EVALUATING THE ROLE OF UV EXPOSURE AND RECOVERY REGIMES IN PAH PHOTO-INDUCED TOXICITY TO *Daphnia magna*

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Polyaromatic hydrocarbons (PAHs) are contaminants synthesized through incomplete combustion of carbon based substances. PAHs are known to be photodynamic and toxicity increases exponentially when in contact with ultraviolet radiation (UV). The effect of UV absent recovery periods and potential for latent toxicity during photo-induced toxicity are previously unknown and are not included within the toxicity model. Results of equal interval tests further support the current reciprocity model as a good indicator of PAH photo-induced toxicity. Interval test results also indicate a possible presence of time-dependent toxicity and recovery thresholds and should be included into toxicity risk assessments. Moreover, results of latent effects assays show that latent mortality is a significant response to PAH photo-induced toxicity and should be included into toxicity risk assessments. The present research demonstrates that UV exposure time rate is a significant driving force of PAH photo-induced toxicity.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Photo-Induced Toxicity

An exponential increase in toxicity of a substance due to exposure to light; usually ultraviolent radiation (UV) is known as photo-induced toxicity. Photo-induced toxicity may occur within an organism (photosensitization) or within the environment (photomodification). Photosensitization is thought to be the major contributor to photo-induced toxicity in aquatic systems and will be the focus of this study (Diamond, 2003).

Photo-induced toxicity occurs when an organism absorbs a photodynamic chemical (sensitizer) into its tissues and is simultaneously exposed to ultraviolet radiation within wavelengths between 320 to 400nm, known as the UVA region (Allred, 1985). An electron promotion is initiated within the sensitizer due to the available light energy causing the molecule to transition from ground state into an excited singlet or triplet state. When the molecule returns from excited state back to ground state the energy is released into the biological matrix. The available energy may induce a Type I reaction when it reacts with biomacromolecules causing the production of reactive oxygen species (ROS) or the energy may induce a Type II reaction where it reacts with oxygen causing oxidative stress to surrounding tissue due to singlet oxygen formation (Diamond, 2003). Due to ROS production, surrounding cells are damaged via lipid peroxidation (Choi, 2000). The overall effect of photo-induced toxicity is largely dependent upon the amount of sensitizer exposed, duration of UVA exposure, and UV

irradiance. Photo-induced toxicity may also be dependent upon the species tested as well as the age of the organism (Landrum, 1987; Peachey, 1996).

Photodynamic compounds include both natural and anthropogenic compounds that range in potency. In the environment, natural substances are quickly broken down, and do not pose additional threats to ecosystems. However, many anthropogenic substances do not break down readily and accumulate within the surrounding land and water systems within an ecosystem. When ecological resources are contaminated with photodynamic compounds there is a threat of photo-induced toxicity to the organisms within the ecosystem. One of the first studies designed to investigate photo-induced toxicity potential between chemicals was performed in 1939 where researchers sought to evaluate toxicity of *Paramcecium caudatumn* when exposed to light as well as series of individual compounds known to be photodynamic. Of these compounds the polyaromatic hydrocarbon (PAH), 3:4-benzopyrene, was found to be a thousand times more potent than the other non-PAH compounds tested (Doniach, 1939). This study provided evidence that some PAH compounds have potential to be highly phototoxic.

Polycyclic Aromatic Hydrocarbons

Polyaromatic hydrocarbons are a class of compounds formed through the incomplete combustion of carbon based substances such as coal, petroleum, natural gas, wood, etc. PAHs are composed of multiple aromatic rings found in over 100 different compounds; however, almost always occur as complex mixtures when in the environment (Diamond, 2003). There are wide physical, chemical, and toxicological differences between PAH compounds but they are all found to be highly lipophilic

having logarithmic octanol-water partition coefficients (log K_{ow}) values of 3.37 – 6.75 (Douben et al., 2003). Because of high lipophilicity PAHs typically have a high binding affinity to soil and dust particles. These particles cycle through the environment until eventually settling within an aquatic ecosystem.

Though there are both natural and anthropogenic sources of PAHs, dependence on fossil fuels energy accounts for a significant source of PAH emissions. Concentrations of PAHs in North American and European wastewater treatment plants (WWTPs) range from < 1µg/L to over 625 µg/L depending on proximity to oil drilling or refineries. WWTPs primarily used for domestic purposes contain PAH concentrations typically less than 5 µg/L (Fathallah et al., 2012). PAHs contribute to the some of the most highly chemically contaminated sites in the world and often PAH concentrations in aquatic environments are found to be well above those shown to cause significant toxicological damage (Diamond, 2003).

Furthermore, because of the high binding affinity to sediments, aquatic ecosystems serve as contaminant sinks for PAH accumulation and pose a potential risk to the ecology of the waterbody. PAHs have been found within tissue of exposed organisms at levels much higher than those found in the external environment with bioconcentration factors (BCFs) ranging from 10-10,000 (Cho, 2003; Douben et al., 2003). In aquatic environments, PAHs become available to organisms through multiple exposure routes. PAHs may be trophically transferred when contaminated sediment or prey is ingested. Also, PAHs suspended within the water column are transferred into tissue across the mucus layer of the gills of aquatic organisms allowing the lipophilic compounds to accumulate within gill tissue in high concentrations (Weinstein et al.,

1997). Many life history trait factors influence uptake and elimination of PAHs such as organism size, temperature, lipid content, and behavior as well as changes in environmental changes and degree of PAH contamination (Douben, 2003)

PAHs are widely used as model compounds to study photoactive chemicals and the mechanisms of photo-induced toxicity. By the 1980's, researchers began realizing the potential impact PAH photo-induced toxicity could have on natural areas, specifically aquatic environments. To understand the potential for photo-induced toxicity the conditions allowing for the phenomena to take place must be determined. Studies were designed utilizing natural sunlight and artificial sunlight to determine the photo-induced toxic effect of anthracene to species of sunfish (Bowling et al.,1983; Oris et al., 1985). In both studies the researchers tested the effect of organisms exposed to both anthracene and UV, organisms exposed only to UV, organisms exposed only to anthracene only, and organisms exposed to anthracene allowing a 144-hour depuration period prior to UV exposure. Results of both studies showed high mortality in organisms exposed to UV combined with anthracene; however, no mortality was observed in organisms exposed to anthracene only or UV only. Additionally, organisms exposed to anthracene and allowed to depurate survived once exposed to UV. These findings demonstrate the critical role of both PAH and UV doses within photo-induced toxicity as well as the potential for recovery when PAHs are metabolically removed during UV absent periods. Similar results were found when the effect of PAH contaminated sediments was evaluated using benthic invertebrates exposed to artificial UV (Ankley, 1994). One study reviewed PAH photo induced toxicity data from previous studies and evaluated effects across an abundance of aquatic organisms such as fish, insects,

microcrustaceans, phytoplankton, and bacteria (Landrum et al., 1987). The analysis revealed the addition of UV exposure to PAH may increase previously determined no effect concentrations of PAH up to 400%. Results also conclude that the prime parameters for photo-induced toxicity are UV and the parent PAH compound rather than photodegradation products and this has been supported in other studies as well (Allred, 1985).

PAH photo-induced toxicity varies depending upon the photodynamic properties and concentration of the PAH compound as well as the intensity and duration of UV received. Within an ecosystem, exposure to PAH may vary due to mobility of the organism or the physical dispersal and spread of the chemical due to natural forces such as wind, waves, or velocity (Douben et al., 2003). Additionally, exposure to UV may differ in aquatic ecosystems due to pigmentation an diet of organisms as well as habitat parameters like cloud cover, shade availability, or depth and turbidity of the water column (Douben et al., 2003; Gevertz et al., 2012; Seckmeyer et al, 2008).

PAH Photo-Induced Toxicity Modeling

There is a large range of photodynamic responses of PAHs greatly due to differences in chemical properties of each compound. A method to predict the degree of response is needed for determination of which PAH compounds are photodynamic and at what dose causes biological harm. Researchers have created an index of potency (RPA) value to classify the photodynamic potential of the different PAHs (Oris et al., 1987; Newsted et al. 1987). RPA value is defined as the index of photodynamic activity in comparison to Benzo [b] anthracene which exhibits a median level of potency when

compared to other PAH compounds. Using the calculated RPA values the photodynamic potential of the different PAH compounds have been classified as nontoxic, moderately toxic, and very toxic.

In addition to potency, understanding physical properties of PAH compounds also provides insight of individual photodynamic potential. Researchers have shown that measuring energy differences between ground state and excited state of PAH compounds can serve as a valuable quantitative structure-activity relationship (QSAR) in predicting photodynamic potential (Newsted et al., 1987; Veith et al., 1995). The energy differences between the highest occupied molecular orbital and the lowest occupied molecular orbital is knowns as HOMO-LUMO gap. The HOMO-LUMO gap differences of 6 parent PAH compounds was measured and results showed this to be a good indicator of molecular stability and the ability of the compound to absorb light (Veith et al., 1995). The PAH compounds with a HOMO-LUMO gap between 6.7-7.5 eV were determined to be photodynamic. Additionally, this study analyzed how their derivative PAH compounds may affect the gap. Daughter compounds showed to have little effect on gap size; however, results did suggest that a showed how a possible additive effect of photo induced toxicity may occur within PAH mixtures with parent compounds which are photodynamic. Because PAHs are typically found in mixtures it is important to consider the interactions each compound may exude when combined. Studies comparing single PAH compounds to binary, tertiary, and quaternary mixtures have found photo-induced toxicity to be consistent in additive effects (Erickson et al., 1999, Willis et al. 2014). Using the concentration addition model, results show as a PAH

compound mixture becomes more complex, photodynamic potential will additively increase based on the potency of each individual PAH within the compound.

