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Photoenhanced Toxicity of Diluent to the Frog, *Rana sphenocephala*

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ABSTRACT

Studies were conducted to determine the interactive toxicity of a water-accommodated fraction (WAF) of total petroleum hydrocarbons (TPH) from diluent and solar radiation to an amphibian, the southern leopard frog (*Rana sphencephala*). Light treatments for the toxicity tests were based on incident sunlight intensity and spectra measured in the vicinity of an abandoned oil field in California. Frog tadpoles were monitored for survival and growth during a seven-day static-renewal exposure to dilutions of WAF of diluent collected in the vicinity of the abandoned oil field. Exposure to UV alone was not lethal to the tadpoles, and 20 % WAF (4.3 mg/L TPH) in the absence of UV was toxic at the highest concentration tested. Exposure to a 10 % WAF solution (2.82 mg/L) under reference (control) irradiance conditions was not lethal to *R. sphenocephala*; however under 17 μW/cm^2 UVB irradiance, 5% WAF (1.52 mg/L TPH) was lethal after 96 hours of exposure, and significant mortality occurred among tadpoles exposed to 10% WAF within 24 hours of exposure. Accordingly, the 7 day lowest observed effect concentration (LOEC) was 4.3 mg/L TPH for the reference (control), low, and medium irradiance treatments. The LOEC declined to 1.52 mg/L for the 17 μW/cm^2 irradiance treatment. Diluent toxicity was also evident through impaired growth, with an estimated EC50 TPH concentrations of 3.5 mg/L under the low irradiance and LOECs of 2.82 mg/L for the reference, low, and medium irradiance treatments. There was a potential photoenhanced effect on growth at the medium irradiance treatment with an NOEC of 0.36 mg/L. Chemical analysis indicated that 100% WAF was composed of 195 μg/L PAHs primarily of naphthalene, as well as other parent and alkyl homologs for 2- and 3- ring PAH compounds, including substantial concentrations of nitrogen-, oxygen-, or sulfur-substituted heterocyclic compounds that may also be photoenhanced. Relatively limited irradiance was necessary to initiate photoenhanced toxicity, thus a range of amphibian habitats may be impacted by UV radiation. These studies indicate the importance of evaluating the interactive influence of environmental stressors present in amphibian habitats.

INTRODUCTION

A number of hypotheses have been proposed as underlying causes of the worldwide decline of amphibian populations, including habitat alteration and habitat destruction (Perhmann and Wilbur 1994), predation (Lefcort and Blaustein 1995), competition from exotic non-indigenous species (Hayes and Jennings 1986), parasites (Sessions and Ruth 1990), disease (Laurance et al. 1996; Carey 1993), ultraviolet radiation (Anzalone et al. 1998; Hayes et al. 1996), climate change (Corn et al. 1989), and environmental contamination (Zaga et al. 1998; Walker et al. 1998; Cooke 1981). Generally, studies supporting these hypotheses have shown each of these factors to be harmful to amphibians and to be present in their natural habitats. Some of these stressors may be solely responsible for injury to certain populations; however, it is more likely that several factors occur simultaneous in most amphibian habitats and induce injury through interactions that significantly increase their impacts. The present investigation explores the potential impact that two environmental stressors, solar ultraviolet radiation and environmental contamination, may have on amphibians. Many amphibians breed and develop through their early life-stages in shallow temporary pools. These habitats are vulnerable to chemical contamination from aerial transport, direct application, runoff from point and non-point sources and recharge through ground water connections with surface water pools. For example, common amphibian habitats such as roadside ditches can receive significant amounts of lead, petroleum hydrocarbons, and other compounds from road drainage. Similarly, wetlands adjacent to agricultural areas would be subjected to agricultural chemicals through runoff, chemical drift, direct application, etc. Such contamination can result in immediate acute responses in amphibians and other organisms, but more likely, injury will occur as a results of sublethal effects manifested over extended periods.

