Using WET test methods to detect phototoxic effects in PAH-contaminated groundwater

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RESEARCH QUESTION

Can phototoxic effects be detected in PAH-contaminated groundwater using acute WET test methods?

STUDY OBJECTIVES

- Conduct measurements of local solar spectral irradiance;
- Design light treatment that best approximates the spectral irradiance of local natural sunlight;
- Assess the toxicity of PAH-contaminated groundwater samples to *Daphnia magna* and *Pimephales promelas* by EPA acute WET test methods using:
  1- Ambient laboratory lighting; and
  2- Ambient laboratory lighting plus UVA (320-400nm) and UVB (290-320nm).

BACKGROUND

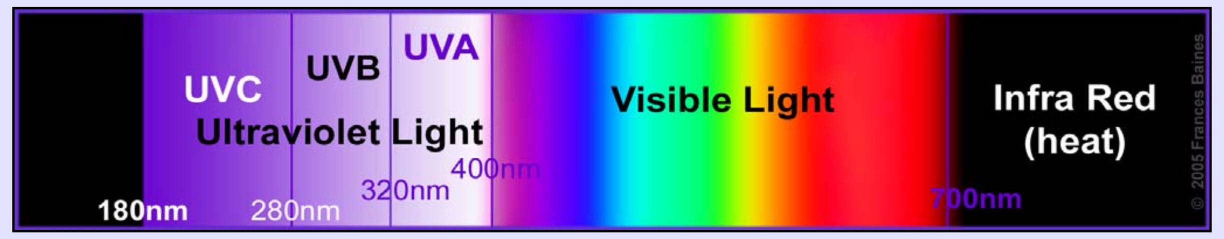

Figure 1. Light spectra. From Baines et. al 2005. With Permission.

PAHs are ubiquitous and persistent contaminants in many aquatic environments (Diamond et. al 2003). WET testing is one mechanism by which state and federal agencies currently regulate the release of these substances to aquatic systems. The current test illumination specifications in USEPA WET test manuals, however, may lead to inaccurate estimates of environmental toxicity when WET tests are conducted on samples containing photoactive PAHs. This is because the current recommendations do not require the use of light sources that emit ultraviolet (UV) light.

Under standard WET test protocols, USEPA currently recommends the use of ambient laboratory lighting during toxicity tests (10-20μE/m2/s or 50-100 ft-c) (USEPA 2002). Little information is provided, however, regarding the quality and quantity of illumination that should be used when conducting these tests. The protocols also indicate that plastic or glass should be used to cover test chambers during testing; a procedural element that could alter the spectral character and amount of light reaching the test solutions.

In particular, WET manuals lack guidance related to:

- Light source spectral distribution;
- Spectral irradiance levels;
- Presence of UVA and UVB light;
- Dose of light over time.

Studies using a variety of methods demonstrate the potential of UV irradiation and natural sunlight to increase the toxicity of PAHs to aquatic organisms (Diamond et. al 2000). In particular, exposure of aquatic organisms to light sources emitting UVA and UVB has been shown to significantly increase the toxicity of many 3-5 ring PAHs (e.g. fluoranthene, pyrene, anthracene, and benzo-a-pyrene) (Weinstein and Diamond 2006). Since light treatment design can strongly influence the outcome of PAH toxicity tests, researchers recommend that light treatments used in aquatic toxicity tests include UVA and UVB radiation to approximate the characteristics of habitat-specific solar radiation (Diamond et. al 2000, Weinstein and Diamond 2006).

Based on toxicity studies conducted using single phototoxic compounds and complex PAH mixtures, the discharge of PAH-contaminated groundwater to adjacent surface waters may result in increased acute groundwater toxicity due to the photoinduced toxicity of PAHs under natural sunlight. Though phototoxicity testing of PAH-contaminated groundwater is not typically conducted using acute USEPA WET test methods, WET test methods may represent a means to characterize the toxicity of PAH-contaminated waters.

The goal of this research is to see if WET methods can detect phototoxic effects in groundwater known to contain photoactive PAHs, using ambient test lighting supplemented with ecologically-relevant levels of UVA and UVB.

MATERIALS AND METHODS

Solar and laboratory light measurements

- Local solar spectral irradiance was measured on April 3, 2007 at the surface of Lake Padden, Bellingham, WA within 2 hours of solar noon, under a cloudless sky, using a Licor LI1800UW scanning spectroradiometer.
- Solar measurements were analyzed and used to develop a light treatment best approximating local solar spectral irradiance to be used in groundwater toxicity tests.
- Laboratory light measurements were performed 100cm below the plane of each light treatment to estimate the spectral irradiance received at the top of each test chamber.

