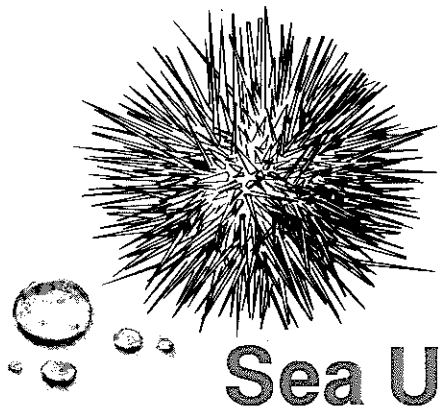

Sea Urchin Embryo Bioassay Methods For Use with Sediment Elutriates

The suitability of using sea urchin embryo and gamete test methods to assess the toxicity of sediment was evaluated during January-May of 1987. This project was funded by the National Oceanic and Atmospheric Administration (NOAA) as part of a nationwide comparison of several promising sediment bioassays. All participants in the project tested the same sediments, which were collected from sites in and around San Francisco Bay (Figure 1). The sites were selected based on previous studies to form a gradient of contamination, ranging from the control site at Tomales Bay to the most contaminated site at Oakland Inner Harbor. The general order of contamination was Oakland > Vallejo > Yerba Buena > San Pablo > Tomales Bay. The sites had various types of contamination, but high molecular weight polynuclear aromatic hydrocarbons (PAHs) were the dominant chemical group at the most contaminated sites (Table 1).

Steven M. Bay, Darrin J. Greenstein, Karen D. Englehart, and Valerie E. Raco performed SCCWRP's part of the project. Their goals were threefold: (1) to compare the biological responses between the stations, (2) to compare the results of several endpoints of the urchin bioassay, and (3) to compare the responses of three different species of urchins for one of the endpoints. Each of these goals was met with varying degrees of success.

Sea urchin embryo and gamete tests have been used at SCCWRP for many years for the testing of aqueous solutions, such as wastewater effluent and dissolved metal and organic contaminant solutions (Oshida et al. 1981). To provide an aqueous solution from the sediments, the scientists added clean laboratory seawater to the sediments and then allowed this to be stirred overnight. The sediment slurry was then centrifuged, and the overlying water (sediment elutriate) was removed for the assays.

Bay et al. used three species of urchins in this test: the purple urchin (*Strongylocentrotus purpuratus*), the white urchin (*Lytechinus pictus*), and the green urchin (*Strongylocentrotus drobachiensis*). These species were chosen to represent differences in geographic distribution and spawning season.

Four possible endpoints to the urchin assay were investigated. The first endpoint was fertilization success of sperm exposed to the elutriates. For this test, Bay and co-workers employed the methods of Dinnel et al. (1987). The second endpoint was normal embryo development after 48 h of exposure. This involved examining the embryos microscopically and rating their development. Subsamples of these embryos were also used for the third and fourth endpoints, the third being the production of the pigment echinochrome by the embryos. This test used the methods of Bay et al. (1983). The fourth endpoint was a microscopic examination of the embryos for cytologic/cytogenetic abnormalities. This part of the project was completed by Dr. Jo Ellen Hose of Occidental College using methods which she

developed (Hose 1985). Only the 48-h development test was performed on the white and green urchins.

The sperm test showed results different from what was expected based on the relative degree of contamination at each station (Table 2). The elutriate samples from the Vallejo and Yerba Buena sites had significantly greater fertilization percentages than the other stations and the laboratory seawater control samples. The 48-h urchin development test showed few differences between stations; however, embryos from the Tomales Bay station had a significantly greater number of abnormalities than the other sites. The echinochrome pigment assay showed that exposure to elutriates from the Oakland, Vallejo, and San Pablo Bay sites resulted in significantly lower pigment production than that for Tomales Bay (Table 2).

The cytogenetic analysis produced the most responsive results and also had patterns which closely followed the contamination gradient. For most of the parameters examined, embryos exposed to Tomales Bay and San Pablo Bay elutriates usually were significantly less affected than those at the other sites (Table 3). The toxicity at Tomales Bay for the 48-h development test was not encountered in cytogenetic analysis, which suggests that the mechanism for the abnormal development was due to something other than genetic damage. The results of the white urchin test were similar to those for the purple urchins, with the Tomales Bay station having the greatest percentage of abnormally developed embryos. The green urchin test was completely unsuccessful as none of the fertilized eggs developed beyond the early cleavage stage. This was probably caused by stress resulting from their shipment from the East Coast.

