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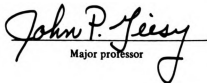
THE CHRONIC EFFECTS OF THE PHOTO-ENHANCED TOXICITY
OF ANTHRACENE ON DAPHNIA MAGNA REPRODUCTION

presented by

Linda Lee Holst

has been accepted towards fulfillment
of the requirements for

M.S. degree in FISH.&WILDL.


Major professor

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THE CHRONIC EFFECTS OF THE PHOTO-ENHANCED TOXICITY
OF ANTHRACENE ON DAPHNIA MAGNA REPRODUCTION

By

Linda Lee Holst

A THESIS

Submitted to
Michigan State University
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ABSTRACT

THE CHRONIC EFFECTS OF THE PHOTO-ENHANCED TOXICITY OF ANTHRACENE ON DAPHNIA MAGNA REPRODUCTION

By

Linda Lee Holst

The chronic effects of polycyclic aromatic hydrocarbons (PAH) and UV-radiation exposure on zooplankton reproduction were investigated. Daphnia magna were exposed to anthracene, a linear 3-ring PAH, in the presence or absence of ecologically relevant intensities of UV-radiation for 21 days. Exposure to anthracene in the absence of UV-radiation significantly reduced the number of neonates produced by D. magna; however, exposure to UV-radiation in the absence of anthracene had no significant effect on the fecundity of D. magna. Concurrent exposure of D. magna to UV-radiation enhanced the toxicity of anthracene and caused a reduction in the number of neonates produced, which was proportional to both anthracene concentration and UV-radiation intensity. An equation was developed which predicts the relative percent reduction in neonate production due to the photo-enhanced toxicity of anthracene given an anthracene concentration and UV-radiation intensity.

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TABLE OF CONTENTS

	<u>Page</u>
LIST OF TABLES	iv
LIST OF FIGURES	vi
INTRODUCTION	1
MATERIALS AND METHODS	12
Zooplankton	12
Algae	13
Exposure System	13
Experimental Design	19
Statistical Analysis	24
RESULTS	25
Anthracene Effects	25
UV-Radiation Effects	25
Combined Effects of Anthracene and UV-Radiation	30
DISCUSSION	48
CONCLUSIONS	54
APPENDIX A	55
APPENDIX B	56
LIST OF REFERENCES	62

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1 Modified Woods Hole-MBL Media for Algae Cultures	14
2 Experimental Conditions of Light Intensity, Anthracene Concentration and Temperature	21
3 Average Number of Neonates per Brood and Total Number of Neonates Within the First 6 Broods Produced by <u>D. magna</u> Exposed to Anthracene in the Absence of UV-Radiation	26
4 Average Number of Neonates per Brood and Total Number of Neonates Within the First 6 Broods Produced by <u>D. magna</u> Exposed to UV-Radiation in the Absence of Anthracene	28
5 ANOVA of Model That Predicts the Relative Percent Reduction in Production of Neonates by Surviving <u>D. magna</u> Given an Anthracene Concentration and UV-Radiation Intensity	40
6 ANOVA of Model That Predicts the Relative Percent Reduction in Production of Neonates by <u>D. magna</u> (Including Adults Which Did Not Survive 21 D) Given an Anthracene Concentration and UV-Radiation Intensity	42
7 Concentrations of Total PAH in Various River Waters (Neff, 1979)	52
8 Concentrations of PAH (ug/L) in Great Lakes Water (Eadie, et al., 1982)	53
A Neonates per Brood Produced in 21 Days per <u>D. magna</u> Fed <u>Chlamydomonas reinhardtii</u>	55

<u>Table</u>		<u>Page</u>
B1	Neonates per Brood Produced per <u>D.</u> <u>magna</u> in 21 Days During Experiment 1 . .	56
B2	Neonates per Brood Produced per <u>D.</u> <u>magna</u> in 21 Days During Experiment 2 . .	58
B3	Neonates per Brood Produced per <u>D.</u> <u>magna</u> in 21 Days During Experiment 3 . .	60

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Structure of Some Commonly Occurring Polycyclic Aromatic Hydrocarbons	2
2	Percent Reduction in TOTL6BRD by Surviving <u>D. magna</u> Exposed to Anthracene (Actual Concentrations) and UV-Radiation Relative to TOTL6BRD by <u>D. magna</u> Not Exposed to Anthracene or UV-Radiation	32
3	Mean Cumulative Number of Neonates Produced by Surviving <u>D. magna</u> Exposed to a UV-A Intensity of 117 uW/cm ² and Anthracene (Actual Concentrations)	34
4	Mean Cumulative Number of Neonates Produced by Surviving <u>D. magna</u> Exposed to 8 ug Anthracene/L (Approximate Concentrations) and UV-Radiation	36
5	Percent Reduction in TOTL6BRD by <u>D. magna</u> (Including Adults Which Did Not Survive 21 D) Exposed to Anthracene (Actual Concentrations) and UV-Radiation Relative to TOTL6BRD by <u>D. magna</u> Not Exposed to Anthracene or UV-Radiation	38
6	Mean Cumulative Number of Neonates Produced by <u>D. magna</u> (Including Adults Which Did Not Survive 21 D) Exposed to a UV-A Intensity of 117 uW/cm ² and Anthracene (Actual Concentrations)	44
7	Mean Cumulative Number of Neonates Produced by <u>D. magna</u> (Including Adults Which Did Not Survive 21 D) Exposed to 8 ug Anthracene/L (Approximate Concentrations) and UV-Radiation	46

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are a class of organic compounds consisting of two or more fused benzene rings with occasional inclusions of heteroatoms or cyclopentene rings. The most commonly studied PAH which are found in the environment include: fluorene, acridine, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]pyrene (BaP), and perylene (Figure 1); however, many other PAH have been recognized as environmental contaminants. Although natural processes such as forest fires and volcanic eruptions release PAH into the environment, inputs from domestic and industrial pyrolytic activities are the major sources of PAH (Laflamme and Hites, 1978). Large quantities of PAH are released into the environment each year due to human activities, such as oil spills, fossil fuel combustion, and incineration, as well as many industrial processes (Mix, 1984; Suess, 1976). Annual emissions of B[a]P in the United States are estimated to be between 900 and 1300 tons (Dipple, 1983). Heat and power generation contribute approximately 38% of the total, open refuse burning 42 to 46%, coke production 15 to 19% and motor vehicles 1 to 1.5% (Dipple, 1983).

PAH can enter aquatic systems by different routes. The total annual PAH input into the aquatic environment has

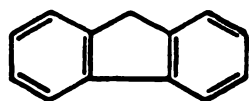
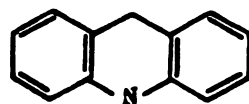
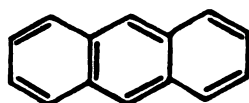
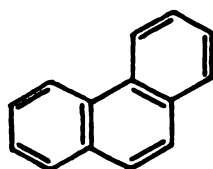
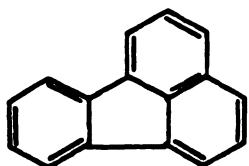
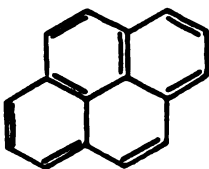
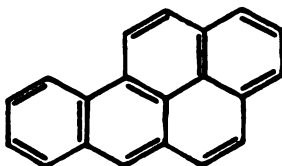
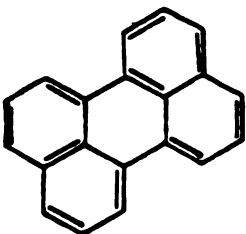
**Fluorene****Acridine****Anthracene****Phenanthrene****Fluoranthene****Pyrene****Benzo[a]pyrene****Perylene**

Figure 1. Structures of Some Commonly Occurring Polycyclic Aromatic Hydrocarbons.

been estimated to be 230,000 metric tons (Neff, 1979). This input is expected to increase substantially due to projected increases in coal benefaction processes (Gehrs, 1976).

Aerial transport is the major source of trace contaminants to the Great Lakes (Eisenreich et al., 1981). The atmospheric deposition of PAH to the Great Lakes is estimated to be 484 metric tons per year (Eisenreich et al., 1981). The flux of total PAH into southern Lake Michigan has been measured to be approximately 1×10^5 - 10^6 kg/yr in dry flux and 1×10^6 kg/yr in wet flux (Strand and Andren, 1980).

In addition to atmospheric deposition, substantial amounts of PAH can enter the aquatic environment from oil spills and runoff. Approximately 6×10^6 tons of oil enter the oceans each year (NAS, 1972). Runoff from coal piles may contain numerous PAH. Simulated rainfall runoffs from model coal piles contained up to 107 ug/L of certain PAH (Stahl et al., 1984).

PAH are nonpolar compounds, therefore, they have low aqueous solubilities, are lipophilic and are readily bioconcentrated by aquatic organisms. A freshwater crustacean, Daphnia pulex, accumulated benz(a)anthracene to concentrations 10,109 times and anthracene 917 times over that of the water concentrations (Southworth et al., 1978). Chironomids were able to bioconcentrate anthracene between 47 and 132 times over the amount dissolved in water

(Gerould et al., 1983). In addition, BCF's for anthracene and B[a]P in bluegill sunfish were determined to be 675 and 490, respectively (Spacie et al., 1983).

PAH are of concern in the aquatic environment not only because they are ubiquitous contaminants that can be bioconcentrated, but also because many PAH are carcinogenic and/or toxic. Much of the research conducted on PAH has been concerned with their carcinogenic properties. Many PAH including benzo[a]pyrene (BaP), dimethylbenz[a]anthracene (DMBA), and methylcholanthrene are potent carcinogens and have caused malignant skin and lung tumors in laboratory animals (EPA, 1980).

Some PAH have been shown to be toxic; however, many of the laboratory tests, which found PAH to be acutely toxic to organisms, used carrier solvents that resulted in PAH concentrations well above their respective aqueous solubility limits (Neff, 1979). Other laboratory tests have indicated that PAH are not acutely toxic within their aqueous solubility limits (Herbes et al., 1976; Applegate et al., 1957), however, many tests of PAH toxicity were conducted under environmentally unrealistic laboratory lighting regimes in order to reduce PAH photodegradation. Recent studies that have incorporated natural or simulated solar ultraviolet radiation have demonstrated PAH to be extremely toxic to aquatic organisms at concentrations well below aqueous solubility limits.