Although guidelines and application factors have been developed to insure the safe use of many chemical products, the interaction of these products with other environmental stressors has not been thoroughly considered in regulatory guidelines for their use. One stressor of concern which can occur simultaneously with environmental contaminants is UV . UV can be directly harmful to developing amphibians, particularly if there is a change in water clarity or shade that would result in an increased duration or magnitude of exposure. Blaustein et al. (1994) found that ultraviolet B radiation reduces egg hatching success of amphibians in a manner consistent with the species capability to utilize photorepair mechanisms. Indirectly, UV can increase the hazards posed by chemical substances through either *in vitro* photomodification (Ren et al. 1994; Zepp and Schlotzhauer, 1979) or in vivo photosensitization (Landrum et al. 1987; Newsted and Giesy, 1987; Boese et al. 1997). For example, the toxicity of polyaromatic hydrocarbons (PAH), present in most petroleum products to marine invertebrates, increased by as much as 50,000 times in the presence of UV radiation (Pelletier et al. 1997). In the presence of sunlight, 125 μg/L of fluoranthrene, a common PAH in petroleum, delayed hatching success of *Rana pipiens* embryos and caused mortality of newly hatched larvae within 24 hours of exposure (Hatch and Burton 1998). Such photomediated toxicity is often not considered in the regulated use of chemical products, and has not been considered as a factor in amphibian habitats. The purpose

of the present study is to examine the interactive toxicity of UV and the dissolved phase of diluent to amphibians and to provide a better understanding of the impact such interactions may have on the status of amphibians. We used total petroleum hydrocarbons (TPH) as the measure of petroleum exposure in these 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

Test Organisms: *Rana sphenocephala* (previously known as *Rana utricularia*) were collected as eggs by a commercial source and tested at approximately 1 week post hatch. The eggs, embryos and subsequent larvae were cultured in aerated well water (pH 7.0, hardness 283 mg/L as $CaCO_3$) of confirmed high purity at 18 °C under static conditions. The well water was replenished daily to maintain water quality. The larvae were held in 50 L aquaria in well water and fed once daily with ground Tetramin^R, (Nature, Wilton, CT). Uneaten feed was siphoned from the aquaria daily. The contaminant exposures were conducted according to procedures described by Lewis et al. (1996) and ASTM 1993, as adapted by Zaga et al. (1998) for simulated solar radiation treatments.

Solar Simulation: The toxicity tests were conducted in a solar simulator (Little and Fabacher 1996) 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 was equipped with cool white, UV-B (313 nm peak irradiance), UV-A (365 nm peak irradiance) fluorescent lamps and halogen flood lamps. The cool white and UV-A fluorescent lamps were controlled by a timer to operate for 14 hours. The UV-B lamps were activated with a second timer to operate for 4 hours. The UV-B photoperiod began five hours after the onset of the white light and UV-A photoperiod. These photoperiods were comparable to an August photoperiod and were of sufficient length to ensure that the exposed organisms received sufficient irradiance to utilize photorepair mechanisms. Water bath temperature was maintained by a recirculating water chiller and the solar simulator was checked daily for lamp function, photocycle intervals, water bath temperature, water bath, water level, and recirculating flow.

Various filtering materials were used to generate the laboratory irradiance treatments used

during the toxicity tests. These treatments were based on solar irradiance values measured in amphibian habitats and were primarily intended to manipulate UV-B intensities since these wavelengths are the most harmful to aquatic organisms. The sides and the tops of the exposure chambers were covered with the filtering materials. The nominal simulated solar radiation treatments ranged from a low of 0.12 $\mu W/cm^2$ to a high of 17 $\mu W/cm^2$. The reference light treatment used as a control in the toxicity tests (UVB-0.002 $\mu W/cm^2$; UVA-3.2 $\mu W/cm^2$; visible-247 $\mu W/cm^2$) was the lowest possible irradiance that provided sufficient visible light within the chambers to allow feeding and provided UVB irradiance somewhat lower, and visible irradiance somewhat greater than average office-like lighting (UVB-0.21 $\mu W/cm^2$; UVA-3.2 $\mu W/cm^2$; visible-98 $\mu W/cm^2$) provided in the laboratory.

