
# PHOTOENHANCED TOXICITY OF A DILUENT TO *MYSIDOPSIS BAHIA*

## FINAL REPORT

Prepared by:

Laverne Cleveland, Edward E. Little, Robin D. Hurtubise
USGS, BRD, Environmental and Contaminants Research Center,
Columbia, MO

Prepared for:

Rob Richer and Kathy Verrue-Slater
California Department of Fish and Game
Office of Spill Prevention and Response
1700 K Street, Suite 250
Sacramento, CA 95814

and

Mace Barron
Stratus Consulting Inc.
1881 9th Street, Suite 201
Boulder, CO 80302

December 12, 1998

## ABSTRACT

The toxicity of a water-accommodated fraction (WAF) prepared from diluent collected from the Guadalupe oil field was assessed in a 7-day static renewal test with *Mysidopsis bahia.* Solar ultraviolet and visible light intensities were measured in various habitats in the vicinity of the diluent sample collection sites, and the resultant measurements were used to reproduce laboratory light treatments that were representative of the on-site quality and intensity of natural solar radiation. Each of five WAF dilutions and a control without WAF was tested under three different simulated solar radiation intensities. During the test, survival and growth of the mysids, irradiance, and total petroleum hydrocarbon (TPH) concentrations in the test treatments were measured. Significant increases ($\leq 0.05$) in mortality occurred among mysids exposed to 0.57 and 1.30 mg/L TPH and the effects were potentiated as irradiance increased. Seven-day LC50 (0.92 to 0.42 mg/L TPH) and LC20 (0.58 to 0.15 mg/L TPH) values decreased as the simulated solar irradiance increased. Calculated EC20 and EC50 values for mysid growth indicate that surviving mysids exposed to 0.1 to 1.0 mg/L TPH would incur significant reductions ($\leq 0.05$) in productivity (biomass). Results of the present study indicate that effects elicited through the interaction of WAF of diluent and solar radiation will substantially increase the toxicity of diluent. Further, the photomediated effects of petroleum compounds, measured as TPH, on mysid survival and growth observed in the present study demonstrate a need to consider the interactions of UV light and contaminant to avoid under estimating toxicity that might occur in field settings.

## INTRODUCTION

Recent environmental research has focused on the toxicity to aquatic organisms elicited by concurrent exposure to natural solar radiation and environmental contaminants. Exposure to natural solar radiation alone, especially wavelengths in the UV-B range (280-320 nm), can cause damage at the molecular, cellular, and organismal levels of biological organization (Tevini 1993). However, concurrent exposure of many different kinds of organisms to ultraviolet radiation and specific contaminants can cause more severe biological injuries (Bowling et al., 1983; Oris and Giesy, 1985, 1987; Allred and Giesy, 1985; Newsted and Giesy, 1987; Cody et al., 1984; Gala and Giesy, 1992; Ren et al., 1994). Ecological effects due to the interactions of natural solar radiation and environmental contaminants may be exacerbated by recent increases in the levels of ultraviolet radiation reaching the earth (Rowland 1991; Smith et al., 1992; Kerr and McElroy 1993) due to ozone depletion.

The photo-mediated toxicity of environmental contaminants can be expressed through two mechanisms. *In vitro* photomodification (direct photooxidation) of a contaminant may occur, with the photoproduct being more toxic than the parent compound (Ren et al., 1994; Zepp and Schlotzhauer, 1979). *In vivo* photosensitization (indirect photosensitized oxidation) may occur, whereby organisms bioaccumulate photoactive chemicals and the toxic effects are manifested upon exposure of the organisms to solar radiation (Landrum et al., 1987; Newsted and Giesy, 1987; Boese et al., 1997). Toxicity resulting from *in vivo* photosensitized oxidation reflects complex interactions of (a) how much compound is accumulated in an organism, (b) the amount of light penetrating the aquatic environment and subsequently an organism, (c) the amount of light absorbed by the photosensitizing molecule (molar absorptivity), (d) the proportion of the sensitizing molecules that are transformed (excited) to a reactive species (quantum yield), and (e) the probability of interaction of the excited sensitizer with a target molecule (Newsted and Giesy 1987). The toxicity of photoactive chemicals can be grossly underestimated if these photo-mediated toxicity mechanisms are not considered in ecological risk assessments. Also, these two mechanisms are extremely important ecologically in that they facilitate the interaction of aquatic contaminants and natural solar radiation at the water surface, within the water column, and at the surface of sediments. For example, effects on aquatic organisms due to the photosensitized oxidation of contaminants are likely to increase as the depth of penetration of natural solar radiation in aquatic systems increases. Thus, highly turbid or colored surface waters, which attenuate the harmful wavelengths of solar radiation, tend to mitigate effects caused by photosensitized oxidation (Ireland et al., 1996; Oris et al., 1990), except in instances where the behaviors of organisms cause them to be active at the water surface (e.g., reproduction or feeding). On the other hand, the turbidity and color of surface waters are not likely to mitigate effects due to *in vitro* photooxidation of contaminants, which can be a function of several variables, including photooxidation rate (Ren et al., 1994), irradiance penetration depth, and the volume and mixing characteristics of the surface water.

Generally, investigations of the photomediated toxicity of petroleum have focused on a few non-alkylated PAHs such as anthracene; however, other petroleum components may also

contribute to the photomediated toxicity. In contrast to unalkylated parent compounds such as anthracene, the alkylated forms of PAHs are predominant in crude oils and many refined products and their water accommodated fractions (WAFs). QSAR modeling suggests that the alkylation of PAHs will have little effect on their photoactivation (Veith et al. 1995). Further, in addition to PAHs, heterocyclic aromatics and their alkylated homologs such as acridine (Oris and Giesy, 1987) and dibenzothiophenes (Kosian et al., 1996) can be photoactivated and are abundant in petroleum. Water soluble fractions of oils are likely to be enriched by these compounds because of their greater solubility compared to other components.

The present study was conducted to provide information on the interactive toxicity of a diluent and solar radiation to estuarine organisms. Early-life-history stages of the mysid shrimp (*Mysidopsis bahia)*, a marine crustacean, were exposed to dilutions of a WAF prepared from a diluent collected from the Guadalupe oil field. Field light intensity and spectral data were used to develop light treatments for the laboratory test. The organisms were exposed to the WAF dilutions under a range of simulated solar radiation treatments in a 7-day static-renewal test. The objective of the test was to quantify the toxicity of the WAF of the diluent under site-relevant conditions of light quality, intensity and exposure durations. Total petroleum hydrocarbon (TPH) was selected as an appropriate measure of exposure in the toxicity test 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 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, 1997a); 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). During the exposure, survival and growth of the mysids were monitored.

## MATERIALS AND METHODS

**General:** A randomized experimental design was used to expose *Mysidopsis bahia* to dilutions of a WAF of diluent collected from the Guadalupe oil field which has been described by Hagler Bailly (1997a). The organisms were exposed in a 7-day static renewal test to five WAF dilutions and a control treatment of dilution water with no WAF added. Each WAF dilution and control was tested under 3 different simulated solar radiation intensities, and three replicates of each WAF dilution/light intensity were tested. The tests were conducted according to procedures described in Klemn et al. (1994) at 20 $^{0}$C in 20 parts per thousand (0/00) saline water prepared with well water and 40 Fathoms Crystal Sea Salt (Marine Enterprises International, Inc., Baltimore, MD).

