Wastewater Toxicity Tests

Toxicity tests with sea urchin gametes and embryos have been used at SCCWRP since the late 1970s. This test system has been used effectively for measuring the toxicity of seawater, sewage effluent, and sediment extracts (this report). Several different responses can be measured with this test system including fertilization success of sperm, occurrence of abnormal embryonic development, and production of echinochrome pigment by developing embryos. Sea urchin bioassays were first used at SCCWRP to test the toxicity of sewage effluents in 1978 (Oshida et al. 1981). Effluent toxicity was measured again in 1982 and included samples from southern California's five largest ocean dischargers: Hyperion (City of Los Angeles), Joint Water Pollution Control Plant (JWPCP; Los Angeles County), Orange County, Point Loma (City of San Diego), and Oxnard (Oshida et al. 1983).

SCCWRP scientists Steven M. Bay, Darrin J. Greenstein, Valerie E. Raco, and Karen D. Englehart repeated these effluent tests in April and May of 1987, with the addition of test samples from two smaller treatment plants, Encina and South East Regional Reclamation Authority (SERRA) (located in San Diego and Orange Counties, respectively). In addition to the urchin bioassay, Bay and co-workers also performed the Microtox (Microbics Corp., Carlsbad, CA) bacterial luminescence test (Bulich 1982) on all of the effluents. The objective of this project was to see if a decrease in toxicity that would be expected after improvements in sewage treatment over the past few years could be detected. In addition, the researchers wanted to compare the relative sensitivities of the sea urchin and Microtox test endpoints to aid in their evaluation for routine use in effluent monitoring.

The samples used in this project were 24-h composites of final effluent collected by the dischargers as part of their routine chemical and biological monitoring. This allowed the SCCWRP scientists to compare their results with the measurements of effluent composition and fish toxicity gathered by the dischargers.

The sea urchin bioassay consisted of two parts with three separate endpoints, all performed by using gametes from the purple sea urchin, *Strongylocentrotus purpuratus*. The first part was the exposure of sperm to seawater dilutions of the effluent by using the methods of Dinnel et al. (1987). This was a 60-min test, with the endpoint being fertilization success of eggs added to the solution.

The other two endpoints were measured after a 48-h exposure of fertilized eggs to dilutions of effluent. After 48 h, purple sea urchin embryos normally attain the prism stage of development (Figure 1). Toxic effects were determined by measuring the percentage of normally developed 48-h embryos. The last endpoint evaluated was the amount of pigment echinochrome produced by the embryos. Toxic effects are expressed by a reduction in the amount of pigment present in an extract of an embryo subsample (Bay et al. 1983). The level of pigment in a sample was measured as light absorbance with a spectrophotometer.

The Microtox bioassay involved the exposure of luminescent marine bacteria to dilutions of the effluents. A sodium chloride solution was used as the

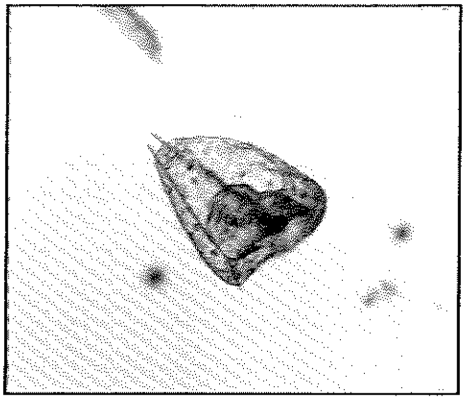

Figure 1. Normally developed sea urchin embryo at the 48-h prism stage.

diluent in this test. Toxicity was indicated by the loss of light output after 30 min of exposure.

The results from this study have been expressed as the percentage of change relative to the dilution water control, facilitating the comparison of results from different experiments and test methods. The effluent concentrations chosen for these tests were selected to include the no observable effect concentration (NOEC). This value is defined as the highest effluent concentration tested not resulting 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.