Predictions of photo-induced toxicity require the use of a model based on the known parameters which determine toxic effect. The foundational photo-induced toxicity model is based on a study which evaluates the effect of light and oxygen on mortality of a bacteria, *Rhodopseudomonas spheroide* (Dworkin, 1957). The author developed this reciprocity model based on the Bunsen-Roscoe law of photochemistry (Bunsen et. al, 1862) which states that a photochemical response may be predicted by calculating the product of radiation intensity and exposure time. The reciprocity model is applied to most PAH photo-induced toxicity models stating that photo-induced toxicity is a product of PAH exposure, UV intensity, and UV exposure time. Researchers investigated the predictive potential of the reciprocity model using benthic and free swimming aquatic vertebrates and invertebrates (Ankley et al., 1995; Oris et. al, 1985). However, it was observed that at high light intensities the model may over predict toxicity and at low light intensities it may under predict toxicity. Discrepancies in the predictive capability of the model have been suggested to be due to biological recovery and fluctuations in UV exposure. These discrepancies are more likely to be present in open air environments where recovery rates and UV exposure are constantly changing.

In a PAH contamination event, the compounds are most likely found in mixtures which have been shown to interact in an additive manner; therefore, it is ecologically relevant to develop a predictive model which incorporates PAH mixture estimations. A PAH photo-induced toxicity model was developed to estimate quantitative risk specifically for PAH mixtures and compared expected LT_{50} values to actual LT_{50} (Sellin

et al., 2013). This research is unique because it incorporated whole body tissue PAH concentration, relative potency (RPA) values, and UV intensity. Because the model utilizes RPA values as well as exposed concentrations of the PAHs potency could be accurately calculated within the PAH mixtures. The model estimated results which fell within confidence limits of low PAH concentrations; however, it exhibited a trend to underestimate risk at high concentrations when compared to actual results. The study illustrated the complexity of assessing actual environmental exposure of organisms based on environmental factors and distribution of contaminants. The authors suggest that photo induced toxicity effects may be different depending on the migration of organisms within the water body due to various exposure to PAH and UV.

PAH and UV doses must be accurately estimated to predict PAH photo-induced toxicity. Researchers have incorporated RPA values to individual PAH compounds present in mixtures and integrated the anthracene equivalent PAH dose with the UV dose (irradiance · time) to calculate overall phototoxic dose. Researchers tested the PAH phototoxic dose model to evaluate effects to blue crab (Callinectes sapidus), mahi-mahi (*Coryphaena hippurus*), red drum (*Sciaenops* ocellatus), and speckled seatrout (*Cynoscion* nebulosus) following the Deepwater Horizon oil spill (Alloy et al., 2015; Alloy et al., 2016; Alloy et al., 2017). Phototoxic dose is a function of molar PAH concentrations with incorporated RPA values compared to anthracene and the integrations of irradiance at λ=380nm within the UVA region (Equation 1). The model proved sufficient at calculating overall dose of both PAH and UV combined when PAH dose and UV intensity are known or estimated.

Equation 1.1. Phototoxic dose= anthracene equivalent(uM/L) · mWs/cm^2

In a natural setting, UV exposure time is a crucial parameter when determining overall UV dose. An organism may experience constant changes in UV exposure time due to physiological traits or habitat parameters offering UV relief (Gevertz et al., 2012; Seckmeyer, 2008). PAH contaminated aquatic ecosystems which have little to no UV relief and those which receive consistently high amounts of UV exposure are at increased risk of photo-induced toxicity (Diamond, 2006; Peachey, 1996). It is believed that once an organism can find refuge from UV exposure, photo induced toxicity ceases and recovery mechanisms may work to alleviate biological damage in UV absence (Bowling et al.,1983, Oris et. al, 1985). It is important to determine if equal effects are seen when total UV exposure is divided into UV absent intervals compared to when UV is exposed at a constant rate.

Post-exposure effects of PAH photo-induced toxicity may occur even after an organism is removed from exposure. Latent effects including mortality and decreased reproductive capability could be potentially significant following an acute PAH photo-induced toxicity exposure. Studies have shown that a delayed mortality may be experienced at sub-lethal PAH and UVA following photo-induced exposure (Ankley, 1995, Oris et al., 1985). However, no studies have specifically investigated these responses following acute exposure.

Latent mortality may be due to gill tissue damage occurring during the exposure causing respiratory distress and ultimately death (Oris, 1985; Weinstein, 1997). If latent mortality is a significant response following a PAH photo-induced toxicity, it should be

included into risk assessment. Additionally, a decrease in reproductive capability may be a significant sub-lethal response to photo-induced toxicity. Using *Daphnia magna,* research has shown a 69% reductions of neonates produced throughout the 21-day lifespan of the organism when chronically exposed to sub-lethal amounts of fluoranthene and UV (Holst et al., 1989). An ecosystem could face major disruptions in population and community structure if reproductive capability is significantly impacted by acute PAH photo-induced toxicity.

Currently, no research has tested the impact defense and recovery mechanisms may have on PAH photo-induced toxicity. Because recovery is expected to occur during times of no UV exposure, understanding its impact in an ecological perspective may alleviate discrepancies seen in risk prediction. This research was designed to evaluate the role of biological repair during a PAH photo-induced toxic event. The first objective evaluates the role of recovery in *D. magna* during time of UV absence. The second objective evaluates latent effects of mortality and reproduction to *D. magna* following an acute PAH photo-induced toxic exposure. This research allows us to better understand the overall significance of UV exposure and UV absence during photo-induced toxicity.

CHAPTER 2

EVALUATING THE ROLE OF UV AND RECOVERY IN PAH PHOTO-INDUCED TOXICITY TO *Daphnia magna*

Introduction

Polycyclic aromatic hydrocarbons (PAH) are contaminants composed of two or more fused benzene rings. PAH are formed by the incomplete combustion of carbon based substances such as fossil fuels, wood, etc. PAH can be found in over 100 different compounds; however, when in the environment almost always occur as complex mixtures (Diamond, 2003). There are wide differences in PAH chemical properties but they are all found to be highly lipophilic (Douben, 2003). Because they are hydrophobic, PAHs typically have a high binding affinity to soils and sediment in the environment and are known to accumulate within the tissues of exposed organisms with bioconcentration factors ranging up to 10,000 (Cho, 2000). Environmental ranges have been found from undetectable limits to >625 µg/L in WWTPs (Fathallah, 2012).

PAH contaminated aquatic ecosystems which receive high amounts of UV exposure are at risk of photo-induced toxicity (Diamond, 2003). Photo-induced toxicity occurs when co-exposure to ultraviolet radiation increases the toxicity of an environmental contaminant. Photo-induced toxicity initiates when an organism with accumulated PAH is simultaneously exposed to UV. The photoreaction increases reactive oxygen species (ROS) resulting in oxidative stress to tissues in gills and liver (Choi, 2000). The magnitude of the effect is largely dependent upon the concentration and potency of the PAH as well as the duration and intensity of the UV exposure

(Bowling et al., 1983; Oris et al., 1985). The effects of photo-induced toxicity are known to impact the health and survival of fish and invertebrates (Landrum et al., 1987).

Models which attempt to predict photo-induced toxicity are generally composed of parameters which include potency and concentration of the PAH and duration and intensity of the UV exposure (Alloy et al., 2015; Sellin et al., 2013; Willis et al., 2014). Injury is generally described as a product of the reciprocal interaction between the PAH and UV parameters. Investigations of the predictive potential of these reciprocity models using various aquatic vertebrates and invertebrates have found generally good agreement between measured and predicted values (Ankley et al., 1995; Oris et. al, 1985). However, it has been observed that at high light intensities, these models may overestimate toxicity and, at low light intensities, underestimate toxicity (Bowling, 1983; Oris et. al, 1985; Wernersson, 1998). This suggests that variation in the rate of the UV dose or rest/recovery periods may add additional uncertainty.

It has been shown that an organism may metabolically remove PAH when UV and PAH exposure are absent; thereby, avoiding photo-induced toxicity once exposed to UV (Bowling, 1983; Oris et al, 1985). Additionally, pigmentation or a diet composition may increase or decrease sensitivity to toxicity (Gevertz, 2012; Wernersson, 1998). Organisms may also avoid toxicity if they can find refuge from exposure. UV exposure may be relieved by habitat parameters such physical shade or turbidity and depth of the water column (Douben et al., 2003; Seckmeyer, 2008). Therefore, because UV exposure time is a crucial parameter when evaluating the potential for photo-induced toxicity, it is important to determine if “rest” periods or the rate the UV dose is

administered affect the potential for toxicity. A slow dosing rate or intermittent rest periods may allow time for repair mechanisms to alleviate biological damage.

