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Sublethal Effects of Photoenhanced Toxicity of Diluent to *Ceriodaphnia dubia* Reproduction

Final Report

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ABSTRACT

Traditionally, the toxic effects of petroleum have been investigated by conducting studies in the absence of UV radiation. Photomediated toxicity is often not considered, and the toxic effects of an oil spill can be grossly underestimated. The toxicity of a diluent to *Ceriodaphnia dubia* was examined in the presence of UV radiation. A solar simulator equipped with UVB, UVA and cool white lamps was used to generate environmentally comparable solar radiation intensities. *Ceriodaphnia dubia* were exposed to six concentrations of water accommodated fractions (WAF) of diluent in conjunction with three levels of laboratory simulated UV radiation (Reference = <0.002 μW/cm^2 UV-B; 3.0 μW/cm^2 UV-A; Low = 0.30 μW/cm^2 UV-B; 75.0 μW/cm^2 UV-A; Medium = 2.0 μW/cm^2 UV-B; 340.0 μW/cm^2 UV-A) and visible light. Seven day static renewal bioassays were used to characterize WAF/UV toxicity. WAF toxicity significantly ($p \leq 0.05$) increased when the organisms were exposed to WAF in the presence of UV radiation. The photoenhanced toxicity of the WAF increased with WAF concentration within each UV light regime. Relative to the reference UV light regime, the average number of neonates from adults exposed to 1.6 mg/L TPH decreased significantly by 20% within the low light regime, and by 60% within the medium light regime. These results indicate that organisms exposed to dissolved-phase diluent in the presence of environmentally realistic solar radiation, exhibit 1.3-2.5 times greater sensitivity, relative to organisms exposed under traditional laboratory fluorescent lighting.

INTRODUCTION

Most studies have focused on looking at the effects of petroleum on marine or estuarine organisms. However, freshwater ecosystems can be contaminated by petroleum through accidental releases, extraction, refining, and transportation. Additionally, the potential interaction between ultraviolet radiation and petroleum products have only recently been evaluated (Pelletier et al., 1997; Little et al.,1998, *Menidia* report). Polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatics (erg; dibenzothiophenes, acridines) are components of oils that contaminate aquatic environments throughout the world, and bioaccumulate in aquatic organisms (Laflamme and Hites, 1978; Southworth et al., 1978). For the most part, laboratory studies have shown that PAHs are not acutely toxic within aqueous solubility limits, because bioassays are usually conducted under normal fluorescent (absent of UV) lighting to prevent photodegradation of the PAH molecule. However, recent studies have shown that PAHs can be phototoxic to aquatic organisms at very low concentrations, well below aqueous solubility limits, and at the low concentrations found in natural waters (Neff, 1979; USEPA, 1980; Geisy and Allred, 1985; Newsted and Giesy, 1987). The PAH composition and the concentration vary with the petroleum product. For example, Fuel Oil #2 a middle distillate, contains limited but detectable amounts of PAHs (2 and 3 ring PAHs), while Fuel Oil #6 is highly enriched, containing large amounts of PAHs (>4 ring PAHs) (NRC, 1985). The acute and chronic effects of petroleum and petroleum products have been examined with freshwater invertebrates, particularly *Daphnia sp.* Several studies have shown that PAH toxicity to these invertebrates increases in the presence of natural or simulated ultraviolet radiation, (Allred and Giesy, 1985; Holst and Giesy, 1989; Davenport and Spacie, 1991; Wernersson and Dave, 1997). However, toxicity depends on the concentrations as well as types of PAHs in the water, therefore effects to aquatic organisms will vary with different PAHs and heterocyclic aromatics.

The phototoxicity of PAHs depends on the amount of ultraviolet radiation that penetrates the water column and the duration of exposure. Increased turbidity (Ireland et al. 1996) and humic acids (Oris et al. 1990) can reduce PAH toxicity by reducing light penetration and also by sequestering PAHs and making toxicants less bioavailable. Holst and Giesy (1989) demonstrated that intensities of ultraviolet radiation at 10 to 12 meter depths in Lake Michigan, in combination with low concentrations of anthracene caused reduced fecundity in surviving *Daphnia magna* in the laboratory.

The phototoxicity of PAHs can occur via two mechanisms. Toxicity can increase through metabolic activation of the PAH compound into metabolites or by-products that are far more toxic than the parent compound (Mekenyan et al., 1994). Toxicity also occurs when the PAHs act as a photosensitizer causing the formation of free radicals or singlet oxygen which may damage cell membranes or DNA (Kagan et al., 1990; Foote, 1991). PAHs associated with petroleum discharges to aquatic environments have the potential to adversely impact aquatic

biota inhabiting surface waters close to production sites if exposed to ultraviolet radiation (Kosian et al., 1998).

The purpose of this study was to examine the effects of exposure to the dissolved-phase of diluent in the presence of environmental intensities of simulated ultraviolet radiation on the survival and reproduction of *Ceriodaphnia dubia*. We used TPH in water as the measure of petroleum exposure because, TPH was selected as an appropriate measure of toxicity in photoenhanced toxicity tests because: (1) TPH accounts for most constituents in diluent and quantifies the complex mixture of hydrocarbons, rather than accounting for only a small fraction (Stratus Consulting, 1998a); (2) specific components of diluent have not been identified as the single or primary determinants of diluent toxicity (Stratus Consulting, 1998b); (3) the most comprehensive exposure data set at the site is TPH in surface water (Hagler Bailly, 1997); and (4) toxicity thresholds and exposure concentrations were developed using the same analytical chemistry methods, thus field and laboratory TPH values are directly comparable. Additionally, in evaluating the toxicity of complex mixtures of petroleum hydrocarbons, rather than evaluating the toxicity of individual analytes, it is common practice to express exposure as a TPH concentration (e.g., Anderson et al., 1974; Markarian et al., 1995).

