Arch. Environ. Contam. Toxicol. 38, 274–282 (2000)
DOI: 10.1007/s002449910037

ARCHIVES OF
Environmental
Contamination
and Toxicology

Phototoxic Evaluation of Marine Sediments Collected from a PAH-Contaminated Site

B. L. Boese, R. J. Ozretich, J. O. Lamberson, F. A. Cole, R. C. Swartz, S. P. Ferraro

Coastal Ecology Branch, Western Ecology Division, U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Hatfield Marine Science Center, 2111 SE Marine Science Dr., Newport, Oregon 97365-5260, USA

Received: 12 July 1999/Accepted: 18 October 1999

Abstract. The phototoxicity potential of PAH-contaminated field sediment was evaluated and compared to standard sediment toxicity test results. Marine sediments were collected from 30 sites along a presumed PAH sediment pollution gradient in Elliot Bay, WA. Standard 10-day acute and 28-day chronic sediment toxicity tests were conducted with the infaunal amphipods *Rhepoxynius abronius* and *Leptocheirus plumulosus* using mortality and the ability to rebury as endpoints. The survivors of these tests were then subjected to 1-h exposures to UV radiation with mortality and reburial again determined. The most highly toxic sediments identified in these experiments were evaluated further for toxicity and phototoxicity by serially diluting them with uncontaminated sediment and repeating the toxicity tests. Standard 10-day toxicity test results indicated that over 70% of the sites sampled in Elliot Bay exhibited measurable toxicity with nine sites being highly toxic to both species of amphipods. Results of standard 28-day chronic sediment toxicity tests were similar. In contrast, almost all of the sites were found to be highly phototoxic. Results indicated that exposure to UV increased toxicity five- to eightfold. This suggests that standard toxicity tests underestimate the potential ecological risk of PAH-contaminated sediments in animals exposed to sunlight. However, only when PAH contamination was between 0.05 and 1.0 toxic units would conducting a phototoxicity evaluation add information to that gained from conducting a standard sediment toxicity test alone.

Photoactivation by ultraviolet radiation (UV) of bioaccumulated sediment-associated polynuclear aromatic hydrocarbons (PAHs) has been shown to cause up to an order of magnitude increase in toxicity (Ankley *et al.* 1994; Monson *et al.* 1995; Swartz *et al.* 1997; Boese *et al.* 1998). This enhanced toxicity poses problems for sediment toxicity testing as standard sediment tests (*e.g.*, ASTM 1996a) are conducted under laboratory lighting conditions, which contain little or no UV, and thus may underestimate the environmental risk of petroleum-contaminated sediments.

Correspondence to: B. L. Boese

Phototoxicity research at our laboratory using the marine amphipod *Rhepoxynius abronius* exposed to a wide variety of parent and alkylated PAHs in 10-day toxicity tests suggested that toxicity increases by about fivefold when amphipods are removed from contaminated sediments and exposed to 1 h of UV radiation (Boese *et al*. 1998; Swartz *et al.* 1997). Our most recent research suggested that the phototoxicity of mixtures of parent and alkylated PAHs was roughly additive if normalized to interstitial water molar concentrations (Boese *et al.* 1999). This result was surprising, as we expected photo-induced toxicity to vary with the contaminants molecular structure (*i.e.,* HOMO-LUMO gap) and bioaccumulation potential (Ankley *et al.* 1995, 1997; Mekenyan *et al.* 1994). However, if PAH photoxicities are roughly additive and related by a constant factor to sediment toxicities derived from standard (no UV) toxicity tests, then evaluating the environmental risk of sediments contaminated with photo-activated compounds would be greatly simplified. In addition, it might be possible to estimate the phototoxic potential of a sediment that contained mixtures of phototoxic and nonphototoxic PAHs by using the existing ΣPAH model (Swartz *et al.* 1995).

The goals of the present research were (1) to determine if marine amphipods exposed to a PAH-contaminated field sediment exhibited enhanced toxic effects when exposed to UV radiation, and (2) to evaluate the relationship between the results of standard (no UV) 10-day amphipod sediment toxicity tests to the phototoxicity of the same sediment.

Materials and Methods

Sediments

Sediment samples were collected subtidally from Elliott Bay (Seattle, WA) within 1 km of the Wyckoff Wood Treatment Facility (West Seattle, WA), a creosote (PAH)-contaminated Superfund site (Cubbage 1989). Grab samples were taken using a 0.1-m^2 van Veen grab at 30 stations (Figure 1). The top 10 cm were sampled for toxicity testing and geochemical analyses using three 7.6-cm diameter plastic corers, the contents of which where extruded into half-gallon glass jars and covered with Teflon-lined lids. These composite samples were then

