber bung to allow the passage of a capillary tube into the sample cell to allow the sample to be aerated or degassed.

Operation of the mercury lamp leads to considerable heat generation. However, the temperature of a carbaryl solution was monitored in the monochromator over a period of three hours by using a digital thermometer and thermocouple. The temperature stayed constant at 20 ± 1°C i.e. room temperature. The monochromator is mounted on the optical bench. Both these items are made of steel and thus they provide an excellent heat sink for any heat transferred to them from the lamp or through the lamp casing and mounting.
Fig. 6. Medium pressure mercury lamp emission spectrum (---) and the sunlight spectrum (-----).
When this photolysis apparatus is used in combination with the fluorescent detection of carbaryl, 1-naphthol and 1-naphtholate, it provides a powerful tool to look at the photolysis of carbaryl and its degradation products. The photolysis of a sample may be conducted at any wavelength using the monochromator, providing the excitation lamp has an emission band at that wavelength.

2.8 Photolysis Procedure

The photocell is placed in the monochromator and the relative light intensity zeroed. The lamp was then turned on and allowed to warm up for approximately 20 minutes. After adjusting the monochromator to give maximum intensity at 313 nm the photocell was removed.

3.5 cm³ of the solution to be irradiated was pipetted into the fluorescent cell and placed in the cell holder which was then positioned in the monochromator. The cell was placed in a light sealed container while being taken to and from the spectrofluorimeter. A control solution was kept in the dark at constant temperature.

Exact timing of the photolysis was needed. A log was kept of the time that the solution was placed in the monochromator and when it was removed. The control solution was re-analysed after every measurement of the irradiated sample in order to measure any decay of the sample due to processes other than photolysis. If decay occurred in the control solution this amount is subtracted from the decay observed in the irradiated sample in order to determine the true decay due to photolysis. No decay was observed in the control solutions except for one special case (section 4.3.2). Before the irradiated sample was placed back in the monochromator, the light intensity was checked using the photocell. No major variations in intensity were detected.

All photolysis studies were conducted at pH 5.5 so that only the direct photodissociation of the sample would be observed. The competing hydrolysis degradation pathways do not occur at this pH. Solutions of 1-naphthol at pH 5.5 will contain minimal 1-naphtholate (0.013%) and thus 1-naphtholate will not effect the photolysis results.

2.9 Potassium Ferrioxalate Actinometer

This was synthesised in the manner described by Calvert and Pitts [18]. The light intensity incident on the reaction cell at 313 nm was measured by placing 3.5 cm³ of the actinometer solution in the sample cell and then into the monochromator. The actinometer solutions were irradiated for two hours.

This actinometer is sensitive to visible light. Manipulations of the actinometer were not carried out in a photographic dark room. However, exposure of actinometer solutions to visible light was minimised at all times, principally by shielding the solutions with aluminium foil. Secondly, care was taken to make sure that the blank solutions were exposed to visible light for the same amount of time as those samples irradiated at 313 nm. Measurement of the solutions with the double beam Shimadzu spectrophotometer would result in the subtraction of any signal due to visible light, from that due to the ultraviolet light at 313 nm.
3.0 Safety Aspects

Carbaryl is a known cholinesterase inhibitor (NZ Poisons & Hazardous Chemicals Database) and possible teratogen. It is absorbed in significant amounts through exposed skin surfaces.

1-Naphthol is also toxic (LD$_{50}$ = 9 mg/kg rabbits) and has been shown to be more toxic than carbaryl to some marine species [14]. Thus care is needed when handling concentrated solutions to minimise the amount of exposure.

The mercury lamp used generates a very intense broad scale spectrum ranging from the visible down to 210 nm. Normal window glass will filter out much of the UV-B radiation (280-320 nm). Pyrex glass (thickness 4 mm) will filter out wavelengths down to approximately 290 nm [36]. The effectiveness of Pyrex safety glasses was assessed by fixing a lens of the glasses in the path of the focused light beam. Significant proportions of the very short wavelength UV around 220 nm were still transmitted and so an arc welding face shield was used for eye and face protection. While the emitted light was tightly focused there was still an appreciable amount of stray reflected light present. Thus all skin surfaces were covered to minimise the amount of exposure, especially when the optics of the system were being maximised. Here it was necessary to adjust the positions of the lenses, while the lamp was in operation and invariably the beam was intercepted by one’s hands.