MATERIALS AND METHODS

Exposure System and Light Treatments: Observations of UV and visible light were measured at 1 nm intervals from 280-700 nm in the laboratory and field (Hagler Bailly, 1997) with an Optronics OL-754 spectroradiometer, equipped with an underwater integrating sphere. Measurements were taken in the water column at several depths in habitats in the vicinity of the Guadalupe oil field to quantify the potential UV radiation of zooplankton such as *Ceriodaphnia*. The UV radiation applied during this laboratory study simulated levels of irradiance measured at the field sites (Hagler Bailly, 1997). The simulated light treatments were selected on the basis of ultraviolet-b (UV-B) intensities because UV-B wavelengths are potentially the most harmful to aquatic organisms. The manipulation of UV-B also reduced the intensity of UV-A and visible light. Generally, this resulted in an irradiance treatment that approximated irradiance that would occur as sunlight is attenuated in the water column of natural aquatic habitats.

All light treatments were manipulated by the use of various filtering materials. The materials were selected based on their filtering capacity and stability over time. Three light regimes were selected that consisted of a reference, low, and medium treatment. The filters used to generate the light treatments along with the corresponding intensities are reported in Table 1. The exposures were performed in an environmental chamber beneath a solar simulator (Little and Fabacher, 1996). The solar simulator consisted of a 0.61m wide by 1.83m long light cap. The light cap was suspended over a water bath of similar dimensions having a water depth of 15 cm. The light cap contained ten 160-Watt cool white lamps (General Electric Co., East Cleveland, OH), four 160-Watt UVB-313 lamps (National Biological Corp., Twinsburg, OH), eight 160-Watt UVA-365 lamps (National Biological Corp., Twinsburg, OH), two 35-Watt high output cool white lamps (Osram Sylvania, Danvers, MA), two 20-Watt SF20 sunlamps (Philips

Lighting, Somerset, NJ), and eight 75-Watt halogen incandescent flood lamps (General Electric Co., East Cleveland, OH). The light cap and water bath were enclosed with reflective specular aluminum to reduce any light loss. The sets of lights were controlled by recycling 24-h timers. The photoperiod was 14 hours of light and 10 hours of darkness, comparable to an August photoperiod along the central coast of California. During the middle portion of the light photoperiod (after 5 hours of UV-A and visible light), the UV-B lamps were turned on for four hours to simulate summer midday UV-B intensities. The air temperature was controlled by an air conditioner and water temperature was regulated at 25 ± 2° by a Remcor ® thermostatic heater/chiller unit that recirculated the water. Temperature in the waterbath was recorded daily during the duration of the exposure.

An integrating light-capture sphere was attached to the spectroradiometer by a glass fiber optic cable to measure the output of the simulator along a two dimensional matrix according to treatment position within the waterbath. Treatment measurements were obtained by covering a glass cylinder positioned on top of the integrating sphere with the various filtering materials used for manipulating light treatments. The light intensities used as treatments in the exposures encompassed the range of intensities that occurred in the field, thus the laboratory simulation was comparable to ambient solar radiation in the natural habitat (Fig. 1).

Test Organisms and Culturing: *Ceriodaphnia dubia* were obtained from a Columbia Environmental Research Center (CERC) laboratory culture. The mass cultures were maintained according to procedures described in Lewis et al. (1994). Individual neonates from clonal cultures were used to start up individual cultures three weeks before the exposures. Organisms were fed 0.5 ml of *Selenastrum capricornutum* and 0.5 ml of YCT (yeast/cerophyll/trout chow) daily. Neonates were removed and discarded daily until needed for testing to maintain optimal culture density. Individuals were acclimated to 2 $\mu W/cm^2$ UV-B (the same level as the medium light treatment, Table 1). This was necessary because preliminary range finding tests showed that organisms cultured under lower light intensities for three weeks exhibited poor control survival in preliminary tests. This finding suggested that lethality associated with UV radiation alone might obscure toxicity test results and hinder our main goal, which was to examine the interaction of WAF and UV on reproduction.

Neonates from pre-acclimated adult *C. dubia* were exposed to a series of dilutions of water accommodated fractions (WAF) prepared from diluent collected from the 5x monitoring well from the 5x underground plume at the Guadalupe Oil Field (described in Hagler Bailly, 1997b). WAF was tested in the presence of three UV light treatments in seven day static renewal tests. The seven-day static renewal tests were conducted according to procedures described by Lewis et al. (1994). Individual neonates less than 24 hours old were used for toxicity testing and one individual per 30 ml beaker containing 25 ml of a WAF dilution was randomly assigned to a group of five beakers (a bundle) in a plexiglass rack which contained four bundles. Three bundles per treatment (15 individuals total) were tested, except for the control WAF treatment in the reference and medium light regimes, in which there were four bundles each (20 individuals total). The bundles within each rack were covered with the appropriate filters to obtain the

desired light treatment and then randomly placed within the simulator water bath which was temperature controlled (Table 1). The organisms were fed 0.5 ml of a solution of yeast, cerophyll, and trout chow, and 0.5 ml of *Selenastrum capricornutum* after the daily renewal of new WAF solution. Adult mortality and offspring per surviving adult were recorded daily, and surviving adults were transferred to new 30 ml exposure beakers containing 25 ml of fresh WAF dilution. The pH, dissolved oxygen, and conductivity were measured daily in nine randomly selected test beakers during the exposure. The filters were replaced each day to control for photolytic decomposition.