UV Measurement: All radiometric measurements during the tests were performed with an Optronic Laboratories Model OL-754 spectroradiometer over a wavelength range of 280 to 700 nm at 1 nm intervals to document the spectral quality and intensity of irradiance treatments. The radiometer was calibrated with an NIST-traceable lamp and radiometer voltage gain and wavelength accuracy were checked during the measurements. The light intensity across the area of simulator water bath was confirmed by measuring surface irradiance through each filter treatment at 12 locations in the water bath. Underwater irradiance was measured at fixed locations in the simulator using all filter combinations used to generate the test light treatments and to ensure that the output of the simulator lamps remained consistent. UV irradiance measurements at field sites were conducted with an underwater integrating sphere at 5 cm intervals through the water column Hagler Bailly 1997a UV Field Report).

WAF/UV Exposure Procedures: Diluent samples were collected from an underground plume at the Sx monitoring well in the Guadalupe oil field and shipped to Environmental and Contaminants Research Center in chilled 1 liter amber glass bottles as described in Stratus Consulting, 1998a. The samples were refrigerated at 4° C prior to use to minimize volatilization. A slow-stir apparatus was used to prepare the water accommodated fraction (WAF) of the oil. A Teflon stir-bar and a 20 mm glass tube was placed into a one-liter screw-top glass jar. Eighty milliliters of well water was added to the jar then 800 ml of oil was added gently to the surface of the water. The jar was sealed with the screw cap and the mixture was stirred slowly to avoid formation of a water oil emulsion (100 ± 20 RPMs) for 24 ± 2 hours in a fume hood at room temperature. A Teflon tube was inserted through the glass tube to siphon off the WAF without disturbing the overlying layer of oil.

Randomized experimental designs were used to expose the larvae to diluted solutions of the WAF in the presence of the simulated solar radiation intensities. Three static-renewal tests were conducted with *R. sphenocephala* according to procedures described by Lewis et al. (1994) and ASTM (1993). In Test 1, larvae less than one week old were exposed to 10, 5.0, 2.5, 1.25, and 0.63 % WAF dilutions and a control treatment in the presence of standard, low, and medium simulated solar radiation treatments for 7 days. A second test was conducted for 6 days using 20 % and 0% WAF under reference, and 0.2 irradiance conditions. A third test was conducted

with 30-day old larvaes exposed over 96 hours to 0, 5, and 10 % WAF dilution in the presence of the reference (0.002 $\mu W/cm^2$) and 17 $\mu W/cm^2$ UVB. Each treatment was replicated three times. The exposure chambers were 600 mL beakers containing 500 mL of WAF dilution prepared with well water were used for each exposure. To begin the test 10 larvaes were pooled into 3 replicate samples (30 total), dried in an oven at 60 °C for 24 hours to obtain initial dry weights. Then 10 larvaes were randomly stocked in the exposure beakers which were placed in a 20 °C temperature-controlled water bath under the solar simulator. A randomization schematic was used to randomly assign each treatment replication to a position in the solar simulator water bath. The beakers were covered with the appropriate light filters to obtain the desired light intensity. The light filters were changed every 2 days to control for photolytic degradation of filter materials.

Temperature in the water bath was recorded daily. The pH and oxygen of the batch dilutions were measured on day 0, then daily on nine randomly selected test. Renewals of WAF dilutions (75 %) were performed daily by siphoning off the old dilutions and adding fresh dilutions. After the renewals, larvaes were fed 1 mL of a solution containing 15 g of Tetramin^R homogenized in 300 mL of well water. Feeding was reduced proportionately as mortality occurred during the test. Mortality in each treatment was recorded daily and on day 7 the dry weights of surviving larvaes in all replicates were obtained.

WAF Chemistry and Sampling: WAF samples were analyzed for semi-volatiles, expressed as total petroleum hydrocarbon (TPH), and volatiles, expresed as and 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 (described in Stratus Consulting, 1998a).

