Solar UV Radiation Enhances the Toxicity of Arsenic in *Ceriodaphnia dubia*

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**Abstract.** Extensive research exists regarding the toxicity of metals (including arsenic) to aquatic invertebrates. However, there has been little consideration of potential synergies between metals and ultraviolet (UV) radiation—despite considerable debate on this topic in human health research. Ultraviolet radiation is nearly ubiquitous in the natural environment, but it is generally overlooked as a confounding variable in toxicological assessments. We evaluate synergies between arsenic and solar UV radiation using the crustacean, *Ceriodaphnia dubia*. Both laboratory (with simulated solar radiation) and outdoor (with natural solar radiation) factorial experiments were performed with two intensities of UV (low and high) and four arsenic concentrations (0, 1, 1.25 and 1.5 mg/l). The laboratory experiment was multigenerational, examining survival and fecundity effects. The combination of high UV + 1.5 mg/l As adversely impacted survival; whereas, High UV + 0 mg/l As and Low UV + 1.5 mg/l As treatments did not. These results suggest synergism. This pattern was consistent for all three generations. Fecundity effects were not consistent across generations, and arsenic was demonstrated to have a greater impact than UV. Outdoor experiments were limited to assessing survival. Exposures in September 1999 resulted in a pattern similar to that in the laboratory exposure. High UV + 1.5 mg/l As treatment elicited diminished survival as compared to high UV + 0 mg/l As and low UV + 1.5 mg/l As. These results indicate that a synergistic effect between arsenic and UV exposure is possible under ambient conditions and within a relatively narrow dose range. The mechanism of this effect is unknown but could include synergistic genotoxic or oxidative stress. These findings point to the importance of using realistic UV exposures when determining criteria for protection of aquatic life.

**Keywords:** arsenic; *C. dubia*; synergy; ultraviolet radiation

**Introduction**

The toxicity of arsenic to aquatic invertebrates has been addressed in numerous studies (Eisler, 1994, for a review). It is known that various factors such as pH (Eisler, 1994), temperature (McGeachy and Dixon, 1990), and co-exposure to other metals (Naddy et al., 1995) can increase or decrease the levels at which arsenic toxicity is observed. However, to our knowledge, there has been no consideration of potential synergies between solar UV radiation (UVR) and arsenic exposure in aquatic organisms.

Researchers in environmental mutagenesis have examined arsenic and UV synergies and elucidated potential mechanisms of interaction. Investigations using *Escherichia coli* (Rossman, 1981), Chinese Hamster Ovary (CHO) cells (Lee et al., 1985;
Okui and Fujiwara, 1986) and human lymphocytes and fibroblasts (Jha et al., 1992) have shown that arsenic inhibits repair of photoproducts induced in DNA by UV light. However, it is unknown whether simple additive DNA damage could result from combined genotoxic effects of arsenic and UV exposure. Arsenic exposure alone elicits direct DNA damage such as clastogenic effects (Nakamura and Sayato, 1981; Sandhu et al., 1989), sister chromatid exchanges (Gomez-Arroyo et al., 1988; Rasmussen and Menzel, 1997), and both DNA-protein crosslinks and DNA strand breaks (Dong and Luo, 1993). Indirect DNA damage may be induced by oxygen free radicals (Hartwig, 1995, for review). UV exposure alone induces a variety DNA photoproducts, principally the cyclobutane pyrimidine dimers (Mitchell and Karenz, 1993). Genetic adaptation to both arsenic (Watkins and Macnair, 1991; Macnair et al., 1992) and UV (Siebeck, 1978) has also been demonstrated.

Combined effects of arsenic and UV exposure are relevant in ecotoxicology, because arsenic is elevated at mining sites in the arid west (Miller et al., 1996) where UV fluxes may also be high due to a large proportion of cloudless days and to low moisture in the air. An important characteristic of many water bodies in the arid west is the abundance of shallow or ephemeral streams and pools in which little attenuation of UV is expected. In addition, increased water clarity or decreased dissolved organic matter and, hence increased UV penetration, is occurring at numerous locales due to effects of acidification (Schindler et al., 1996; Yan et al., 1996). It is important to determine whether predictions of arsenic toxicity derived from laboratory exposure studies reflect the levels of effect that would be observed under realistic UVR conditions.