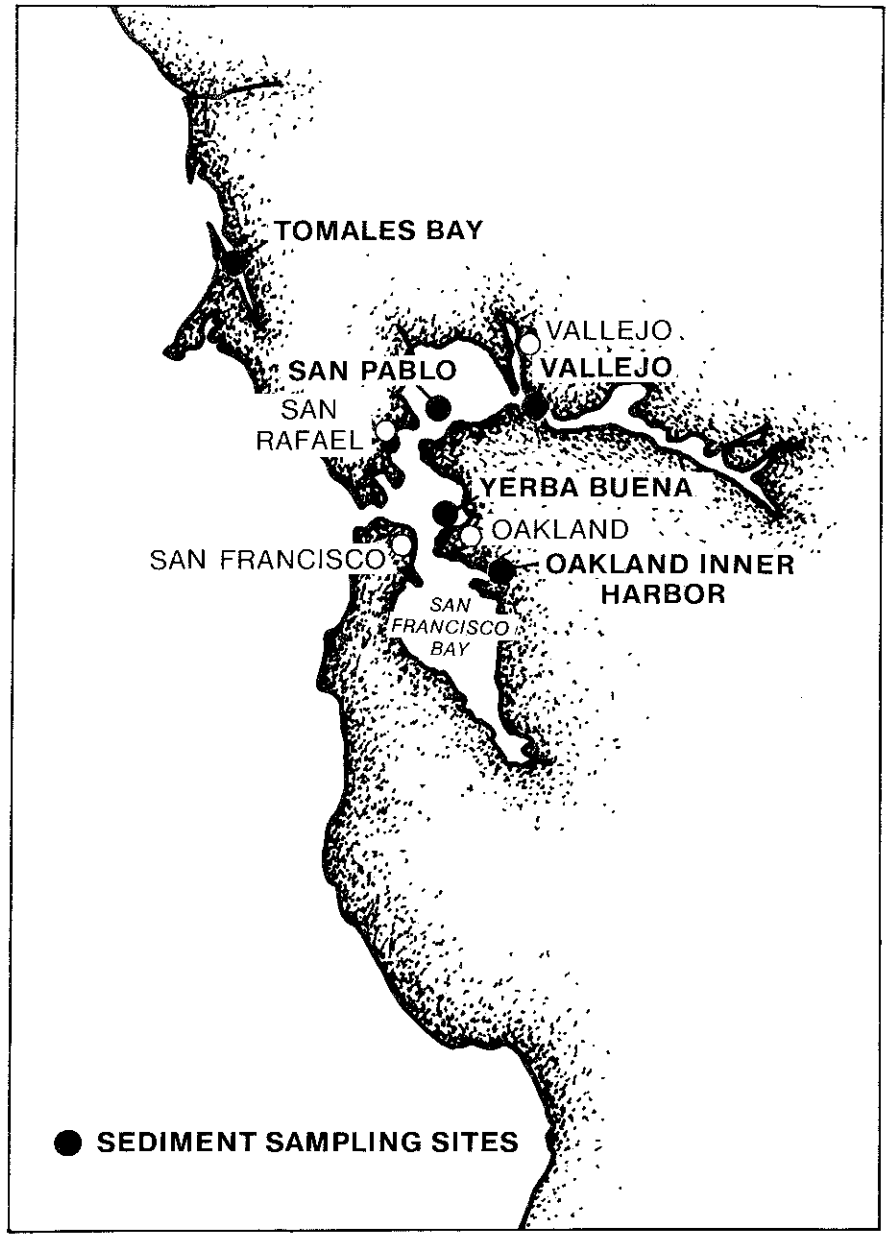

Figure 1. Locations of sediment sampling sites for this study.

One factor affecting the usefulness of an endpoint is the variability of the data between replicates. This variability was expressed as the coefficient of variation (CV, which is the standard deviation divided by the mean). For this project, the

Table 1. Chemical and physical characteristics from the five sediment sampling sites. Values are in dry weight. For each station $N = 3$.

Station	Hydrocarbons (ng/g)					Trace Metals (μg/g)									TOC[a] (mg/g)	Grain Size (%)		
	LPAH[b]	HPAH[c]	Total PCB[d]	Total DDT[e]	Other CHC[f]	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn		Sand	Silt	Clay
Tomales Bay																		
Mean	269	169	6	1	1	0.31	15.5	0.44	206	46.4	0.44	149	27.0	122	19.3	2	29	67
SE	23	22	2	1	1	0.11	1.3	0.02	30	10	0.04	24	6.8	8	0.3	<1	<1	2
San Pablo Bay																		
Mean	177	1215	23	9	4	0.61	16.6	0.29	179	57.7	0.26	116	28.0	135	12.4	4	46	47
SE	28	42	2	1	0.1	0.06	0.4	0.01	1	1.5	0.02	2	0.5	1	0.2	1	2	2
Yerba Buena																		
Mean	234	810	45	21	6	0.62	12.5	0.23	180	52.1	0.31	110	31.9	136	11.3	4	43	50
SE	17	7	23	2	1	0.03	1.4	0.01	3	5.4	0.03	5	2.2	7	0.5	2	3	3
Vallejo																		
Mean	155	462	42	24	9	0.36	18.4	0.44	182	41.6	0.34	120	53.9	126	10.2	37	31	24
SE	45	59	11	3	2	0.16	1.4	0.07	27	6.5	0.06	25	15.1	7	1.2	11	9	7
Oakland																		
Mean	600	4371	361	97	46	1.33	16.2	1.34	187	171	2.31	134	206	331	18.4	6	33	57
SE	139	623	33	26	2	0.11	0.8	0.22	3	8	0.54	2	11	24	0.5	2	4	4

[a]TOC, Total organic carbon.
[b]Sum of low molecular weight (2- and 3-ring) PAH compounds.
[c]Sum of high molecular weight (4- to 6-ring) PAH compounds.
[d]Sum of 2Cl-9Cl polychlorinated biphenyl compounds.
[e]Sum of *o,p'* and *p,p'* DDT, DDE, and DDD compounds.
[f]Sum of hexachlorobenzene, lindane, heptachlor, aldrin, heptachlor epoxide, alpha-chlordane, transnonachlor, and dieldrin.