WAF Preparation and Test Solutions: A slow stir procedure was used to prepare the WAF (Anderson et al., 1974). A Teflon stirbar and a 20 mm glass tube was placed into a one-quart glass mason jar, followed by 800 ml well water and 80 ml diluent, which was added gently to the surface of the water. The jar was sealed and the mixture allowed to stir slowly at approximately 200 RPMs for 24 hours under a fume hood at room temperature (25°C). After 24 hours, a Teflon tube was inserted through the 20 mm glass tube to siphon off the water accommodated fraction. This solution was defined as the 100% WAF, and our test concentrations were made from dilutions of this starting WAF mixture. The following five WAF dilutions were used: 0.31, 0.63, 1.25, 2.5, and 5% along with a control (0% WAF). A new concentrated WAF solution was made daily to prepare dilutions for the duration of the test exposures.

WAF Chemistry/TPH Analysis: WAF samples were analyzed for semi-volatiles, expressed as total petroleum hydrocarbon (TPH), and volatiles, expressed as benzene, toluene, ethylbenzene, and xylene (BTEX). The samples were taken from batch dilutions of new WAF and from the exposure chambers during the toxicity test. Sample volumes ranged from 0.25 to 1.0 L. All samples were gently transferred to pre-cleaned amber glass sample bottles (TPH analysis) or 40 mL volatile organic analysis vials (BTEX analysis) and stored in the dark at 4°C until they were analyzed.

One L samples for TPH analysis were taken from all batch dilutions of new WAF (initial samples) on days 0 and 5 of the test, except the 0.31% dilution. One L samples of new WAF were collected for the 5 % dilution on days 0, 1, 2, 3, 4, 5 and 6 (triplicate samples on day 6), to assess variability in TPH concentrations in newly prepared WAF across preparation days. The triplicate samples taken on day 6 were collected to assess variability due to analytical procedures. WAF dilutions following animal exposures on days 1 and 6 were composited and analyzed for TPH. Composite samples were pooled test solutions from exposure beakers after renewals (final samples) from each light regime within treatments except the 0.31 % dilution. All dilution samples were kept separate and were gently transferred to pre-cleaned amber glass sample bottles, and maintained in the dark at 4°C until extracted (EPA method 3510) and analyzed using full screen gas chromatography/mass spectrometry (GC/MS) (described in Stratus Consulting, 1998a). Initial and final samples for each WAF and light treatment combination were collected at test end and analyzed for BTEX compounds following EPA method 8260 (Stratus Consulting, 1998b). The minimum detection limit was 0.0005 mg/L for each analyte.

Statistical Analysis: The data collected at the end of the exposures (day 7) were analyzed as a UV by WAF factorial arrangement of treatments. Standard ANOVA analysis of cumulative mortality and mean number of neonates produced per surviving adult was performed because the test data were replicated. The one-tailed Dunnett's test was used to compare all treatment means if there was a significant interaction term. ANOVAs were performed for each UV intensity using its 0% WAF as a control. The data were arcsine square root transformed prior to analysis. All computations were performed using Statistical Analysis System (SAS, 1985) computer programs.

RESULTS

Water Quality: Water quality parameters were consistent for the duration of the exposure. Conductivity ranged from 527 to 586 μS/cm; dissolved oxygen ranged from 6.5 to 8.9 mg/L; pH ranged from 8.0 to 8.4; and temperature ranged from 24.5 to 25°C (Table 2).

Spectral Characteristics: The laboratory solar simulation was well below the irradiance observed in aquatic habitats in the vicinity of the oil collection locations (Hagler Bailly, 1997a) (Fig. 1). The filters used to obtain the UV-B treatments resulted in concurrent modifications of the UV-A and visible wavelengths, but these modifications were consistent with the spectral composition of natural sunlight. The total UV-B and UV-A doses for each light regime at days 4 and 7 of the exposure are reported in Table 6.

WAF Analysis: TPH concentrations (mg/L) varied with percent WAF composition used in the exposures (Table 3). Mean concentrations ranged from below the detection limit in the control to 1.6 mg/L TPH in the highest treatment level (5% WAF). The 0.31% WAF dilution was below detection limits, but TPH concentration was estimated as one half of the next higher TPH concentration. Newly prepared 5% WAF sampled on multiple test days ranged from 1.5 to 2.0 mg/L TPH (Table 3) (Stratus Consulting, 1998). Triplicate samples of 5% WAF measured on day 6 all had a concentration of 1.7 mg/L TPH (Table 3). In general, measured test concentrations correspond the percent WAF dilution, and were similar at test initiation and test termination. The volatiles (BTEX) in the WAF samples were all below the detection limit, Little et al. (1998, *Menidia* report) discusses BTEX results in more detail.

