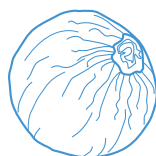

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Use of a Rapid Bioluminescent Test (QwikLite) Using Dinoflagellates to Assess Potential Toxicity of Sediment Pore Waters

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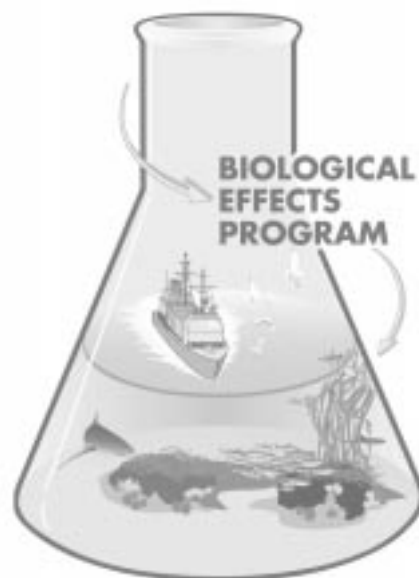
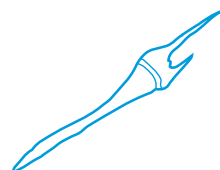
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Abstract

The Navy has developed a rapid bioassay system (QwikLite) that is proving to be a valuable asset for conducting bioassays on other test media (e.g., metals, storm drain discharge, ship hull coatings, and marine sediments). The basis of detection is to measure a reduction in light from the bioluminescent dinoflagellate *Gonyaulax polyedra* following exposure to a toxicant. The toxic response is usually measured within 24 hours from the start of the test and can be conducted for a 4-day acute test or 7-day chronic test. A measurable reduction or inhibition in bioluminescence is an adverse effect. The endpoint used to measure this light reduction is the IC50 (a 50% reduction in light output when compared to control cells).

A series of bioassays were conducted to measure the acute effects of pore water on the light output from the marine bioluminescent dinoflagellate *Gonyaulax polyedra*. Sediment samples were collected from San Diego Bay and San Francisco Bay, and extraction of pore water by centrifugation was analyzed for toxicity. Twenty-four-hour QwikLite bioassays of pore water samples from Treasure Island (San Francisco) showed moderate levels of toxicity. IC50s (inhibition concentration of percent of pore waters required to inhibit bioluminescence by 50%) ranged from 26% to 80%, and total ammonia in the pore waters ranged from 1.7 to 7.1 ppm. Thirty-six percent of the pore water samples showed no toxicity, with total ammonia values less than 1.6 ppm. Similar results were true for pore water collected at Naval Submarine Station (San Diego, CA), however, ammonia levels were as high as 12 ppm and IC50s ranged from 6% to 97% pore water concentrations. Thirty percent of the pore waters showed no toxicity. QwikLite tests are similar in sensitivity as the sea urchin larval development test (48 hr) and the Mysid

shrimp acute survival test (96 hr). High ammonia levels appear to be toxic to the bioluminescent dinoflagellate *Gonyaulax polyedra* and is a confounding influence with respect to identifying toxicity associated from anthropogenic sources which needs to be further addressed.

Introduction

Many species of dinoflagellates produce bioluminescence as part of their daily physiological process. A bioassay has been developed in our laboratory that makes use of this ability to produce light for assessment of toxic effects when the dinoflagellates are exposed to many chemicals, either individually or in compounds, effluents, and antifoulant coatings.

Successful assays using the QwikLite bioassay system have provided data on acute responses as well as chronic effects (3 hours to 11 days) on the bioluminescent dinoflagellate *Gonyaulax polyedra*.

Instrumentation

The QwikLite bioassay system (figure 1) consists of a horizontally-mounted 2-inch-diameter 8575 photomultiplier tube (PMT) attached to the QwikLite test chamber that is connected to the controller box via a combined power and signal cable. The top of the test chamber



Figure 1. QwikLite bioassay system. On the left, the PMT is bolted to the test chamber. The controller box, on the right, provides power to the PMT, stir motor, and displays PMT counts.