Furthermore, the very short wavelength ultraviolet is capable of generating ozone. So the entire photolysis system was maintained in a fume hood. This was then light sealed using black cardboard to prevent stray light entering the laboratory.

3.1 Humic Substance Extraction

Humic and fulvic acid were extracted from the headwaters of the Taieri River [20]. The fulvic acid fraction which comprises approximately 75% of the total humic substances was used for photolysis studies. It had the following microanalysis: 50.19% C; 4.57% H; 1.23% N; 1.72% ash.

3.2 Quartz Fluorescent Tube Calibration

Two different 1 cm x 1 cm quartz fluorescent cells were used and so it was desirable to know if these had any effect on the fluorescent intensity measured. This was assessed by measuring three samples, 0.1 ppm quinine bisulphate, 0.06 ppm carbaryl (pH 5.5) and 0.2 ppm 1-naphthol (pH 5.5), at their respective excitation wavelengths, in each of the two cells. No measurable optical difference was noted between the cells. For completeness, by marking a specific face of each cell, the cells were always positioned in the spectrofluorimeter and in the photolysis apparatus in precisely the same orientation.
Fig. 7. Stability of carbaryl at pH 5.5 in the absence of light.

Fig. 8. Photo-Fries products of carbaryl.
4. Results and Discussion

4.1 Solution Stability Study

To assess the stability of the carbaryl calibration graph at pH 5.5 three carbaryl solutions of different concentrations were held in the dark at 20°C and were monitored over 7-9 days. Fig. 7. shows the variation in the fluorescence of the 0.06 ppm solution. The slight slope observed is well within the range of the errors. Thus carbaryl is stable with respect to hydrolysis at this pH. The same result applied to the other carbaryl solutions monitored.

1-Naphthol is stable at pH 5.5 in the absence of light as it has a pKₐ of 9.40. The stability of 1-naphthol at pH 8.2 was investigated and it was found to degrade significantly over the time scale of this experiment and hence was investigated further.

4.2 Carbaryl

The alkaline hydrolysis of carbaryl provides an alternative degradation pathway which competes with the photochemical degradation process. Thus it is necessary to assess the relative importance of the two processes under conditions applicable to the environment.

4.2.1 Carbaryl Photolysis

Carbaryl photodissociation occurs via a Photo-Fries mechanism in organic solvents [25-26]. Cleavage of the bonds in the α and β positions occurs to give 1-naphthol and naphthalene as the major degradation products (Fig.8).

N-methylcarbamic acid is also formed, which decomposes to methylamine and CO₂. Products with the substitution of the -C-NHCH₃ group at positions 2,4,5 and 7 are also predicted and these have been identified in trace amounts [25]. The photolysis products have been reported to depend upon the excitation wavelength, solvent medium and the photolysis time [13]. One degradation product was found for the photolysis of carbaryl in ethanol [6a] using sunlight or weak ultraviolet light. Exposure to intense ultraviolet light gave rise to five products but only 1-naphthol was identified. In hexane 1-naphthol is the only product formed [4]. Photolysis in water at λ > 220 nm gave five products [6]. Again only 1-naphthol was identified. The unidentified photolysis products may be light sensitive.