To accurately predict ecological risk of PAH photo-induced toxicity, it is important to consider latent responses to PAH photo-induced toxicity following varied UV exposures. Evidence of sub lethal toxicity such as immobility, spiral swimming, and erratic twitching have been recorded in previous studies (Ankley et al., 1995; Wernersson et al., 1997). Additionally, reproductive capability has been shown to significantly reduce when an organism receives chronic exposure to PAH and UV simultaneously (Holst et al., 1989). However, the long-term effects (e.g. latent mortality or reduced fecundity) of a short-term acute exposure followed by relief have not been previously documented.

Latent or long-term toxic response following a phototoxic exposure should be important considerations in ecological risk assessments for PAH photo-induced toxicity. If significant mortality occurs following initial a short-term PAH phototoxic exposure, risk assessments based on initial response may underestimate overall mortality. Reproductive disruption may also be a significant response to PAH photo-induced toxicity which could have dramatic effects on population and community structure following a short-term event.

Currently, it is unknown if recovery periods have a significant effect on overall PAH photo-induced toxicity and the accuracy of the current reciprocity model. Also, the role that latent effects may have is also unknown. The goals of this research are twofold; (1) evaluate the effects of rest periods during UV exposure on photo-induced toxicity to *D. magna* and (2) evaluate potential long-term effects following short

exposures to UV and PAH in *D. magna.* This research allows for a better understanding of how recovery periods may affect toxicity following exposure to PAH and UV.

Methods and Materials

Organisms

The test species used for this study was *Daphnia magna* which were cultured individually in 50ml beakers. Organisms were cultured for a total of 21 days where they were rotated weekly into a neonate generation, 1 week old generation, and 2 week old generation. Each generation was composed of 20 individually separated organisms. The organisms were kept in lab prepared reconstituted hard water which was renewed every other day. Organisms received a photoperiod of 16hrs artificial light and 8hrs darkness. The organisms were fed green algae, *Selenastrum capricornutum,* and neonates were removed daily. Neonates from healthy broods were used to begin new generations each week. For this study, only neonates <24 hr old were used to begin assays and organisms were not fed prior to phototoxic exposure.

Test Chemical

Due to known photosensitivity, the PAH compound used for the study was fluoranthene purchased from Sigma Aldrich. Fluoranthene has a molecular weight of 202.3 g/mol and anthracene equivalent RPA value of 0.447 uM/L (Sellin et al., 2013). Based on mortality trends seen in preliminary work, targeted fluoranthene concentrations in all assays was 0.5ppb, 1ppb, 2ppb, and 4ppb.

To create spiking standards, fluoranthene was dissolved from neat powder form into toluene to create a 10,000ppm stock solution. A sub-stock of fluoranthene was

created at 100ppm in hexane and a 1ppm stock in acetone was used to spike sample water. For all assays, fluoranthene was diluted at target concentrations 0ppb, 0.5ppb, 1ppb, 2ppb, and 4ppb. Serial dilutions were conducted prior to the initial 4ppb spike. Spiking stocks were remade prior to each assay.

Lighting System

The UVA exposure was administered indoors using 45.75” AgroMax UV-A + 10,000K Spectrum bulbs which were hung in a ballast 58” above the test dishes. A Biospherical Instrument BIC radiometer and Logger Lite (version 1.3.0) were used to monitor UVA irradiance at λ= 380nm from start to finish of each test (BioSpherical Instruments, San Diego, CA; Vernier, Beaverton, OR).

UV/Recovery Interval Bioassays

The first goal of this research was to evaluate the significance of biological recovery during times of UV absence when phototoxic doses are equal but interval times of UV exposure differ in duration and frequency. Because the overall phototoxic dose was equal in all assays, the phototoxic effect was expected be equal across all assays regardless of no-UV recovery intervals.

A series of five 48hr assays of equal phototoxic dose were designed where each assay received UV and no-UV recovery intervals of different duration and frequency (see Figure 2.1). Each test received a total of 24hrs UV exposure and 24hrs no-UV recovery. Additionally, fluoranthene concentrations were the same in all tests at 0ppb, 0.5 ppb, 1ppb, 2ppb, and 4ppb. Fluoranthene concentrations were monitored using analytical methods at 0hrs, 12hrs, 24hrs, and 48hrs. Test solutions were renewed at 24hrs. Mortality was checked at the 24hr and 48hr test time.

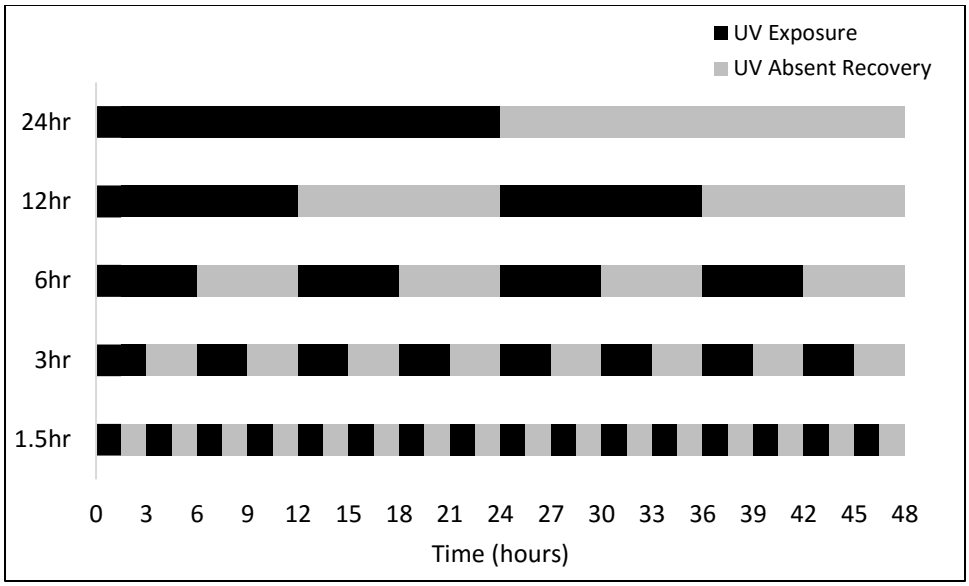

Figure 2.1 UV exposure and no-UV recovery time intervals. Each test received equal amounts of 24hr UV exposure and 24hr no UV recovery intervals.

At the beginning of each test *D. magna* were placed into fluoranthene exposed water in 250 ml crystallizing dishes and immediately exposed to UV. In the first assay, UV exposure was one long interval of 24hrs followed by a 24hr no-UV recovery period (see Figure 1.). The second assay incorporated a UV exposure of 12hrs followed by 12hrs no-UV recovery, another 12hrs of UV, and a final 12hrs of recovery. The third assay followed the same pattern of equal UV exposure and no-UV recovery periods except the intervals were provided in 6hr time frames. The fourth assay was performed in 3hr intervals following the same previous patterns. Finally, the fifth assay was performed in 1.5hr intervals.

Latent Effects Bioassay

The second goal for this research was to evaluate the potential for latent effects in *D. magna* following an acute PAH photo-induced toxic exposure. Latent mortality and reproduction were monitored throughout a 21-day period to determine the organism's ability to recover once being exposed to fluoranthene and UV in various doses.

A total of six tests were performed at different phototoxic doses where target fluoranthene concentrations were equal at 0, 0.5ppb, 1ppb, 2ppb, and 4ppb and UVA exposure durations varied across assays with 0.5hr, 1hr, 2hrs, 4hrs, 8hrs, and 16hr. There were ten individuals tested in each of the four replicates. Analytical samples were taken prior to each exposure. Test media was distributed into 250 ml crystallizing dishes and organisms were immediately placed into the dishes and under the appropriate UV treatment. Organisms were kept in clean 250 ml crystallizing dishes and received daily feedings and water renewals. Following the phototoxic exposure, organisms were immediately assessed for mortality then transferred to clean water. The organisms were maintained for 21 days under white light with daily mortality and reproduction recorded daily. Because of differences in exposure time, each assay has a slightly different total test time with the lowest 0.5hr assay having a total time exposure of 504.5hrs and the highest 16hr assay with a total time of 520hrs.

A separate reproduction assay was conducted following where ten organisms were individually exposed to a PAH phototoxic dose and were maintained individually to avoid competition for resources. Three assays were designed where fluoranthene was spiked only at 0 and 4ppb and analytical samples were taken prior to each exposure. Each assay had a separate UVA exposure duration of 0.5hr, 1hr, and 2hrs. Test media was distributed in 250ml crystallizing dishes and organisms were immediately placed into dishes and exposed to the associated UVA duration. Following exposure, organisms were kept for 21 days in clean 250ml crystallizing dishes under white light and received daily feedings and water renewals. Mortality and reproduction were recorded daily.

Quality Control

Water quality of RHW was inspected prior to each test start and when water was to be renewed. Water quality of RHW was inspected frequently and during water changes. Water quality parameters included were temperature, dissolved oxygen, conductivity, salinity, and total ammonia.