Light treatments

- Groundwater experiments were conducted in duplicate (Test replicate 1 and Test replicate 2) (Figure 4).
- The following light treatments were used in each test replicate:

1. <u>Ambient laboratory lighting</u> **(LL):** This light treatment provides illumination with a spectral irradiance similar to light sources commonly used in WET testing laboratories (Figure 2).

2. <u>Ambient laboratory lighting + UV</u> **(LL + UV):** This light treatment produces light with a spectral distribution similar to that of local natural sunlight and produces ecologically-relevant irradiance levels of UVA and UVB light (Figure 3).

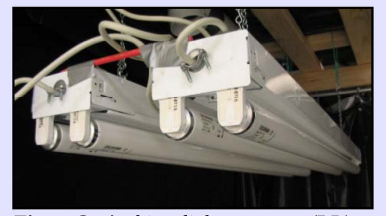

Figure 2. Ambient light treatment (**LL**). Four Sylvania F40 T12 CWP bulbs

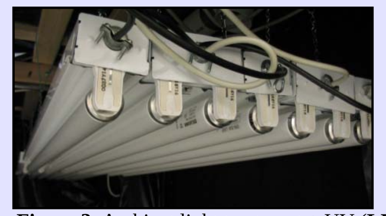

Figure 3. Ambient light treatment + UV (**LL + UV**). Four Sylvania F40 T12 CWP bulbs plus three Q-Panel Corp UVA-340 bulbs

Figure 4. Light treatments suspended over test chambers in water baths.

Reference UV control

- Fluoranthene was used in all WET tests as a reference UV control
- Nominal concentrations of fluoranthene were identified that result in 0% mortality under **LL** treatments and 100% mortality under **LL + UV** treatments for *Daphnia magna* and *Pimephales promelas* (8 μg/L and 18 μg/L, respectively).

Groundwater sampling site

- Little Squalicum Park (Bellingham, WA) is located next to The Oeser Company federal Superfund site.
- Groundwater underlying the Park contains high concentrations of PAHs relative to surrounding areas (START 2002).
- Analytical profiles of water collected from wells at the site show the presence of known photoactive PAHs (START 2002).
- One sampling well was identified for the collection of test water for all WET tests because it contains more photoactive PAHs than water from other wells at the site (Figure 5).

Figure 5. Little Squalicum Park sampling site (START 2002) and groundwater sampling well.

MATERIALS AND METHODS

Whole Effluent Toxicity (WET) tests

Figure 6. *Daphnia magna*

Organisms: *Daphnia magna* and *Pimephales promelas*

Test types: 48 hr static acute (*D. magna*)

96 hr static acute with renewal (*P. promelas*)

Methods: EPA-821-R-02-012, methods 2021.0 [1] and 2000.0 [1]

LL Photoperiod: 16 h light : 8 h dark (ambient light only)

LL + UV Photoperiod: 16 h light (with 12 h UV light) : 8 h dark

[1] Since glass and plastic can attenuate and filter UV light, EPA methods were modified to exclude the use of covers on all test chambers as illustrated in Figures 8 and 9.

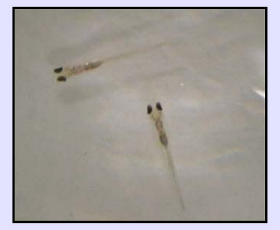

Figure 7. *Pimephales promelas*

Experimental design

Table 1. Experimental units

	Groundwater	Dilution Water Control	Reference Control ($CuSO_4$)	Reference UV Control (fluoranthene)
Concentrations	5	1	5	1
Replicates per concentration	4	4	3	4
Organisms per replicate	10	10	10	10

Figure 8. Experimental units under ambient light (**LL**)

Figure 9. Experimental units under ambient light + UV (**LL + UV**)

RESULTS

Light measurements

- Spectral distribution is similar for both light treatments across visible part of the spectrum (400-700nm) (Figure 10).
- The **LL + UV** treatment emits UV irradiance from 300-400nm (UVA and UVB) (Figure 10).
- The **LL** treatment emits almost no light in the 300-400nm range (Figure 10).
- Test chambers under the **LL + UV** treatment receive UVA and UVB irradiance at much lower levels than those that were measured at the surface of Lake Padden (Figure 10 and Figure 11).
- Total daily UVA dose is 23 times greater under the **LL + UV** treatment than under the **LL** treatment (Table 2).
- Total daily UVB dose is 11 times greater under the **LL + UV** treatment than under the **LL** treatment (Table 2).

