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Carbaryl in Aquatic Systems: A Kinetic Analysis of Degradation Rates

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Carbaryl in Aquatic Systems: A Kinetic Analysis of Degradation Rates

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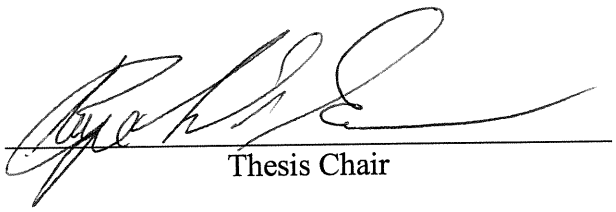
Thesis Committee:



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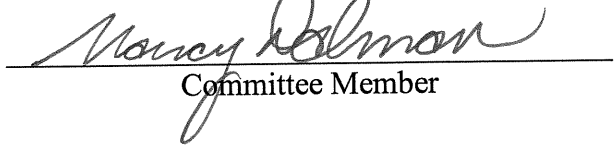

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Abstract:
1-naphthyl methylcarbamate or carbaryl remains one of the most commonly used pesticides. It has neurotoxic properties, thus making it important to understand the long-term effects of its chemical persistence in environmental systems. In this study, fluorescence was used to determine the concentration of carbaryl pesticides in aquatic systems. Samples were collected over a period of 48 hours from an empty fish tank with an initial carbaryl concentration of 28μg/L in both dark and ambient light conditions. Fluorescence was then used to determine the concentration of carbaryl using an emission wavelength of 336nm and an excitation wavelength of 270nm over time. This was a baseline study conducted to be used as comparison values to future experiments on the rate of carbaryl degradation when fish are introduced into the system. Despite photosensitivity, there was only a small difference in the determined rate constants of the light vs. dark trials with carbaryl half-lives of 24.35 and 30.67 days, respectively.

Introduction:

In recent years, pesticide use has become increasingly common. The amount of time these chemicals can stay in the environment, known as chemical persistence, has also become an increasing concern. Carbamate compounds are a specific classification of pesticides commonly used to control insects that live in soil.[1] Carbamates are ubiquitous because they leave low amounts of pesticide residues and are effective insecticides at low application amounts.[2] While carbamates possess many desirable qualities, they are toxic to mammals, birds, and fish. [2] Contamination in water sources can affect humans as well. The Environmental Protection Agency (EPA) specifically classifies carbaryl as being highly toxic to fish in freshwater environments.[3] Carbaryl was also found to be highly toxic to multiple species of earthworms living in contaminated soil, even after extended amounts of time post pesticide contamination.[1] Therefore it is important to understand the way pesticides, specifically carbaryl, interact with the environment and the length of time that they remain in aquatic systems.

Environmental Impact

When released into the air, carbaryl has an estimated half-life of fifteen hours.[4] Due to this relatively short half-life, carbaryl is one of the most commonly used pesticides. However, the half-life is extended in water, which ranges from anywhere from 1.7 to 5.8 days in freshwater to 96 hours in salt water systems.[4] This increased half-life in water makes the exposure time for organisms in aquatic systems much longer. In past testing it was found that rivers flowing from agricultural areas and urban areas had carbaryl concentrations of <0.046-1.5µg/L and <0.15-2.5 µg/L, respectively.[4] These are both well below the EPA standards of 700µg/L in drinking water[4]. The researchers attributed the lower amounts of carbaryl in the agricultural areas to the fact that the environment was only being exposed during planting season and the higher amounts in urban areas to the constant use of carbaryl by the general population as an everyday insecticide in densely populated areas.[4]

Sun light also plays an important role in the effect of carbaryl in the environment. This is due to the photosensitivity of carbaryl's photolytic degradation pathway producing the degradation products of 1,2-naphthoquinone, 1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, and 7-hydroxy-1,4-naphthoquinone as seen in figure 1.[5] Theoretically, light exposure should make carbaryl degrade faster, increasing the degradation rates and lowering the half-life. Carbaryl would therefore degrade slower in areas that don't have a lot of sun exposure. For example, carbaryl would remain in ground water for longer periods of time than in a river system that is exposed to the sun. In the United States, carbaryl levels between 1-500 ng/L have been detected in ground

water when the acceptable amount is 7 ng/L. Whereas the concentrations in river samples range from undetectable to 38ng/L.[6]

