Final Data Report for Newport Bay DPR Antifouling Paint Monitoring Study--January 24, 2007

This report presents the complete data set for samples collected from Newport Bay during the DPR antifouling paint monitoring study. This document contains data from sediment pore water, whole sediment exposures and a whole sediment toxicity identification evaluation (TIE) that were not previously reported, as well as water and sediment toxicity data included in a previous report.

Sampling and testing for this project was conducted in two phases. During the first phase, water and sediment samples were collected from 10 stations in marina areas of Newport Bay (previously reported). Water samples from the first phase were tested with the mussel embryo development assay. Whole sediment from the first phase was tested using a sediment-water interface exposure with mussel embryos and a whole sediment test using amphipods. Based on the results of the first phase, a second round of more targeted sampling of sediment only was conducted. Pore water was tested using the mussel embryo assay on 10 stations. Whole sediment tests using amphipods were conducted on four stations. One station was targeted for a whole sediment TIE.

Toxicity Methodology

Water column

The mussel embryo development test (USEPA 1995) was used to evaluate toxicity on water column, sediment-water interface and pore water samples. This test measures toxic effects on mussel embryos, as a reduction in their ability to normally develop from fertilized eggs. The mussels (*Mytilus galloprovincialis*) used in the tests were obtained from Carlsbad Aquafarms. The test consisted of a 48 h exposure of fertilized eggs to marina water samples. The tests were conducted in glass shell vials containing 10 mL of solution at a temperature of 15°C. Four replicates were tested for each sample. A seawater blank was included as negative control. A copper reference toxicant test was conducted as a positive control.

After 48 h, the embryos were preserved and examined later with a microscope to assess the percentage of normal development. Toxic effects are expressed as a reduction in normal development percentage. The data are presented as percentage normal-alive which was calculated by dividing the number of normal embryos counted by the number of fertilized eggs added at the beginning of the exposure.

Sediment-Water Interface (SWI)

Whole sediment from the 10 stations was loaded into five replicate polycarbonate core tubes, with bottom caps in place, to a depth of 5 cm. The loaded tubes were placed in 1 L beakers of seawater to prevent leakage from within the tubes. Laboratory seawater at approximately 33 g/kg was added over the sediment to a depth of about 7 cm and gentle aeration added. The water and sediment were equilibrated overnight at 15°C. The next day, polycarbonate screen tubes (22 μm mesh) were added on top of the sediment. Fertilized mussel eggs were then added to the screen tubes and given 48 h to develop. After 48 h, the screen tubes were removed from the cores and the embryos were washed

into glass shell vials and preserved. Microscopic examination and data expression were the same as above.

Whole Sediment

For phase one, the whole sediment exposure with amphipods was conducted using a modified procedure due to limited sediment sample size. The exposure was conducted on the same sediment as the SWI testing. Two days after the SWI test was concluded, the overlying water was siphoned from the core tubes and replaced with 20 g/kg seawater and gentle aeration added. After the water had equilibrated overnight, 10 adult amphipods (*Eohaustorius estuarius*) were added to each of the core tubes. Northwestern Aquatic Sciences (Yaquina Bay, OR) supplied the amphipods. The amphipods were exposed for 10 days at 15°C. At the end of the exposure, the sediment was passed through a 0.5 mm screen to remove the amphipods. The number of surviving amphipods was evaluated and the data expressed as percentage survival. A negative control consisting of amphipod collection site sediment (home sediment) was loaded into a core tube and treated as the other stations. A 10 day, water only, reference toxicant exposure with ammonia was conducted as a positive control.

For phase two, whole sediment exposures with amphipods followed the EPA guidelines of 1 L glass jars containing 2 cm of sediment and 800 ml of 20 g/kg seawater (USEPA 1994). Twenty amphipods were added to each jar and the exposure period was 10 days. All other aspects of the testing were conducted as described above.

Whole Sediment Toxicity Identification Evaluation

A reduced volume and duration initial amphipod survival test was performed on two stations to determine if toxicity was present at a high enough level to justify conducting a TIE. This test was performed in 250 ml beakers with 40 ml of sediment and approximately 150 ml of overlying water. Ten amphipods were added to each beaker and the exposure was conducted for 7 days.