WAF Effects

ANOVA conducted on mortality data revealed no significant effect of WAF concentration on the survival of adult *C. dubia* for all light treatments (Table 4a). However, there were significant effects on reproduction. Within the reference light regime (0.002 μW/cm^2 UV-B), the average number of neonates per surviving adult significantly ($p<0.05$) decreased from 21.88 for the 0% WAF (0.0 mg/l TPH) concentration to 11.2 for the 5% WAF (1.5 mg/l TPH) concentration (49% decrease from the control) (Table 4b).

UV-B Effects: ANOVA conducted on mortality data revealed no significant effect of UV-B

intensity on the survival of adult *C. dubia*. Effects on reproduction were significant with increasing UV-B levels. The average number of neonates per surviving adult significantly ($p<0.05$) decreased in the 0% WAF (0.0 mg/l TPH) from 21.88 (reference light regime) to 12.0 (low light regime) and to 10.4 (high light regime) (Table 4b). This is a 45% decrease in the low light regime and a 52% decrease in the high light regime when comparing to the reference light regime.

WAF/UV Effects: ANOVA conducted on mortality data revealed no significant effects of interactions between WAF treatment, duration of exposure and UV treatment (Fig. 2). Significant interactions between effects of WAF concentration and UV treatment on *C. dubia* reproduction were observed during the exposure. WAF toxicity significantly increased when *C. dubia* were exposed to increasing levels of UV. The average number of neonates per surviving adult for the 5% WAF (1.6 mg/L TPH) treatment within the reference light regime was 11.2. Within the low light regime, the average number of neonates significantly ($p<0.05$) decreased to 9.0 at the 5% WAF (1.6 mg/l TPH) concentration, a 20% decrease. For the 5% WAF (1.6 mg/l TPH) treatment within the high light regime, the average number of neonates significantly ($p<0.05$) decreased to 5.11, a 54% decrease for fecundity under the reference UV light treatment (Table 4b).

DISCUSSION

The effects of petroleum spills on aquatic systems has been studied extensively, particularly the PAH toxicity associated with petroleum and petroleum products. Marine systems have been the focus for much research, and thus data on the impacts of oil to various marine organisms predominate the literature (Nounou, 1980; Hollister et al. 1980; Fayad et al. 1995; Wertheimer et al. 1996; Moles, 1980; Korn, 1979). This is especially true for large oil spills such as the Exxon Valdez disaster (Marty et al. 1997; Wertheimer et al. 1994; Dean et al. 1996; Hooten and Highsmity, 1996). Traditionally, the toxicity of oils have been tested in the absence of UV light, with toxicity values generally greater than 1 mg/L (e.g., Rice et al. 1976). The consequences of petroleum spills in freshwater environments has received less attention, and the potential interaction between ultraviolet radiation and PAHs has been largely overlooked. However, a few studies have documented the photomediated toxicity of PAHs to aquatic organisms in the presence of UV radiation. Some specific PAHs are 10 to >50k times more toxic to many aquatic organisms in the presence of UV radiation. The mechanism of PAH toxicity is not well understood, however it seems that photolytic alteration of the PAH molecule by UV radiation causes tissue damage by oxidation of membrane bound contaminants (Newsted and Giesy, 1987). Ongoing research has also identified phototoxic heterocyclic aromatics that occur in petroleum (e.g., acridine, dibenzothiothenes; Kosian et al., 1998; Newsted and Giesy, 1987). Some of these compounds are also present in the diluent used in the present tests (Ricker, pers comm.) WAF prepared from diluent is low in 3 ring and larger PAHs, including known photoactivated chemicals (Stratus Consulting, 1998). Thus TPH was used as the measure of

petroleum exposure in photoenhanced toxicity tests because diluent toxicity was not obviously linked to any specific PAH or total PAH concentration.

Ambient solar radiation was measured in a Marsh Pond C at the Guadalupe Oil Field on August 5, 1996 (Hagler Bailly, 1997). Marsh Pond C is a deep, freshwater pond surrounded by emergent vegetation (*Scirpus*) and limited submergent vegetation. In open water at a depth of 10 cm, total UV-A of 998 $\mu W/cm^2$ and total UV-B of 38$\mu W/cm^2$ was observed. The UV light regimes used in this study were well below the levels measured in the field. The high light regime was 5% of ambient UV-B, underwater at 10 cm depth and 34% of ambient UV-A, underwater at 10 cm depth (Table 1). The low light regime was <1% of ambient UV-B and 8% of ambient UV-A (Table 1). Since less than 1% of ambient water column UV-B radiation was sufficient to cause photoenhanced toxicity of the TPHs, even habitats of low optical clarity will sustain photomediated toxicity.

In this study, the effects of UV, WAF, and UV/WAF in combination on *Ceriodaphnia dubia* were examined. Mortality of adult *C. dubia* was not significantly affected by UV, WAF, or UV/WAF combinations, during the seven day exposures, however many studies have shown that exposure to enhanced levels of UV radiation in combination with PAHs can cause injury to various aquatic organisms such as daphnids (Allred and Giesy, 1985; Holst and Giesy, 1989; Davenport and Spacie; 1991.). Allred and Giesy (1985) showed *Daphnia pulex* were not affected by exposure to anthracene in the dark at concentrations of 3.0, 9.6, and 30.0 $\mu g/L$, but when simultaneously exposed to UV radiation, anthracene was highly toxic, immobilizing organisms at these concentrations well below water solubility (30 to 44 $\mu g/L$ at 25°C) (May et al., 1978) . Another study showed that *Daphnia magna* survival decreased more than additively when exposed to anthracene and UV radiation than by either of the two stressors by themselves (Holst and Giesy, 1989). At a low level of UV radiation (60 $\mu W/cm^2$ UV-A) and an anthracene concentration of 8.5 $\mu g/L$, 10% mortality of *D. magna* occurred. When the UV level was increased to 117$\mu W/cm^2$ UV-A, and the anthracene concentration was reduced to 7.2 $\mu g/L$, 70% mortality occurred, which clearly demonstrates photomediated toxicity. Relatively low concentrations of petroleum hydrocarbons in the presence of UV radiation can elicit adverse effects on survival as shown by the results of others.