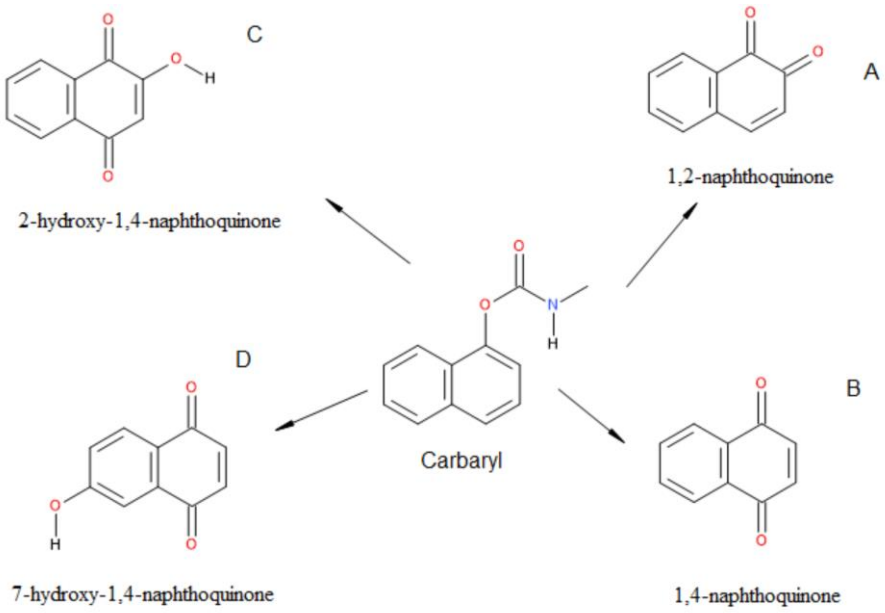

Figure 1: Products of photolytic degradation of carbaryl. The naphthalene ring is hydroxylated and the dihydroxy derivatives get oxidized, resulting in the formation of A and B. Hydrolysis followed by decarboxylation of the oxidized fragments yield hydroxybenzene derivatives, C and D.[7]

Experimental Testing with Kinetics

One way of determining the rate of carbaryl concentration change in a system over time is though chemical kinetics. Kinetics measures the effect that chemical conditions have on the rate of the reaction. Carbaryl undergoes hydrolysis in water because of its carbamate functional group, which acts as both an ester and an amide.[8] The hydroxide ion (OH^-) in the water will displace the carbamate groups forming 1-napthol, methylamine and CO_2.[9] As seen in Figure 2, the concentration of carbaryl should decrease the longer carbaryl is in water due to the formation of degradation products. This mechanism allows for the tracking of carbaryl degradation rates with a UV-vis

spectrophotometer (UV-vis) or fluorescence spectroscopy. By tracking the total absorbance or emissions of the carbaryl response, the concentration change over time can be determined.

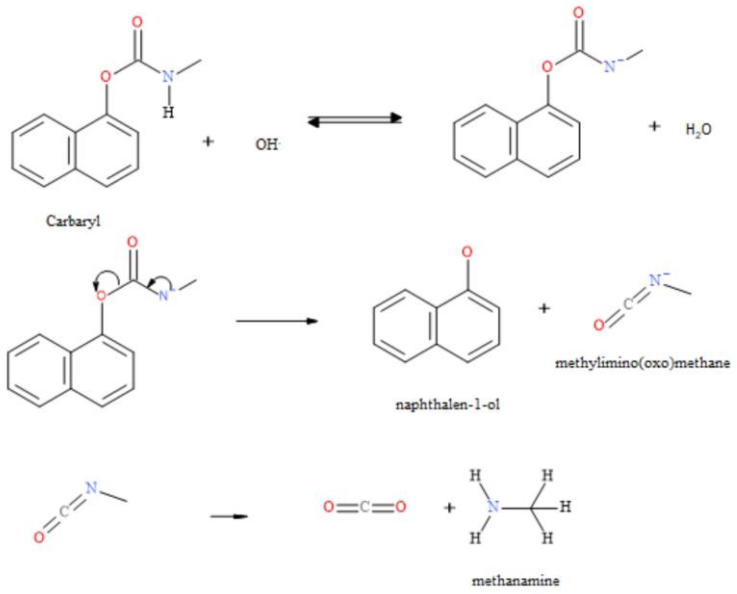

Figure 2: Hydrolysis of Carbaryl shows the decomposition pathway for carbaryl into napththalen-1-ol, CO_2, and methenamine.

