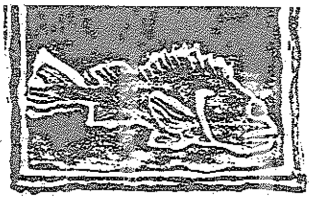
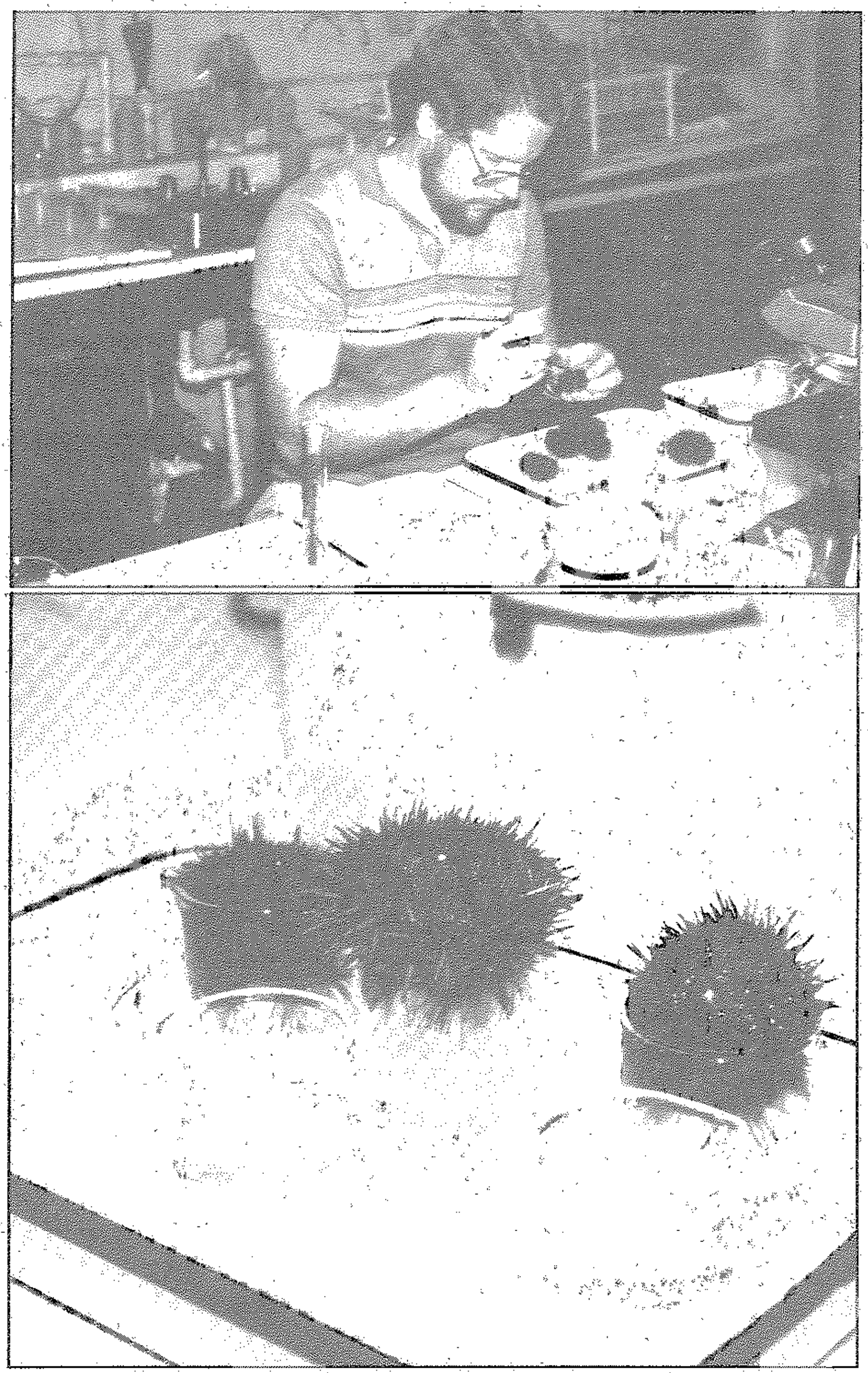


# Wastewater Toxicity Studies

SCCWRP has used sensitive marine tests to measure the toxicity of wastewater for many years. Recent findings from this research program indicated large reductions in wastewater toxicity since 1982, documented the relative sensitivity of various methods for measuring toxicity, and identified effluent from the Los Angeles County Joint Water Pollution Control Plant (JWPCP) as more toxic than other wastewater effluents in southern California (SCCWRP 1989). Similar activities conducted during 1989-90 were designed to: 1) expand the SCCWRP historical database on the effects of effluent on sea urchin gametes and embryos, 2) compare the sensitivity of different test species and endpoints, and 3) examine the variability of the sea urchin sperm test.

