Comparative Wastewater Toxicity Tests

Discharge samples were collected in December 1988 by Steven Bay, Darrin Greenstein, Valerie Raco, and Karen Englehart from the seven major wastewater treatment plants in southern California to document changes in wastewater toxicity at each plant over time and to calculate no observable effect concentrations (NOEC). NOEC represents the highest concentration of effluent used in a test that does not produce toxicity, and is a measure of chronic or sublethal toxicity of sewage effluent. Proposed revisions of the California Ocean Plan will use this parameter as a basis for regulating effluent toxicity; NOEC should be greater than the effluent concentration resulting from the initial dilution upon discharge into the ocean.

Three toxicity tests were used to measure toxicity levels in the wastewater samples: The sea urchin sperm test, sea urchin embryo test (including percent normal development and pigment production), and the Microtox bacteria luminescence test.

This project was developed as a follow-up to a study conducted in April and May 1987 on samples of final effluent from the seven major ocean wastewater dischargers (SCCWRP 1988). The results of the 1987 sperm exposure tests showed unexpectedly high levels of toxicity in most of the effluent samples, and usually showed significant toxicity at the lowest concentrations tested (0.1% to 2%), which in some cases were below the treatment plants' initial dilutions (0.6% to 1.2%). Because the lowest concentration tested was toxic, NOECs could not be calculated for the 1987 samples, leading these researchers to develop the present study.

Methods

Twenty-four hour composite samples of final effluent were collected at the Los Angeles City Hyperion Wastewater Treatment Plant (Hyperion), the Los Angeles County Joint Water Pollution Control Plant (JWPCP), the County Sanitation Districts of Orange County Plant (CSDOC), the San Diego City Point Loma Treatment Plant (Point Loma), the Encina Wastewater Treatment Plant (Encina), and the South East Regional Reclamation Authority Treatment Plant (SERRA). Samples were collected on the same day at each of the seven facilities.

Three tests were used to compare the relative sensitivities of the tests and to review the long-term trends by comparing data analyzed previously: The sea urchin sperm test, the sea urchin embryo test (including percent normal development and pigment production), and the Microtox bacteria luminescence test.

Sea urchin tests were performed on gametes and embryos of purple sea urchins (*Strongylocentrotus purpuratus*). Seawater was added to dilute effluent samples to concentrations ranging from 0.05% to 4% effluent in preparation for lab testing.

The sea urchin sperm test was used to measure toxicity as described by Dinnel et al. (1987). Urchin sperm were exposed to the effluent samples for 60 min, then mixed with urchin eggs to determine the percentage of fertilization success. Effluent samples from all seven wastewater treatment plants were tested with this method.

Effluent samples from JWPCP and CSDOC were tested for toxicity by using the urchin embryo test in which fertilized sea urchin eggs were exposed to the samples for 48 h. Two end points were examined after 48 h: (1) the embryos were examined under 100x microscopic magnification to determine the percentage of normal development, and (2) the pigment echinochrome was extracted from the embryos using the methods described by Bay et al. (1983). For the second end point, toxicity was indicated by a reduction in the quantity of echinochrome produced.

The Microtox Toxicity Analyzer System, which utilizes luminescent marine bacteria (*Photobacterium phosphoreum*), was used to test samples from JWPCP and CSDOC as described by Bulich (1982). Initial light produced by the bacteria was measured with a photometer and recorded, then remeasured and recorded after 30 min exposure to the diluted effluent samples. The loss of light output after 30 min of exposure was the end point for this toxicity test.

In addition to the three toxicity tests, repetitive sperm tests were conducted to determine if there was any change in the toxicity of the samples during the 48 h exposure period of the urchin embryo tests. Diluted samples of JWPCP and CSDOC effluent were prepared exactly like the 48 h samples, and they were tested after 24 h and 48 h for sperm toxicity to document toxicity changes over time.