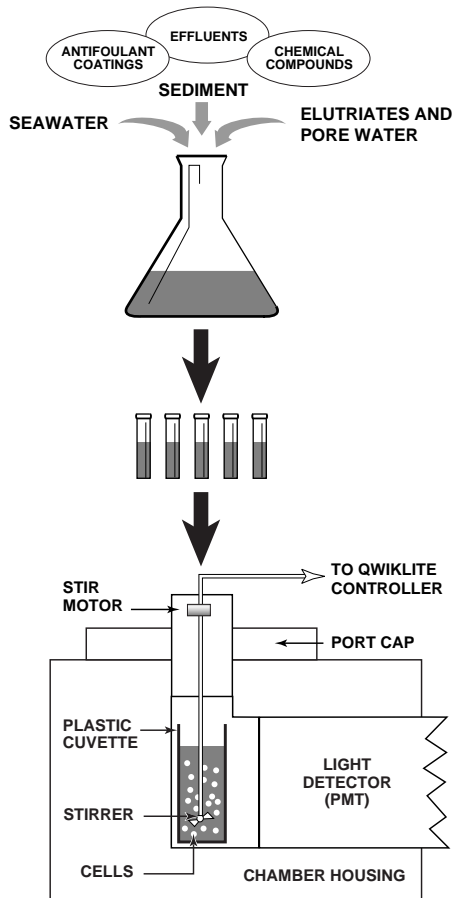


Figure 2. Simplified schematic of the QwikLite test chamber showing a cuvette containing bioluminescent dinoflagellates seated in front of the light detector.

is removable and houses a small adjustable speed motor that drives a plastic stirrer (figure 2). The controller box displays stirring motor and PMT voltages, PMT counts, time countdown, and cycle status. It has manual and automatic switches for system operation, time settings, start, stop, and reset buttons. Neutral density optical filters (ND-1, ND-2, ND-3) can be easily changed to prevent PMT saturation.

The QwikLite bioassay system was used in the automatic mode for light output testing. In this setting, the high voltage and the stir motor were automatically engaged after the start button was pushed. Stirring created shear forces within the cuvette, stimulating the dinoflagellates to emit light that was detected by the photomultiplier tube (PMT). During stirring, PMT counts were accumulated and displayed on a timer-counter.

Testing typically took less than 1 hour to run

five replicates for each of five pore water concentrations and a control.

Bioassay Organisms

Dinoflagellates were maintained in sterile enriched seawater medium (ESM) under 40-

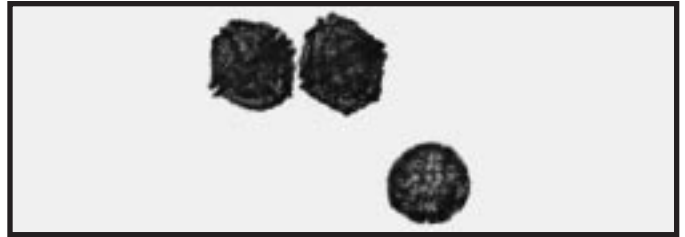


Figure 3. Photomicrograph of the bioluminescent dinoflagellate *Gonyaulax polyedra*. Cell size = 60 μm .

watt cool-white fluorescent bulbs on a 12:12h (light:dark) cycle at 19-20° C. Cells were cultured in ~600 mL ESM in borosilicate Erlenmeyer flasks at 2000-3000 cells/mL. Bioluminescent dinoflagellates (figure 3) are most stimutable and produce maximum light during their dark phase, so the day:night cycle was reversed to accommodate daytime testing.

Bioluminescence inhibition is strongly correlated to inhibition of phototaxis behavior and chlorophyll fluorescence. The impact of toxicity on the mobility of dinoflagellates is serious for an organism restricted to the euphotic zone. Inhibition of all three functions suggests signifi-

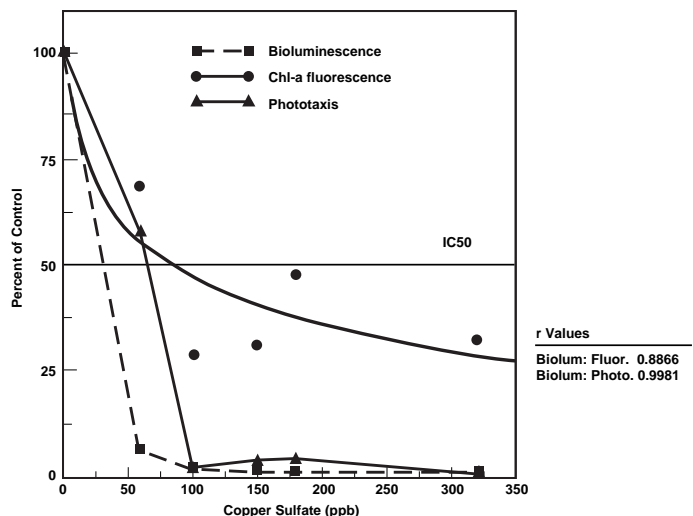


Figure 4. Effect of copper sulfate on bioluminescence, chl-a fluorescence, and phototaxis.