WAF concentrations of 0, 0.63, 1.25, 2.5, 5, and 10% were used in the *Menidia* toxicity test. Initial (newly prepared test solutions, test days 0 and 6) and final (pooled test solution sampled 24 hours after renewal, test days 1 and 7) samples of the 0, 0.63, 1.25, 5, and 10% WAF solution were collected for analysis of TPH. Separate samples were collected from each light treatment (reference , low, medium, high). Initial samples of 10% WAF were sampled once daily on days 0 to 6 to assess variability in TPH concentrations in newly prepared WAF across preparation days. TPH samples were extracted and analyzed for semi-volatiles using a gas chromatography/mass spectrometry (GC/MS) modified from EPA method 3510 (Stratus Consulting 1998a). The minimum detection limit ranged from 0.05 to 0.2 mg/L TPH, depending on the collected sample volume. 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: Data collected at the end of the exposures (day 7) were analyzed as an

irradiance versus WAF factorial arrangement of treatments. The one-tailed Dunnett's test (Dunnett 1955) was used to compare all treatment means. Because of a significant light-WAF interaction term, ANOVAs were performed for each light treatment using its 0 % WAF treatment as a control. ANOVA and the Dunnet's test were used to determine no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs). Arcsine square root transformations were performed on all mortality data before analysis. Daily mortality data were statistically analyzed. The Toxstat^R computer program (TOXSTAT^R V3.5, 1996), which incorporates control mortality corrections, were used to calculate seven-day LC50 and LC20 values as TPH concentrations within each light treatment. EC50 values were estimated by incorporating one-half of the control weights for *R. sphenocephala* into the regression line formula. EC20 values were calculated in a similar manner. All computations were performed using Statistical Analysis System (SAS 1989) computer programs. Confidence intervals were only calculated if regression coefficients were significant (Snedecor and Cochran 1980).

RESULTS

During the test with *Rana* larvae, conductivity ranged from 618 to 634 μS/cm ; dissolved oxygen ranged from 3.5 to 8.5 mg/L; pH ranged from 7.9 to 8.3; and temperature ranged from 20 to 21 °C (Table 1). All measurements were within the range for test acceptability recommended by Klemm et al.(1994).

Measured UV-B irradiance during the larvae tests ranged from 0.002 μW/cm^2 for the reference light treatment to 1.82 μW/cm^2 for the medium light treatment (Table 2). The manipulation of UVB intensities also reduced the intensity of UVA 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. Total UV doses applied during the test are shown in Table 3.

WAF Treatments: 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). Measured TPH test concentrations corresponded to the nominal WAF dilution and ranged from below the detection limit in the control (0. 00) to 4.24 mg/L TPH in the highest treatment level (20% WAF) (Table 4). Measured TPH concentration for the 0.63 % WAF dilution was also below the detection limit and was estimated as one-half of next highest test concentration. Daily TPH concentrations for the 10% WAF are shown in Table 5.

Survival: No significant effects on the mortality of larvae occurred during the seven-day test at concentrations of TPH ranging from 0.10 to 2.82 mg/L and no interactions between TPH and light treatments occurred (Table 6). Larvae exposed to 4.24 mg/L TPH for six days incurred significant mortality in each light treatment tested, but photoenhanced toxicity was evident as mortality that occurred earlier in the exposure under UV treatment than observed in the reference

UV treatment (Table 7). ANOVA conducted on mortality data from the exposures with 17 $\mu W/cm^2$ revealed significant interactions between WAF treatment, duration of exposure, and UV treatment (Table 7). Photoenhanced toxicity was evident with 100% mortality induced among larvae exposed to the 1.52, 2.82, and 4.3 mg/L TPH (Table 8).

Growth: Within the standard, low, and medium light treatment, total biomass and mean individual weight of larvae were significantly reduced in the 2.82 mg/L TPH treatment compared to the control treatments (Table 9). Significant reductions in total biomass and mean individual weight were observed among larvae exposed to 0.75 mg/L TPH under the medium light treatment, but the effects were not present at the next higher TPH concentration of 1.52 mg/L.