Analytical Methods

A gas chromatograph mass spectrometry (GCMS) SIM analysis was performed to monitor fluoranthene exposure concentrations. Samples were analyzed for both control and high fluoranthene concentrations in UV exposed treatments and UV absent treatments. Samples were collected in 10ml volumes with two replicates per concentration. Using known fluoranthene concentrations and a deuterated fluoranthene standard purchased from Sigma-Aldrich a ten-point standard curve was created to compare test fluoranthene concentrations.

Using methods described in previous studies and C-18 cartridges purchased from Sigma-Aldrich, a solid-phase extraction was used to collect fluoranthene from sample water (Martinez et al., 2004). Cartridges were conditioned with 5ml ethyl acetate, followed by 5ml methanol, and then 5ml milliQ water. Next, the 10ml samples were spiked with internal standard and added to the appropriate cartridge. Cartridges were rinsed with 5ml milliQ water and allowed to vacuum dry before being eluted with 3 repetitions of 400µl ethyl acetate into large amber vials. A constant vacuum flow rate of 5ml/min was used for the extraction procedure. Samples were evaporated using nitrogen blow down and then were reconstituted with 110µl hexane. Samples were evaluated using GCMS SIM methods created.

Statistical Analysis

In this study, only one PAH compound was used, therefore, only the molar concentration of fluoranthene was multiplied by its corresponding RPA value (0.447 uM/L) to generate the anthracene equivalent concentration value (Sellin et al., 2013). UVA exposure is reported as the integration of UVA (time and irradiance) at a resolution of 1 second and has units in uM/L · mWs/cm^2. The overall phototoxic dose of each assay was calculated as a function of the anthracene equivalent value of fluoranthene and the integrated UVA dose (Equation 2.1) (Alloy et al., 2015).

Equation 2.1. Phototoxic dose= anthracene equivalent(uM/L) · mWs/cm^2

SAS JMP software (version 10.0.1) was used to generate LC_{50} values and corresponding confidence intervals (SAS, Cary, NC). A logistic fit curve was plotted with dose on the x axis and response (both dead and alive) on the y axis. Inversion prediction was set at 0.5 to determine LC_{50}s and confidence intervals.

A Mann-Whitney U Test was conducted using IBM SPSS (version 20) to determine differences in reproduction (alpha =0.05) (IBM, Dallas, TX).

Results

UV/Recovery Interval Bioassays

Water quality parameters of RHW used for exposure media remained within normal ranges in all assays. The mean measured concentration of fluoranthene in the highest test concentration was 3.5ppb (+/- 0.23 SD); therefore, serial diluted

concentrations were estimated to be 1.8ppb, 0.9ppb, and 0.4ppb. The anthracene equivalent RPA value in the high fluoranthene concentrations was 0.008 uM/L. The integrated UV dose was relatively equal in all assays with a small range of 4418.0-4449.6 mWs/cm^2. In all assays, phototoxic dose ranges from 4.3 uM/L · mWs/cm^2 in the low fluoranthene concentration to 34.7 uM/L · mWs/cm^2 in the high concentration.

There were no significant differences in 24hr LC_{50}s between the 1.5hr, 3hr, and 6hr UV interval assays (Table 2.1; Figure 2.2a & 2.2b). In the 12hr interval assay, the 24 hour LC_{50} was 1.0ppb (95% C.I. 0.9-1.1ppb) which is significantly less than those in the 1.5hr, 3hr, and 6hr assays at 24hrs. In the 24hr interval assay, the 24hr LC_{50} was 0.5ppb (95% C.I. 0.3-0.5ppb) which is up to 65% less than the values in all other assays. However, in the 24hr interval assay at test time of 24hrs, the phototoxic dose was twice the dose of the other four tests due to receiving twice the amount of UV.

Test Duration (hrs)	UVA/no-UVA Interval Time (hrs)	LC_{50} (95% CI) (ppb)
24	1.5	1.3 (1.3-1.3)
	3	1.3 (1.1-1.5)
	6	1.4 (1.2-1.5)
	12	1.0 (0.9-1.1)
	24	0.4 (0.3-0.5)
48	1.5	0.5 (0.4-0.5)
	3	0.5 (0.4-0.5)
	6	0.8 (0.7-0.9)
	12	0.6 (0.5-0.7)
	24	0.2 (0.1-0.3)

Table 2.1 LC50 values for UVA/no-UVA interval assays 1.5hr, 3hr, 6hr, 12hr, and 24hr at test time 24hrs and 48hrs.

At 48hrs, no significant differences in LC_{50}s were observed between the 1.5hr and 3hr interval assays. In both the 1.5hr and 3hr interval assays, the 48hr LC_{50}s were

approximately 60% of their associated 24hr LC_{50}s. In the 6hr interval assay, the highest 48hr LC_{50} was seen at 0.8ppb (95% C.I. 0.7-0.9ppb) and was more than 35% higher than the values in the 1.5hr and 3hr interval assays at 48hrs. The 48hr LC_{50} in the 6hr interval assay was 40% of its associated 24hr LC_{50}. In the 12hr interval assay, the 48hr LC_{50} was about 40% of its associated 24hr LC_{50}. The 48hr LC50 in the 12hr interval assay was found to have no difference in LC_{50} value compared to 1.5hr, 3hr, & 6hr assays and fell between these high and low values. In the 24hr interval assay, the 48hr LC_{50} was 0.2ppb (95% C.I. 0.1-0.3ppb) which is up to 75% less than the values at 48hrs in all other assays. At 48hrs in the 24hr interval assay, the LC_{50} value was slightly less than the value at 24hr.

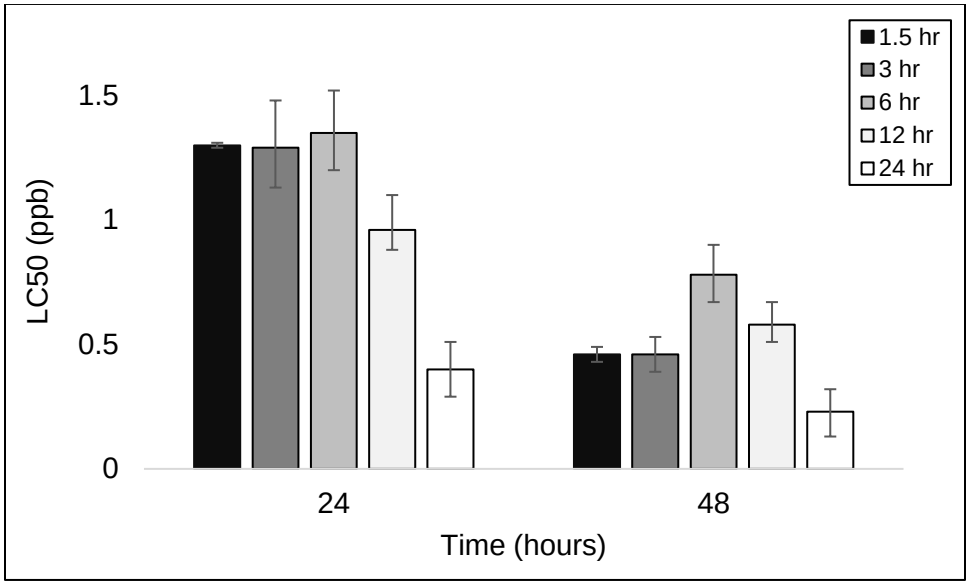

Figure 2.2a LC50 values with 95% confidence intervals of 1.5hr, 3hr, 6hr, 12hr, and 24hr interval assays at 24hrs and 48hrs.

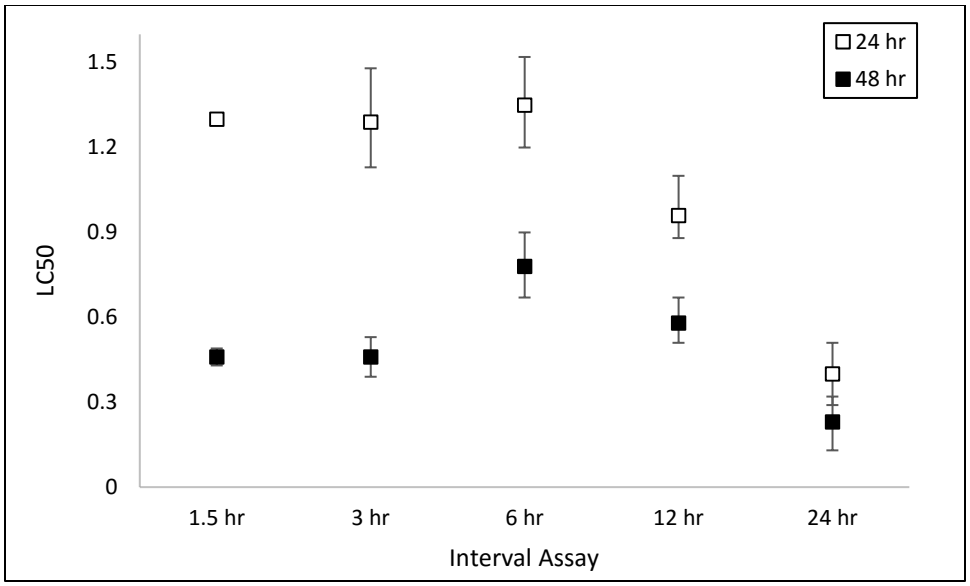

Figure 2.2b LC50 values with 95% confidence intervals of 1.5hr, 3hr, 6hr, 12hr, and 24hr interval assays at 24hrs and 48hrs.