The purpose of this investigation was to determine whether UVR enhances the effects of arsenic exposure on Ceriodaphnia dubia. Exposures to arsenic and UVR were conducted in a laboratory solar simulator and in outdoor tanks to provide a realistic UVR spectrum. Reproduction and survival were assessed. In addition, simulator studies included multigenerational exposures to evaluate whether evidence of decreased reproduction or survival were observed among generations, possibly indicating either lethal DNA damage or induced genetic adaptation. Ceriodaphnia dubia was selected because daphnids are among the most sensitive species to both arsenic (Eisler, 1994) and UVR (Hurtubise et al., 1998). Responses of C. dubia are included in the calculation of USEPA arsenic criterion for the protection of aquatic life (USEPA, 1984), and daphnid species are abundant in surface waters adjacent to mining sites.

**Methods**

**Organisms**

Ceriodaphnia dubia juveniles and adults were obtained from brood stocks maintained by the UC Davis Aquatic Toxicology Laboratory, Davis, CA. Experiments were performed using standard US Environmental Protection Agency culture methods (Lewis et al., 1992) for *C. dubia* with deviations for the more complex experiments noted below.

Brood stock of *C. dubia* was maintained as batch cultures in 1L of EPA moderately hard water (Lewis et al., 1992) in glass jars at 25 °C, and fed daily a combination of *Selenastrum* concentrate (20ml/l final concentration) and Yeast-Cerophyl-Trout chow (YCT) daphnid feed mixture (Aquatic Biosoysms Inc, Fort Collins, CO) (7 ppt final concentration). Selenium, biotin, thiamine, and B12, each to a final concentration of 1 mg/I, were also added. The daphnids were transferred to new media every other day.

**Laboratory exposures**

Two experiments were performed in a solar simulator in the UC Davis Department of Evolution and Ecology during June/July 1998. The solar simulator was composed of a 0.90 m × 1.77 m light cap. The light cap was suspended over a temperature-controlled water bath (25 °C) of similar dimensions. The light cap consisted of twelve 165 W cool white VHO lamps (Marvel Lighting Co. Mullins, SC, USA), four 100 W UVB-313 lamps (FSX72T12, National Biological Corporation, Twinsburg, OH, USA), eight 100 W UVA-365 lamps (F72T12-HOVALITE, National Biological Corporation, Twinsburg, OH USA) and three 1500 W 6500 K metal halide lamps (Iwasaka Electric Company, Tokyo, Japan). The light cap and water bath were surrounded by reflective specular aluminum to reduce light loss. The sets of lights were controlled by a Solar 1000 timer (Niche Engineering, Lakeville, MA, USA) and dimmable ballasts (Model 660 IceCap Inc., Hamilton, NJ, USA).
that allowed for dawn/dusk simulation (1 h each) on a 14-h light:10 h dark photoperiod. During the midportion of the light cycle, UVB lamps were turned on for four hours to simulate summer midlatitude, midday light intensities.

A spectroradiometer (Model 754 Optronics, Orlando, FL, USA) was used to measure the spectral output of the simulator at 1 nm intervals from 290 to 800 nm. Measurements were taken with the optics head of the spectroradiometer at the surface of the water, approximately 60 cm from the light source. Measurements were taken twice during the course of each experiment; once at the beginning of the experiment and once at experiment completion. The spectroradiometer was calibrated to the temperature of the test conditions using a US National Institute of Standards and Technology (NIST) traceable tungsten lamp over the range of 290–800 nm at 1 nm intervals.