**Diluent Receipt and Handling:** Standard techniques (Stratus Consulting, 1998b) were

used to collect the diluent from an underground plume. The diluent samples were shipped to the Columbia Environmental Research Center (CERC) via overnight courier in pint or quart-sized screw-top amber glass bottles maintained at approximately 12-17° C with blue ice during shipment. The caps on the sample bottles were secured with tamper-resistant security tape and a chain-of-custody form was included with each shipment. A strict chain of custody was maintained during all activities in which the diluent was used, stored or handled at the CERC. Upon receipt at the CERC the sample shipping containers were inspected for damage and the security seals on the sample bottles were inspected for evidence of tampering. The chain of custody forms were completed and filed. The diluent was stored in their shipping containers in a walk-in cooler at 4° C. Subsequent usage of the diluent was documented on appropriate CERC data forms. After each usage the diluent was secured with tamper resistant security tape and stored at 4° C.

**WAF Preparation:** A slow-stir apparatus was used to prepare the WAF. A Teflon stirbar and a 20 mm glass tube were placed into a one-quart screw-top glass jar. Then 10 parts (by volume) well water (pH 7.0, hardness 283 mg/L as $CaCO_3$) was added to the jar and one part oil (by volume) was added gently to the surface of the water. The jar was sealed with the screw cap and the mixture was stirred slowly at about 200 $\pm$ 20 RPMs for 24 $\pm$ 2 hours in a fume hood at room temperature. A Teflon tube was inserted through the glass tube and the WAF was removed by siphoning. The solution was considered to be a 100% WAF of the diluent. A fresh WAF was prepared daily. The salinity of the freshly prepared WAF solutions was adjusted daily with appropriate volumes of a 33 parts per thousand (0/00) brine solution.

**Light Treatments:** Light treatments for the toxicity test were developed so that the WAF of the diluent could be tested in the laboratory under light conditions that were representative of the quality and intensity of natural solar radiation in the field. Incident light intensity and spectra were measured in selected habitats in the vicinity of an abandoned oil field on the California coast (described in Hagler Bailly, 1997b). The habitats selected for study were two coastal marsh ponds, a shallow freshwater wetland, an estuary lagoon, and the intertidal area of a high energy sand beach. These locations were selected because of their importance as habitat for aquatic organisms and because they periodically received discharges of dissolved petroleum from the abandoned oil field described in Hagler Bailly (1997a). The radiometric measurements were performed with an Optronics Model OL-754 spectroradiometer over a wavelength range of 280 to 700 nm. Photo multiplier tube voltage gain checks and wavelength calibrations of the spectroradiometer were conducted on the day before measurements were performed. When voltage gains of $\pm$ 10% were observed, the radiometer was calibrated with a NIST-traceable standard lamp before conducting the irradiance measurements. The light intensities used as treatments in the laboratory toxicity test encompassed the range of intensities that occurred in the field and were based on UV-B intensities since the UV-B wavelengths are potentially the most harmful to aquatic organisms. The manipulation of UV-B intensities resulted in concurrent changes in the intensity of UV-A and visible light and the resultant light

treatment approximated irradiance that would occur as sunlight is attenuated in the water column of natural aquatic habitats. All laboratory light measurements were performed with the same Optronics Model OL-754 spectroradiometer. Each light treatment required the use of specific filter materials or combinations of filter materials. The light filtering materials used to generate the laboratory light treatments were (1) 0.79 mm thick polycarbonate, (2) 0.79 mm thick UVF polystyrene, (3) 0.39 and 0.13 mm thick cellulose acetate, (4) 0.13 mm thick Mylar, (5) 51% shade cloth and (6) aluminum foil. The materials were selected based on their capacity to filter simulated solar radiation (i.e., UV-B, UV-A, and visible wavelengths), their stability over time, and their capacity to produce consistent filtering over time. The light filtering materials were used to cover the sides and the top of the exposure chambers to generate the desired light treatments for the test. The nominal simulated solar radiation treatments, based on the UV-B intensities measured in the selected habitats ranged from a low of 0.3 $\mu w/cm^2$ to a high of 2 $\mu w/cm^2$. The reference light treatment (UV-B, 0.002 $\mu W/cm^2$ ; UV-A, 3.2 $\mu W/cm^2$; visible, 247 $\mu W/cm^2$) was the lowest possible irradiance that provided sufficient visible light within the exposure chambers to allow feeding and was lower than office-like lighting conditions (e.g.,UV-B, 0.21 $\mu W/cm^2$; UV-A, 3.2 $\mu W/cm^2$; visible, 98 $\mu W/cm^2$). To ensure consistent lighting conditions with minimal fluctuations during the test, the filter combinations used were changed every other day.

**Solar Simulator:** A solar simulator was used to produce the laboratory light treatments. The solar simulator is a light fixture with dimensions of approximately 1 meter wide times 2 meters long. The simulator was suspended over a water bath of similar dimensions and was enclosed with a highly reflective NIST specular aluminum. The simulator contained fourteen 160-watt cool white lamps, four 160-watt UV-B 313 Lamps, eight 160-watt UV-A 365 lamps, two 20-watt warm white lamps, two 20-watt SF20 sun lamps, and eight 75-watt halogen incandescent flood lamps. The solar simulator was checked daily for lamp function, photocycle intervals, water bath temperature and recirculating flow. The exposure chambers were examined daily to ensure complete coverage by filtering materials.

To maintain the desired photo period, the solar simulator's UV-B lamps and incandescent lamps were controlled by one recycling 24-hour timer and the cool white and UV-A fluorescent lamps were controlled by a second timer. A 14:10 hour (light:dark) photo period was used during the test. This photo period consisted of a four-hour photo period of UV-B which was consistent with a midday UV-B exposure in the selected habitats in August. A 14-hour white light and UV-A photo period was used and was comparable to a photo period in the selected habitats in August. The UV-B photo period began five hours after the onset of the white light and UV-A photo period, except on day zero of the test when the UV-B, UV-A, and white light exposures were started at the same time due to study initiation activities. The white light and UV-A photo period continued for five hours after the end of the UV-B photo period to ensure that the exposed organisms had sufficient irradiance to utilize photo repair mechanisms.

To document the spectral quality and intensity of light during the test, a series of radiometric measurements were conducted within the solar simulator with the Optronics Model

OL-754 spectroradiometer used to conduct the field measurements. Prior to beginning the test, radiometric measurements within the solar simulator were conducted to ensure that the output of the simulator lamps remained consistent. These surface irradiance measurements were performed at a fixed position in the front and center of the simulator water bath. The integrating sphere of the spectroradiometer was inclosed with a glass cylinder to simulate the glass walls of the beakers used for exposure chambers. Also, the light intensity across the area of simulator water bath was confirmed by measuring surface irradiance at 12 locations in the water bath on three different occasions. These measurements were obtained at each location by covering the sides and top of a glass cylinder with each filter combination used to generate the light treatments, then placing the glass cylinder over the integrating sphere of the radiometer. Further, underwater irradiance was measured at fixed locations in the simulator on five different occasions to evaluate all filter combinations used to generate light treatments for the test. During the underwater measurements, the simulator was raised 84 cm above the lens of the optics head of the radiometer to maintain the same distance between the integrating sphere and overhead lamps as between the lamps and the exposure vessels.