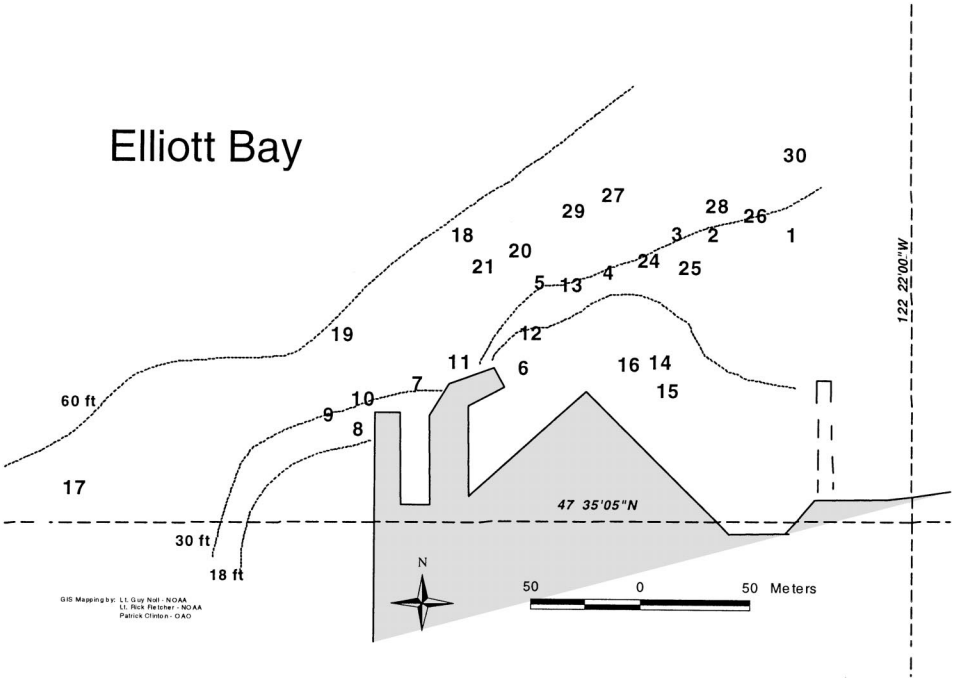

Fig. 1. Locations of 30 Elliott Bay sediment collection sites adjacent to the Wyckoff Wood Treatment Facility (West Seattle, WA), a creosote (PAH)-contaminated Superfund site

transported on ice to the Coastal Ecology Branch Laboratory (Newport, OR) where they were stored at 4°C until testing or analysis.

These sediments were used in two standard sediment toxicity evaluations (ASTM 1996a; DeWitt *et al.* 1997). The first of these was designed to determine which of the collected sediments were the most toxic. For this site survey toxicity evaluation, sediments were mixed by gentle stirring and used directly for toxicity testing within 7 days of collection. In the second experiment, sediments from the most toxic sites were diluted with control sediment to obtain concentration series. This dilution was accomplished by determining the ΣTU of each of these highly toxic sediments using organic carbon (OC) and PAH concentrations (Swartz *et al.* 1995). The concentration and the bulk density of these field sediments where then used with the bulk density and OC content of the diluent sediment to compute initial dilution to four toxic units (TUs) (see Equation 1) assuming equilibrium of the combined OCs and PAHs. These mixtures were then placed in 2,000-ml glass jars and mixed by rolling for 2 days (4°C). These high concentrations were then split into six portions, five of which were diluted using the same methodology with additional control sediment to obtain a series of diluted test sediments. All these sediments were then rolled (2 days) and aged in the rolling mill jars for at least 28 days (4°C) before test initiation.

Uncontaminated sediment, which was used for controls and to dilute field-collected sediments, was collected from McKinney Slough (Waldport, OR). An additional negative control sediment was collected from Yaquina Bay (Newport, OR). These sediments were sieved (0.5 mm) into 28‰ sea water and allowed to settle for 24 h at which time the overlying water was decanted and discarded.

PAH concentrations in sediments were determined using the method of Ozretich and Schroeder (1986). Sediment subsamples (2 g) were spiked with deuterated surrogate compounds at 500 µg/kg (wet weight) and extracted by sonication in acetonitrile with C-18 cleanup, then exchanged into isooctane. Standard reference material (SRM) 1647c, Priority Pollutant Polycyclic Aromatic Hydrocarbons (NIST, Gaithersburg, MD), containing 16 PAHs was spiked into locally obtained sediment (100–500 µg/kg wet weight). Aliquots (1 g) of SRM 1941, Organics in Marine Sediment (PAHs 200–1,000 µg/kg dry weight), were also extracted. Extracts were amended with deuterated phenanthrene (recovery standard) and quantified by GC/MS using the detector response of the analytes relative to the surrogate internal standards. Organic carbon (OC) content of test and control sediments were determined by combustion (Perkin Elmer 2400 CHN Elemental Analyzer, Norwalk, CT) after carbonate removal by acidification (Plumb 1981).

Toxic Unit Calculations

Sediment PAH concentrations of 13 individual PAHs (Table 1) were converted to TU values using the method of Swartz *et al.* (1995). Briefly, a TU for an individual PAH is defined as:

$$TU_i = C_{IW}/\text{10-day } LC50_{IW} \qquad \text{(Eq. 1)}$$

where C_{IW} is freely dissolved interstitial water concentration of an individual contaminant; and 10-day $LC50_{IW}$ is freely dissolved IW concentration needed to kill 50% of amphipods in a 10-day sediment toxicity test. For marine amphipods, Swartz *et al.* (1995) determined the relationship between octanol water partition coefficient (K_{ow}) values and $LC50_{IW}$ for these 13 PAHs (Table 1) to be

$$\text{Log 10-day } LC50_{IW}(\mu M) = 5.92 - 1.33 \log K_{ow} \qquad \text{(Eq. 2)}$$

$$R^2 = 0.96,\ p < 0.001$$

As IW concentrations were not measured directly, IW concentrations for each PAH were estimated from the bulk sediment concentrations by:

$$C_{IW} = C_S/(K_{OC} \times f_{oc}) \qquad \text{(Eq. 3)}$$

where C_s equals sediment concentration (µmol/kg dry weight); K_{oc} is the organic carbon-PAH/water partition coefficient (L/kg OC); and f_{oc} is the fraction organic carbon (kg OC/kg dry weight). K_{oc} values were estimated from K_{ow} values using the relationship of DiToro (1985):

$$\text{Log } K_{OC} = 0.00028 + 0.983 \log K_{OW} \qquad \text{(Eq. 4)}$$

Table 1. Thirteen PAHs and their physical and toxicological characteristics, which were used to determine their toxic unit (TU) values

PAH	Log K_{ow}	Log K_{oc}	Predicted 10-d $LC50_{iw}$ (µg/L)	Solubility Limit (µg/L)	TU Limit	Potentially Phototoxic
Naphthalene	3.37	3.31	3,500	31,690	9.1	No
Acenaphthylene	4.07	4.00	490	3,930	8.0	No
Acenaphthene	3.85	3.78	970	3,420	3.5	No
Fluorene	4.18	4.11	270	1,685	6.2	No
Phenanthrene	4.36	4.29	240	1,002	4.2	No
Anthracene	4.45	4.37	180	44.6	0.25	Yes
Fluoranthene	5.09	5.00	29	206	7.1	Yes
Pyrene	5.32	5.23	14	132	9.4	Yes
Benz[a]anthracene	5.61	5.51	6.6	9.4	1.4	No
Chrysene	5.61	5.51	6.6	1.8	0.27	No
Benzo[b]fluoranthene	6.57	6.46	0.38	1.5	3.9	Yes
Benzo[k]fluoranthene	6.84	6.72	0.17	1.5	8.8	Yes
Benzo[a]Pyrene	6.04	5.94	1.9	3.8	2.0	No

Table from Swartz *et al.* (1995). Potential for phototoxicity estimated from chemical structure (HOMO-LUMU gap) (Mekenyen *et al.* 1994)

If the calculated IW concentration exceeded the solubility limit for that PAH, then the TU limit value was used for that contaminant (Table 1).