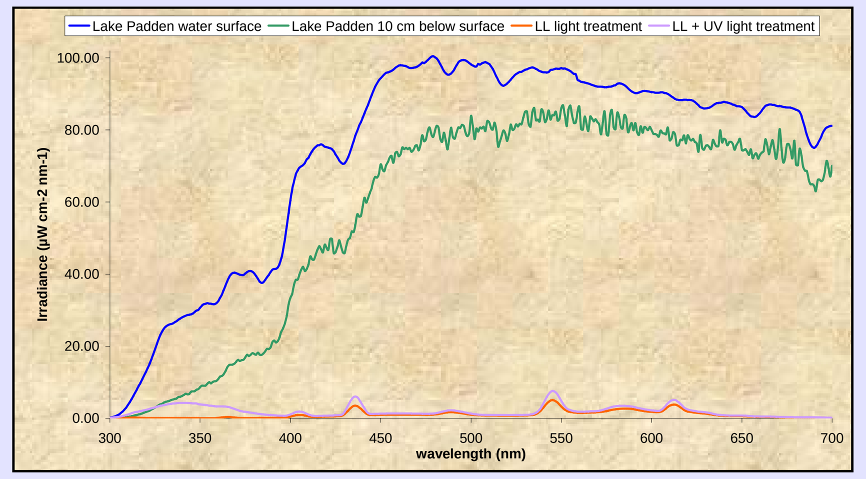

Figure 10. Local solar and laboratory light treatment spectral irradiance

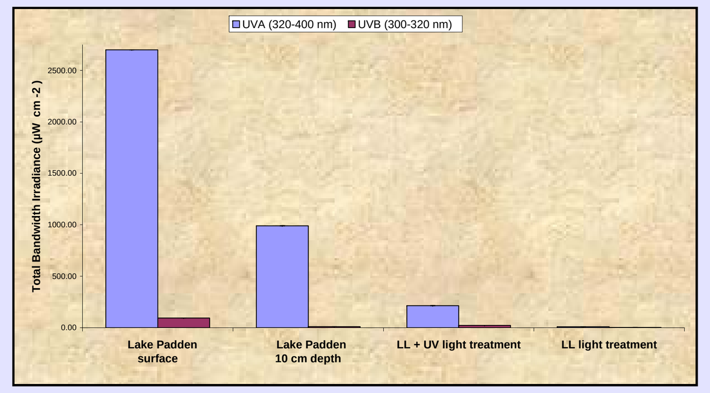

Figure 11. Bandwidth irradiance: Local sunlight (left) and **LL** and **LL + UV** light treatments (right).

Table 2. Total daily UV dose (J/cm^2)

Light treatment	Total UVA	Total UVB
LL	0.11	0.025
LL + UV	2.58	0.27

RESULTS

***Daphnia magna* definitive WET test**

LL treatment (Test Replicate 2)

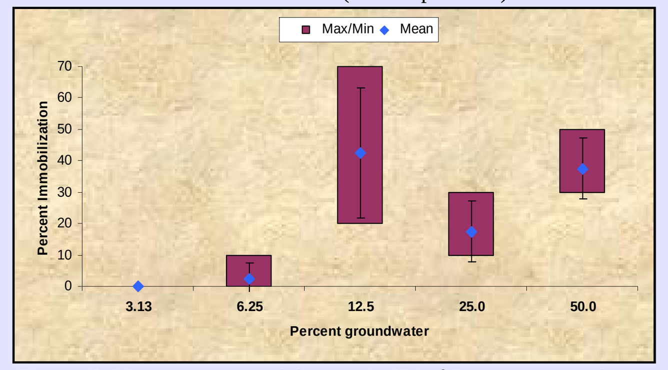

Figure 12. Mean percent immobilization (± SD) of *D. magna* exposed to PAH-contaminated groundwater under ambient light (**LL**).

LL + UV treatment (Test Replicate 2)

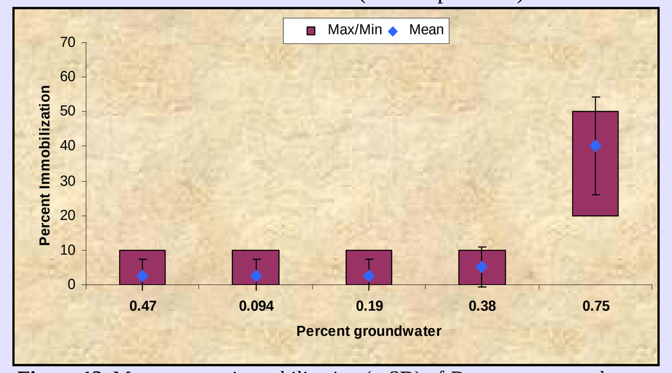

Figure 13. Mean percent immobilization (± SD) of *D. magna* exposed to PAH-contaminated groundwater under ambient light plus UV (**LL + UV**).