Latent Effects Bioassay

Targeted fluoranthene concentrations in the latent effects bioassays were the same as in the UV/recovery interval bioassays. Water quality parameters of RHW used for exposure media and water changes remained within normal ranges in all assays. The mean measured concentration of fluoranthene in the highest test concentration was 4.8ppb (+/- 0.2 SD); therefore, serial diluted concentrations were estimated to be 2.4ppb, 1.2ppb, and 0.6ppb (Table 2.2). The integrated UV dose ranged from 90.1 mWs/cm^2 in the 0.5hr exposure assay to 2770.9 mWs/cm^2 in the 16hr exposure assay. Because UV duration ranged between individual tests, there was a range of phototoxic doses received. Due to reciprocity, many of the phototoxic doses are equal where UV and fluoranthene exposure are in proportionate doses. The phototoxic dose ranged

from 0.09 uM/L · mWs/cm^2 in the low 0.5hr/0.6ppb treatment to 27.7 uM/L · mWs/cm^2 in the high 16hr/4.8ppb treatment.

Exposure Time (hrs)	Integrated UVA (mWs/cm^2)	Anthracene Equivalent (uM/L)	Phototoxic Dose (uM/L · mWs/cm^2)
0.5	90.1	0	0
		0.001	0.1
		0.003	0.3
		0.005	0.5
		0.01	0.9
1	189.0	0	0
		0.001	0.2
		0.003	0.6
		0.005	0.9
		0.01	1.9
2	383.6	0	0
		0.001	0.4
		0.003	1.2
		0.005	1.9
		0.01	3.8
4	729.3	0	0
		0.001	0.7
		0.003	2.2
		0.005	3.6
		0.01	7.3
8	1510.3	0	0
		0.001	1.5
		0.003	4.5
		0.005	7.6
		0.01	15.1
16	2770.9	0	0
		0.001	2.8
		0.003	8.3
		0.005	13.9
		0.01	27.7

Table 2.2 Phototoxic dose with associated parameters integrated UV (mWs/cm^2) and fluoranthene RPA (uM/L) for each assay.

All LC_{50} values for each assay and treatment are reported in Table 2.3 and Figure 2.3. Due to a lack of mortality over the 21 day period in the 0.5hr, 1hr, and 2 hour UV exposure assays, LC_{50} values were unable to be determined. In the 4 hour UV exposure assay, the LC_{50} value was unable to be determined at post exposure time 0hrs and 24hrs due to low mortality; however, the LC_{50} declined from 48 (3.4ppb (95% CI 3.2-3.6ppb)) to 72hrs post exposure (2.5ppb (95% CI 2.3-2.7ppb)). The LC_{50} values did not decline significantly for the remainder of the assay. In the 8hr UV exposure assay, the LC_{50} values also declined over post-exposure time from 3.5ppb (95% CI 3.1-3.9ppb) to 1.4ppb (95% CI 1.2-1.6ppb). In the 16hr UV exposure assay, the LC_{50} values also declined post-exposure from 1.2ppb (95% CI 1.1-1.3ppb) to 0.5ppb (95% CI 0.4-0.6ppb.

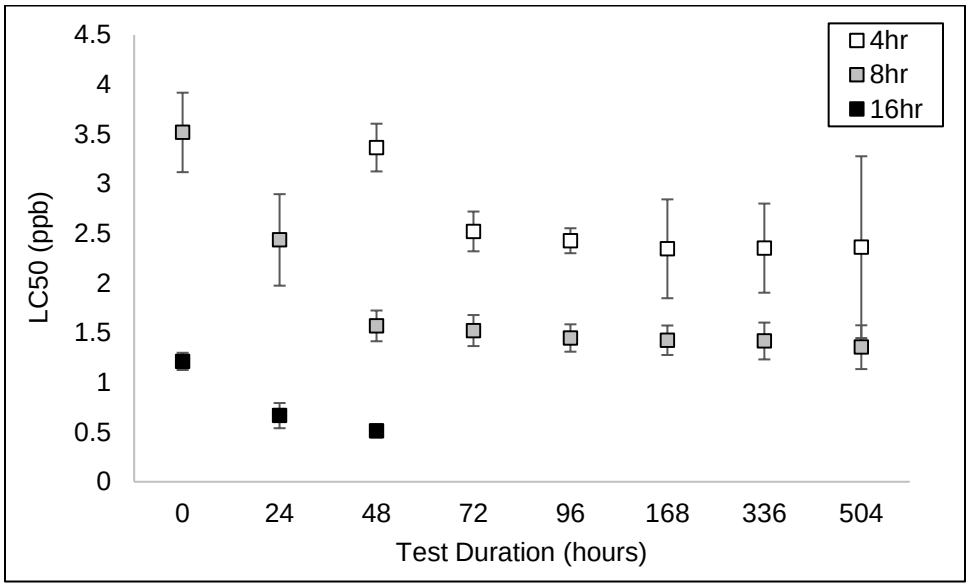

Figure 2.3 Post Exposure LC50 values of 4hr, 8hr, and 16hr assays over test duration time.

UV/PAH Exposure Time (hours)	Post –Exposure Time (hours)	LC_{50} (95% CI) (ppb)
4	0	NC
	24	NC
	48	3.4 (3.2-3.6)
	72	2.5 (2.3-2.7)
	96	2.4 (2.3-2.5)
	168	2.3 (1.8-2.8)
	336	2.4 (1.9-2.9)
	504	2.4 (1.5-3.3)
8	0	3.5 (3.1-3.9)
	24	2.4 (1.9-2.9)
	48	1.6 (1.4-1.8)
	72	1.5 (1.3-1.7)
	96	1.4 (1.2-1.6)
	168	1.4 (1.3-1.5)
	336	1.4 (1.2-1.6)
	504	1.4 (1.2-1.6)
16	0	1.2 (1.1-1.3)
	24	0.7 (0.6-0.8)
	48	0.5 (0.4-0.6)
	72	NC
	96	NC
	168	NC
	336	NC
	504	NC

Table 2.3 Post exposure LC50 values for latent assays 4hr, 8hr, and 16hr. *NC= not calculated, mortality was either too high or did not reach 50%

Reproductive capability of surviving organisms was successfully monitored in all six tests (Figure 2.4. Table 2.4). There were no significant differences in mean reproduction across all treatments in the 1hr and 16hr assays when compared to controls. In the 0.5hr assay, average reproduction in the control treatment was lowest across all assays with 20 neonates (+/- 4.4 SE) and was found to be significantly less than all other treatments within this assay ($p< 0.01$).

In the 2hr assay, mean neonates in the control treatment was 36.5 (+/- 9.9 SE) which is significantly less than the value at 1.2ppb ($p<0.004$). In the 4hr assay, mean neonates in the 2.4ppb treatment were significantly more than in the control ($p<0.001$) and was the highest across all assays with 121 neonates (+/- 8.7 SE). Also, in the 4hr

assay, the mean neonates in the 4.8ppb treatment were found to be significantly less than the control ($p<0.03$). In the 8hr assay, mean neonates in the 0.6ppb treatment were significantly higher than the control ($p<0.01$) and the 1.2ppb treatment was also significantly higher than the control ($p<0.001$).

UVA Duration (hrs)	Fluoranthene (ppb)	Mean Neonates per replicate (+/-SE)
0.5	0	20 (4.4)
	0.6	47.5 (11)
	1.2	73.3 (10.1)
	2.4	49.3 (6.4)
	4.8	54.5 (9.9)
1	0	29.5 (6.6)
	0.6	31.8 (3.9)
	1.2	42.8 (23.8)
	2.4	28.5 (10.4)
	4.8	32.3 (7.5)
2	0	36.5 (9.9)
	0.6	52.8 (5.9)
	1.2	62 (5.4)
	2.4	33 (1.4)
	4.8	55.8 (13.3)
4	0	64 (5)
	0.6	67 (18.7)
	1.2	61.8 (10.8)
	2.4	121 (8.7)
	4.8	28 (18.1)
8	0	44 (7.4)
	0.6	75 (4.1)
	1.2	114 (7.7)
	2.4	60.25 (20.8)
	4.8	NC
16	0	79.5 (5.9)
	0.6	83 (14.6)
	1.2	NC
	2.4	NC
	4.8	NC

Table 2.4 Mean neonates per surviving adult with associated standard error. *NC= not calculated due to high mortality

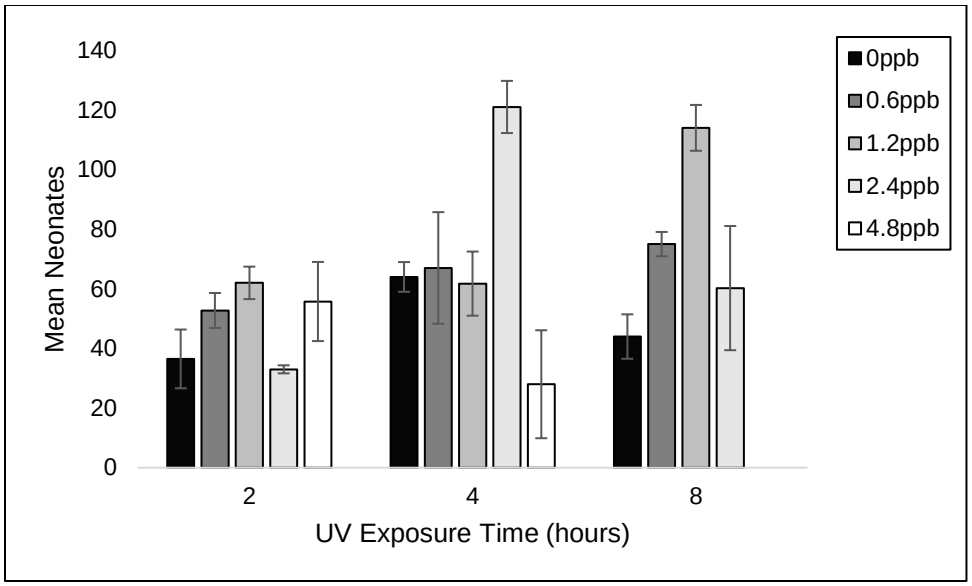

Figure 2.4 Mean and standard error of reproduction in 2hr, 4hr, and 8hr exposure assays.