Chronic and Acute Toxicity Tests

Standard 28-day chronic sediment toxicity tests using *Leptocheirus plumulosus* (DeWitt *et al.* 1997) and standard 10-day sediment toxicity tests (ASTM 1996a) were performed using *R. abronius* and *L. plumulosus.* Each sediment replicate consisted of a 1,000-ml beaker containing test or control sediment (approximately 2 cm deep), which was covered with 775 ml of sea water (28‰). For the site survey test a single replicate was prepared for each species from sediment collected at a given site. An additional contaminated sediment replicate from each site was prepared for chemical analysis. For the sediment dilution experiment, single replicates were again prepared for each species of each of the dilution treatments. Chemistry replicates for these latter tests were prepared for only the highest concentrations used from each site with the exception of site 14, where a chemistry replicate was prepared for all of the sediment dilutions used in the test. For both the site survey and sediment dilution tests, five control sediment replicates were prepared. Each replicate was given a randomly assigned beaker number and placed overnight in a water bath (15 or 25°C), with gentle aeration (via 1-ml glass pipet), and covered with a watch glass.

A maximum of 10 days before test initiation, *R. abronius* were collected subtidally with a small biological dredge from Yaquina Bay (Newport, OR). *L. plumulosus* were collected by sieving (0.5 mm) them from cultures maintained at our facility. Collected amphipods were maintained in sediment from the collection site and acclimated to bioassay salinity (28‰ for *R. abronius*, 25‰ for *L. plumulosus*), temperature (15°C for *R. abronius*, 25°C for *L. plumulosus*), and fluorescent lighting conditions (continuous). At the start of each 28-day or 10-day toxicity test (t_0), 20 amphipods of a given species were added to single contaminated sediment replicates. At the same time 20 amphipods of each species were added to each of the negative control beakers. McKinney Slough sediment was used as a negative control sediment for the *L. plumulosus* tests and a sediment collected from the *R. abronius* collection site in Yaquina Bay was used as the negative control sediment in tests using that species. Control and experimental sediment physical and chemical characteristics are presented in Table 2.

After amphipod additions, beakers were returned to the water bath, covered with a watch glass, and aeration resumed. The remaining test and carrier control replicates were sampled for bulk sediment and interstitial water contaminant concentrations.

Table 2. Physical and chemical characteristics for each of the contaminated and control sediments

Site	%OC	ΣPAH (µM/kg)	%Sand	%Silt	%Clay
1	7.4	378	26.5	62.2	11.3
2	5.8	7,428	30.7	47.0	22.4
3	12.8	15,823	29.8	40.0	29.8
4	8.2	832	45.6	33.8	20.6
5	6.8	899	57.7	35.3	7.0
6	3.1	1,095	53.8	39.1	7.1
7	2.2	344	52.9	42.0	5.1
8	4.4	372	63.1	30.5	6.3
9	2.8	1,517	66.3	29.3	4.4
10	2.1	285	55.2	39.8	5.0
11	2.2	295	19.9	70.5	9.6
12	2.9	367	19.7	69.7	10.6
13	3.6	388	68.4	23.3	8.3
14	12.4	12,015	54.6	23.4	22.0
15	16.0	98,725	22.8	44.5	32.7
16	7.9	6,441	29.8	47.0	23.3
17	0.9	35	62.1	30.9	7.0
18	2.8	151	38.0	47.9	14.2
19	2.0	56	13.6	77.8	8.5
20	2.9	225	33.6	45.2	21.3
21	3.2	685	37.4	43.9	18.7
22	1.5	75	51.0	36.6	12.4
23	1.5	118	50.1	37.4	12.5
24	4.0	3,234	5.5	80.1	14.4
25	5.1	4,092	16.1	62.6	21.3
26	3.4	665	16.5	61.2	22.3
27	2.9	1,321	7.7	77.4	14.9
28	3.8	376	9.5	78.9	11.6
29	3.0	255	25.6	56.0	18.4
30	3.1	237	32.3	53.5	14.2
YB control	0.2[a]	<LLD	98.5	0.3	1.2
MS control	2.3	<LLD	16.0	63.0	21.0

[a] Mean value for this sediment (Cole *et al.* 2000)

%OC = % organic carbon, ΣPAH = total concentration of the 13 PAHs, YB control = Yaquina Bay control sediment collected from *R. abronius* collection site and used in the *R. abronius* 10-day test as a negative control sediment. MS control = McKinney Slough sediment, which was used as a negative control sediment in *L. plumulosus* 10-day and 28-day tests and also used as the dilution sediment in the sediment dilution experiment. <LLD = below lower limit of detection

Positive controls were water-only tests (no added sediment) prepared at t_0. *R. abronius* positive controls consisted of seven 1,000-ml beakers containing $CdCl_2$ (5, 2.5, 1.25, 0.62, 0.31, 0.16, and 0 mg/L) dissolved in 975 ml of sea water (28‰). *L. plumulosus* positive controls were similarly prepared using lower $CdCl_2$ dosage levels (3, 1.5, 0.75, 0.375, 0.188, 0.094, and 0 mg/L) in 25‰ sea water. These concentrations were verified by flame atomic absorption spectrometry (Perkin Elmer Model 5100, Norwalk, CT) using a previously published methodology (Boese *et al.* 1998). As with the sediment-containing beakers, positive control beakers were randomly assigned a number, amphipods (n = 20) were added to each, the beakers covered with a watch glass, placed in the water bath (15 or 25°C) and aerated during the test. Only one replicate was prepared at each $CdCl_2$ concentration.

