Appendix B

TRE Case Study:
Central Contra Costa Sanitary District, Martinez, California

Abstract

TRE Goal:	NOEC ≥ 10%
Test Organisms:	Echinoderms (*S. purpuratus* and *D. excentricus*)
TRE Elements:	TIE
Toxicant Identified:	Copper
Toxicity Controls:	Pretreatment requirements

Summary

Chronic toxicity was detected in a municipal effluent with the echinoderm fertilization assay. *D. excentricus* (sand dollar) appeared more sensitive to the effluent than did *S. purpuratus* (purple urchin). A Phase I TIE was conducted using procedures described by USEPA (1988a) that were adapted to the echinoderm fertilization toxicity test. The Phase I TIE implicated cationic metals as the cause of chronic toxicity, and follow-up investigations suggested that Cu was the primary cation responsible. As part of the TIE, toxicity tests were conducted on ammonia and several cations. No observable effect concentrations for *D. excentricus* were >13.4 µg/L silver (Ag), >9.4 µg/L Cd, 3.8 to 13.1 µg/L Cu, >0.7 µg/L mercury (Hg), and 10 mg/L nitrogen as total ammonia. The data also suggested that inter-specific differences in sensitivity to Cu and ammonia exist between *D. excentricus* and *S. purpuratus*.

Key Elements

1. TIE procedures for freshwater organisms can be successfully modified to apply with the echinoderm fertilization toxicity test.
2. This study demonstrated that Cu could have accounted for the intermittent effluent toxicity observed.
3. Echinoderms exhibited comparatively high sensitivity to Cu with EC50s for both species of approximately 25 µg/L.
4. Source control measures were successful in reducing Cu concentrations by approximately 25%.

Introduction

Permit Requirements

The Central Contra Costa Sanitary District (CCCSD, Martinez, California) was required by the State Water Quality Control Board, San Francisco Bay Region, to conduct a TRE to identify the chemical constituents in their final effluent that were responsible for observed chronic toxicity in the echinoderm fertilization toxicity tests. Results of monthly compliance tests showed frequent exceedance of the discharge permit limit (NOEC ≥ 10% effluent).

Description of the Treatment Plant

The CCCSD WWTP provides secondary level treatment for combined domestic, commercial, and industrial wastewater from a 126-square mile area with a population of approximately 400,000. The treatment plant has an average dry weather design capacity of 45 mgd and currently discharges an annual average flow of 38.7 mgd into upper San Francisco Bay. Treatment facilities consist of screening, primary sedimentation, activated sludge, and secondary clarification followed by chlorination in contact basins. In the treatment process, waste-activated sludge is thickened via flotation thickeners, and lime is added to assist in dewatering with centrifuges. The combined primary and waste-activated sludge is dewatered and incinerated in multiple-hearth furnaces. The effluent TSS and BOD concentrations average <10 mg/L. Total ammonia concentrations range from 10-35 mg/L with an average of 25 mg/L.

Toxicity Identification Evaluation

General Procedures

The echinoderm fertilization toxicity tests were conducted on the final effluent according to published procedures (Dinnel, et al., 1982, as modified by S. Anderson, 1989) using the West Coast species *S. purpuratus* and *D. excentricus*. The purpose of the test is to determine the concentration of a test substance that reduces egg fertilization by exposed sperm relative to fertilization in a control solution. Two species were used in this test because the echinoderms are obtained from feral populations which are gravid at different times during the year. Effluent samples were 24-hour flow-proportional composites. Samples were screened for toxicity within 36 hours of collection. The effluent salinity was adjusted to 30% using hypersaline brine (90%), and the pH was adjusted to 8.0 ± 0.05.

Phase I TIE Studies

The results of this TIE have been published elsewhere (Bailey, et al., 1995). The Phase I TIE included the procedures described by USEPA (1988a). After completing the TIE manipulations, the effluent was salinity and pH adjusted as previously noted.

Table B-1. Summary of Results of Phase I TIE Conducted on Two Effluent Samples with *D. excentricus*

Treatment	Sample 1	Sample 2
pH 3	No effect on toxicity	Increased toxicity
pH 11	Eliminated toxicity	No effect on toxicity
Filtration	No effect on toxicity	No effect on toxicity
Aeration	No effect on toxicity	No effect on toxicity
EDTA	Eliminated toxicity	Eliminated toxicity
Sodium thiosulfate	Eliminated toxicity	Eliminated toxicity
Post C18 SPE column	No effect on toxicity	No effect on toxicity
Methanol eluate add-back	No toxicity	No toxicity

Phase I TIEs were conducted on two effluent samples. The data for both samples (Table B-1) suggested that EDTA and sodium thiosulfate were consistently the most effective treatments in reducing toxicity. Extraction of the sample with SPE columns did not reduce toxicity, suggesting that non-polar organics and weak organic acids and bases were not causes of toxicity. This conclusion is supported by the fact that elution of the columns with methanol did not yield toxicity. The effectiveness of EDTA in eliminating toxicity suggested that a divalent cation(s) was responsible for toxicity in the samples tested. The concurrent effectiveness of sodium thiosulfate in reducing toxicity suggested that the potential suite of cations was limited to Cd, Cu, and Hg (USEPA, 1991). In one case, toxicity also appeared to be increased by temporarily reducing the sample pH to 3; greater toxicity at lower pHs has been associated with Cu (Schubauer-Berigan et al., 1993).