Sublethal endpoints are often used to determine long-term effects on populations. By investigating phototoxic effects on reproduction, the potential impacts to population and community structure within natural aquatic systems can be predicted. In a 21 d exposure of anthracene and UV radiation to *Daphnia magna*, Holst and Giesy (1989) observed a reduction in the number of neonates produced. Exposure to anthracene (8.2 $\mu g/L$; 25% of the aqueous solubility) alone reduced the number of neonates produced by 13.8 % when compared to the controls. When *D. magna* were exposed to anthracene (7.2 $\mu g/L$) and a UV-A irradiance of 117 $\mu W/cm^2$, the number of neonates produced decreased 69%. So the two stressors in combination resulted in photoenhanced toxicity.

WAF prepared from diluent contained a complex mixture of chemicals, including a variety of PAHs, such as anthracene. Detailed chemistry for the 5x diluent and oil is presented in Stratus Consulting (1998a). Although it was beyond the scope of the current study to identify

the specific components of the WAF causing photoenhanced toxicity, it was evident that either specific components or the complex mixture of dissolved oil were phototoxic at environmentally realistic UV irradiances.

Holst and Giesy (1989) found no effects of UV in the absence of anthracene to *D. magna*. This observation may have occurred because they targeted UV-A exposure instead of UV-B and the use of a different species. Many studies have shown that UV-B can injure many aquatic species (Williamson et al., 1994; Siebeck, 1978; Little and Fabacher, 1994; Hurtubise et al., 1998). In our preliminary studies using non-acclimated *C. dubia*, mortality was high in all light treatments, proving these organisms to be very sensitive to UV exposure. However, pre-exposure to the high light regime (2.0 $\mu W/cm^2$ UV-B) for three weeks, adult *C. dubia* survival was high, although reproduction was severely impaired. Our study demonstrated that exposure of *C. dubia* to UV alone, WAF alone , and UV/WAF combinations significantly reduced the average number of neonates produced by surviving adults. Exposure to either WAF or UV alone elicited adverse affects in our study with an average 50% decrease in the number of neonates produced, however, when the two stressors are in combination, the average increases to a 70% reduction. The effects are even more pronounced when looking at mean total number of neonates produced per bundle (Table 5). At the highest WAF treatment in combination with the high light regime there was a 74% decrease in the total number of neonates produced per bundle when compared to the 0% WAF under the reference light regime. Therefore the WAF exposures were significantly more toxic in the presence of UV.

In summary, results from the present study suggest that aquatic organisms exposed to dissolved phase diluent in the presence of ultraviolet radiation may exhibit 1.3-2.5 times greater toxicity than exposure to spilled oil in the absence of UV. This toxicity results in increased mortality and diminished reproductive success which will adversely affect population dynamics and community structure. Studies with amphibians (Little et al., 1998; Rana report), mysid shrimp (Cleveland et al., 1998, Mysid report) and fish (Little et al., 1998; Menidia report) also demonstrate the photoenhanced toxicity of diluent. Although effects were observed in all species tested, threshold levels varied with species and light intensity. The photomediated toxicity of petroleum hydrocarbons to fish, mysid shrimp, amphibians, and the reproductive success of *C. dubia* emphasizes the need to consider the synergistic effects of solar radiation and aquatic contaminants to avoid underestimating effects of petroleum contamination on aquatic populations that are generally exposed to UV radiation in field conditions.

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Table 1. The simulated solar radiation treatments used in the seven-day static renewal toxicity test with *Ceriodaphnia dubia* along with the corresponding filter combinations. All organisms were exposed to five concentrations of a WAF (5, 2.5, 1.25, 0.625, and 0.313) and a control without WAF under each of the light regimes.

		Simulated Solar Irradiance (μW/cm^2)			
Species	**Light Regime**	**UV-B**	**UV-A**	**Visible**	**Filter Combinations**
Ceriodaphnia dubia	Reference	0.002	3.0	257	Side wraps: one piece of 0.030 inch thick polycarbonate and one piece of 0.005 inch thick Mylar D; Top covers: two pieces of 0.030 inch thick polycarbonate and one piece of black, meshed shade cloth
	Low	0.3	75.0	850	Side wraps: one piece of 0.030 inch thick polycarbonate and one piece of 0.005 inch thick Mylar D; Top covers: two pieces of 0.030 inch thick UVF polystyrene, one piece of 0.005 inch thick Mylar D and one piece of black, meshed shade cloth
	High	2.0	340	2180	Side wraps: one piece of 0.030 inch thick polycarbonate and one piece of 0.005 inch thick Mylar D; Top covers: one piece of 0.005 inch thick Mylar D

Table 2. Mean (standard error) conductivity, dissolved oxygen (D.O.), pH, and temperature with standard deviation in parentheses, during exposure of *Ceriodaphnia dubia* to dilutions of a water accommodated fraction of a diluent and simulated UV-B treatments ranging from 0.002 (reference) to 2 ($\mu W/cm^2$) . N= 9 randomly selected treatments per day.