Results

The results for each of the toxicity tests are presented in Table 1. Results are expressed as a percentage of the dilution water control for ease in comparing the results of different end points, effluents, and experiments. Similar to the results of 1987 testing, there were large differences in the sensitivity between the end points, with the urchin sperm test being the most sensitive, followed by the Microtox, 48 h

Table 1. Summary of bioassay data for all effluents sampled in December 1988 (expressed as a percentage of the control value).

% Effluent Concentration	% Fertilized	% Normal 48 h	Echinochrome Absorbance[a]	Microtox
JWPCP				
0.05	94			
0.1	95	101	101	99
0.5	24[b]			
1	3[b]	102	99	77[b]
2	1[b]	99	96	56[b]
4		45[b]	80[b]	40[b]
8		0[b]	61[b]	32[b]
Hyperion				
0.1	99			
0.5	96			
1	94			
2	86			
4	69[b]			
CSDOC				
0.1	85	99	113	99
0.5	74[b]			
1	86	99	116	97[b]
2	84	102	107	97[b]
4	77[b]	99	105	96[b]
8		92[b]	87[b]	93[b]
Point Loma				
0.1	87			
0.5	87			
0.1	85			
1	98			
2	92			
Oxnard				
0.1	89			
0.5	98			
1	97			
2	100			
4	104			
Encina				
0.1	85			
0.5	76[b]			
1	92			
2	88			
4	101			
SERRA				
0.01	85			
0.05	56[b]			
0.1	66[b]			
1	93			
2	97			

[a] Absorbance was measured at 495 nm.

[b] Data are significantly different from the controls (Dunnett's, $p \leq 0.05$).

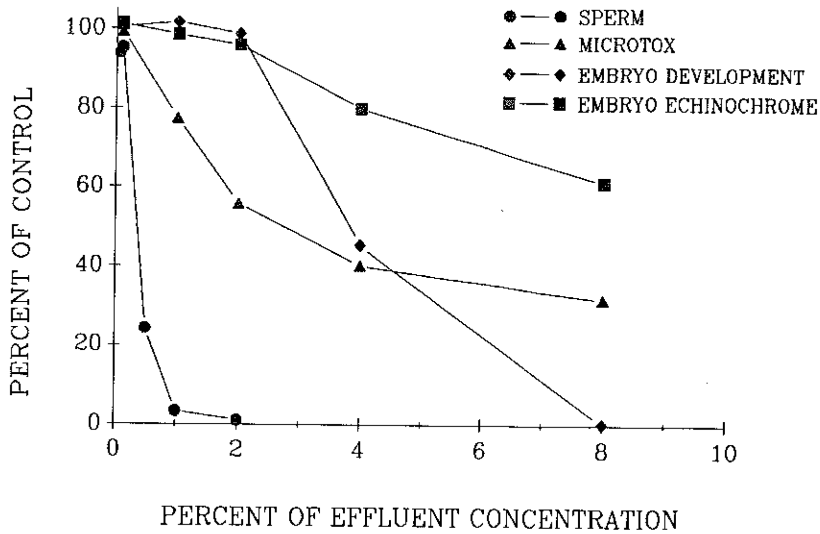


Figure 1. Comparative toxicty of JWPCP effluent (1988) to sea urchin sperm, sea urchin embryos, and Microtox bacteria.