The results for each of the bioassay endpoints are shown in Table 1. Large differences in effluent sensitivity were found between the different test methods. A similar pattern of relative toxicity between effluent samples was indicated by each method, however. Toxicity was strongly related to differences in effluent

Table 1. Summary of data for all effluents, expressed as percentage of control value. Data marked with an * are significantly different from the controls. (Dunnett's $P \leq 0.05$). Note that different dilutions were tested for each sample.

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

[a]Echinochrome absorbance was measured at 495 nm.

Table 2. 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 micrograms per liter unless otherwise noted. Values for 1982 for Encina and SERRA effluents are omitted because we did not perform tests on them at that time.[a]

	JWPCP		Hyperion		Orange Co.		San Diego		Oxnard		Encina	SERRA
Constituent	1987	1982	1987	1982	1987	1982	1987	1982	1987	1982	1987	1987
Flow (MGD)	386	359	369	375	270	223	182	132	20	17	19[b]	15[c]
% Secondary	50	0	25	25	60	60	0	0	100	100	47	100
Susp. Solids (mg/L)	75	164	56	77	52	112	73	126	23	41	58[b]	20[c]
BOD (mg/L)	106	199	111	176	64	158	132	124	26	27	65[b]	11[c]
Ammonia-N (mg/L)	37.5	41.0	15.6	14.8	NA	24.0	23.4	24.2	4.9	13.9	19.5	7.2
Tot. phenol (mg/L)	1.8	2.53	0.048	0.062	NA	0.07	0.007	0.033	<0.02	0.012	0.001	<0.003
Arsenic	7	7	7	<5	NA	2	4	4	<5	11	<5	<5
Cadmium	1	11	12	10	2	16	<5	8	<10	10	7	<1
Chromium	61	190	3	90	17	70	<20	22	<10	8	<5	<50
Copper	44	128	67	140	60	218	50	133	52	28	22	40
Lead	46	81	30	50	20	80	<50	82	<70	26	1	140
Mercury	0.1	0.8	0.2	0.7	NA	0.2	0.5	0.4	<1	NA	<0.2	<1
Nickel	55	150	60	90	30	70	20	69	82	35	38	<40
Silver	8	11	12	20	11	16	<10	<2	<20	19	<1	<10
Zinc	110	510	320	180	70	200	62	292	58	NA	62	140
Cyanide	30	60	26	60	NA	40	5	7	11	<50	10	100
Tot. DDT	0.07	0.45	<0.02	0.06	NA	0.064	ND	0.081	<0.05	ND	ND	NA
Tot. PCB	ND	0.47	<0.1	<0.1	<0.5	1.77	ND	<0.001	<0.15	1.00	ND	NA
Tot. PAH[d]	<0.014	NA	0.024	NA	1.93	NA	<0.009	NA	<0.005	NA	<0.005	NA
96-h LC_{50}[e]	77	22	101	74	132	114	59[b]	96	>100[f]	77	130[g]	NA

[a]Abbreviations: NA, data not available; ND, not detectable or detection limit not available; MGD, millions of gallons per day; PCB, polychlorinated biphenyl; PAH, polynuclear aromatic hydrocarbon; LC_{50}, lethal concentration for 50% of the test organisms.
[b]Average for the month of May.
[c]Average for the month of April.
[d]Analysis done at SCCWRP.
[e]Fathead minnow bioassay, performed by the discharger.
[f]No mortality occurred at any of the dilutions, so LC_{50} cannot be calculated.
[g]Value for different day of same week.

flow at each treatment plant (Table 2). Effluent samples from plants having flows greater than 138 x 10^9 L/yr (100 million gallons per day) usually had much greater toxicity than did 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 highest suspended solids concentration and greatest toxicity of similar sized treatment plants.