PAH photosensitization in organisms has been well recognized for many decades. The photo-induced toxicity of PAH to Paramecium (Mottram et al., 1938) and Drosophila (Maltotsy and Fabian, 1946) by ultraviolet radiation (<400 nm) was observed more than 40 years ago. More recently, photo-induced lethality by PAH has been demonstrated in hamster, mouse and human cells (Utsumi and Elkind, 1979). Photo-toxic reactions of the skin and eyes of outdoor workers exposed to asphalt, roofing materials and creosote-treated wood have been well documented (Kochevar et al., 1982). In addition, products made from crude coal tar which contain PAH are also used in photo-therapy treatment of psoriasis (Tanenbaum et al., 1975; Kochevar et al., 1982).

Although the photo-toxic effects of PAH have been recognized for many years, their effects on aquatic organisms have been ignored until recently. Recent studies have demonstrated variable PAH toxicities which were dependent on the PAH and organism being examined. B(a)P was inhibitory to the growth of the green algae Selenastrum capricornutum in the presence of fluorescent black light (Cody et al., 1984). However, the green alga Chlorella pyrenoidosa was not adversely affected by anthracene exposure in the presence of UV-radiation (Oris et al., 1984). The photo-toxic effects of PAH are much more dramatic for zooplankton. Photo-induced effects of anthracene on the water flea Daphnia pulex were

demonstrated (Allred and Giesy, 1985). Under natural solar radiation, 100% of the D. pulex were immobilized in less than 2 minutes at a concentration of 32.7 ug/L, 100% were immobilized in less than 10 minutes at 7.5 ug/L, and 50% were immobilized in less than 15 minutes at 1.2 ug/L. In addition to these studies, 1 h-LC₅₀ values of <20 ug/L were determined for the photo-toxicity of pyrene, fluoranthene and anthracene to D. magna (Kagan et al., 1985). Mosquito larvae (Aedes aegypti) are also sensitive to the photo-induced toxicity by PAH (Oris et al., 1984; Kagan et al., 1985, 1986) as well as embryonic forms of the frog Rana pipiens (Kagan et al., 1984, 1985). Furthermore, the photodynamic immobilization of brine shrimp nauplii (Artemia salina) by more than 40 PAH has also been examined (Morgan and Warshawsky, 1977).

PAH photo-toxicity has been well demonstrated in fish. Alpha-terthienyl, a natural component of marigolds and many other plants in the family Compositae, has been observed to be more toxic than rotenone to fathead minnows (Pimephales promelas) (Kagan et al., 1986). Acute mortality of bluegill sunfish (Lepomis macrochirus) exposed to 12.7 ug/L anthracene and exposed to natural sunlight was observed in outdoor channels (Bowling et al., 1983). Extensive laboratory studies conducted under well defined lighting conditions also demonstrated that anthracene is acutely photo-toxic to juvenile sunfish and that this

toxicity could be predicted from knowledge of UV intensity and anthracene concentration (Oris and Giesy, 1985, 1986a). PAH other than anthracene are also photo-toxic to fish. Of the 12 PAH tested, six PAH including anthracene caused photo-induced toxicity to fathead minnow larvae (Oris and Giesy, 1986b). Anthracene exhibited a median potency among the PAH that were toxic; therefore, anthracene appears to be an adequate model compound for the examination of PAH photo-induced toxicity (Oris and Giesy, 1986b).

The mechanism for photo-induced toxicity of PAH is unknown, but evidence suggests that disruption of cell membranes is involved (Landrum et al., 1986). Cellular damage may result from the direct binding of PAH to macromolecules in the presence of UV-radiation (Sinha and Chignell, 1983). Exposure to PAH and UV-radiation may alter membrane function by inactivation of enzymes (Wat et al., 1980) or by modification of lysosomal membrane permeability (Allison et al., 1966). Furthermore, upon absorption of UV-radiation, PAH can be excited to triplet states which may react with oxygen to form singlet oxygen (Foote, 1968). Singlet oxygen can react with cell membranes, modify DNA (Gutter et al., 1977) or cause lipid peroxidation (Sinha and Chignell, 1983).

Ultraviolet radiation plays an important role in the photo-induced toxicity of PAH, but UV-radiation alone can be toxic to aquatic organisms. Exposure to UV-radiation can cause lesions and sunburning of the epidermis of fish

(Allison, 1960; Bullock, 1982; Bullock and Roberts, 1981). UV-radiation can also be toxic to zooplankton and can influence their behavior and occurrence in the environment (Damkaer et al., 1980; Barcelo and Calkins, 1979; Karanas et al., 1979). Zooplankton adapt to UV-radiation through increased pigmentation, increased repair capacity and avoidance. The avoidance of damaging UV-radiation present at midday may be an important factor in explaining the phenomenon of diel migration of these organisms (Damkaer, 1982; Barcelo, 1982). Calkins and Thordardottir (1980) concluded that tolerance and exposure of a variety of aquatic organisms (algae, bacteria, protozoa and anthropods) to solar UV-radiation are approximately equal which indicates that many aquatic organisms are currently exposed to UV-radiation intensities at or near thresholds for adverse effects. Any increases in intensities of UV-irradiance or PAH concentrations, which effectively enhance the photo-induced damage, could have drastic effects on the abundance of aquatic organisms.

The purpose of the present study was to examine the chronic effects of sublethal concentrations of anthracene in the presence of environmentally relevant intensities of simulated, solar radiation on reproduction of the microcladoceran, Daphnia magna. Several studies have demonstrated that PAH cause acute photo-induced toxicity to zooplankton, but the chronic effects have not been elucidated.

Research Rationale

Toxicant

Anthracene is a commonly occurring PAH which is found in both oil and coal and is a product of various pyrolytic combustions (Neff, 1979). Anthracene is often used as a model PAH in laboratory toxicity and fate studies because it is noncarcinogenic, inexpensive, ubiquitous in natural environments, and has an intermediate water solubility relative to other PAH (NAS, 1972). Anthracene exhibits a median level of photo-induced acute toxicity to aquatic organisms relative to other PAH which have been tested. In addition, the anthracene molecule is symmetrical so that the number of products formed by photolysis, hydrolysis and biotransformation is smaller than that for other PAH. These products are also well described.

Organism

Zooplankton are important components of aquatic food chains because they are a food link between primary producers and predators of higher trophic levels. Any reduction in zooplankton abundance can significantly alter the structure of aquatic communities. Decreases in zooplankton reproduction due to toxicant exposure are commonly studied using Daphnia in chronic toxicity tests.

Daphnia have been used as indicator organisms for aquatic pollution in both acute and chronic toxicity tests for many years. The advantages of Daphnia as a test

organism are: small size; ease of culture, maintenance and testing; relatively short life-cycle; uniformity of cultures and sensitivity to toxicants (Anderson, 1944; Leonhard, 1979). D. magna, the largest and easiest species of Daphnia to handle, is a standard test organism in toxicity tests and has been shown to be one of the most sensitive aquatic species (Adema, 1978). Daphnia are particularly useful in studying anthracene because they rapidly bioconcentrate anthracene (Eastmond et al., 1984; Herbes and Risi, 1978) but do not biotransform this PAH (Herbes and Risi, 1978). D. magna accumulate anthracene to steady state concentrations in approximately 4 h (Herbes and Risi, 1978).

Chronic Photo-toxicity Tests

Acute toxicity tests provide information about the relative toxicity of a compound to the test organism but do not provide information about the chemical's long-term effects on populations of the organism. Chronic toxicity tests are designed to assess the effects of a compound on the survival, reproduction and growth of an organism in order to assess and predict the possible impact of a compound on populations of organisms in natural environments. Short-term tests have shown anthracene to be acutely toxic to Daphnia in the presence of UV light, however, no studies on the chronic toxicity of anthracene and UV light to Daphnia have been conducted prior to the

work presented here. The study reported on here provides information on both lethality and reduced fecundity of D. magna due to chronic exposure to anthracene and UV-radiation.

Objectives of Research

The objectives of the present study were to:

1. Determine the effects of anthracene exposure on D. magna survival and reproduction in the absence of ultraviolet radiation.
2. Determine the effects of chronic exposure to low intensity, simulated, solar radiation on the survival and reproduction of D. magna.
3. Examine the dose-response relationships between anthracene concentration and UV-radiation intensity necessary to reduce fecundity of D. magna.
4. Use the chronic dose-response information to determine the hazard of present concentrations of PAH and intensities of UV-radiation and set criteria for the protection of aquatic organisms exposed to PAH under ecologically relevant conditions of solar radiation.

MATERIALS AND METHODS

Zooplankton

Daphnia magna were cultured in 20 liter glass aquaria filled with 15-17 liters of aerated well water (temperature = $23 \pm 1^{\circ}\text{C}$, pH = 8.1 ± 0.2 , D.O. = 7.8 ± 0.4 mg O_2/L , hardness = 230 mg CaCO_3/L , alkalinity = 236 mg CaCO_3/L). The D. magna were maintained on a 18 h:6 h (light:dark) photoperiod under a bank of two fluorescent white lamps (Sylvania Gro-lux^R F48T12-GRO-VHO). D. magna were fed Chlamydomonas reinhardtii once a day. Approximately 400 ml of algae were centrifuged at 6000 rpm for 15 minutes, resuspended in 100 ml of well water, and added to each aquarium. D. magna cultures were gently aerated to keep the algae suspended and the water well oxygenated. In order to maintain healthy cultures, D. magna populations were reduced when necessary (every 3-5 days) and the tanks were drained, scrubbed and refilled with aerated well water once a week.

The D. magna cultures were characterized as follows: time to first brood = 7.8 ± 0.4 days, number of broods in 21 days = 6 per female, number of neonates per brood = 15.9 ± 7.4 , interbrood period = 2.4 ± 0.5 days and total number of neonates per female in 21 days = 95.2 ± 8.5 .

Algae

Cultures of Chlamydomonas reinhardtii were grown in sterile 4 L Erlenmeyer flasks filled with 3 liters of autoclaved modified Woods Hole-MBL medium (Table 1; Nichols, 1973). The neck of each flask was fitted with a rubber stopper that contained 2 glass pipettes: one each for air inlet and outlet. The algae cultures were continuously aerated to keep the cells suspended and the media well oxygenated. Air was filtered by a 25 mm extra thick glass fiber filter placed in-line between the air pump and rubber stopper. The top of each flask was loosely covered with aluminum foil to keep foreign matter from collecting on the stoppers and contaminating the cultures. The cultures were grown in a greenhouse.