**Test Organisms:** *Mysidopsis bahia* were obtained from Aquatic Indicators, St. Augustine, Florida. The organisms were shipped in plastic bags on ice to CERC 24 to 48 hours before the test was initiated. The organisms were observed for excessive mortality and signs of shipping stress when they arrived, allowed to warm to 20°C, and then transferred to 38 liter aquaria containing the 20 0/00 saline water described above. Mysids were fed brine shrimp (*Artemia sp.*) daily during the holding period before starting the test.

**Range Finder Test:** A range-finder test was conducted at 20 °C in the reconstituted 20 0/00 saline water. The mysids were exposed to duplicate treatments of a control of reconstituted 20 0/00 saline water and to four concentrations of WAF, each based on an order-of-magnitude dilution (10%, 1%, 0.1%, 0.01%) under the simulated solar radiation intensities. Survival was monitored during the tests. Tests without ultraviolet radiation were conducted in a temperature-controlled water bath under normal laboratory lighting (0.21$\mu$w/cm$^2$ UV-B, 3.2 $\mu$w/cm$^2$ UV-A, and 97.7 $\mu$w/cm$^2$ of visible light). Results of the range finder test were used to select the WAF dilution ranges for the definitive exposure.

**Definitive Test:** In the definitive test, seven-day-old mysids were exposed to five WAF dilutions and a control treatment in the presence of three simulated solar radiation treatments. Organisms were exposed individually to 25 mL of WAF dilution prepared with 20 0/00 saline water in 30 mL glass beakers. The organisms were exposed in bundles consisting of a group of five replicate test vessels that were grouped because they shared the same piece of light filtering material. Three bundles (15 animals total) were tested for each treatment, except the control WAF treatment in the reference and 0.3 $\mu$W/cm$^2$ light treatments in which 4 bundles were tested. The light filters were changed every other day. On day 0 of the test, the initial dry weight of groups of five mysids were determined. To start the test, one organism was placed into each 30-

mL beaker containing 25 mL of WAF dilution and randomly assigned to bundles in plexiglass racks which held 4 bundles. The bundles within each rack were covered with the appropriate filters for the desired light treatment and then randomly placed in the simulator water bath.

Temperature in the water bath was recorded daily. The pH and oxygen was determined according to procedures described by APHA et al. ( 1975), and salinity was measured with a refractometer for batch dilutions on day 0 . The pH, oxygen, and salinity of nine randomly selected test beakers were monitored daily during the test. Seventy-five percent of the exposure volume in each replicate test beaker was removed daily with a small pipette and replaced with fresh dilutions of WAF. Organisms in each test beaker were fed 0.5 mL of concentrated *Artemia* after the daily renewals. Mortality was recorded daily. At the end of the seven-day test, dry weights of surviving mysids in each replicate were determined. Mean biomass was determined from the total dry weight per replicate within each treatment. Mean organism weight was calculated as the total weight per treatment divided by the number of surviving organisms. Mean weight increase was calculated as the final replicate weight minus the initial weight.

**Chemical Analyses:** Water samples for TPH analyses (described in Stratus Consulting, 1998a) were taken from batch dilutions of new WAF. During the toxicity test, samples were taken from the exposure chambers after the mysids had been exposed for 24 hours. Samples of newly prepared test solutions were collected on days 0 and 5, and pooled samples were collected from the test chambers on days 1 and 6 after the daily renewals were performed. Only newly prepared test solution samples were collected for the 0.31% WAF treatment because the concentration of TPH in the test chambers after 24 hours was anticipated to be below the analytical detection limit. Samples of newly prepared test solutions were collected for the 5% WAF treatment on days 0 through 6 to assess variability in TPH concentrations across WAF preparation days. Additionally, triplicate 5% WAF samples were collected on day 4 to assess variability due to analytical procedures. The volumes of test chamber samples ranged from 250 to 500 mL and were dependent on mysid survival within each bundle. One liter samples were collected from the newly prepared test solutions. All samples were gently transferred to pre-cleaned amber glass bottles and stored in the dark at 4°C for 2 to 3 days. Then the samples were packed in coolers on blue or wet ice and shipped via overnight courier to the analytical chemistry laboratory (Zymax, San Luis Obispos, CA).

TPH was quantified by use of the U.S. Environmental Protection Agency method 3510. Sample extracts were analyzed with a gas chromatography/mass spectrometry (GC/MS) combination method (described in Stratus Consulting, 1998b). PAHs and their alkyl homologs were analyzed by GC/MS single ion monitoring (described in Stratus Consulting 1998b).

**Statistical Analysis:** Data were analyzed as an irradiance by WAF factorial arrangement of treatments. The one-tailed Dunnett's test (Dunnett, 1955) was used to compare all treatment means. Because of a significant interaction term, ANOVAs were performed for each light treatment using its 0 % WAF treatment as a control. ANOVA and the Dunnett's test were used to determine no-observed-effect concentrations (NOECs) and lowest-observed-effect

concentrations (LOECs). Arcsine square root transformations were performed on day 7 mortality data before analysis. Toxstat$^{R}$ computer programs (TOXSTAT$^{R}$ V3.5, 1996), which incorporate control mortality corrections, were used to calculate7-day LC50 and LC20 values as TPH concentrations within each light treatment. EC50 values for weight were estimated by incorporating one-half of the control weights into the regression line formula (SAS, 1985). EC20 values were calculated in a similar manner. Confidence intervals were not calculated for non -significant (P>0.05) regression coefficients (Snedecor and Cochran, 1980).

## RESULTS

**Exposure Conditions:** During the test, water quality parameters were maintained within values acceptable for good growth and survival of mysid shrimp. Salinity ranged from 20.1 to 21.7 0/00; dissolved oxygen ranged from 6.5 to 6.8 mg/L; pH ranged from 8.3 to 8.4; and temperature was maintained at 20 °C (Table 1). Measured UV-B irradiance during the test ranged from 0.002 $\mu W/cm^2$ for the reference light treatment to 1.82 $\mu W/cm^2$ for the high light treatment (Table 2). Filtration of UV-B resulted in concurrent reductions in the intensities of UV-A and visible light (Table 2). Mean TPH concentrations ranged from below the analytical detection limit (0.05 mg/L TPH) in the control treatment to 1.3 mg/L in the 5% WAF treatment (Table 3). The TPH concentrations in newly prepared test solutions declined in the test chambers by 32 to 40% after 24 hours (Table 3). The 100 % WAF was analyzed for 41 individual PAH compounds. Of these, naphthalene was predominant, and fluorene, phenanthrene, and anthracene were present at concentrations that could be quantified (Figure 1). The remainder of the PAH compounds were either not detected or were present at concentrations less than the lower calibration limit of the analytical method (Figure 1).

**Mortality:** Significant increases in mortality occurred among mysids exposed to 0.57 and 1.30 mg/L TPH within the reference and high light treatment and to 1.30 mg/L TPH within the low light treatment (Table 4). Mortality increased from 5.0% in the reference light treatment to 20% in the high light treatment in the absence of TPH (Table 4).

**Growth:** Of the three growth endpoints measured, biomass was the best indicator of TPH and UV exposure. Significant ($P \leq 0.05$) reductions of biomass was observed for mysids exposed to 0.57 and 1.30 mg/L TPH in the reference light treatment and for those exposed to 0.31 and 0.57 mg/L TPH in the low light treatment (Table 5). Biomass ranged from 0.40 to 0.83 mg for mysids exposed to TPH in the high light treatment where insufficient survival in the two highest TPH exposure concentrations precluded biomass determinations (Table 5). Within the reference light treatment, significant ($P \leq 0.05$) decreases in mean weight and mean weight increase of mysids were observed for the 0.57 mg/L TPH treatment, but growth in the next higher TPH treatment was similar to that of the control treatment (Table 5). Within the low and high light treatments, TPH did not elicit significant ($P \leq 0.05$) adverse effects on the mean weight or mean weight gain of surviving mysids; however, within the highest TPH treatment in the low

light treatment and the two highest TPH treatments in the high light treatment survival of mysids was insufficient to estimate mean weight and weight gain (Table 5).