Kinetic analysis is useful because it establishes a linear comparison between concentration and time. To determine this relationship, the number of concentration variables in the experiment, known as the reaction order, must be found. There are four common choices for simple reaction orders; zeroth, first, second, or third- order reactions. In each of these the concentration must be manipulated differently to generate a linear relationship.[10] Pseudo first-order kinetics can be used in this case. Carbaryl is being hydrolyzed by water giving two different concentration variables. Usually this would be considered a second-order reaction due to concentration dependence on both water and carbaryl. However since water is the solvent and present in abundance, the water

concentration remains effectively constant. Therefore, first order rate laws can be used in what is known as pseudo first order kinetics.[11] First order kinetics shows linear relationships when the natural log of the concentration is plotted vs time. Linear plots of ln of concentration vs time gives a slope that is equal to the rate constant (k). This constant can then be used along with Equation 1 to then calculate the rate of the reaction from the initial concentration of carbaryl.

$$\text{rate} = k[\text{carbaryl}] \quad (1)$$

Previous work has shown the use of UV-vis to track degradation kinetics of carbaryl. The study used the absorbance of light at 279 nm vs. time to track the degradation rate.[8] This wavelength is at the maximum absorbance for carbaryl, making it the most sensitive wavelength to test for the quantitative data needed for determining reaction kinetics.[8] The graph produced from this data allowed the concentration of carbaryl in the sample to be found based on the absorbance of the sample at any given time. Hawker used a plot of ln (A_t-$A\infty$) vs. Time (s) to compare his trials of carbaryl in water at different pHs in pseudo-first-order kinetic runs with negative rates because the sample was degrading.[8]

Similarly, pseudo first-order kinetics have also been used to calculate the amount of carbaryl in water and vegetable samples.[12] This is another example of how UV-vis has been used to calculate degradation rates and kinetics of carbaryl. It was determined that carbaryl has a weak absorption peak at 279nm in the UV spectrum and its degradation rate was faster than similar pesticides like aminocarb.[12]

Much like UV-Vis, fluorescence is also a useful method for studying reaction kinetics. Fluorescence measures light emitted by the sample after it is excited by a certain

wavelength of light known as the emission and excitation wavelengths respectively. The sample's concentration is directly proportional to the intensity of the emission. Fluorescence has a higher sensitivity for carbamate pesticides than UV-vis methods.[13] Concentrations as low as 10ppb have been detected when using a Xe lamp source or laser induced fluorescence with a fiber optic probe.[13] The relative intensities can be found and the kinetics data can be analyzed using both of these methods. The math for finding this rate constants, rates, and half-lives is the same no matter the method used to gather the data.

Aquatic Life

Testing the kinetics of carbaryl in water shows only the degradation rate changes in the system. To thoroughly assess the effect that aquatic life, specifically fish, has on the system, the effects of carbaryl on fish would also need to be tested. The best method for doing this would be to test how much carbaryl is absorbed by the fish.

One way of seeing how fish affect the rates of carbaryl remediation would be to test tissue samples to see how much is absorbed. Previous studied have compared earthworms (*Metaphire posthuman)* exposed to carbaryl through direct and indirect contact. For indirect and direct exposure, earthworms were placed in contaminated soil or on a piece of filter paper that had been soaked in a carbaryl-acetone solution after allowing the acetone to evaporate, respectively.[1] It was found that the indirect method limited the available concentration for absorption.[1]Similar patterns might hold true for fish. However, testing the effect of direct carbaryl exposure on fish is difficult because they cannot survive out of water. Therefore, the only absorption value for the fish that could be gathered would be from tissue samples of indirectly affected fish. Testing fish

tissue samples for carbaryl or carbaryl metabolites is complex and would require freezing and thawing the samples followed by separation using gas chromatography tandem mass spectroscopy (GC-MS/MS).[14] The GC-MS/MS must be run with a carrier gas of pure helium and electron ionization must be used for mass spectroscopy mesurements.[14] In an effort to simplify that process, a control system without fish was tested to find the degradation rate with planned identical follow-up trials with the addition of a fish. It is proposed that fish absorption values could be estimated based on the change in rates of carbaryl degradation between a tank with the fish and a control without.

