RESULTS OF RECENT WASTEWATER TOXICITY TESTS

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INTRODUCTION

In April and May of 1987, SCCWRP conducted a survey of effluent toxicity from seven southern California sewage treatment plants having ocean outfalls. Three different bioassay methods were used to assess toxicity, each of which is feasible for use in routine monitoring programs. Using these methods we measured the toxic responses of sea urchin sperm, sea urchin embryos, and luminescent marine bacteria (Microtox [Microbics Corp., Carlsbad, CA] system; Bulich 1982) to the effluents.

The objective of this project was to see if we could detect a decrease in toxicity that would be expected following improvements in sewage treatment over the past few years. In addition, we wanted to compare the relative sensitivities of the sea urchin and Microtox test endpoints, as an aid in their evaluation for routine use in effluent monitoring.

This report is not a final presentation of the results. It is intended to serve as a transmission of our test results in response to the current high level of interest in marine bioassay methods. The data contained within this report should indicate the type of responses that will result from the use of similar bioassay tests in effluent monitoring programs.

METHODS

The samples used in this project were 24-hour composites of final effluent collected by the dischargers as part of their routine chemical and biological monitoring. This allowed us to compare our results with their measurements of effluent composition and fish toxicity. Samples from the following treatment plants were tested (collection date of sample in parentheses): SERRA (April 8), Encina (May 3), Oxnard (May 4), Pt. Loma (May 4), Hyperion (May 5), JWPCP (May 5), and Orange County (May 11). Most of the data describing the effluent composition has been extracted from the respective monitoring reports provided by each treatment plant. Measurements of PAH concentrations in these samples were made by SCCWRP.

The sea urchin bioassay consisted of two parts with three separate endpoints, all performed with gametes from the purple sea urchin, Strongylocentrotus purpuratus, at 15^{o}C. The first part was the exposure of sperm to seawater dilutions of the effluent by the methods of Dinnel et al. 1987. In this test, sperm were exposed to dilutions of effluent in culture tubes for one hour. Unfertilized sea urchin eggs were then added to the solution and given twenty minutes for fertilization to occur. Samples of the eggs were then examined with a microscope to determine the fertilization success.

The other two endpoints were measured after a 48-hour exposure of fertilized eggs to dilutions of effluent. Fertilized eggs were exposed in 400-ml beakers containing 250 ml of diluted effluent. After 48 hours, a 10-ml subsample of the embryos was taken and preserved in formalin for microscopic examination. Purple sea urchin embryos normally attain the prism stage of development after 48 hours of development. Toxic effects were determined by measuring the percentage of normally developed embryos. The last endpoint evaluated was the amount of the pigment echinochrome produced by the embryos. Echinochrome was extracted from the embryos contained in a 200-ml sample from each test beaker. The embryos were concentrated by screening and extracted with ethanol to remove interfering pigments from other organisms (e.g., phytoplankton). The embryos were then extracted with acidified ethanol and the light absorbance of this extract was measured with a spectrophotometer. Toxic effects are indicated by a reduction in the amount of pigment present in the extract (Bay et al. 1983).

The Microtox bioassay system is a commercially produced test apparatus which uses luminescent marine bacteria. A sodium chloride solution was used as the diluent in this test. Toxicity was indicated by the loss of light output after 30 minutes of exposure.

The effluent concentrations chosen for these tests were selected to include the no observable effect concentration (NOEC). This value is defined as the highest dilution tested that does not result in a statistically significant toxic response. The NOEC value has been suggested to be the most appropriate way to describe bioassay results for monitoring purposes.

RESULTS AND DISCUSSION

The results for each experiment are shown in Tables 1-4. The results have also been expressed as the percentage of change relative to the dilution water control (Table 5), facilitating the comparison of results from different experiments and test methods.

Large differences in effluent sensitivity were found between the different test methods. However, a similar pattern of relative toxicity between effluent samples was indicated by each method. The toxicity of the samples appeared to be more strongly related to the size of the treatment plant generating it than to the concentrations of individual chemical constituents (Table 6). Effluent from plants having flows greater than 100 mgd usually had much greater toxicity than effluent from facilities with lower flows. This pattern is illustrated most clearly by the sperm test data (percent fertilized eggs).

The suspended solids content of the effluent samples also appeared to have an influence on the degree of toxicity. Among the larger treatment plants, effluent samples with the lowest suspended solids content (Hyperion and Orange County) generally elicited the least toxic response. Similar results were found for the smaller treatment plants. The Encina effluent sample had the greatest suspended solids concentration and greatest toxicity of similar sized treatment plants.