In these experiments, individual C. dubia were placed in 15 ml of test solution in individual 30 ml plastic cups, with 10 cups (replicates) per treatment. Plastic cups were photo-aged, in EPA moderately hard water under UV light for at least four days and rinsed, prior to use in exposures. Individual cups were arranged in floating styrofoam boards in the temperature-controlled water bath. UV filters were then placed over appropriate cups or bags according to treatment. Filters for UV “High” were made of a double layer of mylar sheeting (Interstate Plastics, Sacramento, CA), and UV “Low” filters were made from a double layer of polycarbonate sheeting (Cope Plastics, St. Louis, MO). This resulted in two distinct UV exposure regimes (Fig. 1 and Table 1). The dose of the “Low” treatment is a negligible irradiance and should be considered as zero. The difference in this experiment, where we report actual irradiances for all treatments, versus other experiments that refer to their “control” as “No UV”, is that we have spectro-metrically quantified all of the doses.

The first experiment was a range-finding experiment to determine the appropriate dose range for arsenite. Ceriodaphnia dubia were exposed to high ambient levels of UV irradiance (created through use of a double layer of mylar sheeting) and one of three doses of arsenic (0, 1.25 and 2.0 ppm). This exposure was run for five days, allowing for the generation of three broods. Based on the results of the range-finding experiment, a full experiment was conducted in the solar simulator. The purpose of this experiment was to determine whether arsenite and UV effects were cumulative over multiple generations. For this, C. dubia were exposed to one of eight treatments: four arsenic exposure concentrations (0.0, 1.0, 1.25 and 1.5 ppm, as 2.0 ppm As was found to be lethal to all test organisms within 24 h) combined with two UV irradiance exposures (UV “High” and UV “Low”).

Survival and reproductive success (brood size) of C. dubia were measured as indicators of toxicity. Organisms were scored as dead if they did not respond to probing. Brood size was defined as the sum of live offspring produced by an individual C. dubia over an 8-day period (eight days is the generation time, day 1 of the 8-day period is the beginning of that generation, seeded by a juvenile derived from an adult of the previous generation). The average brood size is the brood size of individuals, averaged across all individuals in a treatment.

In the full experiment, C. dubia were exposed for up to three generations (Fig. 2). The first generation was seeded with juveniles (<24-h-old) from the brood stock. Offspring were counted and removed

![Solar simulator treatment doses](image)

*Figure 1. Comparison of spectral output for the High and Low ultraviolet radiation treatments in the solar simulator laboratory experiment.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total irradiance 295–375 nm (µW/cm²)</th>
</tr>
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<tbody>
<tr>
<td>Laboratory</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>183</td>
</tr>
<tr>
<td>Low</td>
<td>37.69</td>
</tr>
<tr>
<td>Outdoor 1998</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>135</td>
</tr>
<tr>
<td>Low</td>
<td>15</td>
</tr>
<tr>
<td>Outdoor 1999</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>175</td>
</tr>
<tr>
<td>Low</td>
<td>19.7</td>
</tr>
</tbody>
</table>


Figure 2. Experimental design for the laboratory multigeneration experiment. Arrows indicate each treatment cup, as well as the flow of progeny of one exposed generation to the next within treatments. Treatment is shown in the first two rows, indicating the UV and arsenic dose in each replicate.

every 48 h. On Day 8 of each generation, a juvenile from each cup was used to seed the next generation. Survivorship and reproductive output were measured for each individual replicate of each treatment for each generation. There were 10 individuals per treatment. The experiment was terminated after the 3rd generation, after 24 days.

Outdoor exposures

Two experiments were performed outdoors under ambient environmental light conditions in October 1998 (10/24–10/28) and September 1999 (9/16–9/20), respectively on the UC Davis main campus in Davis, CA. The goal of the outdoor experiments was to determine whether interactive effects of arsenic and UV would be observed under both relatively low- and high-ambient lighting conditions. In these experiments, C. dubia were exposed for five days and were seeded by juveniles (<24-h-old) from the brood stock. Individuals were exposed to eight treatments: combinations of four arsenic concentrations (0.0, 1.0, 1.25 and 1.5 ppm), and two UV exposure levels (“High” and “Low”, Fig. 1). Survivorship in each treatment was quantified. There were 10 individuals per replicate, and four replicates per treatment in 1998 and three replicates per treatment in 1999. Unlike the laboratory experiments, C. dubia were placed in 200 ml of test solution in Whirl-pak bags (Nasco, Modesto, CA) placed in a mesh tray. The mesh tray was floated in a water bath maintained at 20.5 ± 0.5°C. In the 1998 experiment, UV “High” filters were made from a double layer of whirl-pak bag plastic, and UV “Low” filters were made from a double layer of polycarbonate sheathing. In the 1999 experiment, UV “High” filters were made from a single layer of Whirl-pak bag plastic, and UV “Low” filters were made from a single layer of polycarbonate sheathing. Ultraviolet irradiance during both outdoor experiments was calculated based on data collected by the USDA UV-B Monitoring Program, University of California, Davis climate station.