C. reinhardtii cultures were diluted with fresh medium every 2 to 5 days, depending on the density of algal cells. All but approximately 500-1000 ml of algae were harvested and refrigerated. The unharvested cells were rediluted with 2-2.5 liters of medium, and the refrigerated cells were fed to D. magna. All algae transfers and dilutions were performed under a laminar flow hood to minimize contamination.

Exposure System

Three 21-day static renewal tests were conducted to determine the effects of various anthracene concentrations

Table 1. Modified Woods Hole-MBL Media for Algae Cultures.

<u>Macronutrients</u>	<u>Concentration (mg/L)</u>
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	36.76
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	36.97
NaHCO_3	12.60
K_2HPO_4	8.71
NaNO_3	85.01
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	28.42
<u>Micronutrients</u>	<u>Concentration (mg/L)</u>
$\text{Na}_2 \cdot \text{EDTA}$	4.36
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.15
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.02
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.01
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.18
<u>Vitamins</u>	<u>Concentration (ug/L)</u>
Thiamine	100
Cyanocobalamin	100
Biotin	5

and ultraviolet radiation intensities on the survival and fecundity of D. magna. These chronic tests were conducted under different UV-radiation intensities on a 16 h:8 h (light:dark) photoperiod. Lights were mounted on a 1.22 x 1.37 m frame on 15.24 cm centers, and the light bank was divided in half by a black plastic curtain. One half of the light bank contained G.E. Chroma F40C50 white fluorescent bulbs, above a 3 mm thick sheet of plexiglas^R that eliminated ultraviolet wavelengths of light (<400 nm). The other half of the light bank contained a combination of G.E. Chroma F40C50 white and FS40T12 ultraviolet fluorescent bulbs. Sheets of 0.005 mm thick Mylar^R plastic were used to eliminate wavelengths less than 315 nm and obtain a UV-A to UV-B ratio similar to what occurs outdoors (approximately 8:1). The desired light intensities were obtained by varying both the height of the light bank over the water bath and the number of Mylar^R sheets.

During each chronic test, D. magna were exposed to various concentrations of anthracene. Stock solutions of anthracene were obtained from a once-through, aqueous, elution column which obviated the need for a carrier solvent. These "generator" columns were prepared by dissolving anthracene crystals in acetone and pouring the solution onto a thin layer of silica sand at 0.2% wt/wt. The solvent was then allowed to evaporate overnight. When dry, the sand was packed into a glass column (400 x 75 mm) and flushed with water to remove any loose anthracene

crystals. Water eluted from the column containing anthracene at a concentration of 33 ug/L and was diluted to the desired concentration before being added to the test vessels.

The test vessels in the chronic exposures were 1.8 L glass baking dishes (250 x 200 x 45 mm) with fiberglass-coated wood covers that contained two rows of five evenly spaced holes (Gersich, 1984). A glass tube (51 x 54 mm) with a 368 um pore size stainless steel mesh bottom was placed in each hole and suspended in the test solution contained in the glass dish. Each vessel contained ten D. magna (1/tube) which were exposed to the same anthracene concentration. The vessels were randomly placed under the lighting system in a water bath in order to maintain the temperature of the test solutions between 22 and 23°C.

Temperature fluctuations occurred during experiment 1 which resulted in fecundity of D. magna which was less than expected. Therefore, the raw data obtained during experiment 1 were multiplied by a weighting factor of 1.3529 in order to allow comparisons of production of neonates among experiments 1, 2 and 3. The weighting factor was calculated by dividing the mean total number of neonates produced in the first 6 broods (TOTL6BRD) by D. magna not exposed to UV-radiation or anthracene in experiments 2 and 3 by the mean of TOTL6BRD by D. magna not exposed to UV-radiation or anthracene in experiment 1. The weighted data were used in all statistical tests.

D. magna were fed C. reinhardtii during each test at a concentration of 2.5×10^5 cells/ml/day which was determined to be an adequate concentration to yield healthy D. magna (Appendix A). Before addition of algae to the test vessels, an excess amount of algae was placed in anthracene solution eluted from the "generator" column and was allowed to sorb anthracene overnight in order to prevent rapid depletion of anthracene from the water by algae when added to the test vessels. Although the algal cells were probably at steady state with the saturated anthracene solution (Herbes and Risi, 1978) before being added to the test vessels, the cells were not an appreciable source of anthracene for D. magna. Even though algal cells rapidly depurate anthracene (Giesy et al., 1978) and quickly come into equilibrium with anthracene concentrations in the surrounding water, measured concentrations of anthracene indicated that the amount of anthracene added to the water by algae was minimal. In addition, ingested algal cells were not a significant source of anthracene for D. magna because D. magna are only able to assimilate approximately one-third or less of PAH which are ingested in food (McCarthy, 1983). Therefore, the major source of anthracene for D. magna was the anthracene dissolved in water.

Cell density of the anthracene-algae solution was determined daily by using a standard regression equation

previously calculated from absorbance readings vs. algal cell densities. Absorbance of algal cells was determined with a Gilford Model 2600 spectrophotometer with the wavelength set at 665 nm. The number of cells necessary to yield a concentration of 2.5×10^5 cells/ml in each test vessel was calculated, and the appropriate volume of algal solution was centrifuged at 6000 rpm for 15 minutes. The cells were resuspended with well water and evenly dispensed to vessels containing anthracene. The control vessels received algal cells that were placed in anthracene-free well water overnight and were centrifuged, resuspended and dispensed in the same manner as the anthracene containing cells.

UV-A (365 +/- 36 nm) and UV-B (310 +/- 34 nm) were quantified with a Macam Photometrics (Livingstone, Scotland) Model UV-103 radiometer equipped with Model SD104, cosine-corrected, photodiodes fitted with water-tight, wavelength selective filters. Photosynthetically active radiation (PAR) was measured by a Techum Instruments QSM-2500 scanning quantum spectrometer, which was coupled to a LI-cor model LI 188-B integrating quantum meter. All light measurements were taken at the surface of the zooplankton test chambers.

Anthracene concentrations in water samples were determined directly by reverse-phase HPLC. Fifty microliters of sample were injected onto a 5 cm, C-18, 2 um guard column that was directly connected to a 15 cm x 4.6

mm Supelcosil LC-PAH, 5 um column, at 22°C. An isocratic elution was performed with 90% acetonitrile: 10% water with a flow rate of 1.0 ml/minute. A Kratos FS-970 fluorescence detector was used at an excitation of 252 nm with a 370 nm emission filter. Peak areas were integrated by a Hewlett-Packard 3390-A integrator. Values for anthracene concentrations in the water samples were obtained by using a regression equation of peak areas vs. concentrations of anthracene in standards.

Experimental Design

At the beginning of each test, ten neonates (<48 h old) were placed in each test vessel (1/tube) and remained in the same tubes for the test duration. Survival of adults and number of neonates produced were monitored and recorded daily (Appendices B1-B3). After enumeration, the young were removed from the test chamber, and the adults were transferred to fresh solutions of anthracene and food. Adults were transferred to clean baking dishes every other day and to clean exposure tubes once a week.

Adult D. magna in the three experiments did not all produce equivalent numbers of broods during 21 day periods. Seventy-eight percent of the adults produced 6 broods during 21 days but 15% of the adults had 7 broods. This difference in number of broods was due to slight differences in the ages of the D. magna, which were placed

in the test vessels at the beginning of each experiment. All of the adult D. magna were <48 h old, but the D. magna born earlier in the 48 h period had more time to have an additional brood than D. magna born later in the 48 h period. In order to compare production of neonates within or between experiments, fecundity was defined as the total number of neonates produced in the first 6 broods (TOTL6BRD) rather than total number of neonates produced during the entire 21 d duration of the experiment.

During each chronic test, half of the D. magna were exposed to four anthracene concentrations, including a control (0.0 ug/L) in the absence of UV-radiation, while the other half were exposed to the same anthracene concentrations under UV-irradiation. The three experiments differed by the UV intensity used, which was either 31, 60 or 117 uW/cm² UV-A. However, the nominal concentrations of anthracene, which were 0 (control), 2.5, 5 or 10 ug/L, remained the same. Actual concentrations appear in Table 2.

Anthracene concentrations in each vessel were measured at least twice a day by HPLC. The daily transfer of adults to fresh test solution occurred at t=0 h. Appropriate volumes of anthracene solution were added to the vessels at t=4, 8, 12 and 14 h to replenish the anthracene removed from the water. Before fresh anthracene solution was added to the test vessel, an equivalent volume was removed and

Table 2. Experimental Conditions of Light Intensity, Anthracene Concentration and Temperature.

	Expt. 1		Expt. 2		Expt. 3	
	w/ UV	w/o UV	w/ UV	w/o UV	w/ UV	w/o UV
UV-A ($\mu\text{W}/\text{cm}^2$)	31.0 (2.0) ¹	<1	59.5 (1.3)	<1	117.3 (4.0)	<1
UV-B ($\mu\text{W}/\text{cm}^2$)	4.7 (0.2)	<1	16.0 (1.8)	<1	31.0 (3.6)	<1
UV-A:UV-B ratio	6.6	--	3.8	--	3.8	--
PAR ($\mu\text{E}/\text{m}^2\cdot\text{sec}$)	19.3 (0.3)	17.3 (0.3)	27.1 (0.3)	20.6 (0.4)	49.3 (0.6)	31.9 (0.2)
Anthracene ($\mu\text{g}/\text{L}$)	0.0	0.0	0.0	0.0	0.0	0.0
	2.2 (0.5)	2.2 (0.5)	2.2 (0.7)	2.2 (0.7)	1.9 (0.6)	1.9 (0.6)
	4.5 (1.1)	4.5 (1.1)	4.1 (1.4)	4.1 (1.4)	3.6 (1.6)	3.6 (1.6)
	8.8 (2.1)	8.8 (2.1)	8.5 (2.8)	8.5 (2.8)	7.2 (3.3)	7.2 (3.3)
Temperature ($^{\circ}\text{C}$)	23.9 (1.6)	23.9 (1.6)	22.7 (0.5)	22.7 (0.5)	22.2 (0.4)	22.2 (0.4)

¹Value within parentheses is standard deviation.

discarded from the dishes to maintain a volume of 1.6 liters. An appropriate amount of algae was added with the replacement anthracene solution to replace the discarded algal cells.