This research will focus on the length of time one commonly used carbamate pesticide, carbaryl, better known as Sevin, remains in aquatic environments. Specifically, the research will track the change in concentration of carbaryl over time in a controlled environment with the aim of determining the kinetics with and without *Fundulus heteroclitus* (killifish). Even though outside factors will be controlled, it is important to use conditions that will support a type of fish that is hardy and able to adapt to the environmental changes. Killifish were chosen for this reason. They are capable of living in harsh environments with fluctuating temperature, salinity, and O_2 levels. Therefore, if they show any kind of response to carbaryl, less hardy species will most likely also be affected. Using a starting concentration of 28µg/L carbaryl in water, the LD 50 for the killifish, an environmentally relevant study can be conducted.[15] Fluorescence spectroscopy is used to determine the amount of carbaryl in the system at different intervals, the rate of decomposition of the carbaryl can be determined from this data.

Methods:

A UV-vis calibration curve for carbaryl was made using Thermo Scientific Evolution 300 UV-vis spectrometer and a max absorbance wavelength of 279nm. Then two different sampling and fluorescence techniques were also utilized to collect a calibration and collect data in this experiment, method 1 and 2 below. For both procedures, the max emission and excitation wavelengths were determined, and a calibration created. This was done by measuring the intensity of the fluorescent response of different concentrations of the pure carbaryl solution. The excitation and emissions wavelengths were measured during the calibration and shown to be 270nm and 336nm, respectively. Both techniques started off with a tank containing brackish water, having an approximate Instant Sea Water concentration of 20 g/L and a carbaryl concentration of 28µg/L. Carbaryl solutions were made by dissolving the solid in a small amount of methanol and then diluting with water. All other factors such as temperature and aeration remained constant in both methods unless otherwise noted. All fluorescence data was collected using the Jasco FP 750 instrument.

Method 1

In the first method, the fluorimeter was used with the aid of a peristaltic pump to transfer analyte samples into and out of the cuvette in order to get continuous real-time data. An automated system was set up to collect emission data at a preset time interval of one sample every 350 sec for 250 cycles to give a total run time of 24.3 hours. Instrument parameters were set as follows: emission and excitation wavelengths 336nm and 270nm respectively, low detector sensitivity setting and a two second response time. The tank was placed under a box to control the light exposure to the sample.

Method 2

In the second method, samples were periodically collected and frozen until they were all analyzed using the fluorimeter at once. This method was a two-tank set up. One tank was kept under a box to provide a dark environment and a second under a lamp with a 100-watt LED bright stik. This allowed for the determination of the effect of light on the degradation rate of carbaryl. The instrument specifications for these trials are as follows: emission spectrum with a fast response time, low sensitivity, excitation wavelength 270nm, emissions wavelength of 336nm, and scan speed of 2000nm/min.

Results and discussion:

The limit of detection (LOD) for the UV- vis method was determined to be 0.0545g/l or 54500 µg/l (figure 3) which is almost 2000 times the desired starting concentration. After it was determined using equation 2 that the LOD for the instrument was too high for carbaryl detection at biologically relevant concentrations, calibrations using fluorescence were determined (Figure 4).

$$\text{LOD} = 3\left(\frac{Standard\ Deviation}{Slope}\right) \quad (2)$$

Similar calculations showed the LOD for the fluorimeter to be 4.061µg/L. As shown in figure 5, Carbaryl was found to have a max excitation wave length of 270nm and a max emissions wavelength of 336nm.