Multivariate statistical analyses are necessary to determine if significant relationships between toxicity and effluent composition exist. These analyses will be performed before these results are submitted for journal publication.

Temporal changes in effluent toxicity can be identified by comparing these current test results with those from our 1982 survey, in which only echinochrome was measured (Oshida et al. 1982). The echinochrome data show that a decrease in toxicity has occurred for the effluent from the five treatment plants studied in 1982 (Table 7). Improvements in sewage treatment and source control practices appear to be responsible for these toxicity changes. Comparison of the current chemistry values with the annual averages for 1982 shows decreases in suspended solids, BOD, and many chemical constituents (Table 6).

An important result of this study was the illustration of the diversity of effluent toxicity estimates that can be obtained through the use of different test methods. The variations in test sensitivity to effluent are demonstrated by a comparison of the various test results for the Pt. Loma effluent sample (Figure 1). For all of the effluents, the sperm test showed by far the greatest sensitivity to effluent; statistically significant reductions in fertilization at effluent concentrations below 1% were found for samples from the largest treatment plants. NOEC levels cannot be determined for many of the effluent samples, because toxic effects were seen at every dilution tested.

The Microtox test also demonstrated toxic effects at low effluent concentrations. Although statistically significant effects were detected at very low concentrations, bacterial luminescence did not change as rapidly with increasing effluent concentration as did the sea urchin test endpoints (Figure 1). As a result, the relative sensitivity of effluent toxicity estimations from Microtox results are dependent upon the data analysis method. If toxicity is expressed in terms of the NOEC, the Microtox test is much more sensitive than the 48 hour embryo test. If EC50 (concentration producing a 50% change) values are used to describe toxicity, the Microtox test would be less sensitive than tests with sea urchin sperm or embryos.

The high sensitivity of the sperm and Microtox test methods are probably due to the rapidity of these methods, rather than to biological differences in contaminant susceptibility. Laboratory tests with individual contaminants usually show that the sperm and Microtox tests have similar or lower sensitivities compared with sea urchin embryo tests. The apparent reduced sensitivity of the sea urchin embryo test observed in this study was probably due to reductions in effluent contaminant levels during the exposure from volatilization and adsorption processes.

The State of California will require the use of sensitive marine bioassay tests for effluent monitoring by 1991. The State Water Resources Control Board is considering regulatory policy that would require effluents not to produce toxic effects in these tests at concentrations below the one resulting from initial dilution in the ocean. Our effluent toxicity study provides an indication of the type of results that can be expected for future monitoring programs. The sperm test is one of the methods recommended by the EPA for effluent testing and will probably be used in some monitoring programs in southern California. Results from our sperm tests indicate that the proposed regulations would be exceeded by most of the effluents tested because toxicity was often found at levels below the treatment plants' assigned initial dilutions, which range from 0.6-1.2%.

REFERENCES

Bay, S. M., P. S. Oshida and K. D. Jenkins. 1983. A simple new bioassay based on echinochrome synthesis by larval sea urchins. Mar. Environ. Res. 8:29-39.

Bulich, A.A. 1982. A practical and reliable method for monitoring the toxicity of aquatic samples. Process Biochem. March/April, pp 45-47.

Dinnel, P. A., J. M. Link and Q. J. Stober. 1987. Improved methodology for a sea urchin sperm cell bioassay for marine waters. Arch. Environ. Contam. Toxicol. 16:23-32.

Oshida, P. S., S. M. Bay, A. Haeckl, T. K. Goochey, and D. Greenstein. 1982. Seawater and wastewater toxicity studies, pp. 217-223. In Southern California Coastal Water Research Biennial Report 1981-1982. W. Bascom. ed., Long Beach, CA.

Table 1. Summary of bioassay results for SERRA effluent dilutions, experiment number 112. Values marked with * are significantly different from controls (Dunnett's $p \leq 0.05$).

Sample	%Fertilized	%Normal 48hrs	Echinochrome Absorbance (A_{495})	Microtox %Control
Control	85.2	80.1	0.043	
25% Art. SW Con.	70.6*	72.0	0.026*	
SERRA 1%	88.1	88.5	0.020*	
SERRA 2%	75.8*	84.0	0.045	
SERRA 4%	74.0*	87.0	0.050	96.5
SERRA 8%	65.3*	87.6	0.053	90.6*
SERRA 15%[a]	80.7	77.5	0.056	83.6*
SERRA 25%[a]	77.6	73.9	0.052	

[a]Concentrated artifical seawater was added to urchin tests to keep salinity at a normal level.