Reproduction was also monitored with organisms separated individually to alleviate the impact of competition on results. Water quality parameters of RHW used for exposure media and water changes remained within normal ranges in all assays. The mean measured concentration of fluoranthene was 4.1ppb (+/- 0.2 SD). The integrated UV dose ranged from 98.6 mWs/cm^2 in the 0.5hr exposure assay to 388.3 mWs/cm^2 in the 2hr exposure assay. Because UV duration ranged between assays, the phototoxic doses received were 0.9 $uM/L \cdot mWs/cm^2$ in the 0.5hr assay, 1.8 $uM/L \cdot mWs/cm^2$ in the 1hr assay, and 3.5 $uM/L \cdot mWs/cm^2$ in the 2hr assay. Mean neonates of *D. magna* were not significantly different compared to controls within or between assays (Figure 2.5).

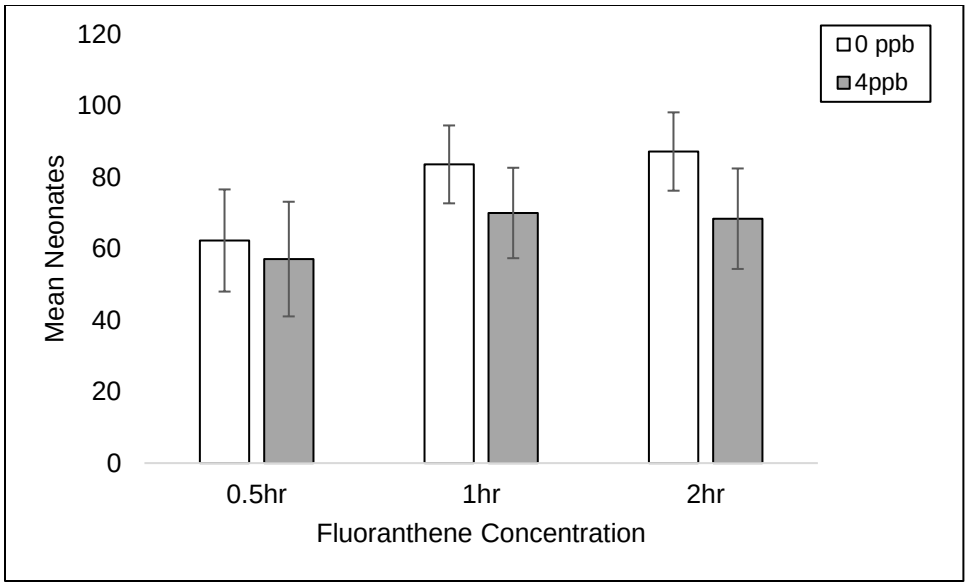

Figure 2.5. Mean with standard error of individual assay reproduction in 0.5hr, 1hr, and 2hr UV at 0ppb and 4ppb fluoranthene

Discussion

UV/Recovery Interval Bioassays

Reciprocity was shown to be an appropriate foundational model for predicting PAH photo-induced toxicity in this study. Mortality was shown to be dose-dependent upon fluoranthene concentrations and UV exposure time while UV intensity remained constant. These parameters are appropriately represented in the reciprocity model. However, due to differences in UV absent recovery interval times, some LC_{50} values were found to differ from expected values when predicted by reciprocity. This determination was consistent with other studies which have found reciprocity to be reliable in PAH photo-induced toxicity modeling; however, data has shown slightly inconsistent effects at certain phototoxic doses (Ankley, 1995; Oris, 1985; Sellin, 2013).

Due to long and continuous UV exposure times, a mortality threshold may exist where the possibility of survival is greatly decreased. In the 12hr interval assay, the 24hr

LC50 was about 20% less than the values of the 1.5hr, 3hr, and 6hr at 24hrs. Because phototoxic doses were equal in these assays, reciprocity states the phototoxic effect would be equal. Additionally, in the 24hr interval assay, though phototoxic dose was twice those of the other assays at 24hrs, the LC50 value was over 50% of the other assays. This data suggests that within a PAH contaminated area, mortality may be significantly increased when UV exposure is 12hrs or more. Therefore, reciprocity may under-predict risk if UV exposure time is long and infrequent extending the 12hr mortality threshold. Studies have shown organisms to avoid UV exposure at high intensities (irradiance and time), *D. magna* will swim deeper into the water column where UV attenuates (Storz, 1998). Within an ecosystem, water depth and mobility of organisms may significantly impact UV exposure. Organisms who are found in shallow water or near the water surface will receive long or constant doses of UV especially if unable to migrate deeper. Artificially weathered oil samples collected from the BP Deepwater Horizon oil spill were used to evaluate toxicity to mahi mahi (*Coryphaena hippurus*) embryos whose buoyancy cause them to receive high UV exposure at the water surface (Alloy et al., 2016; Sweet et al., 2016). Results from these studies show significant toxicity to exposed embryos with total sum PAH concentrations falling within fluoranthene concentrations utilized in the present study. PAH photo-induced toxicity may be exacerbated in organisms bound to surface waters such as during embryonic or larval stages of development. When water is shallow, immobile organisms such as mollusks, aquatic plants, or coral are unable to avoid UV and are at higher risk of toxicity. Studies have shown reproductive toxicity in Carpet Shell Clam (*Ruditapes decussatus*) following coexposure to UV and environmentally relevant concentrations of

PAH (Fathallah et al., 2012). Conversely, mobile organisms are less likely to experience toxicity due to UV avoidance. Benthic organisms with the ability to avoid UV by burrowing into sediments have shown to be less susceptible to photo-induced toxicity within a PAH contaminated ecosystem than pelagic organisms (Ankley, 1994). Furthermore, the presence or lack of some biological traits of organisms may affect UV exposure. Studies have shown that species who are extensively exposed to UV in nature may have evolutionary defenses such as pigmentation against PAH photo-induced toxicity (Boese, 1997; Gevertz et al., 2012). Translucent organisms such as *D. magna*, or those which have little pigmentation may be more susceptible to PAH toxicity.

An additional mortality threshold may occur during PAH photo-induced toxicity due to short and repetitive UV exposure interval times within a 48hr timeframe. In the 1.5hr and 3hr interval assays, 48hr LC50s was up to 69% less than the associated values at 24hrs. The data suggest that, over time, without adequate UV absence, overall effects of PAH photo-induced toxicity may be too severe after just 1.5hrs UV exposure for recovery to be significant. When determining risk to PAH contaminated areas, the reciprocity model may under-predict effect in conditions where organisms are exposed to UV in short and frequent intervals. Other researchers have shown at similar fluoranthene concentrations and UV doses, mortality of *D. magna* increases up to two orders of magnitude within two hours of UV exposure (Wernersson et al.,1998). In nature, UV exposure is constantly changing with exposure depending upon both habitat parameters, meteorological conditions, and time of year (Douben, 2003; Seckmeyer, 2008). It is highly likely that most aquatic organisms are exposed to interspersed UV

due to the abiotic factors of the ecosystem. Areas with a high presence of UV refugia may be less susceptible to PAH photo-induced toxicity.

Data also suggests the presence of a recovery threshold where the chance of survival is greatly increased due to an adequate balance of UV exposure and UV absence. In the 6hr interval assay, the 48hr LC_{50} was between 25%-75% higher than all other values at 48hrs and only 40% of its associated value at 24hrs. This data suggests that in the event of PAH photo-induced toxicity, survival is significantly more likely when UV absent recovery is at least 6hrs following 6hrs UV exposure. The reciprocity model may over-estimate PAH photo-induced toxicity when UV absence is sufficient to allow organisms biological recovery. In, Oris et al., 1986, authors explain that toxicity is a combination of both cumulative and repairable damage which takes place during UV absence. When determining PAH photo-induced toxicity, there is a delicate balance between UV exposure time and UV absent recovery. The results of this study show how UV absent recovery periods could potentially cause inconsistencies in the reciprocity model.