Toxicity Estimates: Seven-day NOEC values for larvae mortality was 2.82 mg/L TPH for the standard, low, and medium light treatments and LOEC values were greater than 2.82 mg/L TPH (Table 10). In the six-day test with larvae exposed to 4.24 mg/L TPH an approximate NOEC and LOEC of less than 4.24 and 4.24 mg/L TPH, respectively, were obtained. In tests with 17 $\mu W/cm^2$, an LOEC of 1.52 mg/L was observed.

The seven-day NOEC value for larval growth was 1.52 mg/L for the reference and low light treatments. (Table 10). For the medium light treatment, a conservative approximation of the NOEC is 0.36 mg/L TPH (Table 10). The LOEC value for larval growth was 2.82 mg/L TPH for the reference, low, and medium light treatments (Table 10). The estimated EC50 values for growth of larvae ranged from 3.5 mg/L TPH in the low light treatment to 8.6 mg/L TPH in the medium light treatment: EC20 values ranged from 1.6 mg/L TPH in the low light treatment to 2.4 mg/L TPH in the medium light treatment (Table 11). EC20 and EC50 values for the reference light treatment were not determined due to non-significant regression coefficients.

DISCUSSION

The results of this investigation clearly indicate that the toxicity of the water accommodated fraction of diluent was significantly increased in the presence of UV radiation. The highest UV exposure of 17 $\mu W/cm^2$ UVB was not harmful to *R. sphenocephala* and was within measured subsurface environmental irradiances ranging from 20 to 97 $\mu W/cm^2$ UVB in the vicinity of the oil field (Barron et al. 1997). The 17 $\mu W/cm^2$ irradiance required to induce photomediated toxicity was considerably higher that the 0.12 $\mu W/cm^2$ UVB required to induce photoenhanced toxicity in fish (Little et al. 1998 *Menidia* Report), cladocerans (Hurtubise et al. 1998 *Ceriodaphnia* Report), or mysid shrimp (Cleveland et al. 1998, *Mysidopsis* Report). The variation in UV sensitivity among different species could be related to differences in epidermal characteristics such as melanin content as well as their abilities to utilize photo-repair mechanisms. The high melanin content in amphibian species such as larval *R. sphenochephala* may mitigate the effects of UV by preventing penetration to reactive sites below the epidermis. Photoenhanced toxicity of the PAH, fluoranthrene, by UV was induced in the lightly pigmented *Xenopus laevis*, but not in *R. pipiens* (Hatch and Burton, 1985). *X. laevis* was also more

sensitive to UV than *R. pipiens*. That photoenhanced toxicity in the frog occurred at elevated UV irradiance levels suggests lower irradiances were blocked by the skin surface. Possibly the epidermal melanin of *R. sphenocephala* provided a measure of photoprotection, and prevented the UV from reaching the membrane-bound oil residues at the lower irradiance levels but were unable to shield the organism from 17 $\mu W/cm^2$. In addition to absorbing UV, melanin precursors can provide antioxidant activity and reduce the formation of lipid peroxidation products that are generated by the UV oxidation of membrane-bound petroleum residues (Schmitz et al. 1995).

Rana sphencephala is likely to be a reasonable surrogate for *Rana aurora*, a threatened species endemic to the vicinity of the Guadalupe oil field. Studies conducted at the University of Missouri have found that the two species are similar in their sensitivity to other compounds. Environmental UV measurements were made in *R. aurora* habitats, and the species was observed in habitats immediately adjacent to the oil field. Thus, the results of this study are likely to be applicable to *R. aurora*.

The results of this investigation are consistent with other studies that have shown oil products and components of petroleum are photoenhanced by UV radiation (Arfsten et al. 1996). The toxicity of #2 fuel oil to fish (Sheier and Gominger 1976) and Arabian light crude to *Mysidopsis bahai* (Pelletier et al. 1997) doubled in the presence of UV light. The PAH composition of crude oil plays a major role in the photoenhanced toxicity of crude and refined petroleum. The toxicity of the PAH, anthracene, to *R. pipiens* increased by 30 times in the presence of UV (Kagen et al. 1984) Oris and Geisy 1985) and the toxicity of individual PAH compounds to marine invertebrates increased by over 50,000 times (Pelletier et al. 1997). In the present study, previously documented photo-modifiable PAHs present in WAF such as anthracene, fluoranthene, and pyrene were at very low levels (175 ug/L total PAH concentration) in the undiluted (100%) WAF. WAF prepared from diluent was 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.