Anthracene concentrations fluctuated in a cyclic manner with the maximum concentrations occurring at the beginning and the minimum concentrations occurring at the end of every four-hour renewal period. The maximum anthracene concentration in each vessel at the beginning of every four-hour cycle was targetted at 20% above the nominal concentration; the minimum concentration in each vessel at the end of every four-hour cycle was targetted at 20% below the nominal concentration. The loss of anthracene during these cycles occurred at a predictable rate which was first-order with respect to anthracene concentration and was described by a rate constant that was specific for each experiment and determined for each test vessel. Since the rate of anthracene loss was first-order, anthracene concentrations could be predicted at any time during the cycle (equation 1).

$$C_t = C_0 e^{-rt} \quad (1)$$

where: r =rate constant (1/h), t =time (h), C_0 =initial concentration (ug/L), and C_t =predicted concentration (ug/L) at time t . Predicted values were calculated for each treatment concentration of anthracene before renewal in order to determine the amount of anthracene to be added to

each test vessel so that concentrations of anthracene could be maintained within the range of $\pm 20\%$ of the desired nominal concentrations. In addition, actual concentrations of anthracene were measured and compared to predicted values to verify that the predicted concentrations calculated from equation 1 were accurately modelling the cyclic fluctuations of anthracene concentrations. The predictive equation did accurately model the cyclic fluctuations of anthracene because the predicted and measured anthracene concentrations were not significantly different (Chi-square, $p > 0.3$) for all three experiments. This allowed the actual concentration of anthracene to be maintained within $\pm 20\%$ of the desired nominal concentrations.

Concentrations of anthracene were continuously changing with time but were only measured 2 to 4 times daily. Therefore, the actual concentration of anthracene that each D. magna was exposed to for 21 days could not be determined. However, an actual concentration of anthracene was estimated for each nominal concentration (2.5, 5 and 10 $\mu\text{g/L}$) by averaging 24 predicted values (1/h) that modelled the daily cyclic fluctuation of anthracene under the specified experimental conditions. It is this integrated, predictive value, which is reported for each anthracene concentration (Table 2).

Statistical Analysis

All data were analyzed with the computer program Statistical Analysis Systems (SAS; SAS Institute Inc., 1986). Means, variance, standard deviation, range, corrected sums of squares, uncorrected sums of squares, standard error of the mean and coefficient of variation were calculated with the MEANS procedure for the dependent variables: number of neonates produced per brood (BRD); the total number of neonates produced within the first 6 broods (TOTL6BRD); and percent decreases of BRD and TOTL6BRD (relative to D. magna not exposed to UV-radiation and anthracene).

Analyses of variance (ANOVA) of all dependent variables were performed with the General Linear Models (GLM) procedure of SAS. The statistical models contained fixed main effects of anthracene and UV-A radiation. The GLM procedure gave results in the form of sums of squares for main effects, interactions, and error, F-values, and associated probabilities. Comparisons of means were performed by Tukey's Studentized Range (HSD) with the MEANS/GLM procedures of SAS.

RESULTS

Anthracene Effects

Adult D. magna exposed to anthracene in the absence of UV-radiation produced significantly fewer neonates than adult D. magna which were not exposed to anthracene (Table 3). The total number of neonates produced within the first 6 broods (TOTL6BRD) was significantly ($p < 0.05$) less when D. magna were exposed to 2.1, 4.0 or 8.2 ug anthracene/L, relative to adults which were not exposed to anthracene. The number of neonates produced in 6 broods by D. magna exposed to an anthracene concentration of 2.1, 4.0 or 8.2 ug/L were 5.3, 8.0 or 13.8% less than the TOTL6BRD by D. magna which were not exposed to anthracene. In addition, significantly ($p < 0.05$) fewer neonates were produced in broods 2 through 6 by D. magna exposed to 8.2 ug anthracene/L, relative to adult D. magna which were not exposed to anthracene.

UV-Radiation Effects

Exposure to UV-radiation did not affect the production of neonates by adult D. magna (Table 4). TOTL6BRD produced by D. magna exposed to a UV-radiation intensity of 31, 60

Table 3. Average Number of Neonates per Brood and Total Number of Neonates Within the First 6 Broods Produced by *D. magna* Exposed to Anthracene in the Absence of UV-Radiation. N=120. Value Within Parentheses is Standard Deviation.

Table 3.

Anth (ug/L)	Brood						Total
	1	2	3	4	5	6	
0	8.3 A (1.4)	15.4 A (1.6)	25.8 A (2.6)	28.7 A (4.0)	28.3 A (2.8)	30.5 A (2.9)	137.1 A (5.4)
2.1	8.4 A (1.5)	15.1 A (1.6)	24.8 AB (2.8)	26.8 AB (3.6)	26.9 AB (2.9)	29.8 AB (3.2)	129.8 B (9.7)
4.0	7.9 A (1.9)	13.1 B (2.3)	23.3 BC (3.4)	26.5 AB (3.5)	27.3 AB (4.1)	29.0 AB (4.0)	126.1 B (8.8)
8.2	7.6 A (1.8)	12.0 B (3.7)	21.7 C (4.3)	24.2 B (4.0)	25.8 B (2.4)	27.8 B (2.9)	118.2 C (12.1)

¹Means with the same letter within a column are not significantly different from one another (Tukey's HSD test).

Table 4. Average Number of Neonates per Brood and Total Number of Neonates Within the First 6 Broods Produced by D. magna Exposed to UV-Radiation in the Absence of Anthracene. N=120. Value Within Parentheses is Standard Deviation.

Table 4.

UV-A ₂ (uW/cm ²)	Brood						Total
	1	2	3	4	5	6	
0	8.3 AB (1.4)	15.4 A (1.6)	25.8 AB (2.6)	28.7 A (4.0)	28.3 A (2.8)	30.5 A (2.9)	137.1 A (5.4)
31	7.8 AB (1.7)	17.1 A (3.2)	26.8 A (2.9)	25.0 B (4.0)	31.8 B (2.6)	30.4 AB (2.3)	139.0 A (11.2)
60	9.5 A (1.4)	15.8 A (1.5)	23.5 B (1.7)	31.8 A (2.4)	27.9 A (2.0)	31.6 A (2.7)	140.1 A (4.8)
117	7.3 B (1.3)	16.5 A (1.6)	26.5 A (2.0)	27.7 AB (1.7)	27.1 A (1.8)	27.4 B (2.8)	132.5 A (6.3)

¹Totals with the same letter within a column are not significantly different from one another (Tukey's HSD test).

or 117 uW/cm² UV-A were not significantly ($p>0.05$) different from D. magna which were not exposed to UV-radiation. Although the mean TOTL6BRD produced by adult D. magna exposed to an intensity of 117 uW/cm² UV-A was 3.5% less than the TOTL6BRD produced by adults not exposed to UV-radiation, the smaller production of neonates by D. magna exposed to this intensity of UV-A was not statistically significant ($p>0.05$).

Combined Effects of Anthracene and UV-Radiation

Survival of adult D. magna was decreased by concurrent exposure to anthracene and UV-radiation. Exposure to 8.5 ug anthracene/L and 60 uW/cm² UV-A resulted in 10% mortality of D. magna, and exposure to 7.2 ug anthracene/L and 117 uW/cm² UV-A resulted in 70% mortality. No mortality was observed at the other treatment combinations of anthracene and UV-radiation. In all cases, mortality occurred after the D. magna had reached reproductive age and had produced at least one brood of neonates. Due to the occurrence of mortality at two treatment combinations, fecundity of D. magna at a specific treatment combination can be examined in two ways: as production per surviving adult or as production which includes adults that died within 21 days. Both cases are included below.

Exposure of D. magna to anthracene in the presence of ecologically relevant intensities of UV-radiation caused a

reduction in the number of neonates produced by D. magna, which was proportional to both anthracene concentration and UV-radiation intensity (Figures 2, 3 and 4). D. magna, which were exposed to 7.2 ug anthracene/L and 117 uW/cm² UV-A and survived for 21 d, had 69.4% fewer neonates than did D. magna which were not exposed to anthracene or UV-radiation (Figure 2). However, if production by D. magna which did not survive the entire 21 d is included, D. magna exposed to 7.2 ug anthracene/L and 117 uW/cm² had 84.3% fewer neonates, relative to D. magna which were not exposed to anthracene or UV-radiation (Figure 5).

An equation was derived to predict the percent reduction in the number of neonates produced within the first 6 broods (TOTL6BRD), relative to production by D. magna which were not exposed to anthracene or UV-radiation, from anthracene concentrations and UV-A radiation intensities (equation 2). This predictive equation was obtained from the Model option of the GLM procedure in SAS (SAS Institute Inc., 1986).

$$Y = -0.212 + 1.972(X) + 0.0226(Z) + 0.0592(X*Z) \quad (2)$$

where: Y = % reduction of TOTL6BRD, X = anthracene concentration (ug/L), Z = UV-A intensity (uW/cm²) and (X*Z) is a term for the interactive effects of UV-A radiation and anthracene. The independent variables of the model explained 78% of the variation of the dependent variable (Table 5). An equivalent equation was derived to predict

Figure 2. Percent Reduction in TOTL6BRD by Surviving D. magna Exposed to Anthracene (Actual Concentrations) and UV-Radiation Relative to TOTL6BRD by D. magna Not Exposed to Anthracene or UV-Radiation.

