Appendix E. Supplemental Bioassay Report Information

Toxicity Bioassays

When oil spills occur in marine environments, concentrations of petroleum constituents dissolved or suspended in the water column are known to result in acute toxicity to marine organisms. Because petroleum products differ in chemical makeup, it is important to design toxicity bioassays that take into account the solubility and toxicity of the specific petroleum products whenever possible. With this in mind, laboratory toxicity bioassays were conducted on April 21-27, 2016, by Pacific EcoRisk Laboratory in Fairfield, CA, at the request of the Refugio Beach Oil Spill Trustees. These toxicity bioassays were conducted using dilutions of a high energy water accommodated fraction (HEWAF) of Line 901 oil that contained polycyclic aromatic hydrocarbons (PAHs), measured as the sum total of 45 PAHs (500, 100, 50, 10, 5, 1, and 0.5 µg/L as $TPAH_{45}$ and a seawater control). The laboratory toxicity bioassays used seawater and were designed to assess the effect of Line 901 oil on three marine species. These organisms were *Mytilus galloprovincialis* (mussels), *Menidia beryllina* (silverside – fish) and *Emerita analoga* (sand crabs). Endpoints included survival and sublethal endpoints (*e.g.* weight). For a detailed discussion of bioassay methods, as well as results and additional information, please see the toxicity bioassay report written by Pacific EcoRisk (2016).

Bioassay Methods

Adult mussels *M. galloprovincialis* were obtained from Taylor Shellfish Company in Shelton, WA. Embryos were generated from gravid adults. Prior to spawning, the adult mussels were held in seawater at a temperature of 12˚C. To induce spawning, the adults were placed into glass trays of clean seawater (filtered Granite Canyon seawater) at 20˚C. This increase in temperature induced the mussels to release sperm and eggs, and embryos were collected. Embryos were exposed to dilutions of the Line 901 HEWAF for 48 hours and survival was monitored.

The larval fish *M. beryllina* were obtained from Aquatic Indicators, St. Augustine, FL (a commercial supplier). Fish were maintained at 25˚C in aerated aquaria containing artificial seawater at a salinity of 34 parts per thousand (ppt) prior to their use in testing. Larval fish were exposed to dilutions of the Line 901 HEWAF for seven days after which the effects on survival and growth were evaluated.

Juvenile sand crabs *E. analoga* (megalopae) were collected from a field population at Salmon Creek Beach, Sonoma, CA, by Dr. Jenifer Dugan (University of California Santa Barbara) and California Department of Fish and Wildlife (CDFW) personnel.

Upon receipt at the Pacific EcoRisk laboratory, the organisms were maintained in aerated tanks of 34.0 ppt and 0.45-µm filtered seawater (collected from the U.C. Granite Canyon Marine Laboratory, Carmel, CA) at 15˚C prior to use in the testing. The sediment used as a substrate for the organisms in this test consisted of 50-70 mesh sized white quartz sand obtained from Sigma-Aldrich Corporation. Sand crabs were exposed to dilutions of the Line 901 HEWAF for six days after which the effects on survival and growth were evaluated.

CETIS

CETIS is a professional toxicity data analysis application developed and published by TidePool Scientific Software in McKinleyville, CA. Because of its comprehensive design, CETIS is frequently used by environmental toxicity laboratories to analyze bioassay dose-response data to estimate the lethal concentration to 50% of the test organisms (LC_{50}) and the effective concentration to 50% of the test organisms (EC_{50}). Definitions of the CETIS terms and results generated for and used in the bioassay report are included as well (Pacific EcoRisk, 2016).

Water Chemistry Conversions

As detailed in the Pacific EcoRisk Report (2016) samples of the HEWAF dilutions were collected daily and analyzed for 45 PAHs (Table 1) by the CDFW Water Pollution Control Laboratory. Detected PAH concentrations were summed to determine the total PAH_{45} ($TPAH_{45}$) concentration. During the Refugio Beach Oil Spill, surf water samples were collected by the Center for Toxicology and Environmental Health (CTEH). Their analyte list included 37 PAHs (Table 1). 1-Methylnaphthalene (1-MN) and 2-methylnaphthalene (2-MN) were summed to estimate the C1-naphthalene group and detected PAHs were summed to determine the total PAH_{37} ($TPAH_{37}$) concentration. For the bioassay chemistry data, the grand mean of the ratio of $TPAH_{37}$ to $TPAH_{45}$ was 0.84. This ratio was applied to adjust $TPAH_{45}$ bioassay endpoints to $TPAH_{37}$ equivalents (Table 2). Before the bioassay was initiated, dilutions of the HEWAF were made using water at 15°C and 25°C. The dilutions were analyzed for both $TPAH_{45}$ and total petroleum hydrocarbons (TPH). There was a linear relationship between the two variables (Figure 1) and the regression equation was used to estimate bioassay endpoints based on TPH (Table 2).

Table 1. PAHs measured by CDFW ($TPAH_{45}$) and CTEH ($TPAH_{37}$)