During the tests, visual observations were made daily and obvious mortalities and unusual conditions noted. Amphipods that had become entrapped at the air–water interface were gently tapped beneath the water surface to allow them a chance to rebury. Amphipods in 28-day chronic tests were fed three times weekly with 400-ml portions of a mixed algal culture consisting of *Pseudoisochrysis paradoxa* and *Phaeodactylum tricornutum* (1×10^6 cells/ml). An equal volume of overlying water was removed from each replicate before algal culture addition.

At the end of each chronic and acute test (t_{10} or t_{28}), amphipods were gently sieved (0.5 mm) from the test and control sediment, the survivors counted and placed into glass culture dishes (10 cm diameter, 4 cm deep), each of which contained approximately 200 ml of control sediment covered by approximately 2 cm of sea water (28‰). After 1 h, the number unable to rebury were counted. Positive controls were similarly sampled and evaluated after 4 days of exposure (t_4 of each bioassay).

UV Exposure

After the initial burial tests, survivors, including those that did not rebury, were sieved (0.5 mm) from the burial sediment with each replicate placed into individual plastic petri dish lids (95 mm diameter, 7 mm deep) containing 30–50 ml sea water at the salinity used in the previous 10- or 28-day toxicity tests. Replicates were exposed to UV light for 1 h in a growth chamber (Model GC15-H, Environmental Growth Chambers, Chagrin Falls, OH) maintained at the temperature used in the preceding toxicity test. Following UV exposures, mortalities and the survivors' ability to bury in control sediment were again determined following the same protocol used for determining the initial test endpoints (ASTM 1996a).

UV radiation and visible light were produced in the growth chamber by a combination of UV-A 340 and UV-B 313 fluorescent lamps (The Q-Panel Company, Cleveland, OH) and standard fluorescent lamps. To reduce UV intensities to levels that mimicked full sunlight, the petri dishes were covered with nylon window screening. An additional layer of cellulose acetate was added to remove any UV-C produced by the lamps. Previous experiments using this apparatus and lighting regimen did not noticeably affect control *R. abronius* survival or reburial (Boese *et al.* 1998; Swartz *et al.* 1997). Light intensities ($\mu W/cm^2$) were measured (250–800 nM at 1-nM intervals) at eight evenly distributed points within the growth chamber using a spectroradiometer (Optronics model 752, Optronics Laboratories, Inc., Orlando, FL). Measurement and calibration procedural details have been previously published (Boese *et al.* 1998).

LC50 and EC50 Calculations

For each species, the results of both the site survey and dilution 10-day tests were combined to determine LC50 and EC50 values by Probit analysis (Finney 1971) using a PC SAS statistical package (SAS Institute Inc., Cary, NC). Statistical comparisons among these values were accomplished using the standard method for comparing LC50 values of the American Public Health Association (APHA 1989).

Results

Initial Acute and Chronic Toxicity Tests

Sediment ΣTUs for the site survey (Table 3) were calculated using measured bulk sediment PAH concentrations for the 13 PAHs of interest (Table 1). These ΣTU values ranged from 0.31 to 28.85, indicating that there was measurable PAH contamination at all of the sites in the study area. Sediment ΣPAH concentrations were highly correlated (r = 0.72) with their OC content. OC content ranged from 0.9 to 16.0% (Table 2) with the sediments collected from the shallowest sites having the greatest OC and PAH contamination (Figure 1 and Table 2). The most highly contaminated sediments contained visible traces of creosote, which likely contributed to their greater PAH and OC content. Control sediment TU values were below detection limits for each of the 13 PAHs used in the TU calculations. The detection limits ranged from 2 to 28 µg/kg for these compounds, which corresponds to 0.00002 to 0.0003 TUs.

Most of the sites sampled in Elliott Bay exhibited measurable toxicity when compared with controls (Table 3). For *R. abronius* using reburial as an endpoint, 13 of the sites were highly toxic (50–100% of amphipods affected), 6 of the sites were moderately toxic (20–45% of amphipods affected), with 11 sites being relatively nontoxic (0–15% of amphipods affected). In the *L. plumulosus* acute test, 17 sites were highly toxic, 8 sites were moderately toxic, and only 5 sites appeared to be nontoxic (Table 3). Data from the *L. plumulosus* 28-day chronic test were not reliable as control survival did not meet the quality control standards of 80% survival (DeWitt *et al.* 1997). However, even given the questionable quality of the data set (*i.e.*, poor control survival), sediments which were the most toxic in the 10-day acute tests also killed all of the amphipods in the chronic test.

Results of the sediment dilution experiment (Table 4), show that as TUs are reduced by dilution, toxicities became progressively less. In the initial survey experiment sediments with $\Sigma TU < 1$ were generally nontoxic, those with $1 < \Sigma TU < 2$ were moderately to highly toxic, and those with $\Sigma TU > 2$ were always highly toxic (Table 3). There were several instances in both tests (especially in the sediment dilution experiment) where sediments with ΣTU values slightly larger than one appeared to be nontoxic (Table 4). In the sediment dilution experiment, all of these anomalies were from sediments in which PAH concentrations were estimated from the sediment dilution factor rather than directly measured (Table 4). Although some errors were undoubtedly introduced by estimating TU values from dilution factors, in those cases where ΣTUs were directly measured, values were generally within 25% of that estimated by dilution.