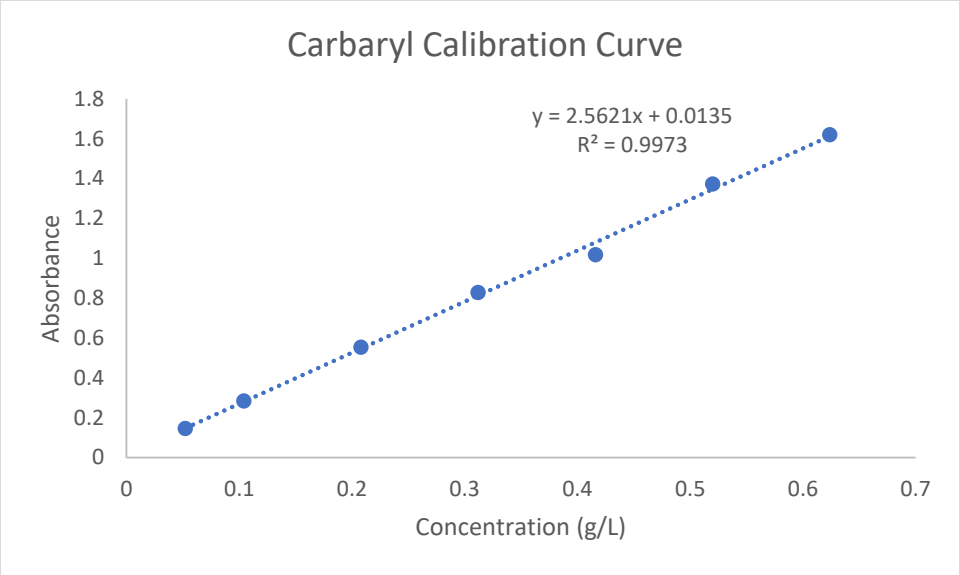

Figure 3: Calibration curve using UV-vis spec, concentration standards between 0.624 g/L and 5.2 mg/L were tested for absorbance at a max wavelength of 279nm.

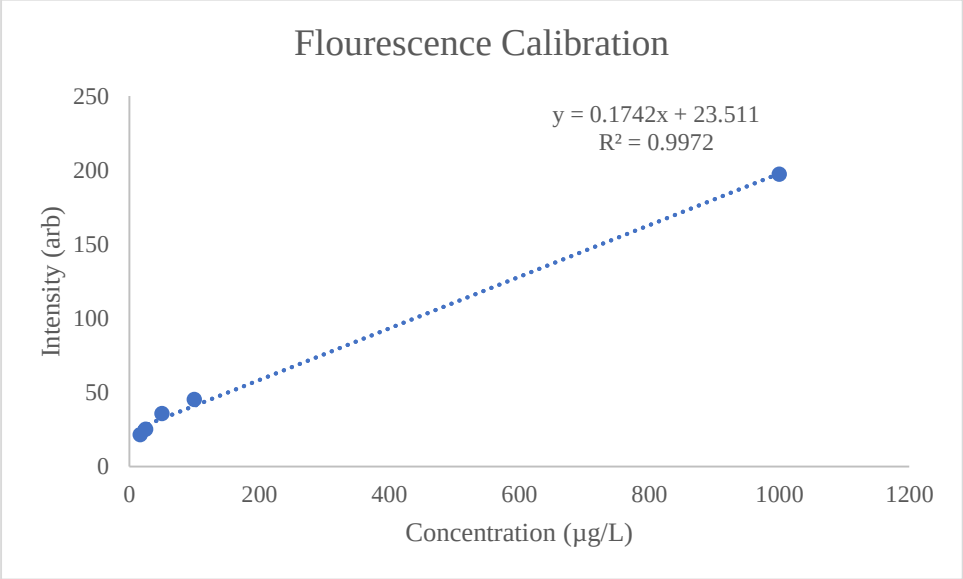

Figure 4: Calibration for fluorescence, concentrations between 1.248 and 41.6 µg/L were used.

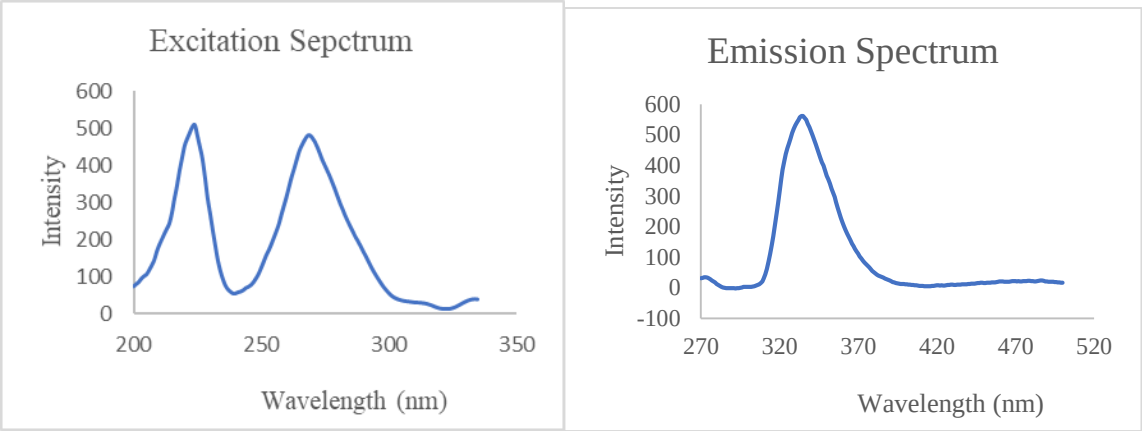

Figure 5: a) Excitation spectrum of carbaryl giving max wavelength of 225nm and 270nm. The 270nm was chosen in favor of known literature values.[13] *b) Emission spectrum of carbaryl at excitation wavelength of 270 nm showing that the max emission wavelength is 336nm*