	Day of exposure							
Variable	0	1	2	3	4	5	6	7
Conductivity (μS/cm)	571 (9.5)	540 (14.2)	550 (11.2)	560 (11.5)	529 (10.2)	586 (8.2)	562 (11.9)	527 (7.9)
D.O. (mg/L)	7.2 (0.25)	6.5 (0.29)	6.5 (0.23)	8.9 (0.20)	8.5 (0.12)	8.1 (0.44)	8.0 (0.39)	8.1 (0.16)
pH	8.2 (0.08)	8.4 (0.11)	8.2 (0.09)	8.0 (0.06)	8.3 (0.09)	8.2 (0.11)	8.3 (0.20)	8.3 (0.12)
Temperature °C[a]	25.0	24.5	25.0	23.0	25.0	25.0	25.0	25.0

[a] Temperature in the exposure waterbath was recorded daily.

Table 3. Mean total petroleum hydrocarbon (TPH) concentrations with standard deviation and number of test samples in parentheses (SD, n) measured during a seven-day static renewal test with *Ceriodaphnia dubia*.*

	Sample type and TPH concentration (mg/L)		
Treatment (% WAF dilution)	Mean Newly prepared solutions	Mean Test chamber solutions	Mean All samples mg/L
0.00	0.00[a]	0.00[a]	0.00[a] (0.0)
0.31	----[c]	----[c]	0.07[b]
0.63	0.15	0.12	0.13 (0.04,4)
1.25	0.26	0.31	0.29 (0.09,3)
2.50	0.73	0.67	0.70 (0.13,4)
5.00	1.60	1.40	1.60[d] (0.29,7)

a. All samples measured below the detection limit (<0.05 mg/L).
b. Mean value estimated as one half of the next highest test concentration.
c. No samples collected.
d. Mean also includes initial samples collected on test days 2 through 4 and day 6[0.062, 1.9, 1.6, 1.7/1.7/1.7 (triplicate samples) mg/L TPH, respectively]. Mean of triplicate samples used as one observation for calculating the overall mean.
* Analytical methods and data are described in detail in Stratus Consulting, 1998b.

Table 4a. Survival data for *Ceriodaphnia dubia* during a 7-d exposure to five concentrations of WAF and three light regimes. Values in parentheses are standard deviations.

SURVIVAL TABLE 4a					
Nominal Light Treatment	**TPH (mg/L)**	**Replicate (bundle)**	**# Adults per bundle (at test beginning)**	**# Adults per bundle (at test end)**	**Percent Mortality**
Reference	0.00 (0.0)	1	5	5	20 (0.40)
		2	5	4	
		3	5	3	
		4	5	4	
	0.07	1	4	5	0 (0.0)
		2	5	5	
		3	5	5	
	0.13 (0.04)	1	5	4	13.33 (0.34)
		2	5	5	
		3	5	4	
	0.29 (0.09)	1	5	5	6.67 (0.25)
		2	5	5	
		3	5	4	
	0.70 (0.13)	1	5	5	0 (0.0
		2	5	5	
		3	5	5	
	1.6 (0.29)	1	5	5	0 (0.0)
		2	5	5	
		3	5	5	

SURVIVAL TABLE 4a Continued					
Nominal Light Treatment	**TPH (mg/L)**	**Replicate (bundle)**	**# Adults per bundle (at test beginning)**	**# Adults per bundle (at test end)**	**Percent Mortality**
Low	0.00 (0.0)	1	5	5	0 (0.0)
		2	5	5	
		3	5	5	
	0.07	1	5	5	13.33 (0.34)
		2	5	3	
		3	5	5	
	0.13 (0.04)	1	5	4	13.33 (0.34)
		2	5	4	
		3	5	5	
	0.29 (0.09)	1	5	5	0 (0.0)
		2	5	5	
		3	5	5	
	0.70 (0.13)	1	5	4	13.33 (0.34)
		2	5	4	
		3	5	5	
	1.6 (0.29)	1	5	5	0 (0.0)
		2	5	5	
		3	5	5	

SURVIVAL TABLE 4a Continued					
Nominal Light Treatment	**TPH (mg/L)**	**Replicate (bundle)**	**# Adults per bundle (at test beginning)**	**# Adults per bundle (at test end)**	**Percent Mortality**
High	0.00 (0.0)	1	5	5	5 (0.25)
		2	5	4	
		3	5	5	
		4	5	5	
	0.07	1	5	4	6.67 (0.25)
		2	5	5	
		3	5	5	
	0.13 (0.04)	1	5	5	6.67 (0.25)
		2	5	5	
		3	5	4	
	0.29 (0.09)	1	5	4	13.33 (0.34)
		2	5	4	
		3	5	5	
	0.70 (0.13)	1	5	5	13.33 (0.34)
		2	5	4	
		3	5	4	
	1.6 (0.29)	1	5	4	13.33 (0.34)
		2	5	5	
		3	5	4	

Table 4b. Reproduction data for *Ceriodaphnia dubia* during a 7-d exposure to five concentrations of WAF and three light regimes. Values in parentheses are standard deviations.