The relationships between toxicity and flow or suspended solids illustrated by these results are to be expected because these parameters serve as general descriptors of the quantity of toxic chemicals likely to be present in the effluent. Large treatment plants (high flow) are often located near areas of increased industrial activity and the suspended solids in the effluent generally contain the highest concentrations of most contaminants. The concentrations of many toxic constituents were measured in these effluent

Table 3. Comparison of 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

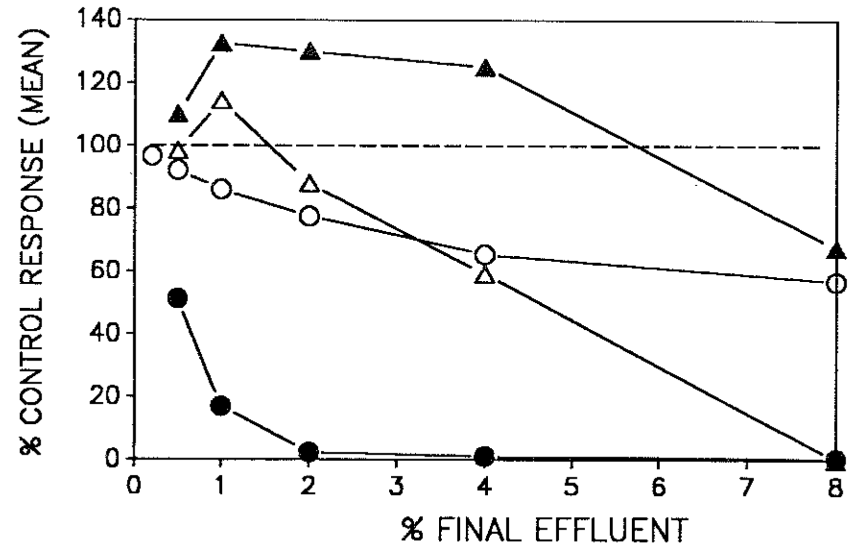

Figure 2. Results of Microtox and sea urchin sperm and embryo tests of Pt. Loma sewage effluent toxicity. The dashed line indicates the control response of 100%. Symbols: ○, microtox; ●, sperm; △, % normal after 48 h; ▲, echinochrome.

samples (Table 2). Multivariate statistical analyses are planned to help interpret these data, with the intent of determining which constituents are most closely associated with the toxicity observed.

Temporal changes in effluent toxicity can be identified by comparing these current test results to those from these researchers' 1982 survey, in which only echinochrome was measured (Table 3). The echinochrome data show that a decrease in toxicity has occurred for the effluent from the five treatment plants studied in 1982. 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, biological oxygen demand (BOD), and many chemical constituents (Table 2).

An important result of this study is 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 Point Loma effluent sample (Figure 2). 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.

The sperm test data cannot be used to determine the NOEC for many of the effluent samples because toxic effects were seen at every dilution tested. The inability to identify a NOEC for a given effluent in this study does not necessarily mean that that sample was the most toxic, since different dilutions were tested in many cases. The fertilization percentages show that the Oxnard and Encina effluents were among the least toxic, even though the NOEC was not bracketed by the concentrations chosen for the test.

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 2). As a result, the relative sensitivity of effluent toxicity estimations from Microtox results is dependent upon the data analysis method. If relative toxicity is expressed in terms of the NOEC, the Microtox test is much more sensitive than the 48-h embryo test. If concentrations producing a 50% change (EC_{50}) are used to describe toxicity, the Microtox test is often less sensitive than the sea urchin sperm or embryo test.

The high sensitivity of the sperm and Microtox test methods is 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 lower than the one resulting from initial dilution in the ocean. This effluent toxicity study provides an indication of the type of results which can be expected for future monitoring programs. The sperm test is one of the methods recommended by the U.S. Environmental Protection Agency for effluent testing and will probably be used in some monitoring programs in southern California. Results from the sperm tests by Bay et al. indicate that the proposed regulations would be exceeded by most of the effluents tested because toxicity was often found at levels below those produced by the treatment plants' assigned initial dilutions, which range from 0.6 to 1.2%.

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