Although most investigations of the photoenhanced toxicity of petroleum have focused on a few non-alkylated PAHs such as anthracene, other petroleum components may also contribute to the photoenhanced toxicity of the petroleum. In contrast to unalkylated parent compounds such as anthracene, the alkylated forms are the dominant PAHs in crude oils and many refined products and their water accomodated fractions. QSAR modeling suggests that alkylation will have little effect on photoactivation. (Veith et al. 1995). In addition to PAHs, heterocyclic aromatics and their alkylated homologs are abundant in petroleum and can be photoactivated. Previous studies have identified acridine (Oris and Giesy 1987) and dibenzothiophenes as likely phototoxic compounds (Kosian et al. 1996). Water soluble fractions are likely to be enriched by these compounds because of greater heterocycle solubility. Bowling et al. (1983) found that a non-toxic concentration of anthracene (12 $\mu g/L$) was toxic at 0.03 $\mu g/L$ to bluegill in sunlight. WAFs also contained a large unresolved complex mixture, which may include unidentified petroleum hydrocarbons or heterocycles contributing to the photoenhanced toxicity of the diluent.

UV radiation at less than 10% of surface irradiance in Lake Michigan was sufficient to photo-enhance anthracene toxicity (Gala 1989). Water quality factors are also important to consider when evaluating the impact of photoenhanced toxicity of petroleum. The toxicity of anthracene was reduced by the presence of humic acids (Oris et al. 1990) presumably because of humic sequestration of the PAH as well as the reduction of UV in the water column. Ireland and Burton (1996) found that photo-induced toxicity of PAHs in storm water runoff at 7.9 $\mu W/cm^2$ UVB and 64 $\mu W/cm^2$ UVA. Pelletier et al. 1997 found that UV levels as low as 9.7 $\mu W/cm^2$ UVA and 3.4 $\mu W/cm^2$ UVB were sufficient to induce photoenhanced toxicity of water soluble fractions of # 2 fuel oil, Arabian light crude, Fuel oil # 6, and Prudhoe Bay crude oil and toxicity of these increased significantly at higher UV fluence (307 $\mu W/cm^2$ UVB; 134 $\mu W/cm^2$ UVA). The fluorescent lighting used for the control conditions of the Pelletier et al. study also photoactivated the petroleum products and caused mortality. This irradiance treatment included UVB levels of 3.4 $\mu W/cm^2$ which was intermediate to the UVB treatments applied during the present study.

A number of factors will influence photoenhanced toxicity in natural habitats. Solar angle associated with time of day, and season, air pollution, clouds, and surface reflection will influence UV irradiance levels. Water quality, especially humic acid concentration, will limit the amount of UV penetrating the water column and may also influence the availability of petroleum to the organism. Chemical concentration is also important, since a threshold concentration is implied.

In conclusion, the results of this study indicate that the toxicity of photoactive compounds can be underestimated if photo-enhanced toxicity is not considered in the assessment of environmental risk. The photoenhanced toxicity of diluent demonstrated for *Rana sphenocephala* is in agreement with responses observed for mysid shrimp (Cleveland et al. 1998,*Mysidopsis* Report), cladocerans (Hurtubise et al. 1998, *Ceriodaphnia* Report), and fish (Little, et al. 1998, *Menida* Report) These results also indicate that photoenhanced thresholds vary with species and light intensity. However photoenhance toxicity was observed at environmentally relevant UV irradiances (Barron et al. 1998, In press). Further, it is apparent from the present study as well as others that contaminant regulatory processes that do not incorporate the interactive effects of contaminants and other environmental stressors are not adequate to protect biological resources.

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Table 1. Mean conductivity, dissolved oxygen (DO), and pH with standard deviation in parentheses, during exposure of *Rana sphenocephala*. N=9 randomly selected treatments per day.