We participated in interlaboratory tests sponsored by local wastewater discharge agencies (City of Los Angeles, County Sanitation Districts of Los Angeles County, and County Sanitation Districts of Orange County) and the State Water Resources Control Board Marine Bioassay Project. Sewage effluent samples were sent to the different laboratories for concurrent testing with a variety of species. Toxicity tests with sea urchins and bacteria (Microtox™) were conducted by SCCWRP; tests with red abalone and giant kelp were conducted by the other laboratories.



**(Above) Biologist Steve Bay conducts bioassay test. (Below) Sperm test using the purple sea urchin <u>Strongylocentrotus purpuratus</u>.**

## Materials and Methods

Single effluent samples from the City of Los Angeles Hyperion Treatment Plant (Hyperion) and the County Sanitation Districts of Orange County (CSDOC) were examined for toxicity in August 1989 and March 1990, respectively. Three effluent samples from the County Sanitation Districts of Los Angeles County Joint Water Pollution Control Plant (JWPCP) were tested in May and June 1990. All effluent samples were 24-hr composites of final effluent.

The sample of CSDOC effluent was treated in a manner similar to samples tested in 1987-88. Samples of Hyperion and JWPCP effluent were treated differently from the past however. The Hyperion sample was stored at ambient temperatures and aerated during the 24-hr collection period; previous samples were stored refrigerated and not aerated. The JWPCP effluent samples were dechlorinated by the addition of sodium sulfite prior to toxicity testing; effluent samples tested in previous years were not dechlorinated.

Each effluent sample was tested with the sea urchin sperm test (Dinnel *et al.* 1987) so results could be compared to previous SCCWRP data. The test uses gametes from the purple sea urchin, *Strongylocentrotus purpuratus*. Sperm were exposed to diluted effluent for one hour and then tested for their ability to fertilize eggs.

Selected effluent samples were tested with sea urchin embryo and bacteria toxicity tests at SCCWRP

**Table 1.**
**Test results for sea urchin sperm (% fertilized), sea urchin embryo (% normal and echinochrome), and Microtox™ bacteria (luminescence) exposed to sewage effluent samples collected in 1989 and 1990. JWPCP=Joint Water Pollution Control Plant (County Sanitation Districts of Los Angeles County); CSDOC=County Sanitation Districts of Orange County; Hyperion=Hyperion Treatment Plant (City of Los Angeles). Test responses are expressed as percent of control response. Data with asterisks (*) are significantly different from the control (Dunnett's test, $p \leq 0.05$).**

| Effluent | % Fertilized | | | % Normal 48-hour | Echinochrome Absorbance[a] | Luminescence |
|---|---|---|---|---|---|---|
| JWPCP | | | | | | |
| 0.56 | | 99 | 94 | | | |
| 1.0 | 100 | 95 | 94 | 96 | 84 | |
| 1.8 | 100 | 94 | 83* | 100 | 99 | |
| 3.2 | 81* | 85* | 56* | 98 | 92 | |
| 5.6 | | | | 26* | 20* | |
| 8.0 | | | | 0* | 46* | |
| Hyperion | | | | | | |
| 0.10 | | 105 | | | | |
| 0.18 | | 113 | | | | 94* |
| 0.32 | | 101 | | | | |
| 0.56 | | 113 | | | | 94 |
| 1.0 | | 99 | | | | |
| 1.8 | | 82 | | | | 84* |
| 3.2 | | 65* | | | | |
| 5.6 | | | | | | 89* |
| CSDOC | | | | | | |
| 0.10 | | 96 | | | | 99 |
| 0.32 | | 100 | | | | 100 |
| 0.56 | | 97 | | | | 99 |
| 0.75 | | 98 | | | | |
| 1.0 | | 97 | | 89* | 95 | 96* |
| 3.2 | | 90* | | 82* | 92 | 90* |
| 8.0 | | 1* | | 1* | 42* | 74* |