A whole sediment toxicity identification evaluation (TIE) was conducted on station 6013 from the Newport Dunes Marina. This station was found to be very toxic to amphipods for the initial sample collected (Table 4) and again when the station was resampled (Table 7). Baseline toxicity tests were performed on untreated aliquots of sediment and sediment that had been diluted 50% by weight with clean sediment from the amphipod collection site. Whole sediments and, in some cases, 50% dilutions were treated with three procedures to reduce or eliminate toxicity in different toxicant classes. Each treatment was performed on a separate aliquot of homogenized sediment. Cation exchange resin was added to the sediment (20% resin by weight) to remove cationic metals. Coconut charcoal was added to the sediment (15% by weight) to sequester organic chemicals. Piperonyl butoxide (PBO) was added to the overlying water to a final concentration of 500 ug/L. This chemical acts on the amphipods to prevent the metabolism of organophosphorus pesticides, thus removing the associated toxicity. There is evidence that the addition of PBO can increase the toxicity associated with pyrethroid pesticides. These TIE exposures were conducted in the same manner as the

initial test with regards to volume and number of animals added, but the duration was 10 days.

Pore Water

Pore water samples were extracted from whole sediment by centrifuging aliquots of homogenized sediment at 3000 X g for 30 minutes. The supernatant pore water was removed from the centrifuge bottle using a glass pipette. The pore water samples were tested using the mussel embryo development test as described above. In addition to the testing of pore water, a "mini-TIE" was performed by adding EDTA to an aliquot of pore water from each station. EDTA is a chelator of metals and was added to the sample to remove toxicity that might be associated with the presence of cationic metals. The concentration of EDTA used in each sample was 5 mg/L.

Data Analysis

Toxicity data within each experimental batch was compared to the control using a T-test, assuming unequal variance. Samples having $p \leq 0.05$ were considered to be significantly different from control. Samples that were significantly different were then compared to thresholds that have been established in our laboratory, based on historical data. For *Eohaustorius* tests, samples had to have control-adjusted survival of less than 82% to be considered toxic. For the mussel tests, samples had to have a control-adjusted %normal-alive of less than 77% to be toxic.

Chemistry Methodology

AVS/SEM

Acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) were measured on sediment samples collected during phase one. Analyses were performed at CRG Marine Laboratories. Extraction and measurement of AVS was performed using the methods of Plumb (1981). Quantification of the SEM was achieved using EPA 6020M.

Pore Water Metals

For the second phase samples, an aliquot of pore water was analyzed for dissolved metals. Samples were filtered at CRG Marine laboratories within 24 hr of pore water collection. Quantification of the metals was performed using EPA method 1640M.

Quality Assurance

Completeness

All of the 10 water and sediment samples collected in the first phase were successfully tested using the mussel embryo test for the water and SWI, and amphipod whole sediment methods. All samples collected in the second phase were also successfully tested using the methods that had been designated for the particular stations.

Test Acceptability Criteria

Test acceptability criteria were met for both batches of mussel embryo tests on marina water in phase one. Acceptable control survival was also achieved in the amphipod test. For the SWI test, the control percent normal-alive value of 72 was below the acceptability criteria of 80. This seems to be due to a systematic loss of embryos during

the recovery process from the screen tubes, as the percentage of normally developed embryos were within the expected range. Therefore, comparison of samples on a percent of control basis should be acceptable. Two of the reference toxicant tests associated with the mussel tests also experienced lower than acceptable control results.

The control acceptability criteria were met for the phase two pore water embryo test. The control survival criteria were also met for both the untreated whole sediment test (Table 6) and the TIE screening test conducted in phase two (Table 7). The control survival for the amphipod TIE (Table 8) was slightly below the EPA criteria of 90%, established for whole sediment tests. However, that criterion is for tests in 1 L jars with 20 animals added. There are no established criteria for the reduced volume and animal number used in the TIE testing procedure. A mean value of 88% should be sufficient to make comparisons between treatments.

Reference Toxicant Data

The effective concentration (EC50) value for the reference toxicant exposure with mussels that passed test acceptability criteria was within normal control chart parameters (within two standard deviations of the mean). The EC50 for the two mussel embryo reference toxicant tests that did not pass acceptability criteria were also within normal control chart parameters. This indicates that the embryos were not more or less sensitive than expected. The reference toxicant exposure with the amphipod was also within control chart parameters.