Because the effluent samples contained moderate levels of ammonia (20–25 mg/L total ammonia), the potential contribution of ammonia to effluent toxicity was determined by comparison with ammonia toxicity tests. This approach was taken because the TIE guidelines evaluate ammonia toxicity by adjusting the pH of the test solution and preliminary data indicated that these pH adjustments adversely affected fertilization success.

Contribution of Ammonia to Toxicity

Ammonia toxicity tests were conducted in natural seawater spiked with ammonia chloride; fertilization success was evaluated using logarithmically spaced concentrations across a range of 1.0 to 100.0 mg/L N as total ammonia. Test solutions were adjusted to pH 8.0 ± 0.05 prior to exposure.

The NOECs for *D. excentricus* and *S. purpuratus* were both 10 mg/L N as total ammonia. Based on the unionized fraction, the NOECs were 0.21 and 0.17 mg/L N for *D. excentricus* and *S. purpuratus*, respectively (calculated per USEPA, 1988a). However, large differences existed between the response of the two species at concentrations higher than the NOEC. For *S. purpuratus*, the IC25 was greater than 100 mg/L N as total ammonia (1.69 mg/L N as unionized ammonia) compared with an IC25 estimate of 16.5 mg/L N (0.34 mg/L N as unionized ammonia) for *D. excentricus*. Because the upper limit of ammonia concentrations in the effluent was 25 mg/L N as total ammonia, these results suggested that ammonia alone could not account for NOECs that were ≤33% effluent, a concentration that would correspond to a maximum of 8.25 mg/L N as total ammonia.

Identification and Confirmation of the Role of Cationic Metals

Sensitivity of echinoderms to cationic metals

Once it appeared that a divalent cation was responsible for the effluent's toxicity, candidate metal ions (Cd, Cu, and Hg) and Ag were evaluated for toxicity with

D. excentricus and *S. purpuratus*. Metal solutions were prepared in moderately hard freshwater (USEPA, 1991) using reagent grade salts of Cu, Cd, and Hg. The CCCSD also was concerned about the potential for Ag to contribute to effluent toxicity; therefore, tests were performed with silver salts. Stock concentrations of metals were confirmed by either graphite furnace (Ag, Cu, and Cd) or cold vapor (Hg) AA spectroscopy (APHA, 1989). Hypersaline brine was then added (1/3 brine:2/3 metal solution) to bring the salinity to 30%, and the pHs of the solutions were adjusted to 8.0 ± 0.05 prior to exposure. This procedure was analogous to the preparation of the effluent samples prior to testing. Serial dilutions that incorporated a 50% dilution factor were made from the stock solutions to achieve exposure concentrations that bracketed those found in the effluent. The NOECs from multiple toxicity tests on Ag, Cd, Cu, and Hg with *D. excentricus* and *S. purpuratus* are summarized in Table B-2. Side-by-side comparisons between the two species are shown by the paired values in the table.

Table B-2. NOECs Obtained for *D. excentricus* and *S. purpuratus* Exposed to Different Metals*

	NOECS (μg/L)	
Metal	***D. excentricus***	***S. purpuratus***
Ag	>13.4	>13.4
Cd	>9.4 >67.0	Not tested >67.0
Cu	10.0 13.1 5.4 3.8 8.0	20.0 19.7 Not tested Not tested Not tested
Hg	>0.7 >2.2	>0.7 Not tested

* When seasonally available, concurrent tests were conducted with both species. Values given as μg metal/L (Bailey et al., 1995).

In some cases, seasonal spawning constraints precluded conducting concurrent tests with *S. purpuratus*. One comparison was conducted with Ag; the NOECs for both species were >13.4 μg/L. Two tests were conducted with Cd; in both cases the highest concentrations tested (9.4 and 67.0 μg/L) failed to produce any measurable effects on fertilization success. Five tests were performed on Cu with *D. excentricus*. The NOECs ranged between 3.8 and 13.1 μg/L with an average of 8.1 μg/L. In two of three concurrent tests with *S. purpuratus*, the NOECs were 1.5 to 2 times greater than those obtained with *D. excentricus*. In two tests with Hg, no effects on fertilization success were found at concentrations up to 0.7 and 2.2 μg/L, respectively.

Comparison of toxic concentrations of metals with concentrations found in the effluent

The NOECs for each of the metals were compared with the discharger's analytical records to determine which metals were present individually in the effluent at concentrations high enough to inhibit fertilization success. Toxicity ratios were calculated for each metal [metal concentration in effluent (μg/L) ÷ NOEC (μg/L)]. A ratio greater than 1 suggested that the metal(s) was present in the effluent at concentrations high enough to produce toxicity. Conversely, a ratio of 1, or less, suggested that the concentration of metal was ≤NOEC and, therefore, probably not directly responsible for toxicity, although some additive effects could possibly exist in combination with the other metals present.