In dilute solutions (absorbance < 0.02) the photolysis of carbaryl, 1-naphthol and 1-naphtholate will follow first order kinetics [27]. The reaction quantum yield (Φᵣ,λ) at a particular wavelength of a compound is defined as

\[ \Phi_{r,\lambda} = \frac{\text{number of molecules decomposed}}{\text{number of quanta absorbed}} \]

It was hoped to obtain a quantum yield for carbaryl photodissociation at 313 nm and then calculate the first order sunlight photolysis rate constant for carbaryl in shallow water bodies using the method of Zepp and Cline [28]. It was also hoped to calculate the sunlight photolysis rate constant in the presence of humic substances.
Fig. 9. Initial carbaryl photolysis using unfiltered light from the mercury lamp.
Before the photolysis apparatus was fully operational, preliminary carbaryl photolysis studies were conducted using unfiltered light (λ>220 nm). All photolysis studies were conducted at pH 5.5 so as only the direct photodissociation of the sample would be observed. The competing hydrolysis degradation pathways do not occur at this pH. The photodissociation of carbaryl followed first order kinetics (Fig. 9).

Humic substances absorb appreciably in the UV-B (280-320 nm) and may cause the photosensitized degradation of pesticides [16]. Using unfiltered light, humic acid was found to decrease the photolysis rate of carbaryl (Fig. 9). This is consistent with the Photo-Fries rearrangement mechanism proposed by Bellus [26]. The mechanism of the photo-rearrangements is not influenced by triplet quenchers, including oxygen, or sensitizers. These results suggest that the humic acid is in fact acting as an optical filter therefore decreasing the photolysis rate of carbaryl.

The absorption spectrum of carbaryl (Fig. 10) has a small spectral overlap with the sunlight spectrum. Therefore one can expect that the rate of carbaryl photolysis will be slow in sunlight. Before any attempts were made to determine the quantum yield of carbaryl in this study at 313 nm, it was discovered in the literature that a value of φ_{ϕ,L} = 0.006 had been reported [29]. The computer program developed by Zepp [28] calculates at half life of 2.2 days at latitude 40°N in mid-summer [38]. There is some discrepancy on this value as Zepp and Baughman [31] report a value for t½ of 6.6 days using the same data.

The amount of ultraviolet radiation incident on the earth’s surface is dependent mainly on the season and latitude, the ozone concentration and the amount of cloud cover. These factors can all contribute to variation in the rate of the sunlight induced photolysis [28]. This may account for the differences in the reported values. Other values reported are t½ = 250 ± 97 hours for sunlight and t½ = 204 ± 24 hours using a filtered Xenon Arc lamp (λ>290 nm) [30].

4.2.2 Carbaryl Hydrolysis

Since the photolysis half life of carbaryl in sunlight is long it was necessary to assess the importance of the hydrolysis degradation pathway.

In alkaline media, carbaryl hydrolyses to 1-naphthol and N-methylcarbamic acid, which then decomposes spontaneously to methylamine and carbon dioxide [37]. The hydrolysis is first order with respect to hydroxide and to carbaryl and is thus second order overall [32-33]. In the presence of excess hydroxyl ions the hydrolysis follows pseudo first order kinetics. The pseudo first order rate constant, k₁, is calculated from the slope (-k₁t) of a ln(C_t/C₀) versus time graph with the half life calculated from the expression:

\[ t_{1/2} = \frac{0.693}{k_1} \]
Fig. 10. Absorption spectra of carbaryl (→) 10 ppm & pH 5.5, 1-naphthol (→→) 7 ppm & pH 5.5 (scale factor 0.8x), 1-naphtholate (→→→) 10 ppm & pH 13 (scale factor 1.2x) and their overlap with the sunlight spectrum (---).
The pseudo first order rate constants and corresponding half lives are shown in Table 1.

Table 1. Rate of Carbaryl Hydrolysis pH 8.2 20°C

<table>
<thead>
<tr>
<th>Solution</th>
<th>Conc /ppm</th>
<th>$r^2$</th>
<th>$K_1 \times 10^{-4}$/min$^{-1}$</th>
<th>$t_{1/2}$/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>0.998</td>
<td>$6 \pm 1$</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.998</td>
<td>$6 \pm 1$</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>0.06</td>
<td>0.997</td>
<td>$4.3 \pm 0.9$</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>0.025</td>
<td>0.960</td>
<td>$8 \pm 2$</td>
<td>14 ± 4</td>
</tr>
</tbody>
</table>