The kinetics of carbaryl degradation was then determined using method 1 and a plot of ln[carbaryl] vs time is shown in figure 6. A degradation rate of $3.88X10^{-6}$ µg/L a second or $2.33X10^{-4}$ µg/L a minute was found. This was done using the slope of the line found in Figure 6 for the rate constant k in equation 1.

$$rate = 1.39\,X10^{-7}[27.986\mu g/L]$$

Where 27.986 µg/L is the starting concentration. The data as seen in Figure 6, is after box car averaging two above and below each point took place. The overall downward slope shows that degradation was occurring, however since this was such a short time frame, only 24hr, the amount degraded was almost insignificant. Another problem was that the lamp in the fluorimeter was not designed to stay on for this length of time. Therefore, the lamp cycled through periods of greater intensity followed by lower intensity explaining the wave like look of the data. It was for this reason that method two was used, it extended the time of the trial as well as allowed for all the samples to be tested without having fluctuation in the lamp output.

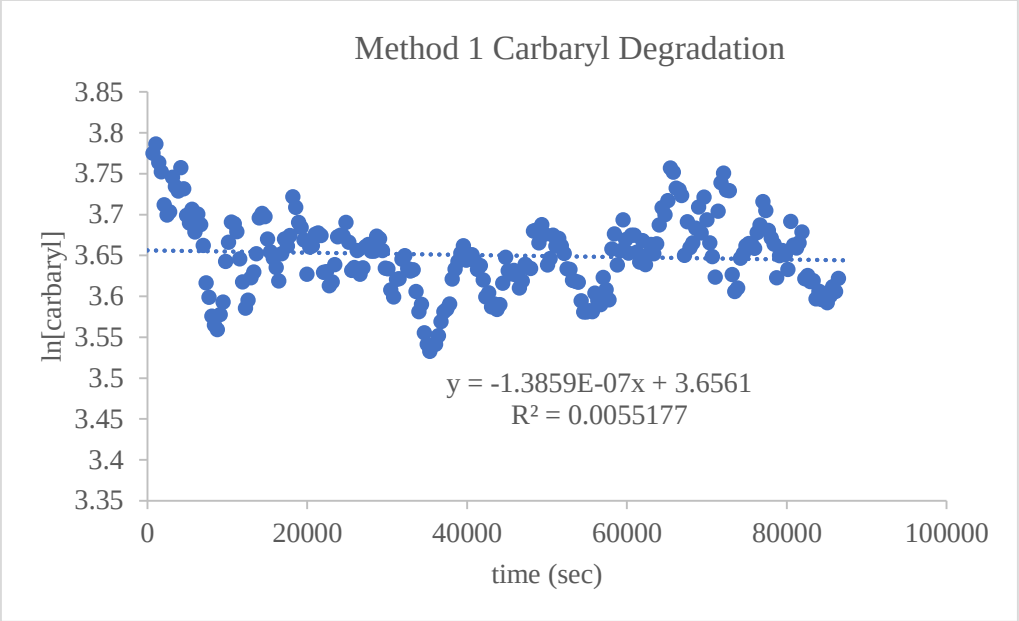

Figure 6: Averaged data for the degradation rate found through method one. This gives a k of 1.3859X10^{-7} /s the sine curve pattern is due to lamp fluctuations not changes in concentration.

In method 2, the degradation rate in the dark was found to be 4.39X10^{-4} µg/L a min. The degradation rate for the reaction under the lamp was found to be 5.53X10^{-4} µg/L a min. This showed that there was still a relatively slow degradation rate, but the sample under the light source did degrade faster due to multiple pathways for degradation (Figure 7). The small variance in the degradation rate between the two trials without light exposure is most likely due once again to lamp fluctuations. Therefore, using equation 3 the half-life of carbaryl can be determined as approximately 24.35 days under light and 30.67 days in darkness. This number has a large error margin because the calibration curves were taken on a separate day than when the data was collected due to time restraints.