Table 2. Summary of bioassay results for Oxnard, Encina, and San Diego effluent dilutions, experiment number 113. Values marked with * are significantly different from controls (Dunnett's $p \leq 0.05$).

Sample	%Fertilized	%Normal 48hrs	Echinochrome Absorbance (A_{495})	Microtox %Control
Control	61.9	81.0	0.040	
Oxnard 2%	43.2*	89.3	0.050	94.4
Oxnard 4%	38.4*	85.1	0.056	90.6*
Oxnard 8%	24.8*	71.8	0.048	85.0*
Encina 2%	36.1*	84.0	0.051	88.6
Encina 4%	15.3*	67.5	0.050	84.6*
Encina 8%	6.9*	5.2*	0.027*	83.0*
San Diego 0.05%				100.0
San Diego 0.1%				97.7
San Diego 0.2				96.7
San Diego 0.4%				91.7
San Diego 0.5%	31.7*	79.7	0.044	92.2
San Diego 1%	10.5*	92.7	0.053	86.1*
San Diego 2%	1.5*	71.3	0.052	77.7*
San Diego 4%	0.7*	48.0	0.050	65.5*
San Diego 8%	0.3*	0.0*	0.027*	56.8*

Table 3. Summary of bioassay results for Hyperion and JWPCP effluent dilutions, experiment number 113. Values marked with * are significantly different from controls (Dunnett's $p \leq 0.05$).

Sample	%Fertilized	%Normal 48hrs	Echinochrome Absorbance (A_{495})	Microtox %Control
Control	65.4	87.9	0.070	
Hyperion 0.1%	54.5	88.7	0.065	97.4
Hyperion 0.2%	43.0*	91.8	0.069	95.7*
Hyperion 0.5%	25.8*	93.8	0.071	91.8*
Hyperion 1%	24.8*	88.8	0.075	86.3*
Hyperion 2%	22.5*	83.6	0.063	80.8*
Hyperion 4%	5.0*	84.6	0.068	
JWPCP 0.05%				100.0
JWPCP 0.1%	25.4*	93.0	0.073	97.9
JWPCP 0.2%	23.9*	90.1	0.074	93.8
JWPCP 0.5	0.6*	87.3	0.076	
JWPCP 1%	0.3*	82.6	0.077	
JWPCP 2%	0.1*	84.6	0.077	
JWPCP 4%	0.0*	14.7*	0.054	

Table 4. Summary of bioassay results for Orange County effluent dilutions, experiment number 113. Values marked with * are significantly different from controls (Dunnett's $p \leq 0.05$).

Sample	%Fertilized	%Normal 48hrs	Echinochrome Absorbance (A_{495})	Microtox %Control
Control	73.1	79.6	0.058	
Orange Co. 0.02%				99.0
Orange Co. 0.2%	42.3*	64.2*	0.046*	97.5
Orange Co. 0.5%	29.0*	75.6	0.057	94.6*
Orange Co. 1%	24.1*	86.1	0.057	88.9*
Orange Co. 2%	3.5*	80.6	0.061	85.4*
Orange Co. 4%	0.2*	61.8*	0.053	
Orange Co. 8%	0.1*	0.5*	0.028*	

Table 5. Summary of bioassay results for all effluents, expressed as percentage of control value. Data with * are significantly different from control seawater (Dunnett's p≤0.05).

Sample	%Fertilized	%Normal 48hrs	Echinochrome	Microtox Absorbance (A_{495})
JWPCP 0.05%				100
JWPCP 0.1%	39*	106	104	98
JWPCP 0.2%	36*	103	106	94
JWPCP 0.5%	1*	99	109	
JWPCP 1%	0*	94	110	
JWPCP 2%	0*	96	110	
JWPCP 4%	0*	17*	77	
Hyperion 0.1%	83	101	93	97
Hyperion 0.2%	66*	104	99	96*
Hyperion 0.5%	39*	107	101	92*
Hyperion 1%	38*	101	107	86*
Hyperion 2%	34*	95	90	81*
Hyperion 4%	8*	96	97	
Orange Co. 0.2%	58*	81*	79*	98
Orange Co. 0.5%	40*	95	98	95*
Orange Co. 1%	33*	108	98	89*
Orange Co. 2%	5*	101	105	85*
Orange Co. 4%	0*	78*	91	
Orange Co. 8%	0*	1*	48*	
San Diego 0.5%	51*	98	110	92
San Diego 1%	17*	114	132	86*
San Diego 2%	2*	88	130	78*
San Diego 4%	1*	59	125	66*
San Diego 8%	0*	0*	68*	57*
Oxnard 2%	70*	110	125	94
Oxnard 4%	62*	105	140	91*
Oxnard 8%	40*	89	120	85*
Encina 2%	58*	104	128	89
Encina 4%	25*	83	125	85*
Encina 8%	11*	6*	68*	83*
SERRA 1%	103	110	46*	
SERRA 2%	89*	105	105	
SERRA 4%	87*	109	116	96
SERRA 8%	77*	109	123	91*