[a]Absorbance was measured at 495 nm

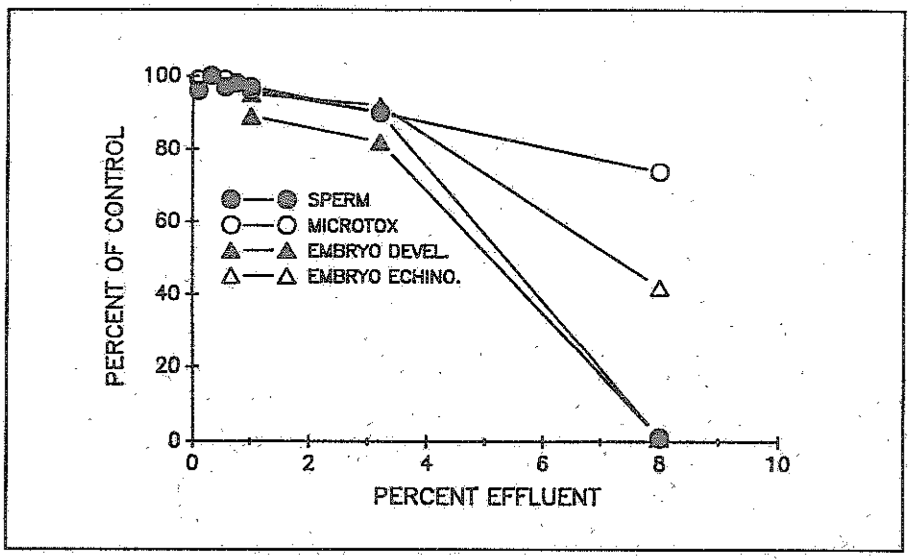
to provide information on the relative sensitivity of the methods. Developing purple sea urchin embryos were exposed for 48 hr to the CSDOC sample and one of the JWPCP samples. The embryos were then examined for developmental malformations and alterations in echinochrome pigment production (Bay *et al.* 1983). Toxicity of the Hyperion and CSDOC effluent samples was also measured using the Microtox™ test, which measures the effects of toxicants on the bioluminescent marine bacterium, *Photobacteria phosphoreum* (Beckman Instruments Inc. 1982). Bacteria were exposed to the effluent samples for 30 min.

Tests with each of these species were conducted at 15°C and a salinity of 34 ppt. Effluent samples were diluted with filtered natural seawater from Redondo Beach to produce a series of four to seven concentrations that bracketed the estimated no observable effect concentration (NOEC), the highest effluent concentration with no statistically significant evidence of toxicity. In most cases, the dilution series also bracketed the calculated maximum effluent concentration at initial dilution (0.6-1.2%) in the ocean for the treatment plants. A reference toxicant exposure (copper chloride dissolved in natural seawater) was included with most sea urchin tests to provide information on organism sensitivity between tests.

The toxicity of Hyperion, CSDOC, and JWPCP effluents was also measured in sublethal tests with red abalone (*Haliotis rufescens*) and giant kelp (*Macrocystis pyrifera*). Tests were conducted at the Marine Pollution Studies Laboratory in Monterey (California Department of Fish and Game) as part of the State Water Resources Control Board's Marine Bioassay Project. The red abalone test is a 48-hr embryo development test and the giant kelp tests are a 48-hr zoospore germination and a 48-hr gametophyte growth test. The methods were developed by the State of California for monitoring effluents for compliance with toxicity limits (Hunt *et al.* 1989). The kelp and abalone tests were usually conducted on the same day as the SCCWRP tests and used natural seawater from Monterey for the effluent dilutions.

Statistical significance ($p \leq 0.05$) of test responses relative to the control was determined with analysis of variance and Dunnett's multiple comparison test. Point estimates of effects were calculated by probit analysis (EC50) and bootstrap (IC25) techniques. Test responses are expressed as percent of the control to allow comparisons between different tests and endpoints.