**Toxicity Estimates**. The NOEC and LOEC values for mortality of mysids exposed to TPH did not vary extensively among the three light treatments. Based on cumulative 7-day mortality the NOECs ranged from 0.31 mg/L TPH for the reference and high light treatments to 0.57 mg/L TPH for the low light treatment (Table 6). The 7-day LOECs ranged from 0.57 mg/L TPH for the reference and high light treatment to 1.30 mg/L TPH for the low light treatment (Table 6). Seven-day LC50s values ranged from 0.92 mg/L TPH for the reference light treatment to 0.42 mg/L TPH for the high light treatment: LC20 values ranged from 0.51 mg/L TPH for the reference light treatment to 0.15 mg/L TPH for the high light treatment (Table 6). In the absence of TPH 1.82 $\mu W/cm^2$ UV-B, the highest intensity tested, did not elicited significant ($\leq 0.05$) effects on the mortality of mysids.

The 7-day NOEC and LOEC were 0.31 and 0.57 mg/L TPH, respectively, for mean biomass of mysids exposed to the reference light treatment (Table 6). In the low light treatment, the 7-day NOEC and LOEC were $\leq 0.05$ and 0.31 mg/L TPH, respectively (Table 6). In the high light treatment the NOEC for biomass of mysids was 0.31 mg/L TPH, and the LOEC was 0.57 mg/L TPH, which is based on insufficient survival for biomass determination (Table 6). For weight increase and mean weight of mysids, respectively, the estimated EC50 values ranged from 0.21 mg/L TPH to 1.20 mg/L TPH in the reference light treatment; from 0.25 to 0.60 mg/L TPH in the low light treatment; and from 0.22 to 0.60 mg/L TPH in the high light treatment (Table 7). Mean weight EC20 values ranged from 0.24 mg/L TPH in the reference light treatment to 0.30 mg/L TPH in the high light treatment. Mean weight increase EC20 values ranged from 0.11 mg/L in the low light treatment to 0.15 mg/L in the high light treatment (Table 7).

Survival and growth data for all replicate (bundle) treatments are presented in Table 8.

## DISCUSSION

Measuring effects on aquatic organisms elicited by the interaction of natural solar radiation and environmental contaminants requires the integration of well-designed field and laboratory studies. Studies based on exposure conditions not likely to exist in the field or on species that are not likely to be exposed to those conditions, provide less useful information for predicting potential ecological impacts. In the present study, the quality and quantity of solar radiation present in natural habitats were measured and used to develop realistic light regimes for simulated light treatments in laboratory tests. The simulated light treatments were tested in the presence of concentrations of WAF typical of petroleum concentrations expected to occur under field conditions of oil-water contacted and natural mixing. Thus, the effects of WAF on mysid shrimp observed in the present study are more likely to represent WAF effects that would occur under natural field conditions with exposure to sunlight.

WAF contains a complex mixture of compounds that represent a fraction of the total

compounds present in the diluent. The TPH concentrations measured during the test served as an exposure indicator for the whole WAF. In general, concentrations of TPH measured during the test corresponded to the nominal WAF dilutions tested. As expected, some variation in TPH exposure concentrations occurred during the test with initial TPH concentrations declining by 32 to 40 % during the 24-hour period between solution renewals. During the test, the solar simulator and filters used to generate the simulated solar radiation treatments functioned well, and measured UV-B intensity was maintained at 91 to 100 % of nominal values.

Exposure of mysids to TPH in the presence of simulated solar radiation significantly increased their mortality. Within each simulated solar radiation treatment, the significant increases in mortality of mysids were directly related to TPH exposure concentrations, and the effects were potentiated in the presence of increased simulated solar radiation. For example, mortality of mysids exposed to 1.3 mg/L TPH under the reference light treatment increased by 33 % under the low and high light treatment. Moreover, the estimated LOECs, LC50s, and LC20s values for mysid mortality (0.1 to 0.9 mg/L TPH) which decreased with increases in irradiance indicate that natural mysid populations would likely experience photomediated TPH toxicity, especially under marine oil spill conditions or in marine habitats receiving continuous point-source inputs of oil. The estimated EC20 and EC50 values for mysid growth indicate that surviving mysids exposed to 0.1 to 1.0 mg/L TPH would incur significant ($\leq 0.05$) reductions in productivity (biomass).

Photomediated toxicity to aquatic organisms has been demonstrated for several classes of environmental contaminants. Among these are organochlorine, organophosphate and carbamate pesticides (Metcalf 1968; Brooks 1980; Zaga et al., 1997) and polyaromatic hydrocarbons [(PAHs) Newsted and Giesy, 1987; Landrum et al., 1987]. The PAHs have generated the highest level of environmental concern due to their toxicity and environmental prevalence (Eisler 1987). Recently, the Agency for Toxic Substances and Disease Registry and the USEPA ranked PAHs among the top 10 environmental contaminants most commonly found at facilities on the National Priorities List and that pose the greatest potential threat to human health (ATSDR, 1997). Annually, about 230,000 metric tons of PAHs enter aquatic environments (Eisler 1987). Human activities such as fossil fuel combustion, petroleum spillage, domestic or industrial waste discharges, and surface runoff account for the largest portion of PAHs that enter aquatic systems (Neff, J.M. 1979; Eisler 1987; Arfsten et al., 1996).

PAHs and natural solar radiation interact to elicit a broad range of effects in aquatic animals and plants. Arfsten et al. (1996) reviewed the literature and concluded that a minimum requirement for the induction of lethal phototoxic effects is coexposure to adequate amounts of sunlight and PAH and that the lethal effects are likely due to massive cellular and tissue damage that cannot be repaired at an adequate rate. Other sublethal effects of the coexposure of organisms to PAHs and sunlight included random cellular damage requiring expenditure of energy by the organism for biological repair (Arfsten et al., 1996). Exposure of juvenile fathead minnows, *Pimephales promelas*, to the PAH fluoranthene and solar ultraviolet radiation resulted in disruptions of the integrity and function of gill tissue, suggesting that lethally was likely due to decreased oxygen diffusion capacity of the gills (Weinstein et al., 1997). Bluegill, *Lepomis*

*macrochirus*, exposed to anthracene and solar ultraviolet radiation for 96 hours exhibited significant increases in hematocrit, decreases in whole blood hemoglobin, evidence of hemolysis, and inhibition of Na,K-ATPase and Mg-ATPase enzyme activity in gill tissue (McCloskey and Oris 1993). McCloskey and Oris (1993) attributed the acute toxic responses of the exposed bluegill to a general disruption of cell membrane function. In the presence of simulated ultraviolet light, the individual PAHs anthracene, fluoranthene, and pyrene were 12 to >50,000 times more toxic to the juvenile marine bivalve, *Mulinia lateralis*, and juvenile mysid shrimp, *Mysidopsis bahia*, compared to toxicity in the absence of simulated ultraviolet light (Pelletier et al., 1997).