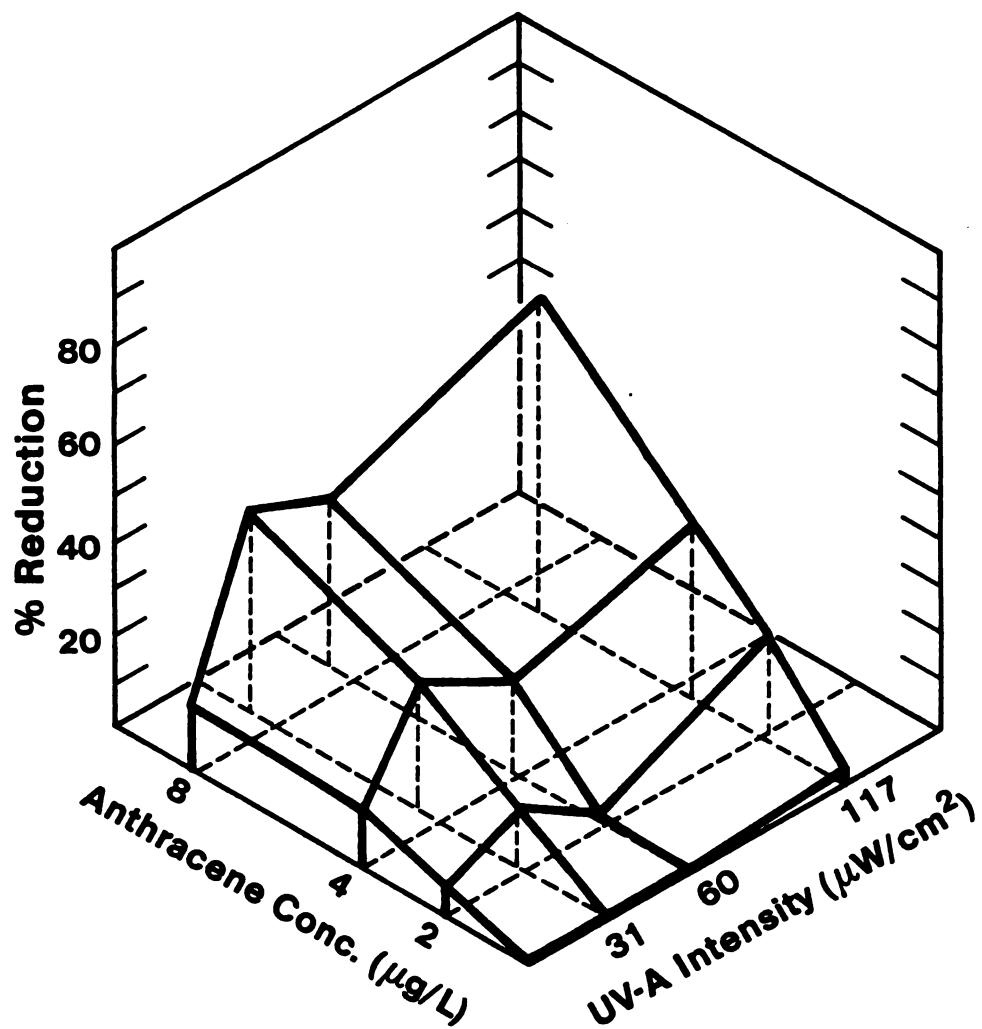


Figure 2.

Figure 3. Mean Cumulative Number of Neonates Produced by Surviving D. magna Exposed to a UV-A Intensity of 117 $\mu\text{W}/\text{cm}^2$ and Anthracene (Actual Concentrations). Vertical Bars Correspond to (Gill. 1978). ($\alpha = 0.05$); $n=120$ and $r=30$ for UV-A=0; $n=40$ and $r=10$ for UV-A=31, 60 or 117 $\mu\text{W}/\text{cm}^2$).

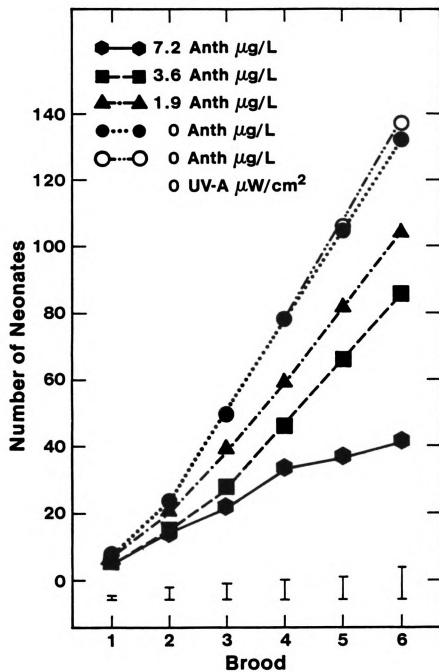


Figure 3.

Figure 4. Mean Cumulative Number of Neonates Produced by Surviving D. magna Exposed to 8 ug Anthracene/L (Approximate Concentrations) and UV-Radiation. Vertical Bars Correspond to Minimum Significant Differences (Gill, 1978). ($\alpha=0.05$; $n=60$ and $r=10$).

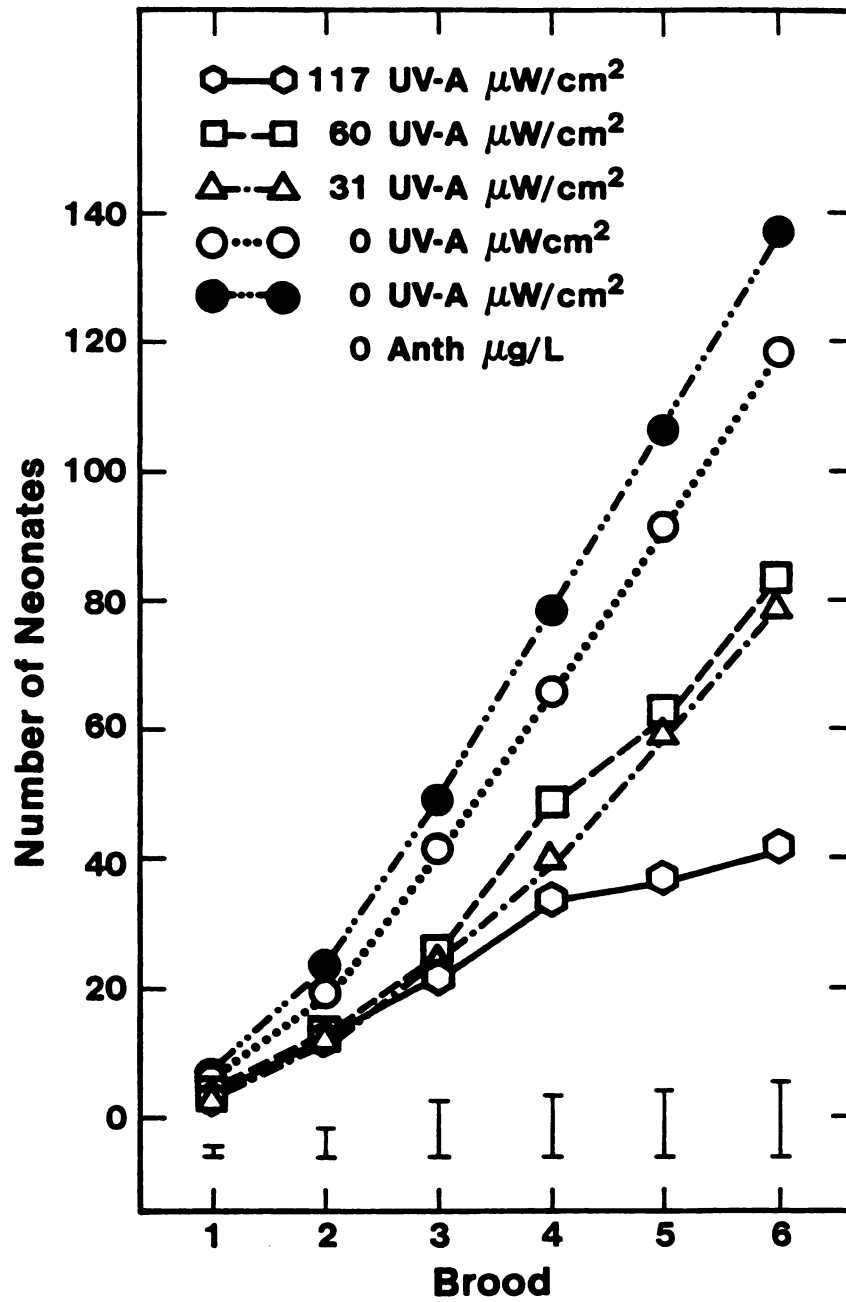


Figure 4.

Figure 5. Percent Reduction in TOTL6BRD by D. magna (Including Adults Which Did Not Survive 21 D) Exposed to Anthracene (Actual Concentrations) and UV-Radiation Relative to TOTL6BRD by D. magna Not Exposed to Anthracene or UV-Radiation.

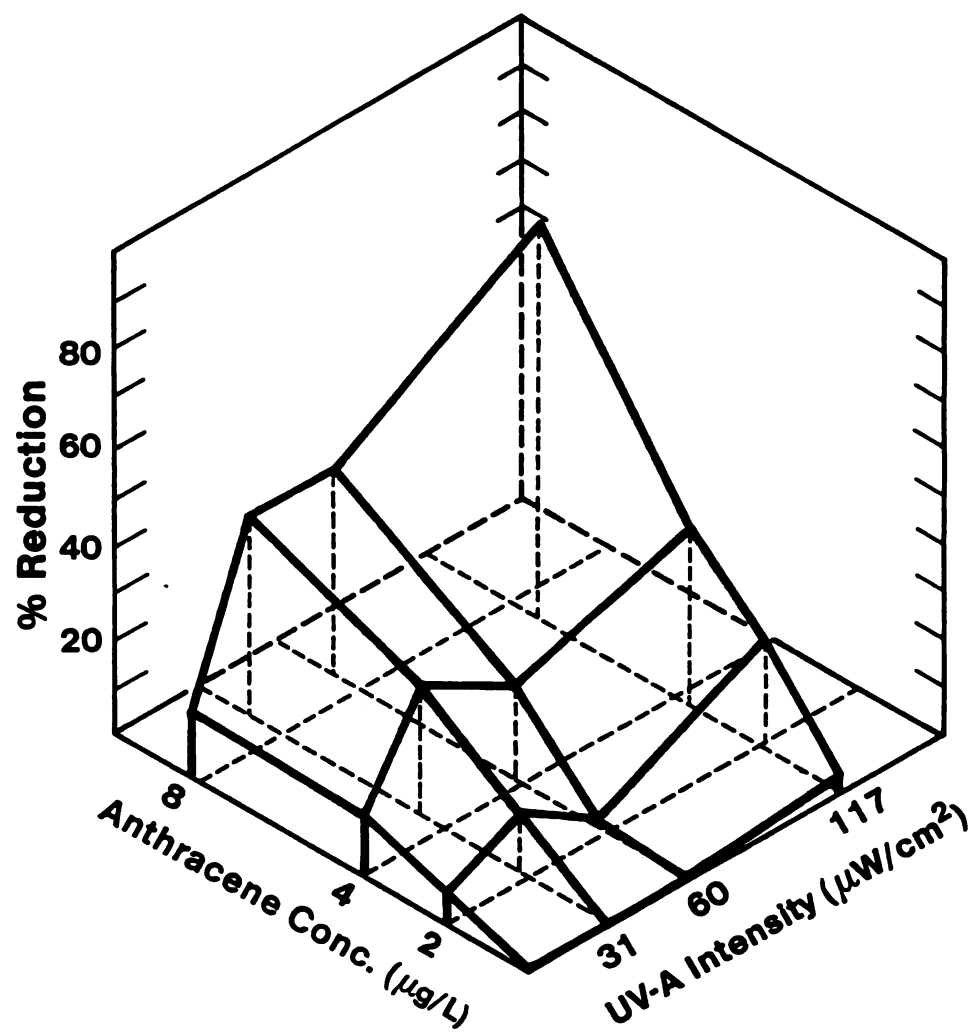


Figure 5.