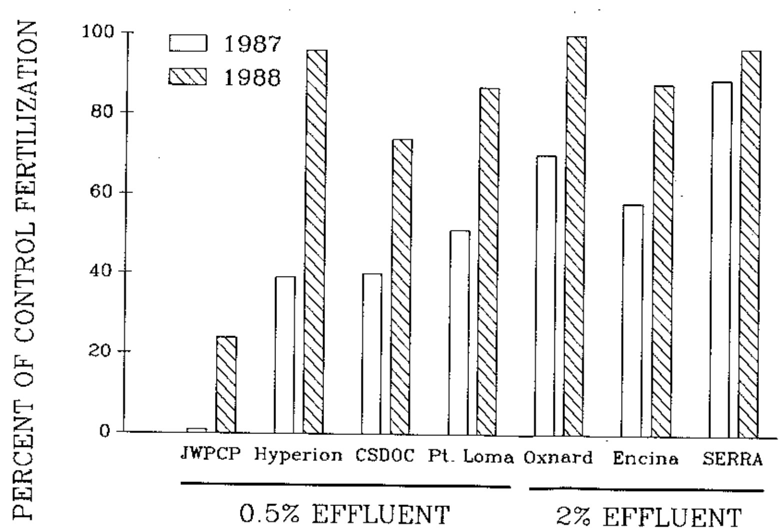


Figure 2. Comparison of sea urchin sperm test results for 1987 and 1988 effluent samples. Fertilization in 0.5% effluent is presented for the larger dischargers and fertilization response to 2% effluent is presented for the other dischargers.

percent normal embroyo development, and echinochrome absorbance tests (Figure 1).

The sperm test indicated differences in relative toxicity between the effluent samples from the seven wastewater dischargers. The toxicity of all discharger effluent samples was reduced in 1988 as compared to the 1987 toxicity test results (Figure 2). JWPCP effluent remained the most toxic, exhibiting a NOEC of 0.1% for the sperm test. Hyperion and CSDOC effluents both exhibited 2% NOECs. The highest concentrations of effluent tested in 1988 from Point Loma, Oxnard, Encina, and SERRA did not exhibit measurable toxicity levels. Some of the intermediate samples from the CSDOC, Encina, and SERRA dilution series exhibited significantly reduced fertilization percentages. However, because these results did not show normal dose-response behavior, contamination of the samples is indicated.

The Microtox assay also indicated less toxicity for the 1988 CSDOC sample than in 1987. The Microtox bioassay showed an NOEC of 0.1% for both effluents tested in 1988 (JWPCP and CSDOC). These NOEC values were similar to, or lower than, any of the urchin end points, even though the response data (Table 1) indicated that the Microtox test was often less affected by the effluents than the sperm and 48 h percent normal values. This discrepancy can be explained by the very low variability of the Microtox data, which allows for the detection of statistically significant differences from

Table 2. Characteristics of effluent samples used for bioassays. Measurement units are μg/l unless otherwise noted.

Constituent	JWPCP		Hyperion		CSDOC		Point Loma		Oxnard		Encina		SERRA	
	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988
Flow (mgd)[a]	386	387	369	368	270	245	182	182	20	17	19[b]	21	15[b]	17[b]
Percent Secondary	50	50	25	40	60	47	0	0	100	100	47	100	100	100
Susp. Solids (mg/l)	75	54	56	28	52	44	73	49	23	30	58[b]	8	20[b]	12[b]
BOD (mg/l)	106	129	111	87	64	79	132	126	26	30	65[b]	12	11[b]	10[b]
Ammonia-N (mg/l)	37.5	35.2	15.6	19.4	28[b]	24[b]	23.4	21.3	4.9	11.6	19.5	26.2[b]	7.2	12[b]
Non-Cl Phenol (mg/l)	1.8	4.9	0.048	0.022	0.62[b]	0.021[b]	0.007	0.002	<0.02	0.012	0.001	<0.010	<0.003	<0.005[b]
As	7	6	7	6	3[b]	3[b]	4	4.5[b]	<5	3	<5	3[b]	<5	<0.005[b]
Cd	1	7	12	ND	2	<1	<5	<5[b]	<10	4	7	6[b]	<1	<0.01[b]
Cr	61	42	3	ND	17	14	<20	<50[b]	<10	<10	<5	6[b]	<50	<0.01[b]
Cu	44	32	67	48	60	46	50	70[b]	52	31	22	12[b]	40	<0.005[b]
Pb	46	38	30	ND	20	20	<50	<50[b]	<70	<10	1	38[b]	140	<0.06[b]
Hg	0.1	0.3	0.2	ND	0.8[b]	0.2[b]	0.5	<0.5[b]	<1	<1	<0.2	0.2[b]	<1	0.002[b]
Ni	55	67	60	20	30	<10	20	<20[b]	82	24	38	46[b]	<40	<0.06[b]
Ag	8	6	12	2.1	11	4	<10	<10[b]	<20	<4	<1	5[b]	<10	<0.05[b]
Zn	110	130[d]	320	54	70	60	62	64[b]	58	29	62	124[b]	140	0.06[b]
Cyanide	30	50	26	ND	<0.02[b]	<0.02[b]	5	3.9	11	16	10	51[b]	100	0.01[b]
Total DDT	0.07	0.03	<0.02	ND	0.06[b]	<0.05[b]	ND	ND	<0.05	<0.02	ND	NA	NA	NA
Total PCB	ND	ND	<0.1	ND	<0.5	<0.3	ND	ND	<0.15	<0.2	ND	NA	NA	NA
Toxicity (Tu)[c]	1.30	1.40	0.99	0.94	0.76	0.59[b]	1.69[b]	1.55	0	0.41	0.77[b]	1.82	NA	NA