Water Quality Analysis

All samples tested were within normal ranges for the measured water quality parameters (pH, salinity, dissolved oxygen and ammonia) during the course of the exposures. Ammonia values for the SWI test were slightly elevated, but were an order of magnitude below the EC50 value for mussel embryos.

Results

For the phase one samples, none of the stations were found to be toxic with either the water or sediment-water interface tests using mussel embryos (Tables 1-3). Three samples from the SWI test had significantly reduced %normal-alive embryos, but the differences did not exceed the 77% of control threshold for toxicity established for this test method. Therefore, no TIEs were performed on these samples. For the whole sediment testing, eight of the ten stations were found to be toxic to the amphipods (Table 4).

For the phase two samples, none of the ten stations where pore water was tested with the mussel embryos was found to be toxic (Table 5), therefore the results of the EDTA addition are mute. Two of the stations (6013 and 6073) had reductions in %normal-alive that were significantly different from the control, but neither were below the toxicity threshold of 77%. All of the four stations tested for whole sediment toxicity using amphipod survival were found to be toxic (Table 6).

The results of the TIE found that the dilution of the sediment by 50% reduced toxicity by approximately half. A small reduction in toxicity occurred after treatment with the cation exchange resin in the 50% sample (Table 8). Since no reduction was observed in the 100% sample, the indication is that metals may be causing some of the toxicity and the amount of metals present in the 100% sample exceeded the resin's capacity to remove enough metals to reduce toxicity. The reduction in toxicity observed with the addition of coconut carbon indicates that organic chemicals are also playing a role in toxicity. The poor blank survival in the coconut carbon addition is an unexpected result that has not been previously experienced in our laboratory. The fact that this treatment greatly reduced toxicity in the field sediment makes the poor blank survival less of a concern. The increase in toxicity observed for the PBO addition may indicate a potentiation of toxicity from pyrethroid chemicals. While no chemistry measurements for pyrethroids were made as part of this study, other researchers have found significant concentrations of pyrethroids in the Newport Bay watershed (Budd *et al.* 2005).

The theory behind AVS/SEM analyses is that if the molar concentration of sulfide exceeds that of the SEM, then the metals are expected to be bound up as insoluble sulfide compounds that are not bioavailable. For all but three stations, the AVS was at a higher concentration than the SEM (Table 9). For the three stations (6063, 6064 and 6074) where the metals exceeded the sulfides, zinc was the most prevalent metal causing the exceedance. There does not appear to be any relationship between this result and toxicity, as these stations were only toxic to the amphipods, but to no greater extent than the remaining stations that were also found to be toxic (Table 4).

The pore water chemistry values did not show any stations to be very elevated for any constituent (Table 10). The two stations having the highest copper concentrations (6073 and 6082) had levels that are below the EC50 for mussel embryos (8.3 µg/L) as determined by our laboratory. This is consistent with the fact that no toxicity was observed in any of the mussel embryo samples. The laboratory seawater blank that was analyzed had a higher concentration of some of the constituents than did any of the samples. At this time we have not determined the cause of the high readings in the blank sample.

References Cited

Budd, R., S. Bondarenko and J. Gan. 2005. Survey for Synthetic Pyrethroids within the San Diego Creek/ Newport Bay Watershed. Southern California SETAC Annual Meeting. Los Angeles, CA.

Plumb, R.A.H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. US Army Corps of Engineers. Vicksburg, VA.

U.S. Environmental Protection Agency USEPA. 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, U.S. Environmental Protection Agency. Narragansett, RI.

U.S. Environmental Protection Agency USEPA. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Office of Research and Development. Cincinnati, OH.

Table 1. Marina water samples tested 8/23/06 in batch MG26 using mussel embryo development test. Asterisk indicates a significant difference from control p≤0.05.

	%Normal-Alive				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Seawater	84	100	4.7	4	
NB 6013W	82	97	8.1	4	
NB 6022W	79	94	8.7	4	
NB 6033W	84	100	4.6	4	
NB 6041W	80	95	9.9	4	

Table 2. Marina water samples tested 8/24/06 in batch MG29 using mussel embryo development test. Asterisk indicates a significant difference from control p≤0.05.

	%Normal-Alive				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Seawater	81	100	4.8	4	
NB 6051	79	98	7.0	4	
NB 6063	85	105	6.6	4	
NB 6064	84	104	8.7	4	
NB 6072	84	103	5.8	4	
NB 6074	84	104	2.1	4	
NB 6082	90	111	1.6	4	

Table 3. Marina sediment samples tested 9/07/06 in batch MG35 using Sediment Water Interface test with mussel embryo development. Asterisk indicates a significant difference from control p≤0.05.