The WAF of petroleum products containing complex mixtures of PAHs demonstrated phototoxicity to marine bivalves and mysid shrimp, and the toxicity appeared to be related to the composition and concentration of PAHs present in the WAF tested (Pelletier et al., 1997). Boese et al., (1997) exposed seven species of marine crustaceans to fluoranthene for four days and then to simulated ultraviolet radiation for one hour: toxicity was enhanced up to tenfold in five of the seven organisms, and the sensitivity of the organisms to photoactivated flouranthene was inversely related to their potential for exposure to sunlight in nature. In other words, organisms that were exposed to sunlight in their natural habitats were the least sensitive to the fluoranthene/simulated ultraviolet light exposure.

WAF prepared from diluent is low in three-ring and larger PAHs, including known photoactivated chemicals (Stratus Consulting, 1998a). Thus, TPH was used as the measure of petroleum exposure in the test because diluent toxicity was not obviously linked to any specific PAH or total PAH concentration. Whereas, it was beyond the scope of the present study to identify specific sources of toxicity within the complex mixture tested, undoubtedly portions of the observed photomediated toxicity were due to the PAHs present, either singly or in concert with other components of the mixture.

## CONCLUSIONS

Results of the present study indicate that effects elicited through the interaction of the WAF of the diluent and solar radiation will substantially increase diluent toxicity. These results are corroborated by results obtained with three other species similarly exposed to the WAF (See companion reports, Little et al., 1998a on Menidia: Little et al., 1998b on Rana, and Hurtubise et al., 1998 on Ceriodaphnia) under site- relevant light conditions (Hagler Bailly 1997b). The photomediated toxicity thresholds vary with species and light intensity. The combined effects of TPH and simulated solar radiation on mysids are probably representative of effects that other small crustaceans inhabiting aquatic habitats would incur. The photomediated effects of TPH on mysid survival and growth observed in the present study underscore the need to consider the interactions of UV and contaminants to avoid under estimating toxicity that might occur in field settings. Accounting for influences such as UV radiation in laboratory tests will provide data that is more useful for predicting injuries to natural resources through ecological risk

assessments.

## ACKNOWLEDGMENTS

This report was prepared through contract with the California Department of Fish and Game, Office of Oil Spill Prevention and Response and Stratus Consulting Inc., Boulder, CO. Contract No. FG 4427 OS.

## REFERENCES

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. and Hightower, G.M., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Marine Biology, 27: 75-88.

Allred, P.M. and Giesy, J.P. 1985. Solar radiation-induced toxicity of anthracene to *Daphnia pulex*. Environ. Toxicol. Chem. 4, 219-226.

APHA (American Public Health Association), American Water Works Association and Water Pollution Control Federation. 1975. Standard method for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.

Arfsten, D.P., Schaeffer, D.J., and Mulveny, D.C. 1996. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: A review. Ecotoxicol. Environ. Safety. 33:1-24.

ATSDR. 1997. Agency for Toxic Substances and Disease Registry: 1997 CERCLA Priority List of Hazardous Substances. ATSDR Information Center, Division of Toxicology. Atlanta, GA.

Barron, M.G., T. Podrabsky, S. Ogle, and R.W. Ricker. **In press**. Do aromatic hydrocarbons determine petroleum toxicity to aquatic organisms? Aquat. Toxicol.

Boese, B.L., J.O. Lamberson, R.C. Swartz, R.J. Ozretich. 1997. Photoinduced toxicity of fluoranthene to seven marine benthic crustaceans. Arch. Environ. Contam. Toxicol. 32:3890.

Brooks, G.T. 1980. Perspectives of the chemical fate and toxicity of pesticides. J. Eviron. Sci. Health. B15:755-793.

Bowling, J.W., Leversee, G.J., Landrum, P.F. and Giesy, J.P. 1983. Acute mortality of anthracene-contaminated fish exposed to sunlight. Aquat. Toxicol. 3, 79-90.

Cody, T.E., Radike, M.J. and Warshawsky, D. 1984. The phototoxicity of benzo [a]pyrene in green alga *Selenastrum capricornutum*. Environ. Res. 35, 122-132.

Dunnett, C. W. 1955. A multiple comparisons procedure for comparing several treatments with a control. Journal of the American Statistical Association. 50:1096-1121.

Eisler, R. 1987. Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Fish and Wildlife Service Biological Report 85 (1.11). 81 pp.

Gala, W.R. and Giesy, J.P. 1992. Photo-induced toxicity of anthracene to green alga, Selenastrum Capricornutum. Arch. Environ. Contam. Toxicol. 23, 316-323.

Hagler Bailly. 1997a. Guadalupe oil field NRDA: Preliminary aquatic injury thresholds and comparison to site exposure data. February 18, 1997.

Hagler Bailly. 1997b. Guadalupe oil field NRDA. Assessment of solar radiation in selected aquatic habitats.

Ireland, D.S., Burton, G.A., Jr., and Hess, G.G. 1996. In situ toxicity evaluations of turbidity and photoinduction of polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 15, 574-581.

Kerr, J.B. and C.T. McElroy. 1993. Evidence of large upward trends of ultraviolet-B radiation

linked to ozone depletion. Science 262:1031-1034.

Klemn, D.J., G.E. Morrison, T.J. Norberg-King, W.H. Peltier, and M.A. Heber. 1994. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. Second edition. EPA-600/R-94/025. U.S. Environmental Production Agency, Cincinnati, Ohio.

Kosian, P.A., E.A. Makynen, P.D. Monson, D.R. Mount, A. Spacie, O.G. Mekenyan, and G.T. Ankley. 1996. Application of toxicity based fractionation techniques and structure-activity relationships models for the identification of phototoxic polyaromatic hydrocarbons in sediment pore water. Environ. Toxicol. Chem. 17:1021-1033.

Landrum, P.F.; Giesy, J.P.; Oris, J.T.; Allred, P.M. 1987. Photo-induced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In: *Oil In Freshwater: Chemistry, Biology, Countermeasure Technology*. Proceedings of a symposium of oil in freshwater, Edmonton Alberta, Canada. Edited by J.H. Vandermeulen and S.E.Hrudey. Pergamon Press, New York. pp. 314-328.

Markarian, R.K., Nicolette, J.P., Barber, T. and Giese, L., 1995. A Critical Review of Toxicity values and an evaluation of the Persistence of Petroleum Products for Use in Natural Resource Damage Assessments. American Petroleum Institute Publication Number 4594, January.

Metcalf, R.L. 1968. The role of oxidative reactions on the mode of action of pesticides. Pages 151-174, In: *Enzymatic Oxidation of Toxicants*, E. Hodgson, ed. North Carolina State University, Raleigh North Carolina. 299 pp.

Neff, J.M. 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates and Biological Effects. Applied Science Publishers LTD, Essex, England. 262 pp.

Newsted, J.L. and Giesy, J.P., Jr. 1987. Predictive models for photoinduced acute toxicity of polycyclic aromatic hydrocarbons to Daphnia magna, Strauss (Cladocera, Crustacea). Environ. Toxicol. Chem. 6, 445-461.

Oris J.T. and Giesy, J.P., Jr. 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (Lepomis spp.). Aquat. Toxicol. 6, 133-146.

Oris J.T. and Giesy, J.P., Jr. 1987. The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow Pimephales promelas. Chemosphere, 16, 1395-1404.

Oris, J.T., Hall, A.T., and Tylka, J.D. 1990. Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia. Environ. Toxicol. Chem. 9, 575-583.