Table 5. ANOVA of Model That Predicts the Relative Percent Reduction in Production of Neonates By Surviving D. magna Given an Anthracene Concentration and UV-Radiation Intensity.

Source of Variation		DF ¹	SS	MS	F	Pr>F	R ²
Model		3	43565.472	14521.824	265.35	0.0001	0.78
Error		228	12477.868	54.727			
Total		231	56043.340				

Source of Variation		DF	Type I SS	F	Pr>F	DF	Type III SS	F	Pr>F
UVA		1	9317.743	170.26	0.0001	1	90.285	11.65	0.200
ANTH		1	25551.133	466.88	0.0001	1	5120.546	93.56	0.0001
UVA*ANTH		1	8696.596	158.91	0.0001	1	8696.596	158.91	0.0001

¹DF=degrees of freedom, SS=sums of squares, MS=mean square, F=F value, Type I SS=sequential sums of squares and Type III SS=partial sums of squares from General Linear Models procedure from SAS.

the percent reduction in the total number of neonates produced within the first 6 broods which included production of neonates by D. magna which did not survive for 21 d (equation 3). This predictive equation contains the same variables as equation 2.

$$Y = 0.0435 + 1.790(X) - 0.00260(Z) + 0.0773(X*Z) \quad (3)$$

The independent variables of the model explained 84% of the variation of the dependent variable (Table 6).

Decreases in production of neonates were manifested in the first brood as well as in successive broods produced by D. magna which were exposed to anthracene and UV-radiation. Rather than including the cumulative effects of all treatment combinations of anthracene and UV-radiation on production of neonates, only the effects of exposure to anthracene concentrations of 0, 1.9, 3.6 or 7.2 ug/L at the greatest UV-radiation intensity (117 uW/cm² UV-A) (Figure 3), and exposure to UV-radiation intensities of 0, 31, 60 or 117 uW/cm² UV-A and the greatest anthracene concentration (8 ug/L) (Figure 4) are presented. At a UV-radiation intensity of 117 uW/cm² UV-A, significantly fewer neonates were produced in the first brood by D. magna which were concurrently exposed to either 3.6 or 7.2 ug anthracene/L (Figure 3) relative to D. magna which were not exposed to anthracene or UV-radiation. D. magna exposed to 1.9 ug anthracene/L and 117 uW/cm² UV-A did not have significantly fewer cumulative number of neonates than D.

Table 6. ANOVA of Model That Predicts the Relative Percent Reduction in Production of Neonates By D. magna (Including Adults Which Did Not Survive 21 D) Given an Anthracene Concentration and UV-Radiation Intensity.

Source of Variation		DF ¹	SS	MS	F	Pr>F	R ²
Model		3	86562.558	28854.186	413.70	0.0001	0.84
Error		236	16460.304	69.747			
Total		239	103022.862				

Source of Variation		DF	Type I SS	F	Pr>F	DF	Type III SS	F	Pr>F
UVA		1	26028.186	373.18	0.0001	1	1.240	0.02	0.894
ANTH		1	39701.549	569.22	0.0001	1	4330.684	62.09	0.0001
UVA*ANTH		1	20832.823	298.69	0.0001	1	20832.823	298.69	0.0001

¹DF=degrees of freedom, SS=sums of squares, MS=mean square, F=F value, Type I SS=sequential sums of squares and Type III SS=partial sums of squares from General Linear Models procedure from SAS.

magna which were not exposed to anthracene or UV-radiation until brood 3. At an anthracene concentration of 8 ug/L, significantly ($p < 0.05$) fewer neonates were produced in the first brood when D. magna were exposed to 31, 60 or 117 uW/cm² UV-A (Figure 4) relative to D. magna which were not exposed to UV-radiation or anthracene. D. magna exposed to 8 ug anthracene and 117 uW/cm² UV-A ug had significantly ($p < 0.05$) fewer cumulative number of neonates in broods 4 through 6 than D. magna exposed to either 0 or 60 uW/cm² UV-A. Cumulative production of neonates by D. magna exposed to 8 ug anthracene/L and either 31 or 60 uW/cm² UV-A were not significantly ($p > 0.05$) different from each other at any brood.

Greater decreases in the mean cumulative number of neonates produced are evident when the production by D. magna which did not survive for 21 d is included (Figures 6 and 7). D. magna exposed to 8 ug anthracene/L and 117 uW/cm² UV-A had significantly fewer cumulative number of neonates in broods 3 through 6 than D. magna exposed to 0, 31 or 60 uW/cm² UV-A and 8 ug anthracene/L. TOTL6BRD for D. magna exposed to 8 ug anthracene/L and 117 uW/cm², including production by D. magna which died during the 21d, was 21.5 (Figure 7) compared to 42.0 by D. magna which survived for the entire 21 d (Figure 4).

Figure 6. Mean Cumulative Number of Neonates Produced by D. magna (Including Adults Which Did Not Survive 21 D) Exposed to a UV-A Intensity of 117 uW/cm² and Anthracene (Actual Concentrations). Vertical Bars Correspond to Minimum Significant Differences (Gill, 1978). ($\alpha = 0.05$; n=120 and r=30 for UV-A=0; n=40 and r=10 for UV-A=31, 60 or 117 uW/cm²).

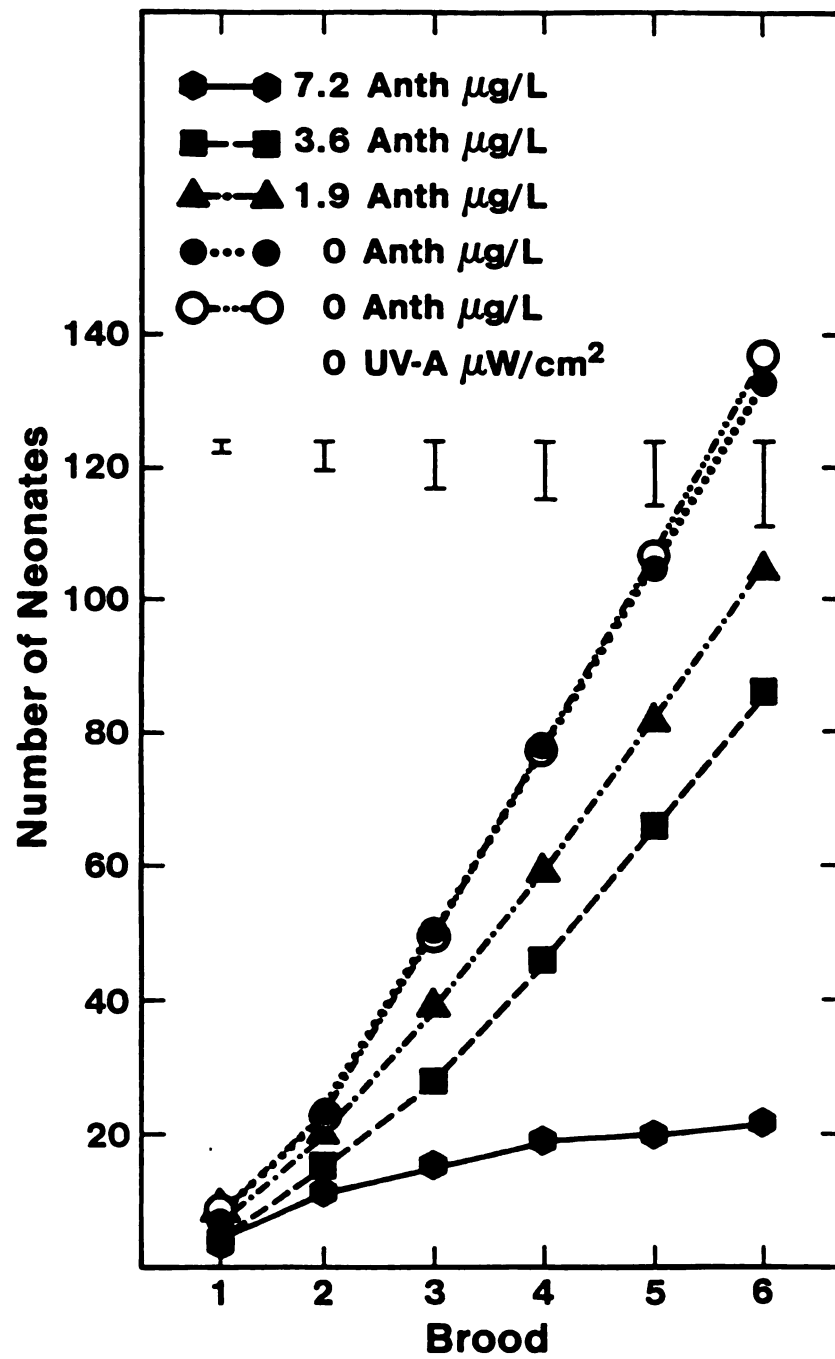


Figure 6.

Figure 7. Mean Cumulative Number of Neonates Produced by D. magna (Including Adults Which Did Not Survive 21 D) Exposed to 8 ug Anthracene/L (Approximate Concentrations) and UV-Radiation. Vertical Bars Correspond to Minimum Significant Differences (Gill, 1978). ($\alpha=0.05$; $n=60$ and $r=10$).