**Figure 1.**
**Toxicity of a County Sanitation Districts of Orange County effluent sample collected in March 1989 to sea urchin sperm, sea urchin embryos, and Microtox™ bacteria.**




## Results and Discussion
### SCCWRP Toxicity Tests

The toxicity of Hyperion, CSDOC, and JWPCP effluents to sea urchin sperm were similar; each had a lowest observable effect concentration (LOEC) of 3.2% (Table 1). Responses of the sea urchin embryo and Microtox™ tests to CSDOC effluent were similar to the sperm test (Figure 1). All of the tests were strongly affected by 8.0% effluent. Sea urchin embryo development was more strongly affected at lower effluent concentrations than the other tests.

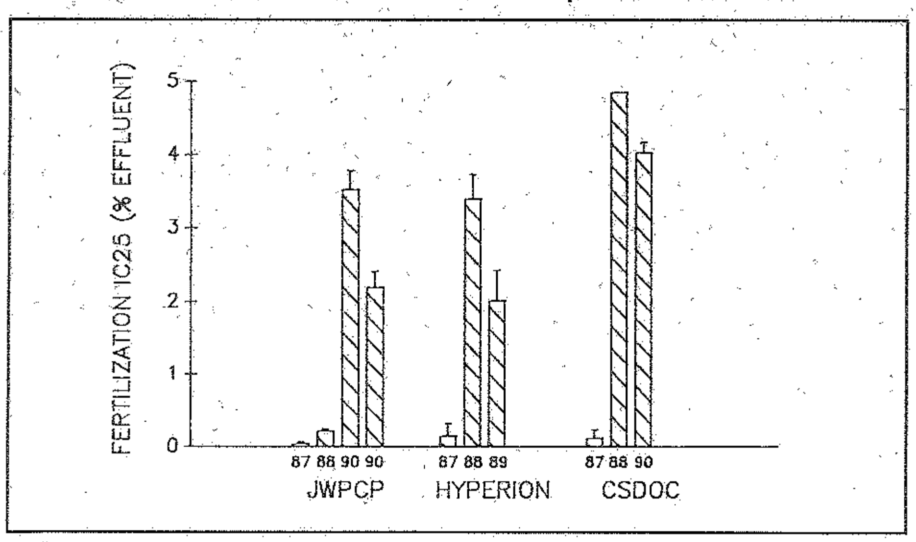
The lowest NOEC was 0.56% in the Microtox™ tests of the Hyperion and CSDOC samples. The relative effect of effluents on Microtox™ bacteria was not greater than the effect on urchin sperm however. The difference in NOEC values between the two methods reflects the lower variability, and thus greater precision, of the Microtox™ method.

The results of three replicate toxicity tests of JWPCP effluent to sea urchin sperm were consistent. Fertilization NOECs ranged from 1.0 to 1.8% and EC50s ranged from 3.5 to 4.5%.

The NOEC is the toxicity parameter of greatest regulatory significance. The State of California's Ocean Plan requires that NOECs for sewage effluents (measured using approved test methods) be equal to or greater than the effluent concentration after initial dilution. The sea urchin sperm test is the only one of these methods that is approved by the State of California for use in compliance monitoring. The NOEC results (1.0-1.8%) indicate that all of the effluent samples were in compliance with the Ocean Plan toxicity limits.

Results of current and previous sperm test data were compared by calculating the concentration that produced 25% inhibition of fertilization (IC25), a technique recommended by EPA (Norberg-King 1988). The IC25 approximates the NOEC, but it is a better description of relative toxicity because it is not as strongly affected by within-test variability. The IC25 values for JWPCP effluent samples in 1990 were much higher than those for 1987 and 1988 indicating a marked reduction in toxicity (Figure 2). Effluent samples from Hyperion and CSDOC had a comparable temporal pattern; 1989 and 1990 values were similar to 1988 values, but much higher than values measured in 1987. The temporal patterns are based on analyses of single effluent samples in most cases and do not reflect the day-to-day variations in toxicity or test organism sensitivity. The data are limited, but they provide the only record of changes in effluent toxicity in marine organism tests.

**Figure 2.**
Toxicity of County Sanitation Districts of Los Angeles County Joint Water Pollution Control Plant (JWPCP); County Sanitation Districts of Orange County (CSDOC), and City of Los Angeles Hyperion Treatment Plant (Hyperion) effluent samples collected between 1987 and 1990 to sea urchin sperm. The IC25 is the effluent concentration inhibiting fertilization by 25%. Higher IC25 values indicate lower toxicity. The upper 95% confidence limit is shown for all samples except CSDOC in 1988. The IC25 for this sample was estimated by a graphical method because the highest concentration (4%) did not produce greater than 25% inhibition. The IC25 could not be estimated for one of the three JWPCP samples for the same reason.