Bacteria did not appear to effect the stability of the carbaryl solutions. However for completeness, sodium azide was added to some solutions to kill any residual bacteria. This resulted in an immediate decrease in the carbaryl fluorescent intensity of approximately 60%, and may be due to azide anion quenching the carbaryl emission. The azide anion may also react chemically with carbaryl to produce non-fluorescent products. Another interesting point to note is that the decay of carbaryl is accompanied by an increase in the amount of 1-naphthol (Fig. 11) to a point where the 1-naphthol achieves what seems to be a steady state. Thus 1-naphthol is unstable at this pH.

The rate of carbaryl hydrolysis increases with increasing pH [11], and so the results obtained here agree well with those previously reported for pH 8 ($t_{1/2} = 1.3$ days) [9]. The hydrolysis rate also increases with temperature [8-9].

The temperature of seawater varies as a function of latitude, season, time of day and of depth. In New Zealand the surface water temperature varies from about 9°C in the winter to 15°C in the summer. Thus the hydrolysis rate of carbaryl in the environment will be somewhat lower than that found in this study. Also as previously noted, the sunlight photolysis half life of carbaryl is $t_{1/2} = 2 - 6.6$ days. This will decrease during the winter months as the amount of ultraviolet light is less. Therefore in seawater, the hydrolysis degradation pathway ($t_{1/2} = 20$ h) will be dominant during the winter months and the photolysis may compete to a greater extent with it during the summer months. Photolysis will be dominant in acidic waters ($t_{1/2}$ hyd = 4.4 months pH 6) all year round.

Once the importance of the hydrolysis degradation of carbaryl was assessed, the emphasis of this study shifted to 1-naphthol.
Fig. 11. Hydrolysis of carbaryl at pH 8.2 in the absence of light.
4.3 1-Naphthol

4.3.1 1-Naphthol Alkaline Degradation

As with carbaryl it was necessary to quantify the rate of the dark alkaline degradation of 1-naphthol that was observed in the initial carbaryl hydrolysis studies. The degradation of 1-naphthol at pH 8.2 was found to follow pseudo zero order kinetics (Fig. 12). The rate constant obtained by regression ($r^2 = 0.980$) is $k = (1.3 \pm 0.2) \times 10^{-5}$ min$^{-1}$ and the half life is $t_{1/2} = 4.3 \pm 0.9$ days. This alkaline degradation process was observed at a fixed pH and thus it is expected to be first order with respect to hydroxide ions. This dark alkaline degradation of 1-naphthol possibly occurs via ring cleavage. The degradation product is unknown.

Sodium azide lead to an enhancement of this degradation process for 1-naphthol. However, on addition, it did not immediately cause a decrease in the fluorescence of 1-naphthol as was observed with carbaryl.

4.3.2 1-Naphthol Photolysis

The UV–VIS spectrum of 1-naphthol exhibits an absorption maxima at $\lambda_{abs} = 292$ nm which is within the sunlight spectrum. It also has a greater overlap with the sunlight spectrum than does carbaryl (Fig. 10) which suggests that it may be more light sensitive than carbaryl. All 1-naphthol photolysis studies were conducted at pH 5.5. At this pH minimal 1-naphtholate is present (0.013%) and thus it will not effect the photolysis results.

Initial studies involving irradiation of a pH 5.5 solution of 1-naphthol with light from a fluorescent tube i.e. room light, showed a marked decrease in the concentration of 1-naphthol with time. Irradiation at 313 nm (pH 5.5) also showed a decrease in the amount of 1-naphthol. However when this data was normalised, it was found that the control was decaying and that the sample being irradiated showed no decay at all. The control which was kept in the dark was contained in a quartz fluorescent cell which had had to be reglued a few weeks before these experiments. The solutions being irradiated with fluorescent light were also contained in this cell. To investigate if contamination in the cell was causing the observed decay, two identical solutions of 1-naphthol were prepared. One was placed in the reglued tube and the other in the second tube. After leaving the two solutions in the dark for 24 hours they were measured. The solution in the reglued tube showed a marked decrease in the amount of 1-naphthol. The other tube showed no change. Thus the glue in the reglued tube was somehow catalysing the breakdown of 1-naphthol.