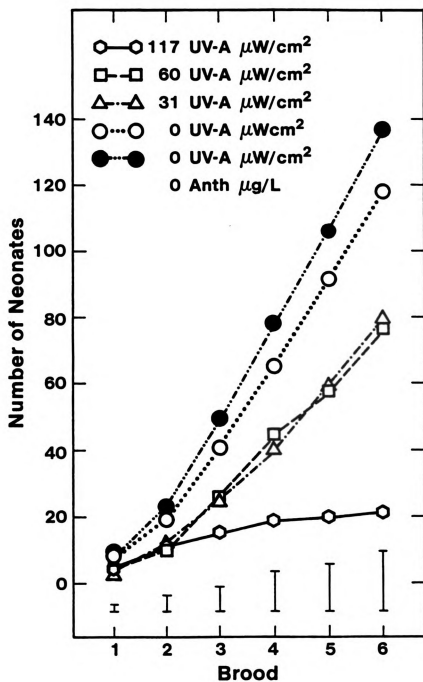


Figure 7.

DISCUSSION

Although PAH have received considerable attention in recent decades, most of the studies have focused primarily on the carcinogenic and mutagenic effects of PAH on mammals. In comparison, relatively little information has been collected on the acute and chronic toxicities of PAH to aquatic organisms. Anthracene, a 3-ring PAH, has been previously considered not to be toxic to aquatic organisms even in supersaturated solutions (Herbes et al., 1976; Applegate et al., 1957); however, the present study has demonstrated that chronic exposure to anthracene in the absence of UV-radiation adversely affects the fecundity of D. magna. TOTL6BRD was reduced by 14% when D. magna were exposed to 8.2 ug anthracene/L (or approximately 25% of the aqueous solubility.)

Solar ultraviolet radiation can have considerable impact on the distribution, survivorship and reproduction of certain aquatic organisms such as bacteria, protozoa, algae, zooplankton and fish (Damkaer, 1980; Calkins and Thordardottir, 1980; Barcelo and Calkins, 1979; Bullock, 1982). UV-B, the most deleterious component of sunlight, is readily absorbed by proteins and nucleic acids and is effective in inducing photochemical reactions in plants and

animals (Damkaer et al., 1980). Prolonged exposure to UV-B radiation can cause lesions in the epidermis of fish (Allison, 1960) and can reduce the survival of algae (Calkins and Thordardottir, 1980) and zooplankton (Damkaer et al., 1980). The ecologically-relevant intensities of UV-A and UV-B radiation used in this study, which were comparable to UV-radiation intensities that routinely penetrate to depths of 10 and 12 meters in Lake Michigan (Gala and Giesy, 1987), did not affect the production of neonates. TOTL6BRD by D. magna exposed to UV-radiation in the absence of anthracene were not significantly ($p>0.05$) different from one another at any UV-radiation intensity.

Recent studies, which have incorporated environmentally realistic lighting regimes with PAH exposure, have demonstrated that anthracene and other PAH are extremely toxic to fish (Oris and Giesy, 1986a, 1986b) and zooplankton (Kagan et al., 1985 ; Newsted and Giesy, 1987; Allred and Giesy, 1985) at concentrations less than aqueous solubility. Results from the study reported here demonstrated that exposure of D. magna to low intensities of UV-radiation and concentrations of anthracene between 6 and 14% of aqueous solubility reduced production of neonates by D. magna up to 69%. If production by D. magna which died during the 21 d is added, the observed maximum decrease in production of neonates was 84%.

As expected, no observable adverse effect concentrations (NOEC) were less when exposure to anthracene

was long-term (chronic) rather than short-term (acute). At UV-A intensities of 29 or 116 uW/cm², the acute NOEC's for D. magna exposed to anthracene for 48 h were between 7.66 and 21.4 and between 2.11 and 6.10 ug/L, respectively (Newsted, 1986). Corresponding chronic NOEC's for D. magna exposed to either 31 or 117 uW/cm² UV-A were between 0 and 1.9 and between 0 and 2.2 ug/L, respectively. The chronic NOEC for D. magna exposed to 60 uW/cm² was between 2.2 and 4.1 ug anthracene/L.

Although no other studies have examined the effects of chronic exposure of PAH and UV-radiation on aquatic organisms, chronic effects have been estimated from laboratory-derived acute dose-response data (Oris and Giesy, 1986a). At a UV-A intensity of 100 uW/cm², the concentration of anthracene necessary to cause lethality in 1% of bluegill sunfish populations during exposure for an infinitely long period of time (LC1) was predicted to be 3.8 ug/L, which is within the range of the NOEC values which were determined for D. magna in the present study. Although D. magna were more sensitive to acute exposure of anthracene and UV-radiation than bluegill sunfish (Newsted, 1986), sensitivity to chronic exposure may not differ. However, reproductive studies which involve chronic exposure of bluegill sunfish to anthracene and UV-radiation have not been conducted to verify previous predictions of chronic effects to bluegill sunfish.

The study presented here has demonstrated that exposure of D. magna to anthracene causes a reduced fecundity in the presence of UV-radiation in the laboratory. The question remains, however, as to what are the current and future impacts of the photo-enhanced toxicity of PAH to zooplankton (and other organisms) in aquatic systems? Concentrations of PAH in some aquatic systems are currently at concentrations (Table 7) which, in this study, reduced production of D. magna neonates by nearly 40% at a UV-A intensity of 117 uW/cm^2 . Therefore, aquatic systems, which have sufficient UV light penetration and concentrations of PAH, may be experiencing increased mortality and reduced reproductive success of aquatic organisms due to the photo-enhanced toxicity of PAH. Concentrations of anthracene presently in the Great Lakes do not appear to be at levels that would result in significant photo-enhanced toxicity (Table 8). Concentrations of anthracene would have to increase by approximately 2 orders of magnitude to elicit a 10% decrease in Daphnia reproduction (equation 2) in the Great Lakes. However, pyrene and B[a]P, two PAH which are two times more photo-toxic than anthracene (Newsted and Giesy, 1987), occur at greater concentrations in Lakes Michigan and Erie than anthracene; therefore, increases in concentrations of these PAH would probably have to occur at lesser amounts to elicit adverse responses in zooplankton reproduction.

Table 7. Concentrations of Total PAH in Various River Waters (Neff, 1979).

Source	Total PAH (ug/L)
River Rhine, GFR	0.500 - 3.000
Thames River, England	0.800 - 2.350
Trent River, England	0.025 - 3.790
Delaware River, PA	0.352
Ohio River, WV	0.058

Table 8. Concentrations of PAH (ug/L) in Great Lakes Water (Eadie et al., 1982) (n=6)¹.

PAH	Mean	Std. Dev.
Phenanthrene	0.024	0.025
Anthracene	0.006	0.006
Fluoranthene	0.015	0.009
Pyrene	0.014	0.006
Chrysene	0.014	0.010
Benzo[a]pyrene	0.012	0.008

¹Six water samples from Lakes Michigan and Erie.

CONCLUSIONS

This study has demonstrated that anthracene, in the presence of UV-radiation, decreases the survival and fecundity of D. magna at concentrations well under aqueous solubility limits. Exposure of D. magna to 7.2 ug anthracene/L and 117 uW/cm² UV-A resulted in 70% mortality or a 69% reduction in fecundity of the D. magna which survived. Although anthracene has been demonstrated to have adverse effects on the survival and fecundity of D. magna, further research needs to be conducted. Elucidation of the mode of toxic action is the next logical step in studying the photo-enhanced toxicity of PAH. In addition to elucidation of the mode of toxic action, effort should be put into the 1) examination of chronic effects of other PAH besides anthracene on the survival and reproduction of D. magna and fish, 2) procurement of more accurate and complete measurements of PAH concentrations in surface waters, and 3) examination of possible synergistic relationships among combinations of PAH.

APPENDICES

APPENDIX A

Neonates per Brood Produced in 21 Days per D. magna Fed Chlamydomonas reinhardtii.

ALGAL CONC (cells/ml)	REP NO*	BROOD						TOTAL
		1	2	3	4	5	6	
1 X 10 ⁵	1	6	17	26	29	13	19	110
	2	8	16	20	22	17	20	103
	3	11	18	27	17	10	20	103
	4	10	13	19	27	21	--	90
	5	4	14	17	19	12	--	66
	$\bar{X}$	7.8	15.6	21.8	22.8	14.6	19.7	94.4
	s	2.9	2.1	4.4	5.1	4.4	0.6	17.4
2.5 X 10 ⁵	1	7	17	19	27	7	17	94
	2	7	13	24	29	14	13	100
	3	10	18	21	29	13	16	107
	4	8	11	20	30	10	10	89
	5	6	12	19	26	5	18	86
	$\bar{X}$	7.6	14.2	20.6	28.2	9.8	14.8	95.2
	s	1.5	3.1	2.1	1.6	3.8	3.3	8.5
5 X 10 ⁵	1	6	12	22	30	10	--	80
	2	5	13	28	28	6	--	80
	3	7	11	23	33	8	--	82
	4	6	11	24	28	7	--	76
	5	3	14	21	29	24	--	91
	$\bar{X}$	5.4	12.2	23.6	29.6	11.0	--	81.8
	s	1.5	1.3	2.7	2.1	7.4	--	5.6

*Each rep. no. corresponds to 1 D. magna that was fed the respective concentration of algal cells daily for 21 days.

APPENDIX B1

Neonates per Brood Produced per D. magna in 21 Days During Experiment 1.