REPRODUCTION TABLE 4b						
Nominal Light Treatment	**TPH (mg/L)**	**Replicate (bundle)**	**# Adults per bundle (at test end)**	**Neonates per bundle**	**Mean Neonates/ Surviving Adult**	**Total Neonates**
Reference	0.00 (0.0)	1	5	100	21.88 (2.17)	349
		2	4	86		
		3	3	63		
		4	4	100		
	0.07	1	5	49	10.93 (1.47)	164
		2	5	52		
		3	5	63		
	0.13 (0.04)	1	4	65	14.78 (1.38)	192
		2	5	73		
		3	4	54		
	0.29 (0.09)	1	5	69	15.87 (3.58)	218
		2	5	69		
		3	4	80		
	0.70 (0.13)	1	5	69	7.93 (0.11)	119
		2	5	40		
		3	5	40		
	1.6 (0.29)	1	5	42	11.2 (2.80)	168
		2	5	56		
		3	5	70		

TABLE 4b Continued
REPRODUCTION TABLE

Nominal Light Treatment	TPH (mg/L)	Replicate (bundle)	# Adults per bundle (at test end)	Neonates per bundle	Mean Neonates/ Surviving Adult	Total Neonates
Low	0.00 (0.0)	1	5	76	12.0 (3.30)	180
		2	5	43		
		3	5	61		
	0.07	1	5	57	10.18 (1.09)	134
		2	3	28		
		3	5	49		
	0.13 (0.04)	1	4	50	10.32 (2.13)	134
		2	4	33		
		3	5	51		
	0.29 (0.09)	1	5	37	8.87 (1.29)	133
		2	5	47		
		3	5	49		
	0.70 (0.13)	1	4	27	6.88 (1.45)	91
		2	4	22		
		3	5	42		
	1.6 (0.29)	1	5	45	9.0 (0.80)	135
		2	5	49		
		3	5	41		

TABLE 4b Continued
REPRODUCTION TABLE

Nominal Light Treatment	TPH (mg/L)	Replicate (bundle)	# Adults per bundle (at test end)	Neonates per bundle	Mean Neonates/ Surviving Adult	Total Neonates
High	0.00 (0.0)	1	5	49	10.44 (1.81)	200
		2	4	35		
		3	5	51		
		4	5	65		
	0.07	1	4	44	9.13 (2.73)	126
		2	5	30		
		3	5	52		
	0.13 (0.04)	1	5	36	6.43 (1.59)	89
		2	5	23		
		3	4	30		
	0.29 (0.09)	1	4	46	10.35 (2.21)	132
		2	4	47		
		3	5	39		
	0.70 (0.13)	1	5	42	5.97 (2.17)	80
		2	4	21		
		3	4	17		
	1.6 (0.29)	1	4	16	5.1 (1.49)	68
		2	5	34		
		3	4	18		

Table 5. Mean number of neonates (SD) produced by surviving adult *Ceriodaphnia dubia* and mean (SD) total neonates per treatment bundle (n=3 except for 0 mg/L in the reference and high light regime where n=4).

TPH concentration (mg/L)	Reference Light Regime		Low Light Regime		High Light Regime	
	Neonates per adult	Total neonates per bundle	Neonates per adult	Total neonates per bundle	Neonates per adult	Total neonates per bundle
0	21.88 (2.17)	87.25 (17.46)	12.0‡ (3.30)	60.0‡ (16.52)	10.44‡ (1.81)	50.0‡ (12.27)
0.06	10.93† (1.47)	54.67† (7.37)	10.18 (1.09)	44.67 (14.98)	9.13 (2.73)	42.0 (11.14)
0.132	14.78† (1.38)	64.0† (9.54)	10.32‡ (2.13)	44.67‡ (14.98)	6.43†‡ (1.59)	29.67†‡ (6.51)
0.29	15.87† (3.58)	72.67† (6.35)	8.87‡ (1.29)	44.33‡ (6.43)	10.35‡ (2.21)	44.0‡ (4.36)
0.698	7.93† (0.11)	39.67† (0.58)	6.88† (1.45)	30.33† (10.41)	5.97† (2.17)	26.67† (13.42)
1.6	11.2† (2.80)	56.0† (14.0)	9.0 (0.80)	45.0 (4.0)	5.11†‡ (1.49)	22.67†‡ (9.87)

†Denote significant ($p<0.05$) difference from control (0% WAF) within individual light regimes.
‡Denote significant ($p<0.05$) difference compared to the same WAF treatment of the reference light regime.