Following this result, all solutions to be irradiated were contained in the cell that had not been reglued. The controls were kept in small (25 cm$^3$) volumetric flasks and measured using the reglued tube. The amount of decay induced by the tube while simply measuring the control solutions will be negligible as this takes approximately 40 seconds.

Photolysis of 1-naphthol at 313 nm for a period of 10 hours showed, within experimental error, that no degradation had occurred (Fig. 13).
Fig. 12. The dark alkaline degradation of 1-naphthol & 1-naphtholate (pH 8.2).
A 1-naphthol solution (0.2 ppm) contained in a 100 cm³ borosilicate glass volumetric flask was left in sunlight for six hours. This showed no decay, as did an identical solution sealed in aluminium foil, which acted as a control.

Nitrate anions are known to photodecompose and generate oxygen ion radicals and hydroxide radicals ($\lambda>300 \text{ nm}$, pH 5-12) [34] via the mechanism:

$$\text{NO}_3^- + \text{h} \nu \rightarrow \text{NO}_2^- + \text{O}^*$$

$$\text{NO}_3^- + \text{H}^+ + \text{h} \nu \rightarrow \text{NO}_2 + \text{OH}^-$$

A 1-naphthol solution (pH 5.5) with 14 ppm nitrate [35] was irradiated ($\lambda = 313 \text{ nm}$) for eight hours. No photodegradation was observed.

The influence of humic substances on the photolysis of 1-naphthol was examined. When excited at $\lambda_{ex} = 295 \text{ nm}$, the fulvic acid has a significant emission at 460 nm which will add to the 1-naphthol signal. A solution of 1-naphthol with 4 ppm fulvic acid was irradiated for 12 hours. Before each measurement, a blank of 4 ppm fulvic acid was measured ($\lambda_{ex} = 295 \text{ nm}$). The CFI for 1-naphthol in the control and the photolysis sample is obtained by subtracting the CFI fulvic acid at 460 nm from the fluorescent intensity of the fulvic acid 1-naphthol mixture at 460 nm. The presence of fulvic acid did not sensitize the photodegradation of 1-naphthol. Also the irradiation of a 10 ppm solution of fulvic acid showed that no photolysis of the fulvic acid occurred under these conditions.

Previous work has found 1-naphthol to be photoactive. It must be noted that 1-naphthol has a very strong absorption maxima at 215 nm. Aly and El Dib [6], using an unfiltered germicidal UV lamps (peak emission 254 nm) found the degradation of 1-naphthol to increase with increasing pH and to photodegrade to two unidentified products. These two products were also observed in the photolysis of carbaryl. This indicates that when identifying the number of photolysis products of carbaryl, the photodegradation products of 1-naphthol and/or 1-naphtholate are also being identified.

Lamberton et.al. [13] note that 1-naphthol degradation in seawater is increased in the presence of fluorescent light ($\lambda>270 \text{ nm}$ depending on the type of fluorescent lamp [36]). They also note that the degradation products are different in the presence and absence of light. Karinen et.al. [8] also indicate that 1-naphthol is unstable in seawater in the presence of fluorescent light. Wauchope et.al. [11] state that 1-naphthol (and carbaryl) are stable to room light in weakly acidic solutions. The choice of light source and the filtering apparatus is clearly of critical importance in the interpretation of these reported photolysis results. If results are to be extrapolated to the environment, wavelengths below 290 nm should not be used for all but very qualitative studies.
Fig. 13. Photolysis of 1-naphthol at 313 nm (pH 5.5).
Measurements of the incident light intensity at 313 nm in the photolysis sample compartment using the potassium ferrioxalate actinometer were made. These indicated an intensity in the range $7 \times 10^{10} - 1 \times 10^9$ E min$^{-1}$. This is approximately 300 times less intense than the intensity obtained at 313 nm by Wolfe et al. [29] using a 450W medium pressure mercury lamp for their photolysis study of carbaryl. The intensity at 313 nm found in sunlight in summer is $0.643 \times 10^{14}$ photons cm$^{-2}$ s$^{-1}$ 2.5 nm$^{-3}$ at 312.5 nm [28]. There is an inherent problem with the units used to specify light intensity. To obtain an intensity value in the above units, the precise cross sectional area of the photolysis cell being irradiated must be calculated.