UV-A	ANTH CONC	REP* NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
0	0	1	5	13	24	18	23	22			105
		2	8	9	20	19	24	21			101
		3	7	12	21	18	23	22			103
		4	5	13	24	19	25	23			109
		5	5	11	19	18	24	24			101
		6	6	11	22	24	27	24	35		149
		7	7	10	19	17	23	22			98
		8	8	12	18	20	19	28	36	20	161
		9	6	11	22	18	20	26	32		135
		10	6	12	18	16	21	22	26		121
	2.2	1	6	13	20	18	24	23			104
		2	5	11	21	17	19	25	29		127
		3	5	10	19	16	18	21			89
		4	6	11	17	18	25	22			99
		5	8	10	17	17	21	19			92
		6	6	13	23	18	23	21			104
		7	4	12	17	17	18	22	30		120
		8	6	11	23	17	20	21	26		124
		9	6	10	16	20	17	22	31		122
		10	5	10	16	16	21	22	28		118
	4.5	1	3	12	18	17	23	23			96
		2	3	8	19	19	25	22			96
		3	4	7	17	19	26	23	29		125
		4	6	7	17	18	23	20			91
		5	10	7	18	18	26	21			100
		6	7	9	16	18	17	19			86
		7	4	7	16	17	18	26	36		124
		8	7	10	16	22	23	21	28		127
		9	5	9	19	20	22	19			94
		10	6	10	21	17	28	22			104
	8.8	1	4	6	15	19	20	19			83
		2	4	10	15	18	19	20			86
		3	7	5	11	16	21	21			81
		4	3	6	15	16	17	18			75
		5	6	7	14	15	20	21			83
		6	7	8	14	15	20	19			83
		7	6	6	13	17	20	25			87
		8	3	6	11	15	18	22	26		101
		9	6	5	12	15	19	18			75
		10	2	4	14	16	18	19	28		101

UV-A	ANTH CONC	REP NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
31	0	1	7	11	22	17	23	22	28		130
		2	6	14	21	24	25	26	25		141
		3	7	17	19	21	25	24	28		141
		4	8	13	20	20	26	24			111
		5	5	12	24	19	23	21			104
		6	7	17	18	23	28	22	30		145
		7	5	12	22	17	23	22	27		128
		8	5	12	21	15	22	21	26		122
		9	5	11	18	17	22	25	22		120
		10	4	10	17	16	23	22	28		120
	2.2	1	7	6	17	15	20	18			83
		2	6	7	19	17	22	19			90
		3	5	10	20	16	22	23			96
		4	5	12	18	15	20	19	28		117
		5	6	10	18	16	19	19	29		117
		6	7	14	21	15	20	20			97
		7	7	6	20	16	21	23			93
		8	6	9	14	16	22	19	29		115
		9	6	8	17	20	24	20	27		122
		10	8	10	16	19	18	18			89
	4.5	1	3	6	14	15	17	17			72
		2	2	8	12	11	16	17	23		89
		3	2	7	14	12	18	20			73
		4	6	11	12	12	17	18	25		101
		5	7	11	11	20	18	25			92
		6	9	9	10	15	16	18			77
		7	5	7	10	14	19	18			73
		8	2	7	13	14	21	18			75
		9	6	7	14	12	18	16			73
		10	6	7	11	13	17	20			74
	8.8	1	3	10	9	13	10	14			59
		2	3	7	8	13	17	16			64
		3	2	6	10	12	16	18			64
		4	2	9	12	14	17				54
		5	3	7	9	12	14	17	22		84
		6	2	5	8	13	14	17			59
		7	3	5	11	9	14	16	20		78
		8	4	8	9	7	16	14			58
		9	3	6	7	10	17	15			58
		10	4	7	11	8	15	16	21		82

* Each rep. no. corresponds to 1 *D. magna* that was exposed to the respective intensity of UV-radiation ($\mu\text{W}/\text{cm}^2$) and anthracene concentration ($\mu\text{g}/\text{L}$) for 21 days.

APPENDIX B2

Neonates per Brood Produced per D. magna in 21 Days During Experiment 2.

UV-A	ANTH CONC	REP* NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
0	0	1	11	15	23	34	26	33			142
		2	8	17	24	31	25	37			142
		3	8	15	22	31	28	30			134
		4	9	14	24	36	29	28			140
		5	10	16	25	32	28	29			140
		6	9	16	26	31	26	32			140
		7	10	15	22	33	25	33			138
		8	8	14	26	36	32	29			145
		9	8	14	23	31	28	28	24		156
		10	10	15	23	34	29	34			145
	2.2	1	9	14	22	30	28	36			139
		2	12	14	24	30	27				107
		3	9	15	21	30	27	34			136
		4	9	17	26	34	30	34			150
		5	8	16	22	29	24	30			129
		6	10	15	24	32	20	29			130
		7	10	14	24	33	30	36			147
		8	9	14	20	29	24	27			123
		9	11	19	25	31	26				112
		10	8	15	22	29	30	32			136
	4.1	1	8	13	20	30	29	32			132
		2	11	12	20	28	27	32			130
		3	8	12	20	29	27	29			125
		4	8	13	21	31	25	35			133
		5	8	13	19	34	30	31			135
		6	7	12	19	30	28	36			132
		7	10	14	19	29	29	29			130
		8	10	13	20	30	24				97
		9	9	12	22	32	29	34			138
		10	7	12	21	32	22	32			126
	8.5	1	6	10	20	27	26	29			118
		2	8	11	19	35	28	28			129
		3	9	10	19	28	27	29			122
		4	10	12	21	27	26				96
		5	9	10	18	31	31	29			128
		6	9	13	22	34	31	30			139
		7	9	11	22	26	27	28			123
		8	8	12	19	26	29	34			128
		9	7	14	23	27	28	31			130
		10	9	14	22	26	27	30			128

UV-A	ANTH CONC	REP NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
60	0	1	11	16	25	32	29	33	37		146
		2	12	16	23	36	32	27			183
		3	8	14	23	34	27	29			135
		4	8	15	24	30	28	36			141
		5	8	16	20	29	29	31			133
		6	10	17	25	34	27	33			146
		7	10	15	24	32	25	32			138
		8	9	16	22	32	29	34			142
		9	9	19	23	31	27	29			138
		10	10	14	26	28	26	32			136
	2.2	1	8	16	24	31	27	27			133
		2	10	14	23	33	25	32			137
		3	9	13	23	30	26	28			129
		4	9	15	22	28	25	29			128
		5	7	14	23	31	24	31			130
		6	8	18	24	34	24	26			134
		7	9	18	23	31	28	36			145
		8	8	15	25	34	29	34			145
		9	8	14	24	33	30	31			140
		10	7	14	26	31	26	30			134
	4.1	1	8	10	19	25	25	26			113
		2	6	9	18	26	24	26			109
		3	9	12	18	28	19	24			110
		4	8	11	18	26	23	24			110
		5	7	8	15	26	25	20			101
		6	7	11	18	28	24	25			113
		7	8	11	18	28	19	27			111
		8	6	11	17	25	20	26			105
		9	8	11	19	28	23	26			115
		10	8	14	17	28	22	23			112
	8.5	1	4	5	18	17	14	22	24		80
		2	4	4	15	19	17	16			99
		3	6	7	16	23	15	20			87
		4	6	4	14	26	13	19			82
		5	5	5	17	18	15	23			83
		6	5	8	18	20	12	22			85
		7	4	5	4						13
		8	7	9	18	23	11	23			91
		9	7	4	17	22	16	21			87
		10	4	9	15	21	15	19			83

*Each rep. no. corresponds to 1 D. magna that was exposed to the respective intensity of UV-radiation ($\mu\text{W}/\text{cm}^2$) and anthracene concentration ($\mu\text{g}/\text{L}$) for 21 days.

APPENDIX B3

Neonates per Brood Produced per D. magna in 21 Days During Experiment 3.

UV-A	ANTH CONC	REP* NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
0	0	1	6	15	24	29	28	33			135
		2	9	19	26	28	31	24			137
		3	8	15	25	33	27	29			137
		4	8	18	26	29	25	27	26		159
		5	7	14	25	27	25	30			128
		6	8	16	28	28	28	31			139
		7	8	18	25	28	27	28			134
		8	7	17	28	27	26	31			136
		9	6	15	30	26	27	28			132
		10	6	14	26	29	26	32			133
	1.9	1	7	16	25	30	25	25			128
		2	8	14	26	27	30	32			137
		3	8	14	28	29	26	31			136
		4	9	15	28	26	28	32			138
		5	10	17	29	27	25	29			137
		6	9	16	26	26	28	30			135
		7	6	13	25	26	29	23	20		142
		8	9	14	24	25	25	28			125
		9	8	15	26	24	26	31			130
		10	9	18	27	26	25	26			131
	3.6	1	7	14	27	26	27	30			131
		2	8	13	26	29	25	31			132
		3	7	15	28	22	24	31			127
		4	8	16	25	26	24	24			123
		5	8	17	26	24	22	26			123
		6	8	17	28	23	24	28			128
		7	9	15	18	22	24	18			106
		8	9	18	28	23	23	31			132
		9	8	14	30	26	23	22			123
		10	6	14	26	24	26	25			121
	7.2	1	9	13	25	23	23	29			122
		2	8	15	26	19	22	23			113
		3	9	15	26	23	21	24			118
		4	9	18	30	23	25	34			139
		5	8	17	26	22	22	25			120
		6	7	17	30	25	24	27			130
		7	6	17	28	24	26	28			129
		8	8	16	27	20	24	28			123
		9	7	14	25	23	26	26			121
		10	8	19	25	24	26	27			129

UV-A	ANTH CONC	REP NO	BROOD								TOTAL
			1	2	3	4	5	6	7	8	
117	0	1	7	16	24	27	26	29			129
		2	8	15	25	28	24	26			126
		3	9	19	26	30	31	27	22		164
		4	9	17	30	29	26	28			139
		5	6	15	28	30	28	27			134
		6	7	19	29	26	27	33			141
		7	9	17	27	28	28	22			131
		8	6	17	26	28	27	28			132
		9	6	15	26	25	28	26			126
		10	6	15	24	26	26	28			125
	1.9	1	6	13	18	21	22	23			103
		2	6	12	16	19	24	25			102
		3	7	12	17	24	24	26			110
		4	8	13	19	19	24	22			105
		5	9	12	23	20	25	24			113
		6	6	15	17	17	23	19			97
		7	6	16	21	23	24	25			115
		8	7	16	18	20	18	18			97
		9	9	17	17	20	19	24			106
		10	7	13	16	18	24	22			100
	3.6	1	7	13	10	17	18	17			82
		2	5	8	15	21	18	23			90
		3	3	9	13	21	19	23			88
		4	5	13	13	19	20	21			91
		5	4	8	8	12	22	24			78
		6	6	13	12	14	20	22			87
		7	3	12	14	19	17	22			87
		8	4	9	15	20	23	24			95
		9	7	12	12	18	18				67
		10	6	10	13	20	24	20			93
	7.2	1	6	13	12	8					39
		2	6								6
		3	6	9	9						24
		4	7	14							21
		5	6	8	6	13	10	14			57
		6	4	6	10						20
		7	3								3
		8	4	8							12
		9	3								3
		10	5	7	4	14					30

*Each rep. no. corresponds to 1 *D. magna* that was exposed to the respective intensity of UV-radiation ($\mu\text{W}/\text{cm}^2$) and anthracene concentration ($\mu\text{g}/\text{L}$) for 21 days.

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LIST OF REFERENCES

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