LC50 and EC50 values were determined on the combined results of acute sediment toxicity tests from the site survey and dilution experiments (Table 5). The initial LC50 and EC50 values were greater than the expected TU value of 1.0 (range: 1.56–2.14), and *L. plumulosus* was slightly but statistically more sensitive than *R. abronius* (Table 4). LC50 and EC50

Table 3. Initial site survey: amphipod initial toxicity using 10-day acute and 28-day chronic sediment toxicity tests followed by a phototoxicity assessment using a 1-h exposure to UV radiation[a]

		R. abronius Acute Test				*L. plumulosus* Acute Test				*L. plumulosus* Chronic Test			
		10-day Initial		1-hour Photox		10-day Initial		1-hour Photox		10-day Initial		1-hour Photox	
Site	ΣTU	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury
1	0.55	5	5	5	35	10	10	15	100	50	50	50	70
2	8.81	100	100	100	100	100	100	100	100	100	100	100	100
3	7.74	100	100	100	100	100	100	100	100	100	100	100	100
4	1.16	0	30	90	100	65	85	100	100	100	100	100	100
5	1.55	5	15	80	100	30	40	65	100	85	85	90	100
6	3.71	25	25	95	95	60	80	85	100	70	70	75	100
7	1.96	0	20	75	95	30	30	45	100	70	70	70	100
8	1.01	15	30	45	100	30	60	90	100	75	75	75	100
9	4.19	65	85	95	100	65	80	95	100	95	95	95	100
10	1.73	5	10	60	100	20	30	35	100	45	45	45	100
11	1.62	75	80	100	100	70	70	100	100	100	100	100	100
12	1.54	20	35	90	100	50	55	70	100	95	95	95	100
13	1.22	0	10	75	100	10	25	100	100	35	35	35	100
14	7.10	100	100	100	100	100	100	100	100	100	100	100	100
15	28.85	100	100	100	100	100	100	100	100	100	100	100	100
16	7.20	100	100	100	100	100	100	100	100	100	100	100	100
17	0.46	0	0	0	15	0	5	5	100	60	60	60	60
18	0.58	5	5	5	85	20	30	20	95	40	40	40	40
19	0.31	30	35	30	65	0	0	0	90	40	40	45	45
20	0.75	5	5	5	85	0	5	10	95	50	50	50	80
21	2.11	55	70	100	100	90	95	100	100	70	80	80	100
22	0.53	0	5	10	90	5	20	15	100	35	35	35	35
23	0.91	10	10	10	50	0	0	0	95	40	40	40	40
24	6.54	100	100	100	100	100	100	100	100	100	100	100	100
25	7.31	100	100	100	100	100	100	100	100	100	100	100	100
26	1.86	90	90	100	100	65	90	100	100	80	80	90	100
27	4.57	100	100	100	100	100	100	100	100	100	100	100	100
28	1.25	75	85	90	100	60	65	70	100	80	80	80	100
29	1.03	10	15	45	100	15	20	30	100	60	60	60	90
30	0.70	5	5	45	100	15	25	30	100	55	55	55	95
Negative controls													
1	< LLD	0	0	0	0	0	0	0	0	45	45	45	45
2	< LLD	0	0	0	0	0	0	0	0	55	55	55	55
3	< LLD	0	0	0	0	0	0	0	0	40	40	40	45
4	< LLD	0	10	0	15	0	0	5	5	30	30	30	35
5	< LLD	5	5	10	10	5	5	5	5	45	45	45	45

[a] Toxicity values are the percent of amphipods that did not survive or were unable to rebury out of the original 20 organisms placed into each beaker at the start (t_0) of the initial 10- or 28-day sediment toxicity test (ΣTU = sum of toxic units; < LLD = below lower limit of detection)

values were not determined from the *L. plumulosus* chronic test because the high mortalities in control sediments precluded reliable LC50 and EC50 determinations.

Cadmium toxicity (positive control) LC50 values for *R. abronius* and *L. plumulosus* in the site survey and sediment dilution experiments were within acceptable quality control limits for these two species (*i.e.*, 95% CI values overlap with previous positive control tests conducted at our laboratory). Control survival in the acute tests met the established criteria for test acceptance (ASTM 1996a).

Phototoxicity Tests

Light measurements within the growth chamber indicated that no radiation was present in the UV-C range (below 280 nm). For the site survey experiments mean UV-B (280–320 nm), UV-A (321–400 nm), and visible (401–700 nm) radiation intensities were 122 ± 1 μW/cm^2 (mean ± SE), 282 ± 6 μW/cm^2, and 3,090 ± 75 μW/cm^2, respectively. For the sediment dilution experiment these same respective values were 96 ± 3 μW/cm^2, 227 ± 18 μW/cm^2, and 2,350 ± 38 μW/cm^2. These values roughly correspond to 85% of UV-B, and 10% of UV-A and visible radiation present in full sunlight measured using the same instrument on a cloudless day (October 3, 1996, 1 PM Pacific Daylight Time) at our location.

Exposure to UV radiation greatly enhanced toxicity in both the site survey and sediment dilution experiments. In contrast to the initial 10-day toxicities, almost all of the sites were found to be highly phototoxic to *R. abronius*, with only one moderately toxic and one nontoxic site identified using reburial as an endpoint (Table 3). In acute tests using *L. plumulosus* all sites

Table 4. Sediment dilution experiment: amphipod initial toxicities using 10-day sediment exposures followed by phototoxicity assessments using 1-h exposures to UV radiation[a]