cant deleterious changes in the physiology of the dinoflagellates. Data indicate that copper sulfate inhibited bioluminescence, chlorophyll fluorescence, and phototaxis in *G. polyedra* at comparable concentrations within 48 hours of exposure. IC50s of 78 ppb, 70 ppb, and 65 ppb copper sulfate were measured in chlorophyll fluorescence, bioluminescence, and phototaxis, respectively (figure 4).

Methods

In previous work, *G. polyedra* (figure 3) has been observed to be sensitive to numerous metals (table 1, figure 5) and to the reference organic toxicant sodium dodecyl sulfate (SDS)

Table 1. Results for several bioassays conducted using the QwikLite bioassay system. Note that bioluminescent dinoflagellates were used for both acute and chronic exposures.

TOXICANT	Duration	IC ₅₀	Mysid* LC ₅₀
Tributyltin	196 hrs.	1.6 ppb	0.5 ppb†
Silver	96 hrs.	13 ppb	249 ppb
Copper Sulfate	96 hrs.	23 ppb	120-140 ppb
Dibutyltin	96 hrs.	34 ppb	—
Lead	96 hrs.	321 ppb	3130 ppb
Zinc	96 hrs.	430 ppb	499 ppb
Chromium	96 hrs.	538 ppb	2030 ppb
Cadmium	96 hrs.	782 ppb	110 ppb

* = Kuhn-Hines et al., 1995 † = Davidson et al., 1986

Table 2. Comparisons of response to reference toxicant sodium dodecyl sulfate (SDS).

SPECIES	ENDPOINT	IC ₅₀ /LC ₅₀ (mg/l)
<i>Gonyaulax polyedra</i> (Dinoflagellate - QwikLite)	Bioluminescence	1.4
<i>Menidia beryllina</i> (Silverside minnow)	Larval Survival	1.8
<i>Cyprinodon variegatus</i> (Sheepshead minnow)	Larval Survival	2.9
<i>Arabacia punctulata</i> (Sea Urchin)	Fertilization	3.2
<i>Mysidopsis bahia</i> (Mysid shrimp)	Survival	9.3

(From Johnston, R.J., 1996)

(table

2). *G. polyedra* appears to be more sensitive to some of these metals when compared to the 96-hour mysid shrimp (*Mysidopsis bahia*) static renewal toxicity test (Table 1). *G. polyedra* is also more sensitive to SDS than silverside minnows (*Menidia beryllina*), sheepshead minnows (*Cyprinodon variegatus*), the sea urchin (*Arabacia punctulata*), and the mysid shrimp (*Mysidopsis bahia*) (table 2).

Recent sediment toxicity studies (QwikSed) were conducted to assess the marine sediment collected at San Diego Bay, CA and San Francisco Bay, CA. Sediment samples were collected at Naval Submarine Base (San Diego Bay) and at Naval Air Station Alameda (San Francisco Bay) for analysis and prepared as sediment leachates by mixing sediment with filtered seawater in a 1:4 ratio for 1.5 hours (EPA, 1991). QwikSed testing was also performed on sediment collected from Treasure Island (San Francisco Bay) with pore waters, which were prepared by centrifugation. The salinity of the leachates and pore waters were checked and adjusted with sea salts to a standard salinity of 33‰ prior to testing. Leachates and pore waters were diluted to 6.25% to find the IC50 of each sample. Total ammonia in each sample was measured with the HACH Spectrophotometer or an Orion Ammonia Electrode. Total Polycyclic Aromatic Hydrocarbons (PAHs) in sediment leachates were also measured by UV-Fluorescence Spectroscopy.