The quality of JWPCP effluent has changed over the years as a result of centrate removal and continuous chlorination. These changes may have contributed to the decline in toxicity in the sea urchin sperm test. Reduction in toxicity of the effluent samples between 1988 and 1990 may have also been caused by changes in handling. At the time of sampling in 1987, 1988, and 1990, JWPCP effluent was chlorinated at levels known to be toxic to sea urchin sperm (Dinnel *et al.* 1981), but only the 1990 sample was manually dechlorinated (by JWPCP) before toxicity tests were conducted.

Chlorine reacts with ammonia in effluent to form chloramines. Chloramines react with a variety of organic and inorganic compounds during transit from the treatment plant to the ocean outfall reducing effluent toxicity. Similar reactions probably occurred during storage (2-24 hr) of the 1987 and 1988 effluent samples before conducting the toxicity tests. Whether these reactions progressed to completion during storage and eliminated chlorine toxicity cannot be determined because we did not measure residual chlorine.

## Interlaboratory Comparisons

Test results with the same effluents on red abalone (embryo development) and giant kelp (spore germination and gametophyte growth) were similar to the sperm test, but the relative sensitivity of the tests varied among effluents. The sea urchin sperm and kelp growth tests responded similarly to the CSDOC effluent;

both tests had an NOEC of 1.0% (Figure 3). The abalone embryo and kelp germination tests were less affected by the effluent; neither test detected toxicity at the highest concentrations (3.2 and 5.6% effluent). The abalone embryo and sea urchin sperm tests responded similarly to the Hyperion effluent sample; both tests had an NOEC of 1.8% (Figure 4). Kelp spores and gametophytes were less affected by JWPCP effluent than sea urchin sperm. Kelp tests had NOECs equal to or greater than 5.6% (data not shown), while the urchin sperm test had an NOEC of 1.8% for the same effluent sample.

**Figure 3.**
Toxicity of a County Sanitation Districts of Orange County effluent sample collected in March 1990 to sea urchin sperm, giant kelp (spores and gametophytes), and red abalone embryos.

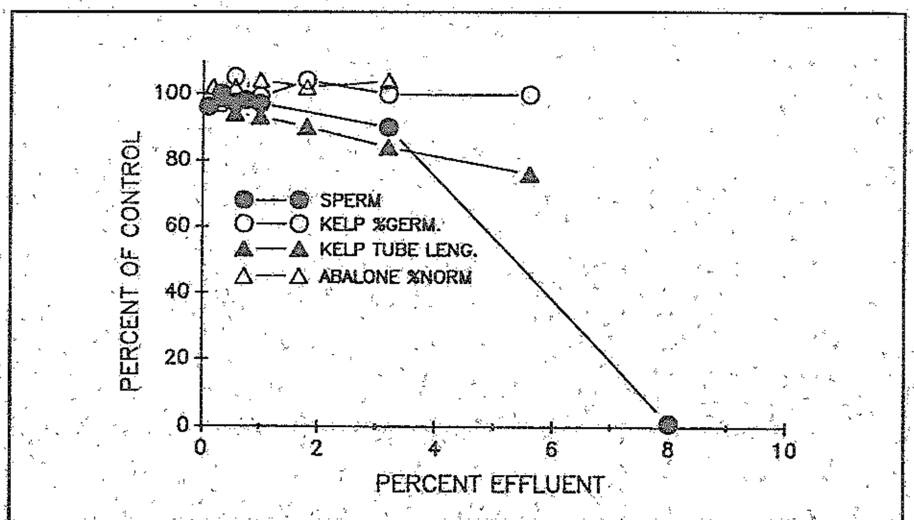
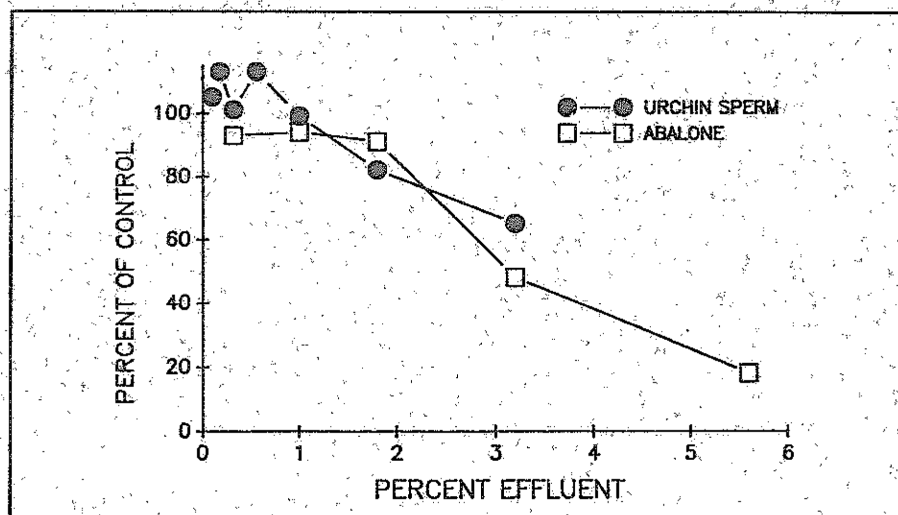