Pelletier, M.C., Burgess, R.M., Ho, K.T., Kuhn, A., McKinney, R.A., Ryba, S.A. 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ. Toxicol. Chem. 16:2190-2199.

Ren, L., Huang, X.D., McConkey, B.J., Dixon, D.G. and Greenberg, B.M. 1994. Photoinduced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene, naphthalene) to duckweed *Lemna gibba* L. G-3. Ecotoxicol. Environ. Saf. 28, 160-177.

Rowland, S. 1991. Stratospheric ozone in the 21st century: the chlorofluorocarbon problem. Environ. Sci. Technol. 25:622-628.

SAS Institute. (1985). SAS[R] Users Guide: Statistics. Version 5 ed. Cary, NC.

*Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Edited by P. A. Lewis, D. J. Klemm, J.M. Lazorchak, T. J. Norberg-King, W.H. Peltier, and M.A. Heber. July 1994. EPA/600/4-91/002).

Smith, R.C., Pcezelin, B.B., Baker, K.S., Bidigare, R.R., Boucher, N.P., Coley, T., Karentz, D., MacIntyre, S., Matlick, H.A., Menzies, D, Ondrusek, M., Wan, Z., Waters, K.J. 1992. Ozone depletion: Ultra-violet radiation and phytoplankton biology in Antarctic waters. Sci, **255**, 952-958.

Snedecor, G.W. and W.G. Cochran. 1980. *Statistical Methods*, Seventh edition. The Iowa State University Press, Ames, Iowa.

Stratus Consulting. 1998a. Guadalupe Oil Field NRDA: Analytical Chemistry of Free-Phase and Dissolved-Phase Diluent.

Stratus Consulting. 1998b. Guadalupe Oil Field NRDA: Chemistry and Toxicity of Diluent, Site Water, and Sediment.

Tevini, M. 1993. Molecular Biological Effects of Ultraviolet Radiation. In: UV Radiation and Ozone Depletion. Lewis Publishers, Boca Raton, FL.

TOXSTAT[R] V3.5. 1996. West, Inc. Western EcoSystems Technology, Inc. 2003 Central Avenue, Cheyenne, WY.

Veith, G.D., O.G. Mekenyan, G.T. Ankley, and D.J. Call. 1995. A QSAR analysis of substituent effects on the photoinduced acute toxicity of PAHs. Chemosphere. 30:2129-2142

Weinstein, J.W., Oris, J.T., and Tayler D.H. 1997. An ultrastructural examination of the mode of UV-induce toxic action of fluoranthene in the fathead minnow, *Pimephales promelas*. Aquat. Toxicol. 39:1-22.

Zaga, A., E.E. Little, C.F. Rabeni, M.R. Ellersieck. 1997. Photoenhanced toxicity of a carbamate insecticide to early life stage amphibians. In Press: Environmental Toxicology and Chemistry.

Zepp, R.G.; Schlotzhauer, P.F. 1979. Photoreactivity of selected aromatic hydrocarbons in water. Pages 141-158 In: *P.W. Jones And P. Leber, Editors, Polynuclear Aromatic Hydrocarbons*. Third International Symposium On Chemistry And Biology--Carcinogenesis And Mutagenesis. Ann Arbor Science Publishers, Inc. Ann Arbor, Mi.

Table 1. Mean with standard deviation in parentheses of water quality parameters measured during exposure of *Mysidopsis bahia* to dilutions of a water accommodated fraction of diluent and simulated solar radiation.

| | Day of exposure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter[a] | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Salinity (0/00) | 20.08 (0.28) | 20.92 (0.64) | 21.0 (0.43) | 21.11 (0.57) | 21.33 (0.67) | 21.45 (0.78) | 21.67 (0.47) | 22.13 (0.33) |
| D.O. (mg/L) | 6.82 (0.16) | 6.49 (0.16) | 6.81 (0.13) | 6.53 (0.13) | 6.71 (0.14) | 6.78 (0.29) | 6.45 (0.19) | 6.68 (0.12) |
| pH | 8.27 (0.05) | 8.43 (0.06) | 8.38 (0.04) | 8.40 (0.0) | 8.38 (0.06) | 8.40 (0.0) | 8.42 (0.11) | 8.39 (0.08) |
| Temperature °C | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |

[a] N= 9 randomly selected treatments per day except for temperature which was measured daily in the exposure waterbath.

Table 2. Mean simulated solar irradiance with standard deviation in parentheses measured during exposure of *Mysidopsis bahia* to dilutions of a water accommodated fraction of diluent.

| Light treatment | Nominal irradiance ($\mu$W/cm$^2$) [a] | | | Measured irradiance ($\mu$W/cm$^2$) | | | Total dose (J/cm$^2$ | | Filter combinations used to obtain light treatments[b] |
|---|---|---|---|---|---|---|---|---|---|
| | UV-B | UV-A | Visible | UV-B | UV-A | Visible | UV-B | UV-A | |
| Reference | 0.002 | 3.0 | 257 | 0.002 (0.00) | 3.0 (0.36) | 262 (44) | 0.0002 | 1.06 | Side wraps - one piece of 0.79 mm thick polycarbonate and one piece of 0.13 mm thick mylar : Top covers - two pieces of 0.79 mm thick polycarbonate and one piece of black, meshed shade cloth |
| Low | 0.3 | 75.0 | 850 | 0.28 (0.06) | 74 (17) | 828 (34) | 0.03 | 26.46 | Side wraps - one piece of 0.79 mm thick inch polycarbonate and one piece of 0.13 mm thick mylar: Top covers - two pieces of 0.79 mm thick UVF, one piece of 0.13 mm thick mylar, and one piece of black, meshed shade cloth |
| High | 2.0 | 340 | 2180 | 1.8 (0.29) | 300 (39) | 2160 (215) | 0.20 | 119.90 | Side wraps - one piece of 0.79 mm thick polycarbonate and one piece of 0.13 mm thick mylar: Top covers - one piece of 0.13 mm thick mylar |

[a] Values represent integrated wavelength-specific intensities as follows: 280-320 nm for UV-B, 320-400 nm for UV-A, and 400 to 700 nm for visible light. Wavelength integrations were performed with scanning spectroradiometer software.

[b]All filter materials were obtained from Cope Plastics, St. Louis, MO: shading capacity of the black, meshed shade cloth = 51 %.

Table 3. Mean total petroleum hydrocarbon (TPH) concentrations with standard deviation and sample size (n) in parentheses (SD, n) measured during a seven-day static renewal test with *Mysidopsis bahia.*

| | Sample type and TPH concentration (mg/L) | | |
|---|---|---|---|
| Percent WAF solution | Newly prepared solutions | Test chamber solutions | All samples |
| 0.00 | < 0.05 | < 0.05 | < 0.05 |
| 0.31 | < 0.05 | NM | < 0.05 |
| 0.63 | < 0.05 | < 0.05 | < 0.05 |
| 1.25 | 0.36 (0.19, 2) | 0.24 (0.04, 2) | 0.31 (0.13, 4) |
| 2.50 | 0.68 (0.34, 2) | 0.46 (0.05, 2) | 0.57 (0.24, 4) |
| 5.00 | 1.43 (0.23, 8) | 0.85 (0.17, 2) | 1.3 (0.30, 9) |

NM = not measured

Table 4. Percent cumulative mortality with standard deviations in parentheses for *Mysidopsis bahia* exposed to total petroleum hydrocarbon (TPH) and simulated solar radiation treatments for seven days.

| Light treatment | Percent mortality for each TPH exposure concentration (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | <0.05 | <0.05 | <0.05 | 0.31 | 0.57 | 1.30 |
| Reference | 5.00 (0.23) | 6.67 (0.27) | 0.00 | 6.67 (0.27) | 53.33† (0.33) | 66.67† (0.46) |
| Low | 13.33 (0.27) | 6.67 (0.27) | 40.00 (0.81) | 20.00 (0.35) | 33.33 (0.56) | 100† |
| High | 20.00 (0.40) | 0.00 | 40.00 (0.21) | 33.33 (0.56) | 73.33† (0.44) | 100† |

† Denote significant difference from control (0.00 mg/L TPH) within each light treatment, ($P \leq 0.05$, Dunnetts Test).