			R. abronius Acute Test				*L. plumulosus* Acute Test			
			10-day Initial		1-h Phototoxicity		10-day Initial		1-h Phototoxicity	
Site	%OC	ΣTU	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury	% Mortality	% Unable to Rebury
2	3.3	2.98	95	95	95	95	100	100	100	100
	ND	*1.19*	65	70	85	100	90	95	100	100
	ND	*0.48*	20	20	35	40	30	30	25	100
	ND	*0.19*	5	20	30	95	10	15	25	90
	ND	*0.08*	0	0	0	10	5	5	10	50
	ND	*0.03*	20	20	20	20	0	0	0	0
3	3.1	3.48	100	100	100	100	100	100	100	100
	ND	*1.39*	5	10	25	100	20	35	80	100
	ND	*0.56*	0	0	15	100	20	20	20	75
	ND	*0.22*	5	5	5	50	5	5	5	40
	ND	*0.09*	5	5	10	10	10	10	20	20
	ND	*0.04*	5	5	20	50	0	0	5	25
14	2.6	1.92	45	75	80	100	60	60	85	100
	2.5	0.69	0	0	0	100	35	35	60	100
	2.5	0.23	5	10	5	70	0	0	0	95
	2.3	0.13	15	15	15	15	25	25	30	60
	2.5	0.05	5	5	5	20	0	0	5	15
	2.5	0.03	5	5	5	65	0	0	0	0
15	3.1	3.45	100	100	100	100	100	100	100	100
	ND	*1.42*	75	80	100	100	45	45	75	100
	ND	*0.57*	5	5	15	100	5	5	5	95
	ND	*0.23*	10	10	10	70	0	0	0	85
	ND	*0.09*	0	0	5	20	10	10	10	35
	ND	*0.04*	15	15	15	15	0	0	0	5
16	3.5	3.88	100	100	100	100	100	100	100	100
	ND	*1.55*	70	80	100	100	60	70	85	100
	ND	*0.62*	10	10	15	95	5	5	5	100
	ND	*0.25*	5	5	15	65	15	15	20	65
	ND	*0.10*	15	15	15	20	5	5	5	5
	ND	*0.04*	15	15	15	15	5	5	15	15
24	2.5	3.22	100	100	100	100	95	100	100	100
	ND	*1.29*	10	10	25	70	45	55	85	90
	ND	*0.52*	10	10	20	95	10	15	20	100
	ND	*0.21*	0	0	0	15	5	5	20	100
	ND	*0.08*	0	0	0	0	0	0	0	15
	ND	*0.03*	10	10	10	10	0	0	10	10
25	3.6	2.61	100	100	100	100	80	100	100	100
	ND	*1.04*	5	15	65	100	20	30	40	100
	ND	*0.42*	20	20	20	85	15	15	15	95
	ND	*0.17*	5	5	15	20	10	10	15	65
	ND	*0.07*	15	15	15	15	10	10	15	30
	ND	*0.03*	10	15	10	20	0	0	0	25
27[a]	2.4	1.27	15	25	40	100	15	15	75	100
	ND	*0.51*	5	10	5	75	5	10	20	100
	ND	*0.20*	25	25	25	35	0	0	0	85
	ND	*0.08*	10	10	10	10	0	0	0	5
	ND	*0.03*	10	5	5	5	0	0	0	0
Negative controls										
1	ND	ND	0	0	0	0	0	0	0	0
2	ND	ND	5	5	5	5	5	5	5	5
3	ND	ND	10	10	10	10	5	5	5	5
4	ND	ND	5	5	5	5	0	0	0	0
5	ND	ND	0	0	0	0	0	0	0	5

[a] Toxicity values are the percent of amphipods that did not survive or were unable to rebury out of the original 20 organisms placed into each beaker at the start (t_0) of the initial 10- or 28-day sediment toxicity test

[b] Only five dilutions tested due to lower toxicity of site 27 whole sediment and the limited amount of sediment collected from each site

%OC = % total organic carbon, ΣTU = sum toxic units, italicized ΣTU values are estimates based on sediment dilution factors, ND = no data as %OC was not measured on all samples

Table 5. Initial and photo-induced LC50 and EC50 (reburial) values for *R. abronius* and *L. plumulosus* exposed to PAH contaminated Elliott Bay sediments in acute 10-day sediment toxicity tests

	R. abronius 10-day Acute	*L. plumulosus* 10-day Acute
Initial 10-day LC50	2.14	1.87[a]
95% fiducial CI	2.01–2.30	1.76–1.99
Initial 10-day EC50	1.88	1.56[a]
95% fiducial CI	1.76–2.02	1.47–1.66
Phototoxicity 1-h LC50	1.15[b]	1.13[b]
95% fiducial CI	1.08–1.22	1.07–1.20
Phototoxicity 1-h EC50	0.37[b]	0.20[b]
95% fiducial CI	0.33–0.41	0.18–0.22

Values are in toxic units, which were determined using the combined results of the site survey and sediment dilution bioassays

[a] Significant difference between *L. plumulosus* and *R. abronius* 10-day acute toxicity responses using the standard method for comparing LC50 values of the American Public Health Association (APHA 1989)

[b] Significant difference between initial and phototoxicity LC50 and LC50 values (APHA 1989)

were highly phototoxic. Unexpectedly high phototoxicity was observed in *R. abronius* reburial in the most diluted replicate of Site 14 sediment (Table 4). However, this high phototoxicity was not observed in the *L. plumulosus* replicate using the same sediment, leading us to suspect that the *R. abronius* replicate was mislabeled and was most likely an intermediate dilution. Although the *L. plumulosus* exposed in 28-day chronic tests were less sensitive to UV enhanced toxicity, 76% of the replicates in which there was at least one survivor from the 28-day exposure, exhibited an increase in toxicity following 1 h of UV exposure (Table 3).

Using the combined results of the site survey and dilution experiments, the 1-h exposure to UV increased toxicity as measured by both mortality and the ability to rebury. LC50 values decreased by 1.9- and 1.6-fold, respectively for *R. abronius* and *L. plumulosus* while EC50 TU values decreased 5.1- and 7.8-fold (Table 5).

Discussion

Although most of the sediments tested in the present experiments were highly phototoxic, this UV-enhanced toxicity was most apparent in reburial (EC50) comparisons. Mortality (LC50) was only moderately enhanced (Table 5). This observation is consistent with previous experiments conducted at our laboratory. In general, test organisms that survived initial (before UV) 10-day tests usually survived the 1-h exposures to UV radiation (Boese *et al.* 1998, 1999). However, if they have bioaccumulated an effective dose of a phototoxic contaminant, they exhibited characteristic symptoms of phototoxicity (Boese *et al.* 1998, 1999), are unable to rebury in control sediment, and usually die within 24 h (Boese *et al.* 1998). Even if these affected amphipods could recover, infaunal amphipods that cannot bury in nature would be easy prey items and are thus ecologically dead.