**Figure 4.**
Toxicity of a Hyperion Treatment Plant effluent sample collected in August 1989 to sea urchin sperm and red abalone embryos. Toxicity tests with giant kelp were not conducted on this effluent sample.




## Reference Toxicant Tests

The results of multiple tests of sea urchin sperm exposed to copper, a reference toxicant, varied among experiments (Table 2). Fertilization EC50s ranged from 5 to 31 µg Cu/l (mean 17 µg/l); NOECs ranged from 3.2 to 18 µg/l. The variation is substantial, but within the range reported by other laboratories for similar test methods (Weber *et al.* 1988). The EC50s for our tests with copper are similar to EC50s reported by Dinnel *et al.* (1989; 25 µg/l)—who used the same method on the same species—and by Weber *et al.* (1988; 30 µg/l)—who used the same method on a different urchin.

The coefficient of variation for the copper EC50 data (59%) is near the upper end of the range reported for chronic effluent toxicity test methods (15-62%; U.S. E.P.A. 1991). This intra-laboratory variability is somewhat higher than many E.P.A.-approved chemistry methods (10-50%; Rue et al. 1988). The reference (and effluent) toxicity tests were conducted according to generally accepted modifications of the procedure recommended in the Ocean Plan (Dinnel *et al.* 1987). This procedure was not written specifically for monitoring programs and does not provide strict guidelines for aspects of the method that may have important effects on test reproducibility (e.g., sperm to egg ratio, control performance, and reference toxicant use). Some of the variability in sperm test response may have been due to changes in these parameters as well as variations in gamete sensitivity and copper bioavailability among tests. For example, the sperm to egg ratio in least sensitive test (NOEC = 18 µg/l) was three times greater than the ratio in most of the other tests. (The concentration of sperm is varied to insure that the control response is within desired limits.) This may have reduced the sensitivity of the test by masking small reductions in sperm viability caused by copper exposure. The most sensitive test (NOEC = 3.2 µg/l) had low percent fertilization in control seawater. This may have enhanced the sensitivity of the test because small reductions in sperm viability due to copper exposure would be more likely to have a measurable impact on percent fertilization.

## Conclusions

The results reported here support our earlier findings that the toxicity of Hyperion and CSDOC effluent samples collected in recent years was less than the toxicity of effluent samples collected before 1988 (SCCWRP 1989). Using test species recommended by the Ocean Plan, NOEC values for Hyperion and CSDOC effluents are above the effluent concentrations expected in the ocean. These effluents are unlikely to cause toxicity to planktonic marine organisms.

The data are insufficient to determine if the reduction in toxicity of JWPCP effluent samples from 1988 to 1990 was due to differences in sample handling or to reductions in effluent toxicity. Chlorine toxicity in effluent can persist for more than 24 hr under storage conditions similar to those used in this study (Nacci *et al.* 1989). The initial chlorine content of the JWPCP effluent was probably much greater than that investigated by Nacci *et al.* (1989); it is therefore possible that chlorine contributed to the toxicity in the 1987 and 1988 JWPCP effluent samples.

