Newport Coast ASBS
Kelp Toxicity Study

Prepared For:

Public Works Department
City of Newport Beach
3300 Newport Boulevard
Newport Beach, CA 92663

Prepared By:

Weston Solutions, Inc.
2433 Impala Drive
Carlsbad, California 92010

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Introduction

In 2006, the cities of Newport Beach and Laguna Beach initiated a program designed to assess the impacts of urban runoff, harbor contamination, stormwater runoff, and public trampling and scavenging activities on rocky intertidal communities living within the Newport Coast Area of Special Biological Significance (ASBS) and the Heisler Park ASBS. As part of this program, a germination and growth bioassay for the giant kelp (*Macrocystis pyrifera*) was conducted to confirm the results of a previous study in which toxicity to giant kelp had been observed in exposures to Buck Gully effluent. In addition to conducting the kelp bioassay test under the standard protocol (EPA/600/R-95/136) (USEPA, 1995), a modified version of the test was also conducted in an effort to determine if any observed toxicity was due to the presence of dissolved chemical contaminants in the water or the result of the physical debris or large particulates, to which contaminants may be bound.

Kelp forests provide vital habitat to a variety of marine animals. (Photo: Robert Schwemmer). http://sanctuaries.noaa.gov/sos2006/channelislands.html

Key Issues

M. pyrifera is the dominant canopy-forming Laminarian alga in Southern and central California and forms extensive subtidal forests along the coast. Because kelp forests support a rich diversity of marine life, providing food and habitat for hundreds of marine invertebrate and vertebrate species, their health is critical to maintaining the diversity of species currently residing the Newport Coast ASBS. In February 2006, effluent from Buck Gully was evaluated for acute and chronic toxicity using three standardized marine toxicity tests with *M. pyrifera, Mysidopsis bahia* (mysid shrimp), and *Strongylocentrotus purpuratus* (purple urchin). While no toxicity was observed in shrimp or urchin exposures to water collected from the Buck Gully mixing zone, significant reduction in kelp spore germination was observed (WESTON, 2006).

Study Design

Grab samples of water were collected on April 20, 2007, from the base of Buck Gulley (BG-Fresh), the nearshore mixing zone (BG-Mix), and the outer edge of the mixing zone (BG-Edge) (Figure 1). Each of the samples was placed on ice in coolers and transported to Weston Solutions, Inc. (WESTON®) in Carlsbad, CA where they were stored at 4°C until the start of the giant kelp germination and growth test.

Figure 1. Buck Gully Mouth and Mixing Zone

The giant kelp (*M. pyrifera*) germination and growth test was conducted in accordance with EPA/600/R-95/136 (USEPA, 1995). Zoospores from *M. pyrifera* were exposed for 48 hours to different concentrations of Buck Gully effluent (0%, 6.25%, 12.5%, 25%, 50%, and either 60%, 90%, or 100% sample water, depending upon the sample) in a static test (Table 1 and Table 2). A reference toxicant test using copper chloride was simultaneously conducted with concentrations of 0 µg/L, 5.6 µg/L, 10 µg/L, 18 µg/L, 32 µg/L, 100 µg/L, and 180 µg/L dissolved copper. For those samples in which salinity was below the protocol limit of 34 ppt (BG-Fresh and BG-Mix), a hypersaline brine was added to increase the salinity of the test to the appropriate level (34 ppt)[1]. Because it was necessary to add brine to these samples, the highest concentrations of freshwater (BG-Fresh) and mixing zone (BG-Mix) test waters that could be achieved were 60% and 90%, respectively. Brine controls were included in the test procedure to ensure that any observed effects of toxicity were properly attributed to either the effluent or the brine.

A modified version of the giant kelp germination and growth test was run concurrently with the standard test due to the extent of physical debris associated with the stormwater samples and its potential to interfere with test performance. In the modified test, particulates were allowed to settle for 12 hours. The supernatant, with fine particulates still in suspension, was then siphoned off and tested according to standard procedures.

[1] Brine was prepared by freezing natural seawater, then collecting the hypersaline liquid as the frozen seawater thawed.

Results

Test water from BG-Fresh, BG-Mix, and BG-Edge did not produce an effect on the germination of kelp at 48 hours in either the modified or standard kelp test (Table 1). The no observed-effect concentration (NOEC) was equal to the highest concentration tested for each of the test samples. Because the addition of brine limited the maximum sample concentration that could be tested (i.e., 60% for BG-Fresh and 90% for BG-Mix), the Toxic Units calculated for these samples were greater than 1.0 despite no observed effects on germination as the toxic endpoint. The copper chloride reference toxicant test indicated that the sensitivity of *M. pyrifera* used in the assessment of the test samples fell within the normal range.