	Day of exposure							
Parameters[a]	0	1	2	3	4	5	6	7
Conductivity (μS/cm)	618 (2.49)	627 (4.64)	634 (4.14)	624 (3.52)	624 (3.20)	624 (6.36)	619 (3.56)	625 (6.04)
D.O. (mg/L)	8.5 (0.19)	3.5 (0.48)	4.8 (0.88)	6.0 (0.99)	5.5 (0.75)	5.6 (0.38)	6.4 (0.37)	6.2 (0.51)
pH	7.9 (0.07)	8.0 (0.08)	8.1 (0.18)	8.3 (0.12)	8.2 (0.17)	8.2 (0.08)	8.0 (0.12)	8.3 (0.13)
Temperature °C	21.0	21.5	20.5	21.0	21.0	21.0	21.0	21.0

Table 2. Ultraviolet radiation measured during the *R. sphenocephala* exposure.

Nominal Treatment	**UV-B** $\mu W/cm^2$	**UV-A** $\mu W/cm^2$	**Visible** $\mu W/cm^2$
Reference	0.00175 (0.0004)	3.05 (0.357)	261.75 (44.21)
Low	0.2825 (0.0560)	74.5 (17.04)	828.5 (34.53)
Medium	1.82 (0.2930)	300 (38.97)	2160.2 (214.97)

Table 3. Total UV-B and UV-A doses for each light regime for *Rana sphenocephala* at day 4 and 7 of the exposure.

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

Table 4. WAF dilutions (%) and corresponding mean total petroleum hydrocarbon (TPH) concentrations measured during the definitive test with *Rana sphenocephala.*

%WAF Dilution	TPH (mg/L)	Standard Deviation	N
0	0	—	4
0.625	0.100	0.04	4
1.25	0.358	0.09	4
2.5	0.750	0.19	4
5.0	1.52	0.30	5
10	2.82	0.46	5
20	4.3	0.20	2

[a] Analytical methods and data are described in detail in Stratus Inc. 1998b.

Table 5. Daily total petroleum hydrocarbon concentrations (mg/L) measured in the 10% WAF during the *R. sphenocephala* definitive test.

DAY	TPH (mg/L)
1	3.2
2	3.0
3	2.9
4	2.7
5	3.3
6	2.6

[a] Analytical methods and data are described in detail in Stratus Inc. 1998b.

Table 6. Percent cumulative mortality with standard deviations in parentheses for *Rana sphenocephala* tadpoles exposed to total petroleum hydrocarbon (TPH) and simulated solar radiation.

Light treatment and TPH concentration (mg/L)[a]	Day of exposure						
	1	2	3	4	5	6	7
Standard							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.36	0.00	0.00	0.00	0.00	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)
0.75	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)
1.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Low							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.52	0.00	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)
2.82	0.00	6.67 (0.94)	0.10 (0.82)	0.10 (0.82)	13.33 (0.94)	20.00 (0.00)	23.33 (0.47)
Medium							
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)
1.52	0.00	0.00	0.00	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)	3.33 (0.47)
2.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] TPH concentrations were obtained from dilutions of a water accommodated fraction of the diluent. N=30 organisms per treatment.

Table 7. Percent cumulative mortality with standard deviations in parentheses for 30 day old *Rana sphenocephala* tadpoles exposed to 4.24 mg/L TPH and simulated solar radiation treatments for six days.

Light Treatment and TPH concentration (mg/L)[a]	**Days of Exposure**					
	1	2	3	4	5	6
Standard 4.24	0.0	6.0 (0.47)	10.0 (0.0)	33.0 (1.9)	56.0 (1.7)	86.6† (1.9)
Low 4.24	0.0	93.4 (.94)	100.0†	100.0†	100.0†	100.0†
Medium 4.24	0.0	3.0 (0.47)	26.6 (1.7)	30.0 (1.4)	53.3 (2.1)	96.6† (1.9)
High 4.24	0.0	26.6 (1.2)	56.7† (0.47)	86.7† (0.94)	100.0†	100.0†
Extra High[b] 0.00	0.0	0.0	6.7 (0.94)	6.7 (0.94)	13.3 (1.2)	13.3 (1.2)

[a] TPH concentrations were obtained from dilutions of a water accommodated fraction of the diluent.
[b] 17.0 $\mu W/cm^2$ UV-B light treatment.
† Indicate significant difference from high light treatment with 0.00 mg/L TPH.