Considering effects of PAH photo-induced toxicity, both recovery and mortality thresholds may exist at specific UV exposure and UV recovery times. These thresholds could potentially affect the accuracy of the reciprocity model. It is not only important to calculate direct risk of toxicity to an ecosystem, but equally imperative to consider traits of organisms and of the habitat which may regulate sensitivity (Hook et al., 2014). Within the ecosystem, UV exposure may vary depending on biological traits of the organisms, meteorological condition, time of year and parameters of the habitat. The availability of UV refugia may have a significant effect on the likelihood for PAH photo-

induced toxicity (Bowling et al., 1983). Data in this thesis suggests that the ability to limit UV exposure decreases the magnitude of injury. Ecosystems most at risk of PAH photo-induced toxicity are those which receive long or continuous intervals of UV exposure with little or no presence of UV refugia. Due to seasonal changes, the Arctic regions experience continuous UV exposure during certain times of the year. Additionally, climate change is causing polar ice caps to decrease which significantly limits UV refuge available for organisms within these ecosystems (Leu et al., 2016). Also at risk are ecosystems which receive high UV exposure but have low UV refuge such as alpine lakes or open-water marine environments (Laurion, 2000; Peachey, 1996).

Latent Effects Bioassay

Results of this study show in addition to initial mortality, latent mortality occurred in a UV/PAH dose-dependent manner. Following exposure, reductions in LC_{50} values over time in the 4hr, 8hr, and 16hr exposure assays are indicative of a latent mortality response. If mortality is only monitored or predicted following the initial response, overall mortality may be greatly underestimated. Most notably, in the 4hr assay, initial mortality was too low to generate a LC_{50} value until 48hrs post exposure where the value continued to drop until reaching stability at 72hrs. If calculated risk is determined based on initial mortality, lethal phototoxic dose estimates may be greatly underestimated. This trend of increased latent mortality was consistent in the three high exposure tests before eventually stabilizing. Latent mortality may occur when phototoxic dose surpasses the point of where biological recovery can remediate damage. During PAH photo-induced toxicity, mortality is due to lipid peroxidation within gill epithelial tissues where PAH

accumulates (Choi et al., 2000; Weinstein, 1997). Therefore, latent mortality is most likely due to irreparable damage to gill tissue which ultimately leads to respiratory distress and death. Sub-lethal signs of toxicity prior to mortality included erratic swimming and twitching which corresponds with toxic responses in other studies (Ankley et al., 1995). In studies with comparable phototoxic doses of fluoranthene and UVA combinations, latent mortality most likely would have been observed as well if the researchers would have carried out the monitoring period longer (Wernersson et al., 1998).

In assays testing multiple organisms per replicate, results showed no significant reduction in reproduction when compared to controls. However, some treatments exhibited significantly higher reproduction compared to the associated control. Because multiple organisms were used in replicate dishes, significant differences in reproduction may be due to differences in competitive stress. A study of the competition of *Daphnia sp.* illustrates how the rate of energy expended toward reproduction is proportional to the rate at which energy inputs are received (Schoener, 1972). In dishes where survival was high, competition was likely high which caused decreased reproduction. Oppositely, exposures with significantly higher reproduction corresponded with those with high mortality. This finding is important because it shows that following a high acute phototoxic dose, recovery may be sufficient with no reproductive loss.

Reproduction was not significantly reduced when organisms were individually separated and exposed to fluoranthene at three different UVA co-exposures. In these assays, phototoxic doses were too low to cause significant mortality and may have also fallen just below the range causing reproductive damage. The results of the present

study are in contrast with Holst et al., 1989 where reproduction of individually separated *D. magna* was found to be significantly less in chronic phototoxic exposure treatments when compared to controls. However, differences in exposed phototoxic doses of anthracene were slightly more at 4.68 uM/L * mWs/cm^2, while the highest phototoxic dose of fluoranthene in the present study was at 3.45 uM/L * mWs/cm^2. Therefore, to effectively monitor individual reproductive effects of *D. magna* following acute PAH photo-induced toxicity, it is necessary to test at phototoxic doses higher than those in the present study.

Acute exposure to PAH photo-induced toxicity may produce significant impacts at environmentally relevant phototoxic doses. Toxicity was increased as phototoxic dose increased in all assays. Though UV exposure time varied, UV irradiance was constant across assays. In the environment, UV exposure time may be highly regulated by various factors. Additionally, factors of the environment may govern PAH exposure concentrations and distribution in an aquatic ecosystem. Following a PAH contamination event in an aquatic ecosystem, water turbulence caused by stream velocity, waves, and wind have significant influences of the distribution of PAHs (Douben, 2003). Exposure concentrations to PAH vary depending upon the environmental distribution and chemical properties. One study has shown fluoranthene to have an environmental half-life between 268-377 days when bound to soil (Park et al., 1990). In the present study, the fluoranthene loss at 12hrs UV exposure was about 68% under continuous UV, while UV absent treatments reduced 34%. Exposure concentration of PAH may also be dependent upon water quality parameters. Numerous studies have shown dissolved organic matter, containing mostly humic

material, may reduce bioavailability and toxicity of PAH (Hessen, 2001; Laurion; 2000). Another study shows that the presence of humic acid in an aquatic system may reduce the body burden of environmentally relevant anthracene concentrations from 86% in fathead minnow (*Pimephalespromelus promelas*) to 90% in *D. magna* (Oris et al., 1990). Therefore, it is environmentally relevant to consider acute exposure to both UV and PAH within an aquatic environment when evaluating PAH photo-induced toxicity.

Results of this study show that it is appropriate to consider latent mortality and reproductive output as biomarkers of acute PAH photo-induced toxicity. The data shows that acute phototoxic doses may cause high initial mortality, but doses showing little or no initial effect may still endure significant latent mortality. It is important for risk predictions to incorporate cumulative latent mortality. The present study shows when acute PAH photo-induced toxicity causes high mortality, surviving individuals may experience complete reproductive recovery. More work should be conducted to evaluate reproductive effects in the absence of competitive stress.

Conclusion

PAHs are distributed globally primarily due to heavy dependency on fossil fuels for energy. Many PAHs are considered to have low toxicological risk; however, many compounds are highly photodynamic leading to exponential increases in toxicity. The effect of PAH photo-induced toxicity in aquatic freshwater systems has been extensively researched and has shown to cause of significant ecological stress and toxicity to marine biota (Barron, 2008, Calfee, 1999, Peachey, 1996, Alloy et al., 2015, Alloy et al., 2016). Recovery from PAH photo-induced toxicity has had little attention, but results

from this study show it could influence predictive capability of the reciprocity model. Additionally, when organisms are removed from acute exposure, latent PAH photo-induced toxicity may still occur in exposures which show no initial effect. Finally, reproductive results in this study illustrate the ability for organisms to recover from highly lethal exposure through exhibiting high reproductive capability.

The mechanisms involved in photo-induced toxicity are still being understood, and the need for a reliable model is constantly increasing as PAH contamination worsens. This research will provide helpful insight for future researchers concerned with PAH photo-induced toxicity and the impact of environmentally relevant recovery.

In a PAH contaminated area, it is more likely that organisms receive a chronic exposure rather than an acute exposure. Surviving organisms which receive continuous PAH exposure along with daily UV exposure may develop adaptations to PAH photo-induced toxicity which help to mitigate damage and sensitivity to organisms such as *D. magna*. Following chronic exposure to anthracene and daily UV exposure, *D. magna* have been shown to survive up and reproduce through their lifespan when PAH concentration and light intensities were low and survive up to reproductive maturity when exposures were higher (Holst et al., 1989). Therefore, during chronic exposures there may be a variety of adaptations within the organism which work to alleviate toxicity to allow for reproduction before energy is expended and the organisms can no longer survive. It has been shown that *D. magna* within PAH contaminated water may avoid UV exposure by swimming into areas where UV attenuates (Storz et al., 1998). Other physical responses to UV exposure such as development of pigmentation may increase survival of organisms within a chronically exposed PAH environment (Gevertz et al.,

2012). Future research involving survival mechanisms within a long-term PAH contaminated area may focus on potential genetic biomarkers which may indicate response or recovery when exposed to UV.

To further investigate the impact of UVA absent intervals, it may be beneficial to monitor latent mortality following the exposure regime as described in the UV/recovery assay in the present study. Also, it may be beneficial to evaluate effects when equal phototoxic doses are administered at different, yet proportional PAH and UV doses. Additionally, I believe investigations involving higher organism such as fathead minnow or zebrafish could determine consistency in threshold and latent mortality trends seen in the present study. Reproductive assays should be investigated where organisms are individually separated to avoid competitive stress.

This research will be beneficial to business professional and scientists who work to manage and conserve fossils fuel emissions by providing more supportive research of the photodynamic risk of PAH contamination. Those who work in environmental conservation and risk assessment will benefit from the present study by increasing understanding of how ecological inputs may influence toxicity. Finally, this research may offer a better understanding of toxicological risk to the environment and to human health.

REFERENCES

Alloy, Matthew M., et al. "Photo-induced toxicity of Deepwater Horizon slick oil to blue crab (Callinectes sapidus) larvae." *Environmental Toxicology and Chemistry* 34.9 (2015): 2061-2066.

Alloy, Matthew Michael, et al. "Ultraviolet Radiation Enhances the Toxicity of Deepwater Horizon Oil to Mahi-mahi (Coryphaena hippurus) Embryos."*Environmental science & technology* (2016).