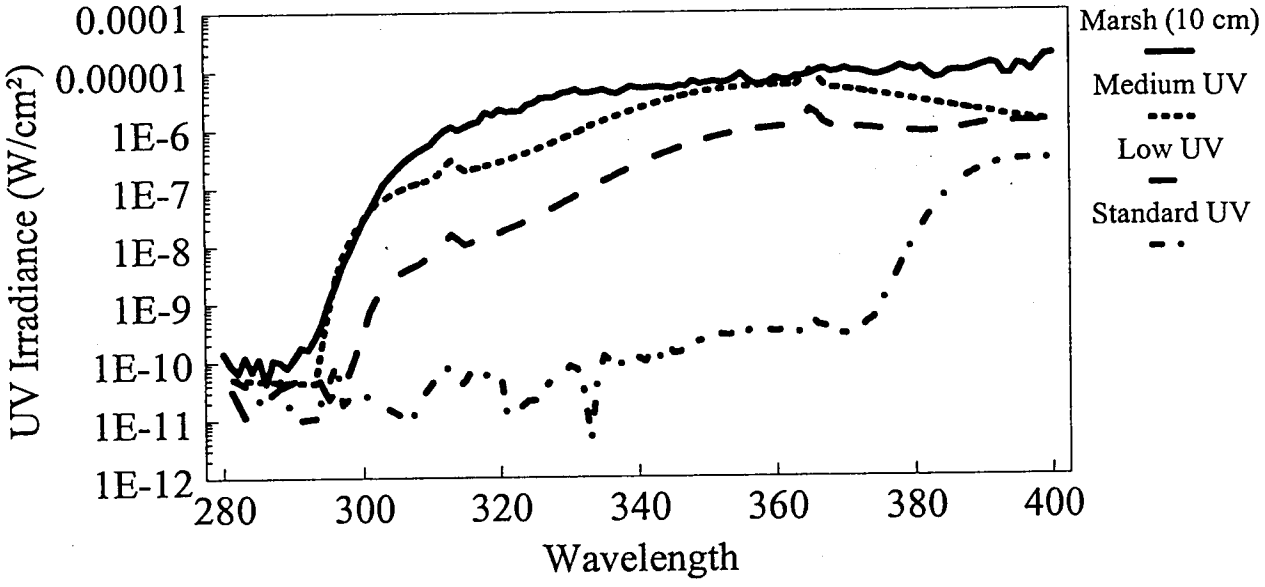
Table 6. Total UV-B and UV-A doses for each light regime at days 4 and 7 of the *Ceriodaphnia dubia* exposure.

Light Regime	**Total Dose (J/cm^2)**			
	Day 4		**Day 7**	
	UV-B	**UV-A**	**UV-B**	**UV-A**
Reference	.0001	.6048	.0002	1.058
Low	.0173	15.12	.0302	26.46
High	.1152	68.54	.2016	119.9

FIGURE LEGENDS

Figure 1. Spectral composition of simulated light treatments compared to natural sunlight at 10 cm in a freshwater pond near the site of an oil spill.

Figure 2. Percent survival for *Ceriodaphnia dubia* exposed to total petroleum hydrocarbons (TPH) in combination with three simulated UV light treatments at seven days.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0.00001
1E-6
1E-7
1E-8
1E-9
1E-10
1E-11
1E-12
UV Irradiance (W/cm²)
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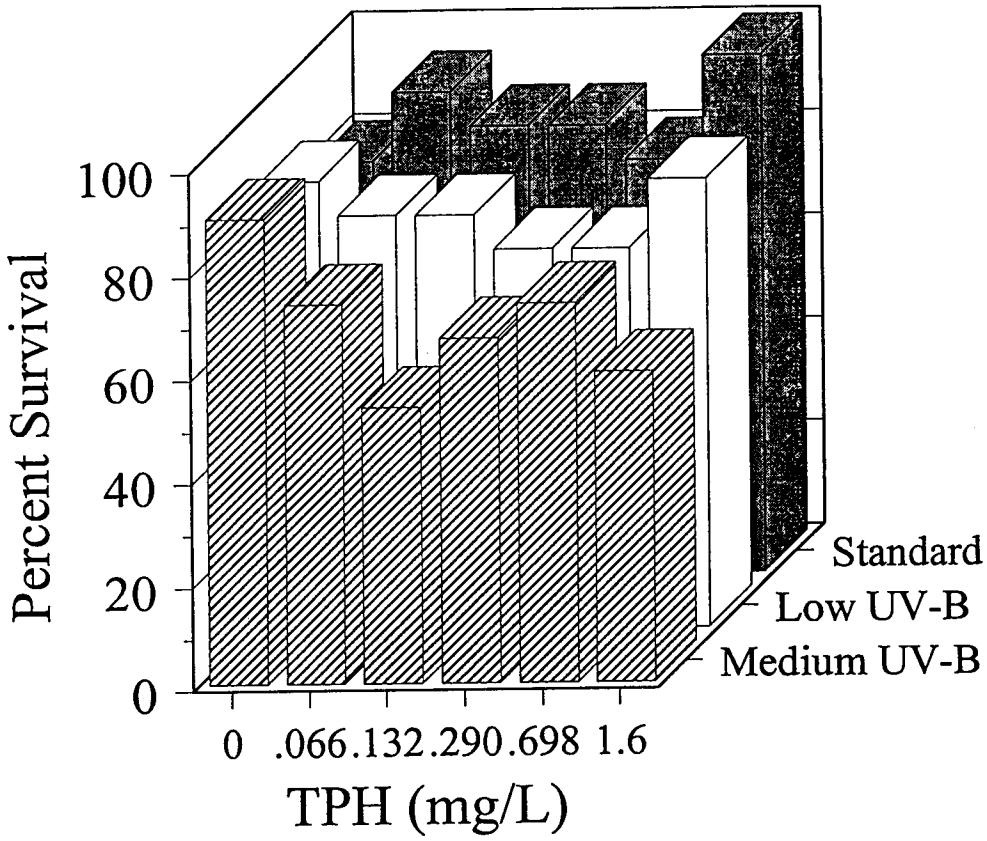
300
320
340
360
380
400
Wavelength
Marsh (10 cm)
Medium UV
Low UV
Standard UV


Percent Survival
100
80
60
40
20
0
0
.066
.132
.290
.698
1.6
TPH (mg/L)
Standard
Low UV-B
Medium UV-B