The increase in toxicity following UV exposure was approximately five- to eightfold, which is consistent with the results of other studies that utilized marine amphipods exposed to sediments spiked with single and multiple PAH contaminants and exposed under similar experimental conditions (Boese *et al.* 1998, 1999; Swartz *et al.* 1997). This degree of enhancement is also similar to that observed in freshwater crustacean species (Ankley *et al.* 1994; Monson *et al.* 1995).

Although the results of the *L. plumulosus* chronic toxicity test are suspect due to low control survival, amphipods exposed for 28 days to sediments appeared to be less sensitive to photo-induced toxicity than those initially exposed for 10 days to the same sediment (Table 2). This result was surprising as increasing the duration of the initial exposure from 10 to 28 days would tend to increase tissue residues of most contaminants, especially those with high K_{ow} values, which are not likely to attain steady-state tissue residues in acute tests (ASTM 1996b). All other factors equal, greater tissue residues of phototoxic compounds should have resulted in increased phototoxicity (Ankley *et al.* 1997). However, it is possible that these amphipods did not attain higher tissue residues in the longer duration tests. In past comparisons conducted at our laboratory using 10-day and 28-day tests, there was little difference in LC50 values (Richard Swartz, personal communication), suggesting that increased exposure time did not result in increased tissue residues. Amphipods in chronic tests were fed algae and, as a result, some overlying water was exchanged. Feeding uncontaminated food and exchanging overlying water would tend to lower contaminant exposures to the gills and gut when compared to the unfed amphipods in 10-day tests, which are conducted without overlying water exchanges. Fed amphipods grew during the 28-day test and were noticeably larger than the unfed adults that survived the 10-day acute tests suggesting the possibility that tissue concentration may have been diluted by growth (see ASTM 1996b). Test organisms that survived and grew in the 28-day tests may have done so because they were able to depurate contaminants at a faster rate, resulting in relatively lower tissue residues. These larger amphipods were also noticeably more pigmented than the nearly transparent and smaller 10-day acute test survivors. Their larger size and pigmentation may have served to reduce the penetration of UV into tissues were phototoxicity occurs. Unfortunately, as tissue residues were not determined in the present study, none of these hypotheses can be further evaluated.

Although standard 10-day sediment toxicity tests do not address phototoxicity, the consistent five- to tenfold enhancement between 10-day toxicity and subsequent phototoxicity observed in this and past experiments (Swartz *et al.* 1997; Boese *et al.* 1998, 1999) suggests that it might be possible to predict which sediments may need to be tested for phototoxicity using ΣTU calculations. As indicated in Figure 2A and 2B, it may be unnecessary to photo-evaluate test sediments which have $\Sigma TU > 1.0$ as standard 10-day toxicities would indicate that these sediments are highly toxic. Although outliers are present (especially in the *R. abronius* sediment dilution experiment), contamination levels below 0.05 TU were generally not phototoxic and little information beyond that obtained from standard sediment tests is gained. Only when the ΣTU values are between 0.05 and 1.0 would conducting an evaluation of the phototoxicity potential of a PAH-contaminated sediment appear to add useful information.

Although this idea is promising, it needs further study for a variety of reasons. Sediments used in the present study were contaminated from a single source and likely contained the same suite of contaminants in similar ratios. PAH-contaminated

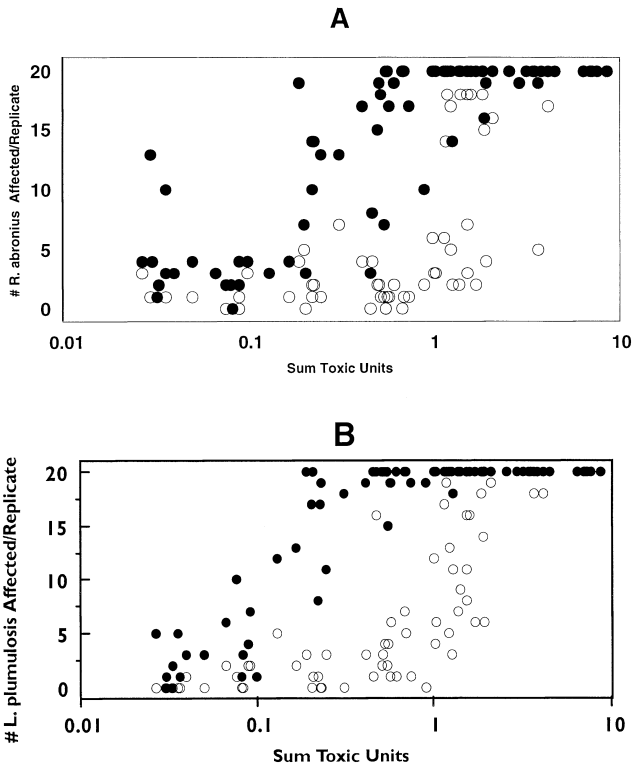

Fig. 2. Relationship between initial reburial following 10-day exposures to contaminated sediment (○) and reburial of survivors following subsequent exposure to 1 h of UV radiation (●) for *Rhepoxynius abronius* (A) and *Leptocheirus plumulosis* (B)

sediments at other sites may have different ratios of PAH constituents, especially if they are pyrogenic in origin (Baumard *et al.* 1998). ΣTU concentrations are calculated using only 13 PAHs (Table 2). Many other phototoxic and nonphototoxic contaminants are likely to be present in contaminated sediments. In addition bioaccumulated PAHs are not equally phototoxic (Ankley *et al.* 1997).