Chemicals and dose determination

The sodium arsenite (Sigma Chemical Co., St. Louis, MO) stock solution was made with 21.7 mg NaAsO₂/25 ml of distilled/deionized Milli-Q water in a volumetric flask. Test media was then generated by adding 1.0, 1.25 and 1.5 ml of the NaAsO₃ stock solution to 500 ml volumetric flasks, then brought to volume with EPA moderately hard water (plus Selenastrum, YCT and nutrients, as discussed above in “Organisms”). Concentrations of arsenic in the test media were determined at the Analytical Chemistry Laboratory, Division of Hydrologic Sciences, Desert Research Institute, Reno, NV, using the US Environmental Protection Agency Method 200.7 (USEPA, 1994).

Solar simulator vs. ambient UV measurements

In order to better compare the measurements of UV irradiance in the solar simulator (Optronics 754 spectroradiometer) and in the outdoor exposures (USDA UV-B Monitoring Program, UC Davis Climate Station, Yankee Environmental Systems UV Multifilter Rotating Shadowband Radiometer) we have chosen to sum them over the available dose ranges. Due to limitations with the USDA monitoring system we are summing only the portion of the spectrum between 295 and 375 nm. Additionally the values presented (both laboratory and outdoor) are representative of the average irradiance over the highest four hours of each day.

Water chemistry analyses

Dissolved oxygen, pH and conductivity were measured every other day, when test organisms were transferred to new media. Measurements were made of both the new media, prior to transfer of the C. dubia, and the old media after the removal of
the *C. dubia*. During the course of all experiments, water chemistry parameters were maintained within the following ranges: pH = 7.29–9.27, dissolved oxygen = 3.94–7.61 mg/l and conductivity = 353–668 µS (with a single measurement of 1304 µS).

**Statistical analyses**

Two-way nested ANOVA (SAS Institute, Cary, NC) was used for analysis of brood size in the laboratory experiment and survival in the outdoor 1998 experiment. Data presented in percent format were square-root transformed to meet ANOVA assumptions. Due to experimental design constraints in the laboratory experiment leaving survival data without variance, these data were analyzed by contingency table with a Fisher’s Exact Test. Outdoor 1999 data was also analyzed by a contingency table by Chi-square, with a t-test for means comparison between treatments suspected of significant difference. Results were considered to be significant when *P* < 0.05.

**Results**

**Laboratory exposures**

The range-finding experiment indicated a narrow dose-range for the deleterious effects of arsenic on *C. dubia* in the presence of UV radiation. A significant decrease in both survivorship and brood size was observed at 1.25 mg/l arsenic, followed by complete mortality and no brood production at 2.0 mg/l arsenic (Fig. 3). As a result of these findings, exposure concentrations of 0, 1.0 1.25 and 1.5 mg/l arsenic were selected for the full experiment.

In the full experiment, decreasing survival was evident in the interaction of UV and arsenic exposure (Fig. 4). For all three generations, contingency table analysis using Fisher’s Exact Test indicated that there was a pronounced arsenic effect only under conditions of High UV irradiance (*P* < 0.0001 for all three generations). Arsenic concentrations in the test media were determined, by ICP analysis, to be “not detected”, 0.35, 1.0 and 1.4 mg/l for the 0, 1.0, 1.25 and 1.5 mg/l treatments, respectively.