Table 8. Percent mortality of *Rana sphenocephala* exposed to 5 and 10% WAF concentrations under an increased UV-B intensity (17 $\mu W/cm^2$) for 96 hours.

Percent WAF	Percent Mortality
10	100
5	100
0	0

Table 9. Mean dry weights with standard deviations in parentheses for *Rana sphenocephala* tadpoles exposed to total petroleum hydrocarbon (TPH) and various simulated solar radiation for seven days.

Light treatment and TPH concentration (mg/L)[a]	Mean Total Biomass (mg)	Mean organism weight (mg)	Mean weight increase (mg)
Standard			
0.00	27.1 (0.4)	2.7 (0.4)	1.0 (0.4)
0.10	25.0 (1.9)	2.5 (0.2)	0.8 (0.2)
0.36	24.8 (1.7)	2.5 (0.2)	0.8 (0.2)
0.75	26.6 (2.7)	2.7 (0.3)	1.0 (0.3)
1.52	25.8 (1.4)	2.6 (0.1)	0.9 (0.1)
2.82	20.9 (2.4)†	2.1 (0.2)†	0.4 (0.2)†
Low			
0.00	26.4 (3.0)	2.6 (0.3)	0.9 (0.3)
0.10	26.6 (3.1)	2.7 (0.3)	1.0 (0.3)
0.36	28.5 (3.5)	2.8 (0.3)	1.1 (0.3)
0.75	23.6 (3.4)	2.4 (0.3)	0.7 (0.3)
1.52	26.1 (2.9)	2.9 (0.3)	0.9 (0.3)
2.82	13.6 (2.2)†	1.5 (0.2)†	-0.3 (0.2)†
Medium			
0.00	29.1 (5.6)	2.9 (0.6)	1.2 (0.6)
0.10	26.3 (2.4)	2.6 (0.2)	0.9 (0.2)
0.36	24.7 (2.3)	2.5 (0.2)	0.8 (0.2)
0.75	24.1 (2.6)†	2.4 (0.3)†	0.7 (0.3)†
1.52	25.3 (2.1)	2.8 (0.2)	0.8 (0.2)
2.82	23.0 (1.7)†	2.3 (0.2)†	0.6 (0.2)†

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

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

Table 10. No-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) for mortality and growth of *Rana sphenocephala* exposed to TPH and simulated solar radiation for seven days.

	Mortality		Mean organism weight	
Light treatment	Day 7 NOEC (mg/L TPH)[a]	Day 7 LOEC (mg/L TPH)	Day 7 NOEC (mg/L TPH)	Day 7 LOEC (mg/L TPH)
Standard	2.82	> 2.82	1.52	2.82
Low	2.82	> 2.82	1.52	2.82
Medium	2.82	> 2.82	0.36 [b]	2.82
High	<4.24[a]	4.24[a]	ND	ND

[a] determined from a six-day exposure of 30-day-old *Rana sphenocephala*.

[b] Conservative approximation based on significant adverse effects occurring at the 0.75 mg/L TPH treatment but not at the next higher treatment of 1.52 mg/L TPH.

ND = Growth was not measured for the 4.24 mg/L TPH treatment during the six-day test.

Table 11. Seven-day EC20 and EC50 values for growth of *Rana sphenocephala* exposed to TPH and simulated solar radiation.

	Mean weight	
Light Treatment	EC20 (mg/L TPH)	EC50 (mg/L TPH)
Standard	ND	ND
Low	1.6 (-0.9 - 6.1)	3.5 (1.3 - 13.0)
Medium	2.4 (ND)	8.6 (ND)

ND = Not determined due to non-significant regression coefficient.