We do not know if chlorine contributes to the toxicity of JWPCP effluent at the point of discharge. The reactiveness of chlorine and the location of the point of discharge make it difficult to obtain a representative sample. The closest sampling access to the outfall diffusers is a manifold 3 km away. Residual chlorine concentrations in daily samples of effluent collected at this location are usually below the limit of detection (0.1 mg/l). However, occasional samples do contain chlorine at potentially toxic concentrations. Between March and June 1990, 30% of the manifold effluent samples contained detectable concentrations of chlorine (CSDLAC 1990). Most (75%) of the detectable concentrations were less than 0.7 mg/l (median = 0.3, maximum = 2.1). Samples taken at the manifold are not representative of the discharged effluent. Substantial reductions in effluent chlorine concentration occur in the 1.5-2 hr transit time from the manifold to the offshore diffusers.

Potential toxicity is further

**Table 2.**

**Test results for sea urchin sperm (% fertilized) exposed to copper chloride (reference toxicant). NOEC=no observable effect concentration (highest toxicant concentration that was not statistically significant from the control). LOEC=lowest observable effect concentration (lowest toxicant concentration that was statistically significant from the control). EC50=estimated concentration producing a 50% reduction in percent fertilization.**

| Date | Test Response (µg Cu/l) NOEC | LOEC | EC50 | Control % Fertilized | Number of Sperm per egg |
|---|---|---|---|---|---|
| 3/13/90 | 18 | 32 | 31 | 95 | 600 |
| 5/23/90 | 10 | 18 | 24 | 98 | 200 |
| 6/5/90 | 5.6 | 10 | 15 | 90 | 400 |
| 6/7/90 | <10 | 10 | NC[a] | 82 | 200 |
| 6/21/90 | 3.2 | 5.6 | 5 | 51 | 200 |
| 6/26/90 | 5.6 | 10 | 12 | 88 | 200 |

[a] Insufficient data to calculate an EC50

reduced as effluent mixes with seawater during discharge. Chlorine is unlikely to cause persistent toxicity in the ocean. The variability in chlorine content at the manifold suggests that laboratory tests on manually dechlorinated samples may underestimate the contribution of chlorine to effluent toxicity.

Sea urchin sperm are sensitive to chlorine. Reduced viability occurs at chlorine concentrations as low as 0.001 mg/l (total residual oxidant) in clean seawater (Dinnel *et al.* 1981). This estimate may not be applicable to diluted sewage. Chlorinated effluent is less toxic to sea urchin sperm than equivalent levels of chlorine in clean seawater (Nacci *et al.* 1989).

Our study demonstrates that the sea urchin sperm test is a sensitive indicator of sewage effluent toxicity and that sperm test results are comparable to other species. Sensitivity of the sperm test was similar to bacteria (Microtox™), kelp spores, red abalone embryos, and sea urchin embryos exposed to effluent samples. Anderson (1989) and Nacci *et al.* (1991) obtained similar results. The sensitivity of red algae, mollusc embryos, mysids, and juvenile fish is similar to, or greater than, the sensitivity of the urchin sperm to samples of municipal wastewater.

There is concern over the reproducibility of chronic toxicity tests such as the sea urchin sperm test. The reference toxicant data for the sperm test presented in this report illustrate the variability in test results. The data are limited and may not accurately represent the reproducibility expected with this test in a monitoring program. Between-test variability in response can be reduced by doing more tests, by deleting data that do not meet quality assurance standards for control performance (within-test precision), and reference toxicant sensitivity.

Quality assurance criteria are not presently available for the sperm test as they are for other toxicity tests in the California Ocean Plan. In some cases, decisions about the acceptability of test data are left to the discretion of individual laboratories. The U.S.E.P.A., the State of California, and the Southern California Toxicity Assessment Group are developing quality assurance criteria for the sea urchin sperm test that should reduce variability.

Chronic tests are important measures of effluent quality because test organisms are sensitive to changes in toxicant bioavailability, integrate multiple toxicant effects, and demonstrate sublethal effects on aquatic biota. These characteristics increase the potential for variable results compared to conventional analytical chemistry procedures. The advantages and limitations of toxicity tests should be carefully considered when regulations are established and when monitoring data are interpreted. ■

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