Exposure to both arsenic and UV radiation over three generations revealed variable effects on brood size. There was a decrease in brood production with the increasing concentration of arsenic and higher UV irradiance in the first generation (ANOVA *P* = 0.0001 and 0.0012, respectively) (Fig. 5). In the

![Laboratory survival (Generation 1)](image)

![Laboratory survival (Generation 2)](image)

![Laboratory survival (Generation 3)](image)

Figure 4. Percent survival for laboratory experiments for each of three generations. Dark lines indicate Low-UV treatments. Hatched lines indicated High-UV treatments. Arsenic data are presented for all four treatment levels (0, 1.0, 1.25 and 1.5 mg/l). In Generations 2 and 3, there are no data for some High-UV and higher arsenic treatments due to complete mortality of the adults.

*Figure 3.* The range finding experiment consisted of three arsenic and one UV treatment level. Average brood size for ten replicates ± SE. Survivorship is out of ten initial individuals.
second generation, there was no statistically significant change in brood size in response to either arsenic or UV treatment ($P > 0.05$). In the third generation, there was again a strong arsenic effect ($P = 0.001$) and a slight UV effect ($P = 0.0478$).

**Outdoor exposures**

1998 Exposure. This exposure was conducted under relatively low ambient UV conditions in October 1998 (77% of the high treatment irradiance in 1999 and 74% of the high treatment irradiance in the laboratory exposure) (Table 1). This experiment revealed no statistically significant difference between High and Low UV treatments among arsenic concentrations ($P = 0.3074$) (Fig. 6), although there was an effect due to arsenic alone ($P < 0.001$). No analysis was conducted to confirm arsenic concentrations during this experiment.

1999 Exposure. This exposure was conducted in September 1999, under late summer ambient light conditions (Table 1). Greater mortality was observed in the High UV irradiance compared to the Low-UV treatments (Chi-square = 0.0001; Fig. 7). The contingency table analysis and the data suggested that this difference was largely due to the Low UV + 1.5 mg/l arsenic and the High UV + 1.5 mg/l arsenic treatments. To confirm this difference, a $t$-test was employed to compare the Low UV + 1.5 mg/l arsenic and the High UV + 1.5 mg/l arsenic treatments. This comparison revealed a significant difference between the two treatments ($t$-test $P = 0.037$).
Arsenic concentrations were determined, by ICP analysis, to be “not detected”, 0.963, 1.26 and 1.51 mg/l (based on an average of three measurements per treatment) for the 0, 1.0, 1.25 and 1.5 mg/l treatments, respectively.

Discussion

Many factors govern arsenic toxicity, but modification by UV has not been examined in the realm of ecotoxicology. Our results indicate that UV irradiance alters arsenic toxicity in *C. dubia* — with effects at environmentally significant concentrations. It is also of interest that the effects of this combination occur within a narrow dose range (1–1.5 mg/l). Previous studies on UV and arsenic, as independent stressors, are in concurrence with our results and provide insight into the potential real-world environmental impacts the stressors may have when present in concert.

Work by Hurtubise et al. (1998) examined the impacts of UV radiation on two species of cladoceran, *C. dubia* and *C. reticulata*. In their study, *C. dubia* was the more sensitive of the two species, having an LD50 of 9.4 μW/cm² UVB. They also observed significantly greater mortality in their 4.2 μW/cm² UVB treatment than in their control (0.001 μW/cm²). To properly compare our laboratory results to theirs, we recalculated our summation of irradiance for the same spectral range they used (280–320 nm). These calculations confirmed that our low UV irradiance (0.188 μW/cm²) and our high irradiance (3.7 μW/cm²) were in a dose range below that which is independently lethal to *C. dubia*. Other studies of cladoceran sensitivity to UV lack dosimetry for direct comparison. However, they do indicate that current ambient mid-summer levels of UV-B alone can be detrimental to cladoceran survival (Siebeck, 1978; Zellmer, 1995). In these studies, pigment production was also examined as an adaptation to UV exposure.