[a] Abbreviations: mgd = millions of gallons per day; NA = data not available; ND = not detectable, no detection limit, or not available.
[b] Value is not from the sample on which the bioassay was performed, but from sample collected at the closest available time.
[c] Fathead minnow bioassay was performed by the discharger. Toxicity is expressed in toxicity units, where Tu=100/96 hr LC_{50} or (log(100-S))/1.7 if an LC_{50} cannot be calculated. S=%Survival in 100% effluent.
[d] Mean value from January through September 1988.

Steve Bay collecting gametes for the sea urchin embroyo test.

the control with relatively small decreases in light output.

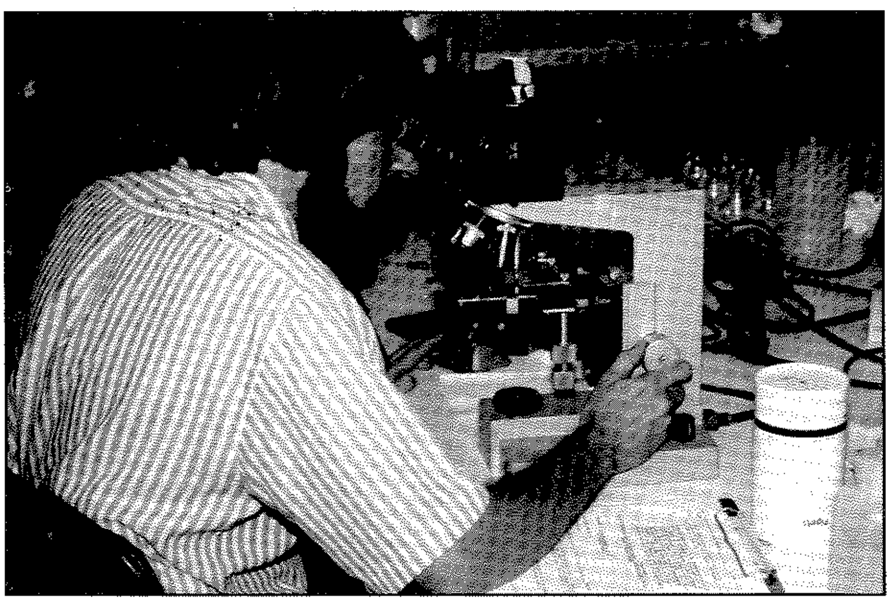
The 48 h embryo endpoints of percent normal development and echinochrome production responded similarly to each effluent type, yielding NOECs of 2% and 4% for JWPCP and CSDOC respectively. When compared with the 1987 results, these data indicated a reduction in toxicity for CSDOC, and similar toxicity for JWPCP.

The decrease in toxicity to sea urchin sperm observed when comparing the 1987 and 1988 samples indicate an improvement in effluent quality between these two samples. These data are based on the analysis of single 24 h composite samples, however, and the day to day variability in the toxicity of these samples to sperm is unknown. Consequently, we cannot determine if these results represent a consistent reduction in toxicity or reflect typical variations in effluent quality.