However, one would expect that the intensity at 313 nm obtained from the photolysis equipment designed in this study to be greater than that found in sunlight. On the basis of this and the sunlight photolysis study, I propose that weakly acidic solutions of 1-naphthol are not photoactive in the region of ultraviolet light that is found in sunlight.

This does not mean that 1-naphthol does not photodegrade in the environment. Karinen et al. [8] note that 1-naphthol is sensitive to sunlight in seawater, suggesting that photolysis may occur in weakly alkaline conditions, but one must note the involvement of 1-naphtholate (6% present in seawater pH 8.2) which has been shown to be very light sensitive (Section 4.4.2).

Lamberton et al. [13] note that 1-naphthol is relatively stable in a light exposed pH 7.8 phosphate buffer solution in the absence of oxygen. The addition of oxygen resulted in a decay of 1.6% per day, indicating that photo-oxidation rather than photodecomposition was occurring. However they did not run a control solution in the dark. Therefore the degradation they observed may be due to the presence of oxygen affecting the alkaline degradation of 1-naphthol. Wolfe et al. [29] also note that oxygen may be important in the decay at 1-naphthol. Again the possible involvement of 1-naphtholate must be noted.
4.4 1-Naphtholate

1-Naphthol is in acid base equilibrium with its anion 1-naphtholate. Given a $pK_a = 9.40$ there will be $6\%$ 1-naphtholate present in seawater (pH 8.2). Due to the conflicting reports on the photoactivity of 1-naphthol it was decided to investigate whether it was actually 1-naphtholate that was responsible for the observed alkaline decay and the possible photoactivity of 1-naphthol.

4.4.1 1-Naphtholate Alkaline Degradation

The dark alkaline degradation of 1-naphtholate at pH 8.2 was observed in this study to occur by a pseudo first order process (Fig. 12). Regression analysis gave a rate constant of $k = (2.1 \pm 0.4) \times 10^6 \text{ min}^{-1}$ ($r^2 = 0.979$). The half life for the decay is $t_{1/2} = 4.3 \pm 1.1$ days.

Again sodium azide on addition to some solutions, did not immediately cause a decrease in the fluorescence of 1-naphtholate as was observed with carbaryl. Sodium azide also caused the enhancement of this degradation process for 1-naphtholate.

1-Naphtholate may degrade by a similar mechanism as 1-naphthol to give an unknown decay product. Since both species decay with the same half life it may be that only one species is decaying and that the observed decay of the other is due to the changing acid base equilibria between them. If the degradation of both species were to decrease with decreasing temperature then the half lives presented here will be higher than that found in the New Zealand environment.

4.4.2 1-Naphtholate Photolysis

The absorption spectra of 1-naphtholate is shown in Fig. 10. It has an absorption maxima at 333 nm which is well within the sunlight spectrum. In view of this enhanced overlap with the sunlight spectrum, it is reasonable to assume it will be more photoactive in sunlight than carbaryl and 1-naphthol.

A solution of 1-naphtholate (10 ppm, pH 13) was exposed to fluorescent light i.e. room light. This solution turned visibly yellow after a day indicating the photodissociation of 1-naphtholate. An identical solution kept in the dark (wrapped in aluminium foil) showed no change. The occurrence of this has also been reported elsewhere [11,21]. One of the photodissociation products of 1-naphtholate has been identified as 2-hydroxy-1,4-naphthoquinone [11].