Table 6. Effluent chemistry values from the actual effluent samples on which the bioassays were performed (unless otherwise noted) and annual averages for 1982. All units are mg/l unless otherwise noted. 1982 values for Encina and SERRA are omitted because we did not perform bioassays on them at that time.

Constituent	JWPCP 1987	JWPCP 1982	Hyperion 1987	Hyperion 1982	Orange Co. 1987	Orange Co. 1982	San Diego 1987	San Diego 1982	Oxnard 1987	Oxnard 1982	Encina 1987	SERRA 1987
Flow (MGD)	386	359	369	375	270	223	182	132	20	17	19[a]	15[b]
% Secondary	50	0	25	25	60	60	0	0	100	100	30	100
Susp. Solids	75	164	56	77	52	112	73	126	23	41	58[a]	20[b]
BOD	106	199	111	176	64	158	132	124	26	27	65[a]	11[b]
Ammonia-N	37.5	41.0	15.6	14.8	NA	24.0	23.4	24.2	4.9	13.9	19.5	7.2
Arsenic	0.007	0.007	0.007	<0.005	NA	0.002	0.004	0.004	<0.005	0.011	<0.005	<0.005
Cadmium	0.001	0.011	0.012	0.01	0.002	0.016	<0.005	0.008	<0.01	0.01	0.007	<0.001
Chromium	0.061	0.19	0.03	0.09	0.017	0.070	<0.02	0.022	<0.01	0.008	<0.005	<0.05
Copper	0.044	0.128	0.067	0.14	0.060	0.218	0.05	0.133	0.052	0.028	0.022	0.04
Lead	0.046	0.081	0.03	0.05	0.02	0.08	<0.05	0.082	<0.07	0.026	0.001	0.14
Mercury	0.0001	0.0008	0.0002	0.0007	NA	0.0002	0.0005	0.0004	<0.001	NA	<0.0002	<0.001
Nickel	0.055	0.15	0.06	0.09	0.03	0.07	0.02	0.069	0.082	0.035	0.038	<0.04
Silver	0.008	0.011	0.012	0.02	0.011	0.016	<0.01	<0.002	<0.02	0.019	<0.001	<0.01
Zinc	0.11	0.51	0.32	0.18	0.07	0.20	0.062	0.292	0.058	NA	0.062	0.14
Cyanide	0.03	0.06	0.026	0.06	NA	0.04	0.005	0.007	0.011	<0.05	0.01	0.10
Tot. Phenol	1.8	2.53	0.048	0.062	NA	0.07	0.007	0.033	<0.02	0.012	0.001	<0.003
Tot. DDT(ug/l)	0.07	0.45	<0.02	0.06	NA	0.064	ND	0.081	<0.05	ND	ND	NA
Tot. PCB(ug/l)	ND	0.47	<0.1	<0.1	<0.5	1.77	ND	<0.001	<0.15	1.00	ND	NA
Tot. PAH(ug/l)[c]	<0.014	NA	0.024	NA	1.93	NA	<0.009	NA	<0.005	NA	<0.005	NA
96 Hour LC50[d]	77	22	101	74	132	114	59[a]	96	>100[e]	77	130[f]	NA

[a]Average for the month of May.
[b]Average for the month of April.
[c]Analysis done at SCCWRP
[d]Fathead minnow bioassay, performed by the discharger.
[e]No mortality occured at any of the dilutions, so LC50 cannot be calulated.
[f]Value for different day of same week.
NA = Data not available.
ND = Not detectable, detection limit not available.

Table 7. Comparison of no observable effect concentrations (NOEC) for echinochrome data between 1982 and 1987 samples.

Discharger	NOEC (%Effluent) 1982	1987
JWPCP	0.2	>4
Hyperion	0.2	>4
Orange Co.	0.2	4
San Diego	1	4
Oxnard	7	>8

Figure 1. Results of Microtox and sea urchin sperm and embryo test of Pt. Loma sewage effluent toxicity.