Table 3. Definitive WET test summary statistics completed using CETIS v1.6.3revB for *D. magna* exposed to PAH-contaminated groundwater under ambient lab lighting (**LL** treatment) and ambient lab lighting plus UV (**LL +UV**)

	Treatment	NOEC	LOEC	MSDp	Analysis	LC 25	LCL	UCL	Analysis
Test Replicate 1	**LL** treatment	6.25	12.5	9.74	Steel Many-One Rank	22.1	14.9	35.9	Probit
	LL + UV treatment	N/A [a]	N/A [a]	N/A [a]	N/A [a]	N/A [a]	N/A [a]	N/A [a]	N/A [a]
Test Replicate 2	**LL** treatment	6.25	12.5	12.0	Dunnett's multiple comparison	21.3	10.7	56.3	Probit
	LL + UV treatment	0.38	0.75	12.3	Dunnett's multiple comparison	0.658	N/A [a]	N/A [a]	Probit

[a] Values could not be determined for test data using standard EPA decision-tree analysis framework

***Pimephales promelas* range-finding WET test**

LL treatment (Test Replicate 2)

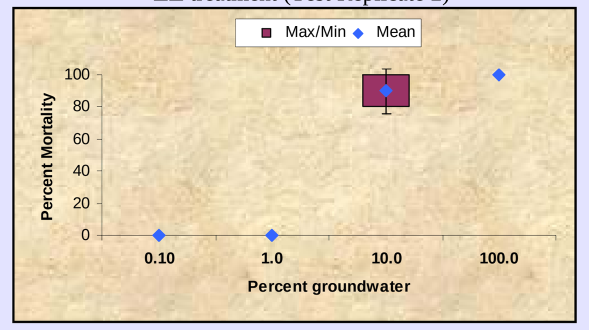

Figure 14. Mean percent immobilization (± SD) of *P. promelas* exposed to PAH-contaminated groundwater under ambient light (**LL**)

LL + UV treatment (Test Replicate 2)

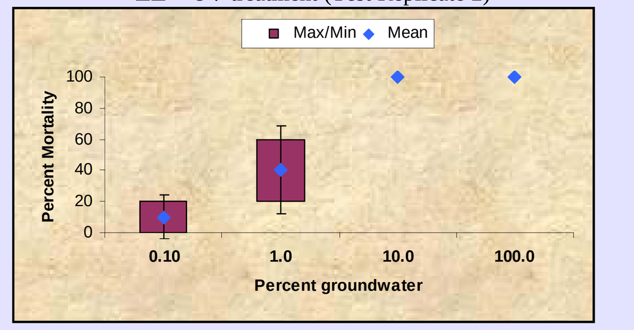

Figure 15. Mean percent immobilization (± SD) of *P. promelas* exposed to PAH-contaminated groundwater under ambient light plus UV (**LL + UV**)

DISCUSSION

The ***D. magna*** **definitive WET test results indicate that the acute toxicity of PAH-contaminated groundwater is greater under ambient light plus UV (LL + UV) than under ambient light (LL) alone.** From the graphs (Figures 12 and 13), the highest concentration of groundwater (0.75%) used in the **LL + UV** treatment resulted in over 40% immobilization on average. In contrast, 40% immobilization was observed at groundwater concentrations between 12.5 and 50% in the absence of UV light (**LL** treatment). In other words, 17-67 times less concentrated groundwater was required in the presence of UV light to produce the same range of effect observed under ambient light alone. This difference is also reflected in the statistical endpoints. NOEC and LOEC values are 17 times higher under the **LL** treatment than under the **LL + UV** treatment (Table 3). The LC25 estimate is 32.4 times higher under the **LL** treatment than under the **LL + UV** treatment (Table 3).

The *P. Promelas* range-finding WET test results suggest that the acute toxicity of PAH-contaminated groundwater is greater under ambient light plus UV (LL + UV) than under ambient light (LL) alone. The average percent mortality was greater for each of the four groundwater test concentrations in the presence of UV (**LL + UV** treatment) than in the absence of UV lighting (**LL** treatment) (Figure 14 and 15). Though these results support greater groundwater toxicity under the **LL + UV** treatment than under ambient lab lighting, the magnitude of toxicity difference between the two treatments appears to be less for *P. promelas* than that observed for *D. magna*. This suggests that increases in toxicity resulting from the use of the **LL + UV** treatment may be less for *P. promelas* than for *D.magna*.

The ambient light plus UV treatment produced enough UV irradiance to elicit phototoxic effects, even though the treatment produces only 10% of the UVA and 25% of the UVB of local solar conditions. **From these results we conclude that, when ambient lighting conditions required by WET test specifications are supplemented with ecologically relevant levels of UVA and UVB, phototoxic effects can be detected in PAH-contaminated groundwater using acute WET test methods**.

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