	PAH	**CDFW-Total PAH_{45}**	**CTEH Total PAH_{37}**
1	Naphthalene	•	•
2	Naphthalenes, C1 -	•	1-MN + 2MN
3	Naphthalenes, C2 -	•	•
4	Naphthalenes, C3 -	•	•
5	Naphthalenes, C4 -	•	•
6	Acenaphthylene	•	•
7	Acenaphthene	•	•
8	Fluorene	•	•
9	Fluorenes, C1 -	•	•
10	Fluorenes, C2 -	•	•
11	Fluorenes, C3 -	•	•
12	Phenanthrene	•	•
13	Anthracene	•	•
14	Phenanthrene/Anthracene, C1 -	•	•
15	Phenanthrene/Anthracene, C2 -	•	•
16	Phenanthrene/Anthracene, C3 -	•	•
17	Phenanthrene/Anthracene, C4 -	•	•
18	Pyrene	•	•
19	Fluoranthene	•	•
20	Fluoranthene/Pyrenes, C1 -	•	•
21	Fluoranthene/Pyrenes, C2-	•	•
22	Fluoranthene/Pyrenes, C3 -	•	•
23	Fluoranthene/Pyrenes, C4 -	•	Not Included
24	Benz[a]anthracene	•	•
25	Chrysene	•	•
26	Chrysenes, C1 -	•	•
27	Chrysenes, C2 -	•	•
28	Chrysenes, C3 -	•	•
29	Chrysenes, C4 -	•	•
30	Benzo(a)pyrene	•	•
31	Perylene	•	•
32	Benzo(e)pyrene	•	•

	PAH	CDFW-Total PAH_{45}	CTEH Total PAH_{37}
33	Benzo(b)fluoranthene	•	•
34	Benzo(k)fluoranthene	•	•
35	Benzo(g,h,i)perylene	•	•
36	Indeno(1,2,3-c,d)pyrene	•	•
37	Dibenz(a,h)anthracene	•	•
38	Dibenz(a,h)anthracene, C1-	•	Not Included
39	Dibenz(a,h)anthracene, C2-	•	Not Included
40	Dibenz(a,h)anthracene, C3-	•	Not Included
41	Dibenzothiophene	•	Not Included
42	Dibenzothiophenes, C1 -	•	Not Included
43	Dibenzothiophenes, C2 -	•	Not Included
44	Dibenzothiophenes, C3 -	•	Not Included
45	Biphenyl	•	Not Included

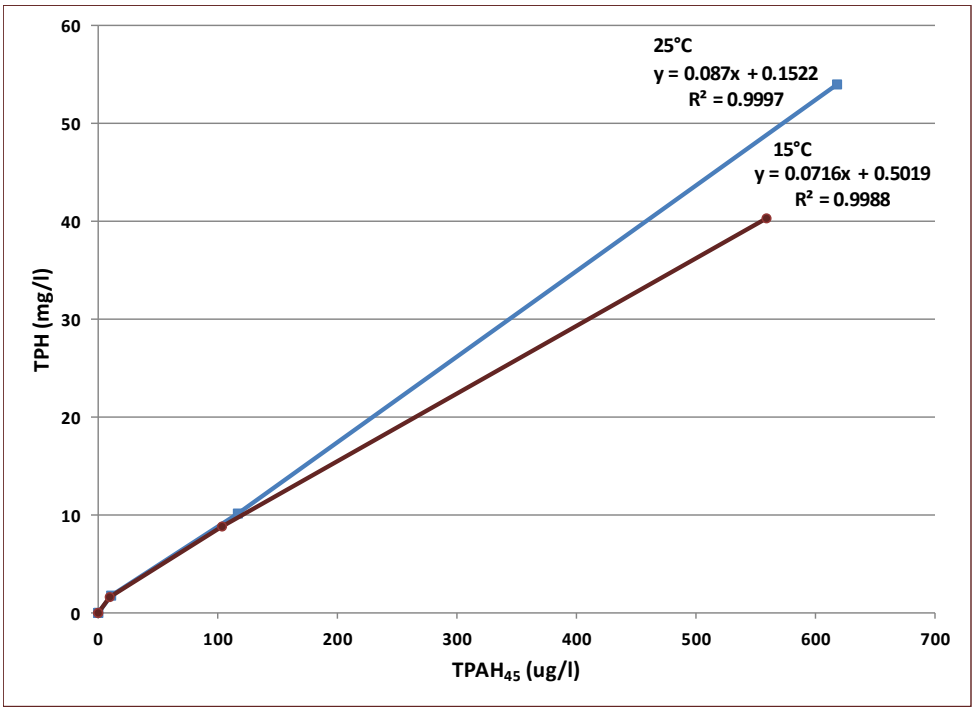

Figure 1. Relationship between total petroleum hydrocarbons (TPH mg/l) and total PAHs ($TPAH_{45}$ µg/l) for water accommodated fraction dilutions prepared at 25°C and 15°C.

Results

LC50's were calculated for bioassay endpoints in Table 2 (see below) for *E. analoga*, and *M. beryllina.* An EC50 (based on larval development) was calculated for *M. galloprovincialis*. In water-only exposures, where both $TPAH_{45}$ and $TPAH_{37}$ were measured, the order of increasing sensitivity is *E. analoga>M. beryllina >M. galloprovincialis.* Similarly, with TPH the order of increasing sensitivity is *E. analoga>M. beryllina* (Pacific EcoRisk, 2016).

Sand crabs (*E. analoga*) data in Table 2 were subsequently compared to porewater and surf water data collected during shoreline assessments. Silverside fish (*M. beryllina*) and mussel embryos (*M. galloprovincialis*) were subsequently compared to surf water data evaluated during subtidal habitat assessments.

Table 2. Bioassay endpoints expressed at total PAHs ($TPAH_{45}$ and $TPAH_{37}$) and total petroleum hydrocarbons (TPH).

Bioassay Endpoint	**$TPAH_{45}$ (µg/l)**	**$TPAH_{37}$ (µg/l)**	**TPH (mg/l)**
E. analoga LC_{50} – 6 days	40.9	34.4	3.4
M. beryllina LC_{50} – 7days	75.6	63.5	6.7
M. galloprovincialis EC_{50} 48 hours	381	320	Not available at 20° C

Reference

Pacific EcoRisk, 2016. Acute and Chronic Toxicity Testing in Support of the Natural Resource Damage Assessment of the Refugio Oil Spill 2 September 2016. 160 pp. NRDA Technical Report. RBOS Administrative Record.