	%Normal-Alive				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Seawater	72	100	7.5	5	
NB 6051	62	86	5.3	4	*
NB 6063	61	85	12.5	5	
NB 6074	61	84	7.5	4	*
NB 6082	58	80	12.5	5	*
NB 6072	67	93	14.0	5	
NB 6064	62	86	16.5	5	
NB 6013	66	92	7.5	5	
NB 6022	68	94	8.0	5	
NB 6033	71	99	7.7	5	
NB 6041	72	100	5.9	5	

Table 4. Marina whole sediment samples tested 9/12/06 in batch EE76 using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisk indicates a significant difference from control p≤0.05.

	%Survival				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Home Sediment	90	100	7.1	5	
NB 6051	44	49	5.5	5	*
NB 6063	60	67	10.0	5	*
NB 6074	60	67	18.7	5	*
NB 6082	58	64	21.7	5	*
NB 6072	84	93	16.7	5	
NB 6064	58	64	11.0	5	*
NB 6013	8	9	13.0	5	*
NB 6022	34	38	15.2	5	*
NB 6033	88	98	11.0	5	
NB 6041	58	64	17.9	5	*

Table 5. Marina pore water samples tested 11/20/06 in batch MG38 using mussel embryo development test. Asterisk indicates a significant difference from control p≤0.05.

	%Normal-Alive				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Seawater	93	100	7.7	4	
EDTA Blank 5 mg/L	90	96	0.7	4	
6011	85	91	7.1	4	
6013	72	77	5.4	4	*
6021	94	101	11.1	4	
6022	93	100	4.7	4	
6032	93	100	6.5	4	
6042	91	97	5.2	4	
6051	87	94	3.5	4	
6063	82	88	9.2	4	
6073	75	80	10.5	4	*
6082	87	94	10.4	4	
6011 EDTA 5 mg/L	95	102	7.0	4	
6013 EDTA 5 mg/L	30	33	24.1	4	*
6021 EDTA 5 mg/L	89	96	4.0	4	
6022 EDTA 5 mg/L	94	101	5.0	4	
6032 EDTA 5 mg/L	83	89	4.9	4	*
6042 EDTA 5 mg/L	95	102	12.0	4	
6051 EDTA 5 mg/L	83	89	7.0	4	*
6063 EDTA 5 mg/L	88	95	4.7	4	
6073 EDTA 5 mg/L	82	88	7.6	4	*
6082 EDTA 5 mg/L	89	96	7.2	4	

Table 6. Marina whole sediment samples tested11/27/06 in batch EE79 using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisk indicates a significant difference from control p≤0.05.

	%Survival				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Home Sediment	90	100	6.1	5	
6011	11	12	7.4	5	*
6012	29	32	8.2	5	*
6014	41	46	30.7	5	*
6021	11	12	8.9	5	*

Table 7. Marina initial whole sediment samples tested 11/20/06 in batch EE77, using the amphipod *Eohaustorius estuarius* with the exposure period reduced to 7 days to determine if sediment TIEs were justified. Asterisk indicates a significant difference from control p≤0.05.

	%Survival				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Home Sediment	90	100	14.1	4	
NB 6013	28	31	15.0	4	*
NB 6022	18	19	5.0	4	*

Table 8. . Marina whole sediment TIE tested 11/27/06 in batch EE78 using the amphipod *Eohaustorius estuarius* 10 day survival test. Asterisk indicates a significant difference from control p≤0.05.

	%Survival				
Sample	Mean	%of Control	Standard Deviation	Number Counted	Sig. Diff.
Home Sediment	88	100	11.0	5	
NB 6013S baseline 50%	26	30	13.4	5	*
NB 6013S baseline 100%	10	11	7.1	5	*
Cation Exchange Blank	97	110	5.8	3	
Cation Exchange 50% 6013	47	53	25.2	3	*
Cation Exchange 100% 6013	7	8	11.5	3	*
Coconut Carbon Blank	33	38	11.5	3	*
Coconut Carbon 100% 6013	77	87	11.0	3	
PBO Blank	97	110	5.8	3	
PBO 50% 6013	3	4	5.8	3	*
PBO 100% 6013	0	0	0.0	3	*

Table 9. Acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) from Newport Bay Marina sediment samples.