Alloy, Matthew, et al. "Co-exposure to sunlight enhances the toxicity of naturally weathered Deepwater Horizon oil to early lifestage red drum (Sciaenops ocellatus) and speckled seatrout (Cynoscion nebulosus)." *Environmental Toxicology and Chemistry* 36.3 (2017): 780-785.

Allred, P. M., and J. P. Giesy. "Solar radiation-induced toxicity of anthracene to Daphnia pulex." *Environmental Toxicology and Chemistry* 4.2 (1985): 219-226.

Ankley, Gerald T., et al. "Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate." *Environmental science & technology* 29.11 (1995): 2828-2833.

Ankley, Gerald T., et al. "Influence of ultraviolet light on the toxicity of sediments contaminated with polycyclic aromatic hydrocarbons." *Environmental toxicology and chemistry* 13.11 (1994): 1791-1796.

Barron, Mace G., et al. "Temporal and spatial variation in solar radiation and photo-enhanced toxicity risks of spilled oil in Prince William Sound, Alaska, USA." *Environmental Toxicology and Chemistry* 27.3 (2008): 727-736.

Boese, Bẋ L., et al. "Photoinduced toxicity of fluoranthene to seven marine benthic crustaceans." *Archives of Environmental Contamination and Toxicology* 32.4 (1997): 389-393.

Bowling, J. W., et al. "Acute mortality of anthracene-contaminated fish exposed to sunlight." *Aquatic Toxicology* 3.1 (1983): 79-90.

Bunsen, R. W., and H. E. Roscoe. "Photochemical Researches.--Part V. On the Measurement of the Chemical Action of Direct and Diffuse Sunlight." *Proceedings of the Royal Society of London* 12 (1862): 306-312.

Calfee, Robin D., et al. "Photoenhanced toxicity of a weathered oil onCeriodaphnia dubia reproduction." *Environmental Science and Pollution Research* 6.4 (1999): 207-212.

Cho, Eun-ah, A. John Bailer, and James T. Oris. "Effect of methyl tert-butyl ether on the bioconcentration and photoinduced toxicity of fluoranthene in fathead minnow larvae (Pimephales promelas)." *Environmental science & technology* 37.7 (2003): 1306-1310.

Choi, Jonghoon, and James T. Oris. "Evidence of oxidative stress in bluegill sunfish (Lepomis macrochirus) liver microsomes simultaneously exposed to solar ultraviolet radiation and anthracene." *Environmental Toxicology and Chemistry* 19.7 (2000): 1795-1799.

Diamond, Stephen A. "Photoactivated toxicity in aquatic environments." *UV Effects in Aquatic Organisms and Ecosystems. New York: Johm Wiley & Sons, Inc* (2003): 219-250.

Diamond, Stephen A., et al. "Photoactivated polycyclic aromatic hydrocarbon toxicity in medaka (Oryzias latipes) embryos: relevance to environmental risk in contaminated sites." *Environmental toxicology and chemistry* 25.11 (2006): 3015-3023.

Doniach, I. "A comparison of the photodynamic activity of some carcinogenic with non-carcinogenic compounds." *British journal of experimental pathology* 20.3 (1939): 227.

Douben, Peter ET, ed. *PAHs: an ecotoxicological perspective*. John Wiley & Sons, 2003.

Dworkin, Martin. "Endogenous photosensitization in a carotenoidless mutant of Rhodopseudomonas spheroides." *The Journal of general physiology* 41.6 (1958): 1099.

Erickson, Russell J., et al. "Additive Toxicity of Binary Mixtures of Phototoxic Polycyclic Aromatic Hydrocarbons to the OligochaeteLumbriculus variegatus." *Toxicology and applied pharmacology* 154.1 (1999): 97-105.

Fathallah, Salem, Mohamed Néjib Medhioub, and Mohamed Mejdeddine Kraiem. "Photo-induced toxicity of four polycyclic aromatic hydrocarbons (PAHs) to embryos and larvae of the carpet shell clam Ruditapes decussatus." *Bulletin of environmental contamination and toxicology* 88.6 (2012): 1001-1008.

Gevertz, Amanda K., et al. "Differential tolerance of native and nonnative fish exposed to ultraviolet radiation and fluoranthene in Lake Tahoe (California/Nevada), USA." *Environmental toxicology and chemistry* 31.5 (2012): 1129-1135.

Hessen, Dag O., and Per J. Færøvig. "The photoprotective role of humus-DOC for Selenastrum and Daphnia." *Responses of Plants to UV-B Radiation*. Springer Netherlands, 2001. 261-273.

Holst, Linda L., and John P. Giesy. "Chronic effects of the photoenhanced toxicity of anthracene on Daphnia magna reproduction." *Environmental Toxicology and Chemistry* 8.10 (1989): 933-942.

Hook, Sharon E., Evan P. Gallagher, and Graeme E. Batley. "The role of biomarkers in the assessment of aquatic ecosystem health." *Integrated environmental assessment and management* 10.3 (2014): 327-341.

Landrum, P. F., et al. "Photoinduced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms." *Oil in freshwater: chemistry, biology, countermeasure technology. Elmsford, NY: Pergamon Press: 304œ318* (1987).

Laurion, Isabelle, et al. "Attenuation of ultraviolet radiation in mountain lakes: Factors controlling the among-and within-lake variability." *Limnology and Oceanography* 45.6 (2000): 1274-1288.

Leu, Eva, Martin Graeve, and Angela Wulff. "A (too) bright future? Arctic diatoms under radiation stress." *Polar Biology* 39.10 (2016): 1711-1724.

Martinez, Elena, et al. "Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels." Journal of Chromatography A 1047.2 (2004): 181-188.

Newsted, John L., and John P. Giesy. "Predictive models for photoinduced acute toxicity of polycyclic aromatic hydrocarbons to Daphnia magna, Strauss (Cladocera, Crustacea)." *Environmental Toxicology and Chemistry* 6.6 (1987): 445-461.

Oris, James T., A. Tilghman Hall, and Jacqueline D. Tylka. "Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia." *Environmental Toxicology and Chemistry* 9.5 (1990): 575-583.

Oris, James T., and John P. Giesy. "The photoenhanced toxicity of anthracene to juvenile sunfish (Lepomis spp.)." *Aquatic Toxicology* 6.2 (1985): 133-146.

Oris, James T., and John P. Giesy. "Photoinduced toxicity of anthracene to juvenile bluegill sunfish (Lepomis macrochirus Rafinesque): Photoperiod effects and predictive hazard evaluation." *Environmental toxicology and chemistry* 5.8 (1986): 761-768.

Oris, James T., and John P. Giesy. "The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow (Pimephalespromelas)." *Chemosphere* 16.7 (1987): 1395-1404.

Park, Kap S., et al. "Fate of PAH compounds in two soil types: influence of volatilization, abiotic loss and biological activity." *Environmental toxicology and chemistry* 9.2 (1990): 187-195.

Peachey, Rita L., and Donald G. Crosby. "Phototoxicity in tropical reef animals." *Marine Environmental Research* 42.1 (1996): 359-362.

Schoener, Thomas W. "Population growth regulated by intraspecific competition for energy or time: some simple representations." *Theoretical Population Biology* 4.1 (1973): 56-84.

Seckmeyer, Gunther, et al. "Variability of UV irradiance in Europe." *Photochemistry and Photobiology* 84.1 (2008): 172-179.

Sellin Jeffries, Marlo K., et al. "Quantitative risk model for polycyclic aromatic hydrocarbon photoinduced toxicity in Pacific herring following the Exxon Valdez oil spill." *Environmental science & technology* 47.10 (2013): 5450-5458.

Storz, U. C., and R. J. Paul. "Phototaxis in water fleas (Daphnia magna) is differently influenced by visible and UV light." *Journal of Comparative Physiology A* 183.6 (1998): 709-717.

Sweet, Lauren E., et al. "Exposure to ultraviolet radiation late in development increases the toxicity of oil to mahi-mahi (Coryphaena hippurus) embryos." *Environmental Toxicology and Chemistry* (2016).

Veith, Gilman D., et al. "A QSAR analysis of substituent effects on the photoinduced acute toxicity of PAHs." *Chemosphere* 30.11 (1995): 2129-2142.

Weinstein, John E., James T. Oris, and Douglas H. Taylor. "An ultrastructural examination of the mode of UV-induced toxic action of fluoranthene in the fathead minnow, Pimephales promelas." *Aquatic Toxicology* 39.1 (1997): 1-22.

Wernersson, Ann-Sofie, and Göran Dave. "Effects of different protective agents on the phototoxicity of fluoranthene to Daphnia magna." *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 120.3 (1998): 373-381.

Wernersson, A-S., and Göran Dave. "Phototoxicity identification by solid phase extraction and photoinduced toxicity to Daphnia magna." *Archives of environmental contamination and toxicology* 32.3 (1997): 268-273.

Willis, Alison M., and James T. Oris. "Acute photo-induced toxicity and toxicokinetics of single compounds and mixtures of polycyclic aromatic hydrocarbons in zebrafish." *Environmental Toxicology and Chemistry* 33.9 (2014): 2028-2037.