Table 5. Mean and standard deviation (in parentheses) for total biomass, weight per organism, and weight increase of *Mysidopsis bahia* exposed to TPH and simulated solar radiation for seven days.

| Light treatment and TPH concentration (mg/L)[a] | Mean Biomass (mg) | Mean organism weight (mg) | Mean weight increase (mg) |
|---|---|---|---|
| **Reference** | | | |
| <0.05 | 0.88 (0.04) | 0.18 (0.01) | 0.07 (0.01) |
| <0.05 | 0.77 (0.05) | 0.16 (0.009) | 0.05 (0.009) |
| <0.05 | 0.67 (0.09) | 0.14 (0.02) | 0.03 (0.02) |
| 0.31 | 0.73 (0.02) | 0.15 (0.03) | 0.04 (0.03) |
| 0.57 | 0.17† (0.02) | 0.08† (0.07) | -0.04† (0.07) |
| 1.30 | 0.30† (0.10) | 0.12 (0.02) | 0.002 (0.02) |
| **Low** | | | |
| <0.05 | 0.80 (0.22) | 0.19 (0.08) | 0.08 (0.08) |
| <0.05 | 1.00 (0.14) | 0.21 (0.03) | 0.10 (0.03) |
| <0.05 | 0.70 (0.14) | 0.16 (0.06) | 0.05 (0.06) |
| 0.31 | 0.50† (0.22) | 0.13 (0.04) | 0.01 (0.04) |
| 0.57 | 0.33† (0.17) | 0.11 (0.02) | -0.003 (0.02) |

| | | | |
|---|---|---|---|
| 1.30 | ND | ND | ND |

Table 5 continued

| Light treatment and TPH concentration (mg/L)[a] | Mean Biomass (mg) | Mean organism weight (mg) | Mean weight increase (mg) |
|---|---|---|---|
| **High** | | | |
| <0.05 | 0.70 (0.13) | 0.15 (0.03) | 0.03 (0.03) |
| <0.05 | 0.83 (0.12) | 0.17 (0.03) | 0.05 (0.03) |
| <0.05 | 0.57 (0.12) | 0.17 (0.05) | 0.06 (0.05) |
| 0.31 | 0.40 (0.22) | 0.11 (0.01) | 0.001 (0.01) |
| 0.57 | ND | ND | ND |
| 1.30 | ND | ND | ND |

[†] Denote significant difference from control (0.00 mg/L TPH) within each light treatment, ($P \leq 0.05$, Dunnetts Test).

[a] TPH concentrations were obtained from dilutions of a water accommodated fraction of a diluent sample.

ND = Not determined due to insufficient survival for weight determination.

Table 6. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs) for mortality and growth and LC50 and LC20 values for *Mysidopsis bahia* exposed to TPH and simulated solar radiation for seven days.

| | Mortality | | | | Mean biomass | |
|---|---|---|---|---|---|---|
| Light treatment | NOEC (mg/L TPH)[a] | LOEC (mg/L TPH) | LC50 (mg/L TPH) | LC20 (mg/L TPH) | NOEC (mg/L TPH)[a] | LOEC (mg/L TPH) |
| Reference | 0.31 | 0.57 | 0.92 (0.71-1.14) | 0.51 (0.31-0.71) | 0.31 | 0.57 |
| Low | 0.57 | 1.30 | 0.62 (0.45-0.79) | 0.29 (0.13-0.44) | <0.05 | 0.31 |
| High[b] | 0.31 | 0.57 | 0.42 (0.29-0.54) | 0.15 (0.02-0.28) | 0.31 | 0.57[c] |

[a] TPH = total petroleum hydrocarbon from a water accommodated fraction of a diluent sample.

[b] In the absence of TPH, exposure of *M. bahia* to the high light treatment for seven days did not elicit significant effects on mortality or growth.

[c] based on insufficient survival for biomass determination.

Table 7. Seven-day EC20 and EC50 values with 95% confidence intervals (in parentheses) for growth of *Mysidopsis* exposed to TPH and simulated solar radiation.

| | Mean weight per surviving adult | | Weight increase | |
|---|---|---|---|---|
| Nominal UV-B Treatments ($\mu$W/cm$^2$) | EC20 (mg/L TPH) | EC50 (mg/L TPH) | EC20 (mg/L TPH) | EC50 (mg/L TPH) |
| Reference | 0.24 (-2.5 -2.7) | 1.2 (-0.4 - 6.6) | ND | 0.21 (-2.6 - 2.6) |
| Low | 0.25 (-2.9 - 3.5) | 0.60 (-2.1 - 4.4) | 0.11 (-3.2 - 3.2) | 0.25 (-2.9 - 3.5) |
| High | 0.30 (ND) | 0.60 (ND) | 0.15 (ND) | 0.22 (ND) |

ND = Not determined due to non-significant regression coefficient (P> 0.05).

Table 8. Summary of survival and growth responses of *Mysidopsis* exposed to TPH and simulated solar radiation.

| Nominal Light Treatment | TPH (mg/L) | Replicate (bundle) | # Mysids per bundle (test end) | Biomass/ Bundle (g) | Mean Biomass (mg) | Mean Weight/ organisms (mg) | Mean weight increase/ organism (mg) |
|---|---|---|---|---|---|---|---|
| Reference | <0.05 | 1 | 5 | 0.0009 | 0.88 (0.04) | 0.18 (0.01) | 0.07 (0.01) |
| | | 2 | 5 | 0.0009 | | | |
| | | 3 | 4 | 0.0008 | | | |
| | | 4 | 5 | 0.0009 | | | |
| | <0.05 | 1 | 5 | 0.0008 | 0.77 (0.05) | 0.16 (0.009) | 0.05 (0.0009) |
| | | 2 | 4 | 0.0007 | | | |
| | | 3 | 5 | 0.0008 | | | |
| | <0.05 | 1 | 5 | 0.0006 | 0.67 (0.09) | 0.14 (0.02) | 0.03 (0.02) |
| | | 2 | 5 | 0.0006 | | | |
| | | 3 | 5 | 0.0008 | | | |
| | 0.31 | 1 | 5 | 0.0009 | 0.73 (0.02) | 0.15 (0.03) | 0.04 (0.03) |
| | | 2 | 5 | 0.0008 | | | |
| | | 3 | 4 | 0.0005 | | | |
| | 0.57 | 1 | 1 | 0.0001 | 0.17 (0.02) | 0.08 (0.07) | -0.04 (0.07) |
| | | 2 | 2 | 0.00 | | | |
| | | 3 | 4 | 0.0004 | | | |
| | 1.30 | 1 | 0 | ND | 0.30 (0.10) | 0.12 (0.02) | 0.002 (0.02) |
| | | 2 | 2 | 0.0002 | | | |
| | | 3 | 3 | 0.0004 | | | |