Toxicity ratios calculated for each metal are presented in Table B-3 for *D. excentricus*. The comparatively small ratios associated with Ag, Cd, and Hg suggest that effluent concentrations of these metals were not high enough to produce the intermittent toxicity associated with the effluent. Cu was the most promising of the metals to be identified in this analysis as effluent/toxicity ratios frequently exceeded 1.

Table B-3. Comparison of Effluent Concentration of Selected Metals with NOECs Derived from Laboratory Studies with *D. excentricus*

Metal	Effluent concentration*	NOEC*	Ratio
Ag	<0.2 – 4.0	>13.4	≤0.3
Cd	<0.2	>9.4; >67	≤0.2
Cu	5.0 – 20.0	3.8 – 13.1	0.4 – 5.3
Hg	<0.2 – 0.4	>0.7; >2.2	≤0.6

* Values given as 7.5 (80.0 ± 2.0)μg metal/L.

Confirmation of the role of Cu in effluent toxicity

The next confirmation step compared fertilization success in an effluent sample against that in seawater spiked with copper sulfate ($CuSO_4$) to the same concentration found in the effluent. These exposures were conducted simultaneously using the same gametes from *D. excentricus.* Fertilization success also was evaluated in an effluent sample spiked with different concentrations of Cu, such that subsamples of the effluent contained 1, 2, and 3 times the amount of Cu (measured concentrations) as the original sample. Serial dilutions, which incorporated a 50% dilution factor, were then prepared from the unspiked and 2x spiked samples and fertilization success evaluated with *D. excentricus*. Depending on the results, it could be determined whether Cu was responsible for toxicity in the effluent. The reasoning was if Cu was the primary factor controlling toxicity, then the LOECs and NOECs obtained for the spiked and unspiked samples should be the same, based on Cu concentration. Similarly, based on percent effluent, the NOEC and LOEC associated with the spiked sample should be one dose level lower than in the unspiked sample.

The results of parallel toxicity tests with *D. excentricus* on effluent and seawater spiked with Cu at concentrations found in the effluent are summarized in Table B-4. Based on Cu concentration, the NOECs and LOECs were the same between the effluent sample and the concurrent toxicity test with seawater spiked with Cu. Furthermore, the percent fertilization was similar at corresponding Cu concentrations in both toxicity tests. These data suggested that Cu accounted for the reduction in fertilization success associated with this effluent sample. Fertilization success in an effluent sample and the same sample spiked with Cu is shown in Table B-5.

Table B-4. Comparison of NOECs, LOECs, and Percent Fertilization Obtained with *D. excentricus* Exposed to Effluent and Seawater Spiked with Cu

Treatment	NOEC*	LOEC*
Effluent	3.8 (89.3 ± 3.0)	7.5 (73.3 ± 6.1)
Seawater Cu spike	7.5 (80.0 ± 2.0)	7.5 (80.0 ± 2.0)

* Percent fertilization given in parentheses (mean ± SD).

Discussion

The data demonstrated that procedures for conducting TIEs with freshwater organisms can be successfully applied to the echinoderm fertilization toxicity test.

The results of this study suggest that Cu could have accounted for the intermittent toxicity demonstrated by the echinoderm fertilization test. Of the four metals identified in the Phase TIE, Cu was the only one that occurred in the effluent at concentrations that overlapped the toxic range. Confirmatory studies conducted with two different effluent samples also showed that Cu could account for the adverse effects observed with the whole effluent. Paired tests also suggested that Cu exhibited greater toxicity to *D. excentricus* than to *S. purpuratus*. This is important because *S. purpuratus* generally exhibited less sensitivity to the effluent.

Source control measures implemented by the CCCSD successfully reduced Cu concentrations in the effluent by 25%. This reduction made it difficult to obtain samples with sufficient toxicity to fully complete the confirmatory phase of the TIE. In fact, nearly all the samples tested at the end of the TIE failed to produce a measurable response with *S. purpuratus*.

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Table B-5. Percent Fertilization Obtained with *D. excentricus* Exposed to Effluent and Effluent Spiked with Cu*

Unspiked effluent			Effluent spiked with Cu		
Effluent (%)	µg/L Cu	Fertility (%)	Effluent (%)	µg/L Cu	Fertility (%)
0.0	0.0	96.0 ± 2.5	0.0	0.0	96.0 ± 2.5
8.4	0.8	96.7 ± 3.1	8.4	1.6	90.7 ± 2.3
16.8	1.6	97.3 ± 1.2	16.8	3.3	90.3 ± 2.3
33.5	3.3	91.3 ± 1.2	33.5 (1×Cu)	6.6	83.3 ± 2.7†
67.0 (1×Cu)	6.6	82.0 ± 4.7†	67.0 (2×Cu)	13.2	74.8 ± 2.2†
			67.0 (3×Cu)	19.8	71.7 ± 12.9†

* Fertilization data are the means and standard deviations of three replicates.
† Significantly less than controls; $p < 0.05$.

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