This experiment was duplicated in sunlight. The 1-naphtholate showed visible decay after only half a day in cloudy conditions. The control solution showed no decay. This indicates that it is 1-naphtholate that is the most photoactive of the three compounds investigated in the present study.
5. **Conclusions**

A sensitive analytical technique has been developed to detect carbaryl, 1-naphthol and 1-naphtholate in aqueous solutions using the natural fluorescence of these compounds. This technique does not require the alkaline hydrolysis or derivatisation steps that other techniques for the determination of these compounds require. All these species can be detected in the one solution by changing the excitation wavelength and monitoring at the appropriate emission wavelength.

The photolysis system designed allows irradiation of a sample at any chosen wavelength, provided that the light source has an emission band at this wavelength. When combined with the detection technique developed it allows the progression of the photolysis experiment to be monitored in the reaction cell itself.

Carbaryl photodissociates via first order kinetics using unfiltered light emitted from the mercury lamp. Humic acid slowed the rate of photolysis by acting as an optical filter. In most natural waters, hydrolysis is the dominant pathway by which carbaryl degrades. Because of this the photolysis of carbaryl in sunlight and at 313 nm was not investigated.

1-Naphthol degrades in the absence of light, by what seems to be a pseudo zero order process at pH 8.2. This is worthy of future investigation. 1-Naphthol appears to be stable (in sunlight and at 313 nm) with respect to photodissociation in slightly acidic solutions. It may however be light sensitive in slightly alkaline solutions, but here the involvement of 1-naphtholate must be taken into account.

The presence of fulvic acid did not sensitise the photodissociation of 1-naphthol at 313 nm. The generation of hydroxide radicals via nitrate photolysis also had no effect on the degradation of 1-naphthol.

Photo-oxidation of 1-naphthol in seawater may occur rather than its photodissociation.

1-Naphtholate decays in the dark in alkaline solutions as does 1-naphthol. Further work is necessary to determine whether it is 1-naphtholate, 1-naphthol or both species that are responsible for the observed decay.

1-Naphtholate is very light sensitive. Therefore the photodegradation of this species would seem to be the most important non-biological route for the decay of carbaryl, after its hydrolysis to 1-naphthol.
Appendix I. Error Analysis

The error in each fluorescence measurement is 5 fluorescence units and does not vary with concentration. This equates to an error of 4% for a 0.01 ppm carbaryl solution, a 1% error for a 0.04 ppm solution and a 3% error when measuring the quinine bisulphate standard. The absolute errors in the NCFI [carbaryl] values are shown in Fig. 3. The actual percentage error in each value decreases with increasing concentration but the effect of the increasing concentration causes the absolute errors to increase with increasing concentration.

A maximum and minimum slope were calculated for the carbaryl calibration graph (Fig. 3) using the error bars. The error in the slope of the calibration graph was taken to be the difference of the maximum and minimum slopes divided by two. This gave an error of approximately 5 percent.

The equation used to calculate the concentration of carbaryl in any solution

\[
[\text{carbaryl}] \text{ ppm} = (\text{NCFI [carbaryl]} - \text{INTERCEPT}) / \text{SLOPE}
\]

introduces two further errors. The error in NCFI [carbaryl] varies in the range of 1-4 % and the error in the intercept adds approximately 1% to the total error. The total percentage error in the concentration of each solution was calculated on a individual basis, with a mean value being 11%.

The precise error analysis for the rate constants of the carbaryl hydrolysis is a long and complicated procedure as logarithms and then regression analysis are involved. To avoid this the method of calculating maximum and minimum slopes to the graphs was employed. This resulted in an error of approximately 16-25 % in the rate constants and half lives.

The same procedures were applied to estimate the errors for all the 1-naphthol and 1-naphtholate data. All other sources of error, for example, in the concentration of the stock solutions or in the timing of the reactions are insignificant when compared to the errors associated with the spectrofluorimetric measurements. This error analysis assumes that all the errors are positive, that is, they all add to one another. This may account for the seemingly high level of error in the results presented in this report.
References