Table 8 continued

| Nominal Light Treatment | TPH (mg/L) | Replicate (bundle) | # Mysids per bundle (test end) | Biomass/ Bundle (g) | Mean Biomass (mg) | Mean Weight/ organisms (mg) | Mean weight increase/ organism (mg) |
|---|---|---|---|---|---|---|---|
| Low | <0.05 | 1 | 4 | 0.0007 | 0.80 (0.22) | 0.19 (0.08) | 0.08 (0.08) |
| | | 2 | 4 | 0.0011 | | | |
| | | 3 | 5 | 0.0006 | | | |
| | <0.05 | 1 | 5 | 0.0009 | 1.0 (0.14) | 0.21 (0.03) | 0.10 (0.03) |
| | | 2 | 4 | 0.0009 | | | |
| | | 3 | 5 | 0.0012 | | | |
| | <0.05 | 1 | 0 | ND | 0.70 (0.14) | 0.16 (0.06) | 0.05 (0.06) |
| | | 2 | 4 | 0.0008 | | | |
| | | 3 | 5 | 0.0006 | | | |
| | 0.31 | 1 | 4 | 0.0003 | 0.50 (0.22) | 0.13 (0.04) | 0.01 (0.04) |
| | | 2 | 5 | 0.0008 | | | |
| | | 3 | 3 | 0.0004 | | | |
| | 0.57 | 1 | 1 | 0.0001 | 0.33 (0.17) | 0.11 (0.02) | -0.003 (0.02) |
| | | 2 | 5 | 0.0005 | | | |
| | | 3 | 4 | 0.0004 | | | |
| | 1.30 | 1 | 0 | | ND | ND | ND |
| | | 2 | 0 | | | | |
| | | 3 | 0 | | | | |

Table 8 continued

| Nominal Light Treatment | TPH (mg/L) | Replicate (bundle) | # Mysids per bundle (test end) | Biomass/ Bundle (g) | Mean Biomass (mg) | Mean Weight/ organisms (mg) | Mean weight increase/ organism (mg) |
|---|---|---|---|---|---|---|---|
| High | <0.05 | 1 | 3 | 0.0004 | 0.70 (0.13) | 0.15 (0.03) | 0.03 (0.03) |
| | | 2 | 3 | 0.0004 | | | |
| | | 3 | 5 | 0.0006 | | | |
| | | 4 | 5 | 0.0007 | | | |
| | <0.05 | 1 | 5 | 0.0007 | 0.83 (0.12) | 0.17 (0.03) | 0.05 (0.03) |
| | | 2 | 5 | 0.001 | | | |
| | | 3 | 5 | 0.0008 | | | |
| | <0.05 | 1 | 3 | 0.0004 | 0.57 (0.12) | 0.17 (0.05) | 0.06 (0.05) |
| | | 2 | 2 | 0.0007 | | | |
| | | 3 | 4 | 0.0006 | | | |
| | 0.31 | 1 | 4 | 0.0005 | 0.40 (0.22) | 0.11 (0.01) | 0.001 (0.01) |
| | | 2 | 5 | 0.0006 | | | |
| | | 3 | 1 | 0.0001 | | | |
| | 0.57 | 1 | 0 | ND | ND | ND | ND |
| | | 2 | 1 | ND | | | |
| | | 3 | 3 | ND | | | |
| | 1.30 | 1 | 0 | ND | ND | ND | ND |
| | | 2 | 0 | ND | | | |
| | | 3 | 0 | ND | | | |

ND = Not determined due to insufficient survival for weight determination.

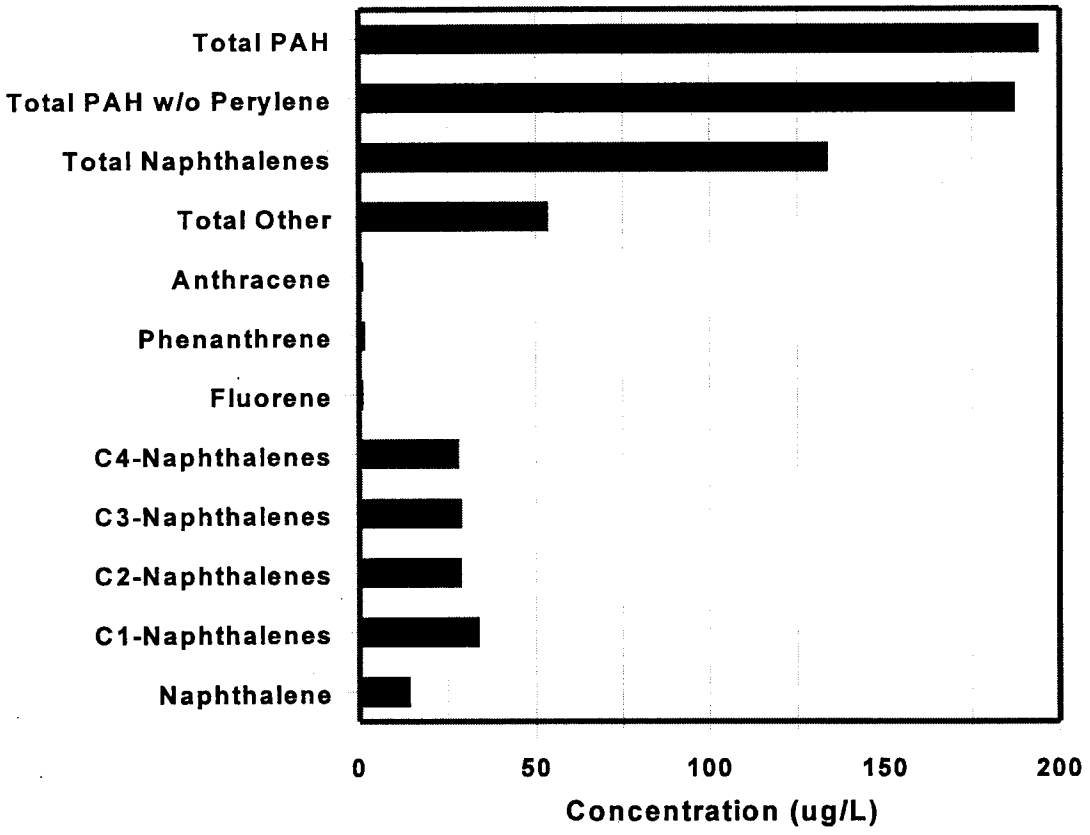


Figure 1. Concentrations of PAHS in the 100% water accommodated fraction (WAF) of the diluent. "Total Other" represent total PAHS without perylene minus total naphthalene. PAHS not present in the 100% WAF include C-3 and C-4 phenanthrenes and anthracenes and C-1 through C-4 chrysenes. PAHS present in the 100% WAF at concentrations less than the lower calibration limit of the analytical method included biphenyl, acenaphthylene, and acenaphthene, C-1 through C-3 fluorenes, C-1 and C-2 phenanthrenesand anthracenes, C-0 through C-4 dibenzothiophenes, fluoranthene, pyrene, C-1 fluoranthenes and pyrenes, benzo(a) anthracene, chrysene, benzo(b) and benzo(k) fluoranthene, benzo(e) and benzo(a) pyrene, perylene, indeno (1,2,3-c,d) pyrene, dibenzo(a,h) anthracene, benzo(g,h,i) perylene and 1-methylphenanthrene.