Table 1. Summary of Results – Kelp Germination

Sample	Test Type	NOEC	LOEC	EC_{50}	Toxic Units	Toxicity Observed?
BG-Fresh	Modified	60%	>60%	>60%	1.67	No
	Standard	60%	>60%	>60%	1.67	No
BG-Mix	Modified	90%	>90%	>90%	1.11	No
	Standard	90%	>90%	>90%	1.11	No
BG-Edge	Modified	100%	>100%	>100%	1	No
	Standard	100%	>100%	>100%	1	No
Copper Chloride	Reference toxicant	100 ppb	180 ppb	157 ppb	N/A	N/A

Test substance BG-Fresh produced an effect on the growth of kelp at 48 hours in the modified test. The modified test NOEC for BG-Fresh was 25%, whereas the EC_{50} was greater than 60% test substance. In the standard test, the NOEC for BG-Fresh was 12.5%, whereas the EC_{50} was greater than 60% test substance (Table 2). Toxicity, expressed as Toxic Units Chronic, was calculated to be 4 in the modified growth test and 8 in the standard test. BG-Mix and BG-Edge did not produce toxic effects in the modified test. In the standard test, exposures to BG-Fresh, BG-Mix, and BG-Edge produced toxic effects on kelp growth.

The copper chloride reference toxicant test indicated that the sensitivity of *M. pyrifera* used in the assessment of the test samples fell within the normal range.

Table 2. Summary of Results – Kelp Growth

Sample	Test Type	NOEC	LOEC	EC_{50}	Toxic Units	Toxicity Observed?
BG-Fresh	Modified	25%	50%	>60%	4	Yes
	Standard	12.5%	25%	>60%	8	Yes
BG-Mix	Modified	90%	>90%	>90%	1.11	No
	Standard	6.25%	12.5%	>90%	16	Yes
BG-Edge	Modified	100%	>100%	>100%	1	No
	Standard	50%	>100%	>100%	2	Yes
Copper Chloride	Reference toxicant	5.6 ppb	10 ppb	95.4 ppb	N/A	N/A

Summary of Findings for Kelp Toxicity Study

Kelp germination and growth bioassays were conducted using unaltered water and water that had been allowed to settle prior to testing from three locations (the Buck Gully discharge point, the nearshore intertidal mixing zone, and the outer edge of the mixing zone). A summary of the findings of the kelp toxicity study are listed below:

- Exposure to freshwater collected from Buck Gully, from the near-shore intertidal mixing zone, and from the outer edge of the mixing zone did not produce toxic effects on kelp germination in either the modified test (large particulates removed) or the standard test.

- Growth of kelp germ tubes was inhibited in exposure to freshwater from Buck Gully in both the standard and modified test procedures. The inhibition of kelp growth was also observed in exposures to mixing zone and outer mixing zone water in the standard test in which no particulates were removed.

The observed effects on germ tube growth may be due to contaminants in the water column that are associated with fine particulates or may be due to contaminants that are freely dissolved. Because a significant reduction in germ tube growth was observed in the brine control relative to the control, the addition of brine may have contributed to observed toxicity of the BG-Fresh and BG-Mix test samples. It should be mentioned that the giant kelp growth and germination test was not intended for testing freshwater test samples, but was instead designed to test marine and brackish water, where only a slight addition of brine would be required to bring the sample water into the salinity range of the test protocol.

The reduction in toxicity in the modified tests may be the result of removing the large physical debris that prevented the kelp spores from adhering to the surface of the petri dish used in testing, or may have inhibited light from reaching the spores. Alternatively, the reduction in toxicity in the modified test may have been associated with the removal of the large particulates and any associated contaminants.

References

USEPA. 1995. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*. EPA/600/R-95/136.

WESTON (Weston Solutions, Inc.). 2006. *Quality Assurance Project Plan (QAPP).* Newport Coast Flow and Water Quality Assessment, Addendum 2. Public use, Biological Surveys, Bioaccumulation and Restoration Monitoring. For Newport Coast and Laguna Beach, ASBS Protection and Restoration Program, Grant Agreement No. 05-230-550-0. November.