The result of this study suggests that the spatial extent of potentially deleterious sediments at the Superfund study site was considerably greater than indicated by standard 10-day sediment toxicity tests. However, the methodology used to evaluate phototoxicity is flawed in that test organisms are removed from the sediment and exposed to UV in minimal water. In nature, infaunal amphipods generally do not emerge in daylight and are therefore unlikely to experience phototoxic effects. Infaunal organisms that do emerge into full sunlight are likely to have evolved means to protect themselves from the direct effects of UV radiation (*e.g.*, pigmentation), which would also tend to protect them from photoinduced toxicity (Boese *et al.* 1997). The unanswered question of this and other research into the phototoxicity of contaminated sediments is whether phototoxicity is ecologically significant or merely an interesting laboratory artifact. Further research is needed in which infauna are exposed to sunlight while buried in contaminated sediments and in relating field measures of infaunal biotic integrity to phototoxicity bioassay results. Until these research areas are addressed, inclusion of phototoxicity tests in the assessments of the ecological risk of contaminated sediments to infauna is of questionable value.

Acknowledgment. Thanks go to Jill Jones, Judy Pelletier, John Sewall, Sally Noack, and Mitch Vance for considerable technical support. Thanks also to Craig McFarlane, Marguerite Pelletier, and Steve Ferraro for reviewing the manuscript. The work discussed in this document has been funded by the U.S. Environmental Protection Agency. This document has been subjected to agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation of use by the Environmental Protection Agency.

References

American Society for Testing and Materials (1996a) Guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. E 1367–92. In: Annual book of ASTM standards, vol. 11.05. American Society for Testing and Materials, Philadelphia, PA, pp 769–794

American Society for Testing and Materials (1996b) Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates. E 1688–95. In: Annual book of ASTM standards, vol. 11.05. American Society for Testing and Materials, Philadelphia, PA, pp 1110–1159

Ankley GT, Collard SA, Monson PD, Kosian PA (1994) Influence of ultraviolet light on the toxicity of sediments contaminated with polycyclic aromatic hydrocarbons. Environ Toxicol Chem 13:1791–1796

Ankley GT, Erickson RJ, Phipps GL, Mattson VR, Kosian PA, Sheedy BR, Cox JS (1995) Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. Environ Sci Technol 29:2828–2833

Ankley GT, Erickson RJ, Sheedy BR, Kosian PA, Mattson VR, Cox JS (1997) Evaluation of models for predicting the phototoxic potency of polycyclic aromatic hydrocarbons. Aquat Toxicol 37:37–50

APHA (1989) Standard methods for the examination of water and waste water, 17th ed. American Public Health Association, Washington, DC

Baumard P, Budzinski H, Garrigues P (1998) Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. Environ Toxicol Chem 17:765–776

Boese BL, Lamberson JO, Swartz RC, Ozretich RJ (1997) Photoinduced toxicity of fluoranthene to seven marine infaunal crustacean species. Arch Environ Contam Toxicol 32:389–393

Boese BL, Lamberson JO, Swartz RC, Ozretich R, Cole F (1998) Photoinduced toxicity of PAHs and alkylated PAHs to a marine infaunal amphipod (*Rhepoxinius abronius*). Arch Environ Contam Toxicol 34:235–240

Boese BL, Ozretich RJ, Lamberson JO, Swartz RC, Cole FA, Pelletier J, Jones J (1999) Toxicity and phototoxicity of mixtures of highly lipophilic PAH compounds in marine sediment: can the ΣPAH model be extrapolated? Arch Environ Contam Toxicol 36:270–280

Cole FA, Boese BL, Swartz RC, Lamberson JO, DeWitt TH (2000) The effect of sediment storage on the toxicity of fluoranthene to the amphipod, *Rhepoxynius abronius*. Environ Toxicol Chem 19: (in press)

Cubbage JC (1989) Concentrations of polycyclic aromatic hydrocarbons in sediment and ground water near the Wyckoff Wood Treatment Facility, West Seattle, Washington. Washington Department of Ecology, Olympia, WA

DeWitt TH, Niewolny LA, Nieukirk SL, Gruendell B, Gardiner W, Borde B (1997) Support for development of a standard chronic sediment toxicity protocol with the estuarine amphipod, *Leptocheirus plumulosus*. Final report prepared for US Environmental Protection Agency, Office of Science and Technology, Washington, DC, under contract 68-C2-0134 by Battelle Marine Sciences Laboratory, Battelle Memorial Institute, Pacific Northwest Division, Richland, WA

DiToro DM (1985) A particle interaction model of reversible organic chemical sorption. Chemosphere 14:1503–1538

Finney DJ (1971) Probit analysis. Cambridge University Press, New York, NY

Mekenyan OG, Ankley GT, Veith GD, Call DJ (1994) QSARs for photoinduced toxicity: I. Acute lethality of polycyclic aromatic hydrocarbons of *Daphnia magna.* Chemosphere 28:567–582

Monson PD, Ankley GT, Kosian PA (1995) Phototoxic response of *Lumbriculus variegatus* to sediments contaminated by polycyclic aromatic hydrocarbons. Environ Toxicol Chem 14:891–894

Ozretich RJ, Schroeder WP (1986) Determination of selected neutral priority organic pollutants in marine sediment, tissue and reference materials using bonded-phase sorbents. Anal Chem 58:2041–2048

Plumb RH (1981) Procedures for handling and chemical analysis of sediment and water samples. Technical report EPACE-81-1. US Army Engineers Waterways Experiment Sation, Vicksburg, MS

Swartz RC, Ferraro SP, Lamberson JO, Cole FA, Ozretich RJ, Boese BL, Schultz DW, Behrenfeld M, Ankley GT (1997). Photoactivation and toxicity of mixtures of PAH compounds in marine sediment. Environ Toxicol Chem 16:2151–2157

Swartz RC, Schults DW, Ozretich RJ, Lamberson JO, Cole FA, DeWitt TH, Redmond MS, Ferraro SP (1995) ΣPAH: a model to predict the toxicity of polynuclear aromatic hydrocarbons mixtures in field-collected sediments. Environ Toxicol Chem 14:1977–1987