	NB6013		NB6022		NB6033		NB6041		NB6063	
	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg
Cadmium	ND	ND	ND	ND	ND	ND	ND	ND	0.0041	0.461
Copper	0.0325	2.07	ND	ND	0.192	12.2	ND	ND	0.0703	4.47
Lead	0.0253	5.24	ND	ND	0.0434	8.99	ND	ND	0.0679	14.1
Nickel	0.0379	2.23	0.05	2.94	0.0298	1.75	0.0426	2.50	0.0517	3.04
Zinc	1.01	66.3	1.77	116	1.65	108	1.76	115	2.57	168
Total SEM	1.11	75.8	1.80	119	1.90	131	1.80	118	2.76	190
AVS	5.00	160	9.56	306	6.88	220	7.19	230	1.92	61.6

Table 9. (continued)

	NB6064		NB6072		NB6074		NB6082		NB6051	
	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg	umoles/dry g	mg/kg
Cadmium	0.0021	0.236	ND	ND	0.0028	0.315	0.0028	0.315	0.005	0.562
Copper	0.0314	2.00	ND	ND	0.0157	1.00	0.0161	1.02	ND	ND
Lead	0.0335	6.94	0.0217	4.50	0.036	7.46	0.0254	5.26	0.0835	17.3
Nickel	0.0209	1.23	0.0489	2.87	0.0423	2.48	0.0397	2.33	0.0556	3.26
Zinc	0.652	42.6	3.17	207.3	2.36	154	1.83	120	3.66	239
Total SEM	0.740	53.0	3.24	214.6	2.46	165	1.91	129	3.80	260
AVS	0.516	16.5	18.9	606	0.741	23.7	1.92	61.5	7.91	253

ND = Not Detected

Table 10. Pore water dissolved metals from Newport Bay marina sediment samples. All values are expressed in µg/L.

MDL	RL		6011	6013	6021	6022	6032	6042	6051	6063	6073	6082	Lab Blank
3	6	Aluminum (Al)	11	12	12	9	11	14	11	11	11	14	ND
0.01	0.015	Arsenic (As)	4.33	6.71	4.47	2.57	2.02	2.38	2.98	1.30	2.59	2.49	3.32
0.005	0.01	Beryllium (Be)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.261
0.025	0.05	Chromium (Cr)	0.38	0.44	0.40	0.44	0.40	0.38	0.41	0.37	0.51	0.39	3.19
0.005	0.01	Cobalt (Co)	0.46	0.438	0.424	0.457	0.392	0.341	0.343	0.369	0.336	0.356	0.263
0.01	0.02	Manganese (Mn)	505.5	332.5	198.3	382.3	115.6	85.83	127.2	87.46	51.4	118.5	0.580
0.02	0.04	Silver (Ag)	0.624	0.641	0.674	0.639	0.609	0.596	0.569	0.555	0.511	0.478	0.590
0.005	0.01	Thallium (Tl)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
0.035	0.07	Titanium (Ti)	0.529	0.977	0.739	0.674	0.498	0.455	0.540	0.408	1.047	0.327	2.949
0.02	0.04	Vanadium (V)	1.03	1.51	1.27	0.50	0.34	0.39	0.93	0.24	3.04	0.4	3.61
0.005	0.01	Zinc (Zn)	3.149	3.784	4.135	3.710	3.256	3.605	3.059	2.926	3.760	3.173	8.835
0.005	0.01	Cadmium (Cd)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.135
0.01	0.02	Copper (Cu)	1.48	1.84	1.86	1.95	1.60	1.60	1.52	1.44	4.56	6.20	3.16
0.005	0.01	Lead (Pb)	0.03	0.037	0.037	0.011	0.013	0.057	0.045	0.01	0.028	0.012	ND
0.005	0.01	Nickel (Ni)	1.185	1.26	1.207	0.979	0.837	1.054	0.957	0.981	0.673	0.925	ND
0.01	0.015	Selenium (Se)	1.22	1.48	1.32	1.38	1.28	1.15	1.12	1.29	1.74	1.13	5.87
0.005	0.01	Tin (Sn)	0.025	0.026	0.033	0.033	0.027	0.021	0.032	0.026	0.14	0.14	0.051

ND = Not Detected