A previous study on the effects of arsenic on *C. dubia* survival and fecundity is of note. Naddy et al. (1995) conducted exposures of *C. dubia* to arsenic alone and in combination with other metals (selenium and molybdenum). At their highest exposure concentration, 1.42 mg/l arsenic (from sodium arsenate), no statistically significant mortality occurred. Combinations of arsenic and selenium elicited statistically significant mortality at 1.42 mg/l arsenic with 0.51 mg/l selenium. Arsenic has statistically significant mortality at 1.02 mg/l when selenium was at 0.87 mg/l. A puzzling result of this study was a lack of mortality at any arsenic concentration when selenium was added at 0.61 mg/l. There was no significant morality when arsenic and molybdenum were combined. Combinations of all three were significantly lethal at most dose combinations. They also demonstrated that arsenic alone, at their exposure levels, was not affecting fecundity. In combination with other metals, the pattern of the fecundity response was similar to survival. The exception to this observed pattern was an interaction between arsenic and molybdenum at the highest molybdenum concentration, resulting in decreased fecundity. Our dose range for arsenic is a close match to that of Naddy et al. (1995) and also indicates that arsenic alone (below 1 mg/l for brood size effects) does not have an adverse impact. This study makes the important point that single chemical exposures often underestimate toxicity as arsenic may often occur in complex mixtures.

Our results, combined with those of previous studies on UVR and arsenic, illustrate the need for evaluation of the sources of subtle variations in arsenic toxicity. While surface waters near mines typically contain less than 1 ppm arsenic, higher levels are occasionally found (Eisler, 1994). More important may be the possible biological effects of exposure to UVR when combined with metals mixtures. Mine drainage is generally a cocktail of metal compounds. As a result, the interactions of UVR with such a mixture may further reduce threshold effects levels. Changes in environmental variables, such as altered DOM profiles, acidification and ozone depletion, may alter UVR intensities, and thereby change the dose at which enhanced effects are observed in field exposures.

It is also important to consider indirect and sublethal effects and what role UVR and arsenic are playing. In the case of fecundity, our study shows variability between generations in brood production with respect to arsenic and UVR dose. Arsenic exposure in the first generation elicited decreased brood size; yet UVR had little impact. In the second generation, there were no significant differences in brood size among any of the treatments. In the third generation, arsenic exposure again resulted in a
significant decline in brood size. The effect level for reduced fecundity, in the two generations that show trends, occurs at 1 mg/l arsenic which is lower than the survival effect level. Sanders (1986) found that copepods exposed to arsenate did not demonstrate direct mortality effects until higher dose levels (1–100 mg/l). These copepods did respond (with increased mortality and decreased fecundity) to indirect effects (altered food type and phytoplankton assemblages) that are predicted to be caused by enhanced arsenic concentrations. Zellmer (2000) has also demonstrated that if a food item is pre-exposed to a stressor (here it was exposure of phytoplankton to UV) the consumer (Daphnia pulex) will be adversely impacted in both survival and altered reproductive strategy. It has also been shown that Daphnia pulex are affected by reduced survival and fecundity, as well as altered reproductive strategy upon UV exposure, if the availability of food is diminished (Zellmer, 1996).

The mechanism of interaction between arsenic and UV is unknown. It may be a synergistic response, possibly genotoxic, oxidative stress or an inhibition of DNA repair mechanisms. It is unlikely that it is a form of photoenhanced toxicity such as that exhibited in UV interactions with polycyclic aromatic hydrocarbons (PAHs) and other organic contaminants. Although the environmental mutagenesis literature suggests that arsenic and UV synergy may be the result of inhibition by arsenic of repair of UV-induced lesions in DNA, we are reticent to speculate on a mechanism. Highly damaging UV-C irradiance was used in those studies (Rossman, 1981), and irradiation under a realistic solar spectrum may produce a different balance of effects, especially in eukaryotes with well-developed compensatory responses.

This study points to the need for additional research in this area. The question of mechanism requires study in order to better determine the likelihood of additional interactions between other environmental stressors. Additional laboratory studies would be beneficial to examine other sublethal endpoints (protective pigment induction, growth rates, DNA damage), as well as to better understand fecundity effects. Finally, in situ experiments at mine drainage sites are critical for assessing the potential interactive effects of all environmental stressors present in the surface water habitat of these invertebrates.

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