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

Previous studies have shown that carbaryl has a half-life of 96 hours in a salt water sample and 1.7 and 5.8 days in freshwater.[4] These values are much shorter than the calculated 24.35 days. A possible reason for the discrepancy could be due to several

factors, carbaryl samples in this study were made from pure carbaryl solids and not the Sevin that is used as a pesticide. Another difference could be that these trials were conducted in brackish water vs. salt or fresh water. It was also found, that carbaryl degrades slower in the dark than under light, as shown in Figure 4. This was expected because the carbaryl is photosensitive and therefore expected to degrade faster when under constant light. This data was collected from one trial therefore more data would be needed to have comprehensive results.

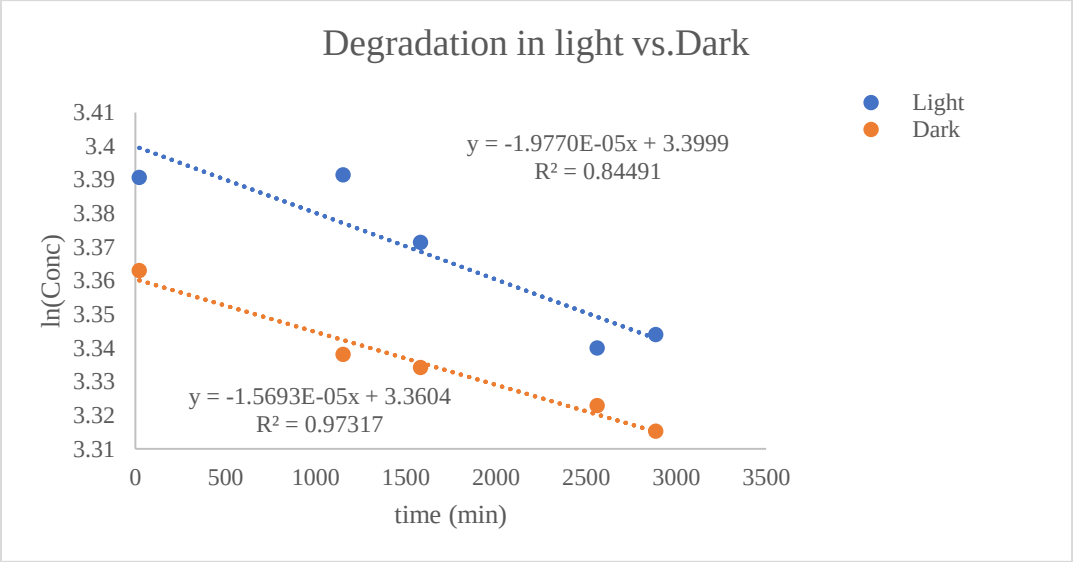

Figure 7: Light to dark comparison of method 2. The orange line is the dark trial which has a k value of 1.569310^{-5} and the blue line is the light trial which has a k value of $1.9770X10^{-5}$ /min

Conclusion:

Over all it was determined that ambient light does play a small role on the degradation rate of carbaryl in a 48hr period and that it degrades in the controlled system slower than expected. The ultimate goal of this study was to compare these experiments with tanks containing killifish. In doing so, the rate will hopefully change and the amount of carbaryl absorbed and/or metabolized by the fish could be estimated.

While it is important to understand the environmental implication of using pesticides and how they affect aquatic life, carbaryl's environmental impact makes it

important to also study the affect that aquatic life has on the pesticide remediation. Previous studies have determined that it would be possible to find the effect that fish have on the remediation of carbaryl in water using a combination of techniques. Since fluorescence spectroscopy proved to be the best method to use to measure the kinetics of carbaryl in water in an empty tank, it could also be used in a tank containing fish to calculate the rate difference.

Many of the previous experiments completed to determine environmental effects were done in one of two ways: a complete ecosystem, or in a controlled environment. When a complete ecosystem was used, samples were collected from rivers and therefore already had the effect of the aquatic life absorption of the carbaryl included in the results. The controlled environments did not account for any outside variables, therefore are not as good of a representation of how carbaryl acts in the real world. By running future test in controlled environments both with and without fish the results should show if the fish in the tank will increase the speed of carbaryl remediation.

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