Chemical analyses of the effluents tested in 1988 (or similar samples) indicated some improvement in effluent quality. Large reductions in trace metal concentrations, suspended solids, and other constituents were found for Hyperion effluent when compared to 1987 values (Table 2). These changes were the result of several factors, including improved plant operations and increased secondary treatment. Reductions in some constituents (primarily suspended solids content) were also found in the other effluent samples, although such changes were much less widespread. These changes reflect overall improvement in some effluent characteristics and may have accounted for some of the reduced toxicity indicated by the sperm test.

The toxicity tests conducted for this study were not designed to identify which specific effluent components were responsible for the differences in relative toxicity observed in 1988. However, the chemical analysis data provided by the treatment plants (Table 2) do indicate that the relative concentrations of most measured constituents bear little resemblance to differences in relative toxicity between the effluents.

Toxicity was greatest for JWPCP effluent, although



Darrin Greenstein measures the percentage of fertilized sea urchin eggs for the sea urchin sperm test.

the concentrations of trace metals and other constituents in this effluent were usually not markedly elevated compared with effluent samples from the other dischargers. The most distinctive characteristic of JWPCP effluent was the concentration of non-chlorinated phenol. The phenol concentration in JWPCP effluent was more than 100 times greater than phenol levels found in effluent samples from the other dischargers. However it is presently unknown if the high phenol levels in JWPCP effluent account for the higher overall toxicity level found in JWPCP effluent.

The repetitive sperm test results for JWPCP indicate a marked decrease in toxicity of a 1% effluent sample over 48 h of incubation at 15°C (Figure 3). These data suggest that there was a loss of some of the toxic effluent components over time, possibly through degradation, volatilization, or adsorption to the exposure beaker. A similar series of tests for the CSDOC effluent showed a decrease in toxicity at 24 h, but a large increase at 48 h, suggesting possible contamination of the sample.

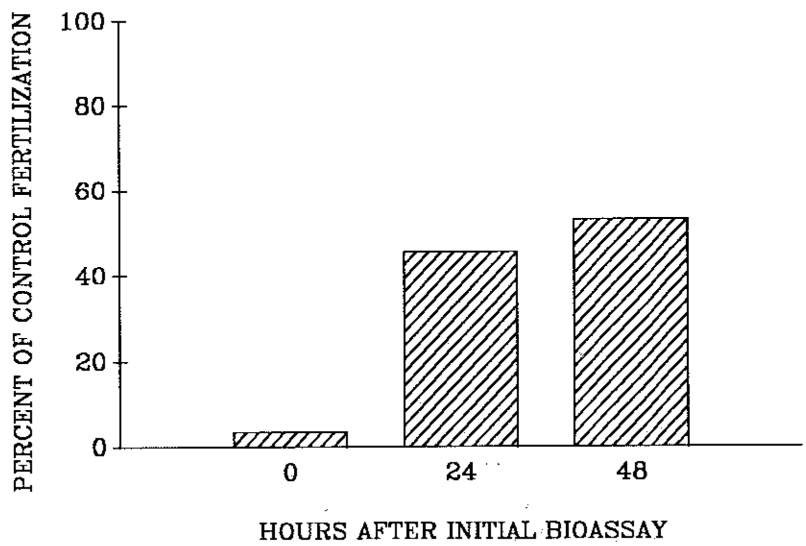


Figure 3. Change in toxicity of 1% JWPCP effluent to sea urchin sperm during storage at 15°C.

Discussion

This study verified the relative sensitivities of three marine toxicity test methods used to analyze sewage effluent samples collected in 1987. Results from the repetitive tests indicate that test duration is a significant factor to be considered in the design and interpretation of toxicity tests. The tests with the shortest exposure time ($\leq$1 h) may indicate greater toxicity because less alteration of the sample occurs before a toxic effect is expressed. The 1988 data indicate that the reduction in toxicity observed between 1982 and 1987 is continuing. In some cases these results correspond to improvements in effluent quality indicated by chemical measurements. To interpret this trend better, we recommend that additional studies should be conducted to measure short-term variability of effluent toxicity and to determine which constituents are causing the effects observed to date.

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