Solutions of pore waters or leachates were prepared with an enriched seawater medium (ESM) and dinoflagellates at a concentration of approximately 200 cells/mL. Three mL aliquots from each test concentration (ESM volume, pore water volume, dinoflagellate cell stock volume) were dispensed into five repli-

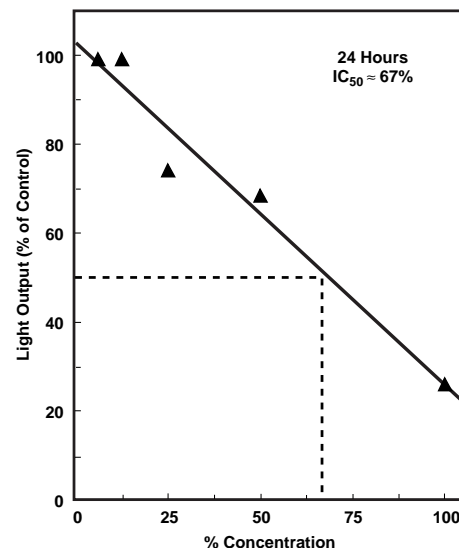


Figure 5. Representative IC₅₀ plot showing toxicity of DBT to *G. polyedra* after 24 hours. The DBT was found in a leachate solution from an experimental coating.

brates for each test concentration and controls. Cells were cultured directly in disposable optical-grade spectrophotometric plastic cuvettes at 19°C under a light intensity of 4000 lux. Bioluminescence measurements were conducted in consecutive 24-hour increments following test setup. For some of the earlier metals toxicity work, testing was conducted throughout a 96-hr period. Sediment leachate and pore water toxicity testing was conducted with only 24 hours of exposure to the cells.

At the conclusion of each bioassay, mean light outputs were calculated and graphed as bioluminescence (percent of control) versus concentration of a particular metal or pore water. The IC50 can be calculated using statistics software or graphically plotting concentration of a test material (pore water, metals, etc.) against bioluminescence (figure 5).

Results

Use of QwikLite for Sediment Testing (QwikSed) at San Diego Bay and San Francisco Bay

Results from QwikSed tests at San Diego Bay (Naval Submarine Station) and at San Francisco Bay (Naval Air Station Alameda and Treasure Island) showed effects of total ammonia and/or polycyclic aromatic hydrocar-

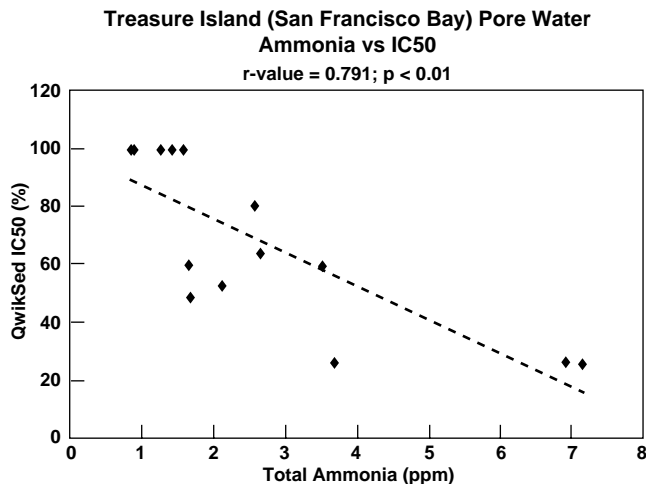


Figure 6. Relationship of total ammonia and toxicity in QwikSed.

bons (PAHs) on assessing sediment toxicity. At Treasure Island in San Francisco Bay, IC50s correlated with total ammonia measurements in pore waters (figure 6). Much of the demonstrated toxicity at the 14 sampled sites

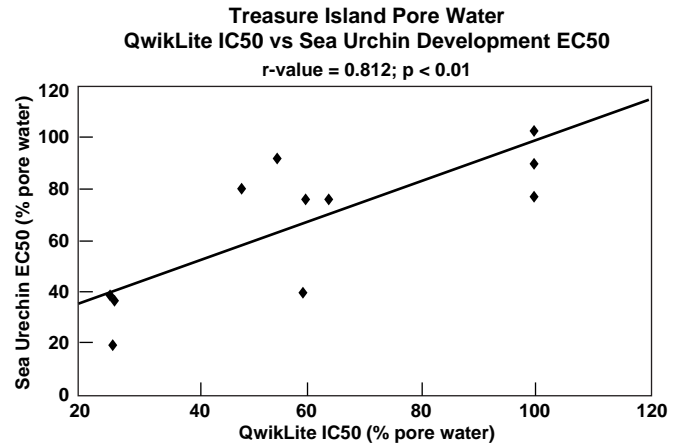


Figure 7. Relationship of QwikSed IC50 vs. sea urchin embryo development EC50.

is believed to be due to ambient levels of ammonia present in the pore waters. QwikSed IC50s were only observed in pore waters exceeding 1.6-ppm total ammonia. Independent toxicity tests conducted at the same sites with the sea urchin embryo development test (*Arabacia*) showed high correlation with the QwikSed IC50s (figure 7) demonstrating that both toxicity tests are sensitive to ammonia.

Another factor that needs to be addressed in sediment toxicity is the role of total PAHs on QwikSed tests. In the sediment leachates

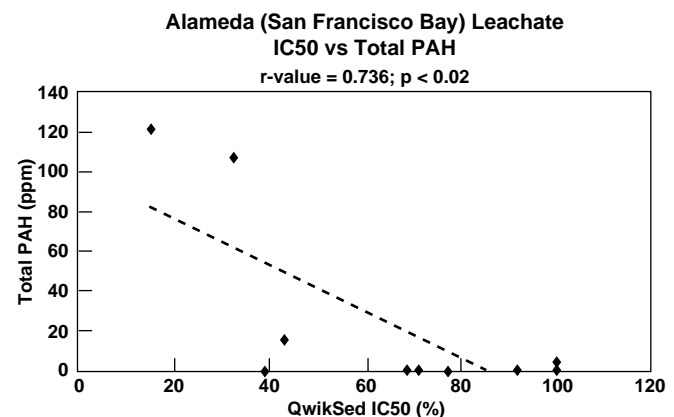


Figure 8. Relationship of QwikSed IC50 vs total PAH.

tested from Alameda in San Francisco Bay, QwikSed IC50s correlated with PAHs (figure 8). Low measurements of total PAHs reflected low levels of toxicity. Toxicity (IC50s 15-30%) was detected at sites with high measurements of total PAH (100-135 ppm).

The influence of total ammonia and total PAH on sediment toxicity is also evident in the study at San Diego Naval Submarine Station (figure 9). The majority of the samples showed low levels of ammonia with variable amounts

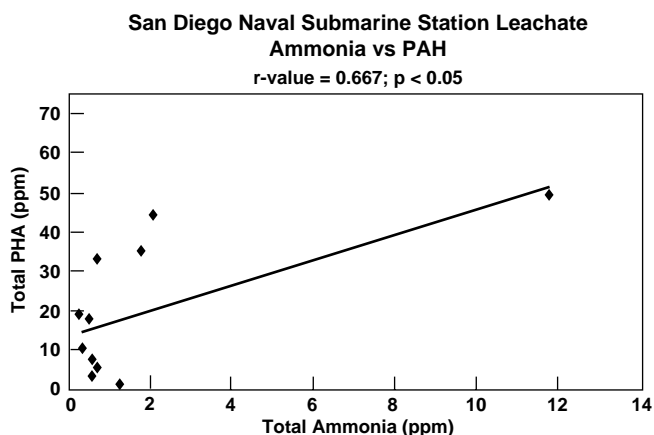


Figure 9. Relationship of total ammonia and total PAH in sediment leachate.

of PAH. However, ammonia and PAH levels were influential to QwikSed tests at 2 sites; ammonia levels (2-12 ppm) and PAH levels (45-50 ppm) corresponded with IC50s (6-10%). This toxicity is believed to be a result of both ammonia and PAH contamination. These observations are critical in understanding their role of confounding influences on sediment toxicity. It is important to determine if sediment toxicity is a result of one or both of these influences.

Conclusions

- QwikLite bioassays can evaluate both acute and sublethal chronic effects from exposure to a variety of toxicants.
- QwikLite and QwikSed have a quick and easy initial setup and testing can be accomplished in less than 1 hour per day.
- QwikLite and QwikSed toxicity tests are as sensitive or more sensitive than mysid shrimp, minnows, chain diatoms, and sea urchins.
- QwikLite toxicity tests can monitor both acute and chronic effects from exposure to a variety of toxicants.
- More work is currently being planned to investigate “false positives” from contaminated marine sediments. Ammonia has demonstrated confounding influence on conventional and QwikSed toxicity test results

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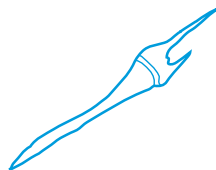
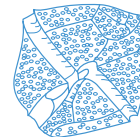
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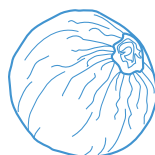
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This subject matter is related to one or more patent applications assigned to the U.S. Government, including U.S. Patent number 5,565,360 (NC 76613)



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