Toxicity Evaluation of Metal Plating Wastewater Employing the Microtox® Assay: A Comparison with Cladocerans and Fish

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Received 3 December 1999; revised 10 April 2000; accepted 4 August 2000

ABSTRACT: The relative sensitivity of the Microtox assay is closely related to the type of toxicant, and hence its utility in biomonitoring effluents is better evaluated on a case-by-case basis. The Microtox® assay, employing the marine bacterium *Vibrio fischeri*, was evaluated for its applicability in monitoring metal plating wastewater for toxicity. The results of the Microtox assay after 5, 15, and 30 min of exposure, were compared with data obtained from conventional whole effluent toxicity testing (WET) methods that employed *Daphnia magna*, *Ceriodaphnia dubia*, and the fathead minnow (*Pimephales promelas*). The Microtox assay produced notably comparable EC50 values to the LC50 values of the acute fathead minnow toxicity test (< 0.5 order of difference). The Spearman's rank correlation analyses showed that the bacterial assay, regardless of exposure duration, correlated better with the acute fish than the daphnid results ($p < 0.05$). These observations were consistent to other studies conducted with inorganic contaminants. The relative sensitivity of the 30-min Microtox assay was within the range of the two frequently used acute daphnid/fish toxicity tests. In conclusion, the Microtox assay correlated well with the acute fathead minnow data and is well suited for toxicity monitoring for these types of industrial wastes. Environ Toxicol 16: 136–141, 2001

Keywords: Microtox; plating wastewater; *Daphnia magna*; *Ceriodaphnia dubia*; fathead minnows; whole effluent toxicity

INTRODUCTION

In toxicity evaluations one test cannot replace all the other tests, because the organisms' sensitivity varies considerably depending on the type of pollutant (Wängberg *et al*., 1995). The toxicological profile of an environmental toxicant is better understood when its impact is measured by organisms that represent different trophic levels. In the majority of aquatic ecosystems, the most important trophic level in terms of energy flow and nutrient cycling is the bacteria (Ross, 1993). Hence, it is important to include representatives from this trophic level in a series of tests designed for protecting the aquatic ecosystems. The Microtox® assay, which employs the marine bacterium *Vibrio fischeri*, has been widely applied as a rapid, economical monitoring tool for toxicity of environmental contaminants. It has a considerably lower coefficient of variance than other bioassays because of the highly formalized, standardized reagents that are less susceptible to variation (McFeters *et al*., 1983).

Many comparative studies with more widely used biological testing procedures, such as acute daph-

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Contract grant sponsor: Aquatic Toxicology and Microbiology Laboratory, University of Michigan.

nid/fish tests, have been conducted to evaluate the applicability of the Microtox assay in environmental pollution monitoring (Dutka and Kwan, 1981; Lebsack *et al*., 1981; Curtis *et al*., 1982; Qureshi *et al*., 1982; Vasseur *et al*., 1984b; Miller *et al*., 1985; Toussaint *et al*., 1995; Wängberg *et al*., 1995; Sweet *et al*., 1997; Doherty *et al*., 1999). However, studies carried out with site specific wastewater have seldom been reported. In addition, analyses of the chemical composition were rarely provided (Lebsack *et al*., 1981; Sweet *et al*., 1997; Doherty *et al*., 1999). Bulich *et al*. (1981) compared the Microtox assay with acute invertebrate and fish test results derived from several species (i.e., *Daphnia*, mysid shrimp, fathead minnows, rainbow trout, bluegill, and sheepshead minnow) with varying exposure durations (24, 48, and 96 hr) to various municipal and industrial wastewaters. While the Microtox assay did not correlate well with the *Daphnia* results, a good agreement with fish tests was observed. However, it should be noted that test specifics and exposure materials might be confounding factors in generalizing a conclusion (Munkittrick *et al*., 1991). The sensitivity of a test organism is dependent on the components associated with the individual industrial sites (Vasseur *et al*., 1984b). Therefore, the utility of the Microtox assay in screening potential environmental impacts of effluents would be better elucidated by specifying the type of wastewater under investigation.

The purpose of this study was to determine the use of the Microtox assay in monitoring the toxic effect of metal plating wastes. Acute *Daphnia magna* and fathead minnow toxicity tests were concurrently conducted with the Microtox assay evaluating a total 11 metal plating wastewater samples. In addition, short-term chronic *Ceriodaphnia dubia* and fathead minnow toxicity tests were carried out with 5 samples.

MATERIALS AND METHODS

Wastewater Samples and Dilution Water

Metal plating wastewater samples ($n = 11$) were collected from two plating facilities on four separate occasions. Samples were taken from two to three points along the batch treatment process to secure a broad range of toxicant concentrations. Upon collection, the samples were transported at 4°C to the laboratory (Ann Arbor, MI). A portion of the sample was utilized for physical–chemical analyses and the remainder was used for toxicity testing. Moderately hard water (MHW) was prepared according to the U.S. EPA guidelines (1993, 1994) and was employed for the control and for dilution water of the respective exposure concentrations.

Microtox Assay and Whole Effluent Toxicity Testings

The wastewater samples were analyzed for total residual chlorine and pH, and when found present the residual chlorine was oxidized with sodium thiosulfate. The extreme pH values, if observed, were adjusted to fall between 7 and 8 by adding 1N HCl or 1N NaOH (U.S. EPA, 1993).

For the bacterial assay, the Microtox Model 500 toxicity analyzer was used. The lyophilized *V. fischeri* bacteria were obtained from Azur Environmental (Carlsbad, CA). Either the "Basic Test" or the "100% Test" protocols was utilized based on the level of toxicity (Microbics, 1992). The bacterial luminescence, the endpoint of this assay, was measured for 11 samples after 5, 15, and 30 min of exposure at 15°C.

Two acute WET tests employing *D. magna* and fathead minnows, and two chronic WET tests with *C. dubia* and fathead minnows were carried out. Daphnids were cultured and maintained in-house, but the larval fish were purchased from a commercial source (Aquatox, Hot Springs, AR). All aspects of testing were performed following the U.S. EPA guidelines (1993, 1994). Test temperature was 21 and 25°C for acute and chronic tests, respectively. Water quality parameters such as pH, temperature, dissolved oxygen, and specific conductivity of the test solutions and control were measured and recorded daily during the experimental period. The dissolved oxygen and specific conductivity were determined following American Public Health Association, American Water Works Association, and Water Pollution Control Federation standard methods (1992).

Acute tests were conducted for 11 samples; however, chronic evaluations were performed for only the first 5 samples. In addition, standard reference toxicity tests with sodium chloride were run to assure comparable sensitivities of each species over time.

Data Analyses

The median effective concentrations of the Microtox assay were determined with the "Data Collection and Reduction Software" (version 7.82, Azur, Carlsbad, CA). The E(L)C50s of the acute toxicity tests were calculated with the TOXSTAT program (version 3.5, West, Cheyenne, WY). The IC25 values derived for the chronic toxicity tests were obtained by the ICp Approach of the U.S. EPA (version 2.0, U.S. EPA, Duluth, MN). The correlation of the Microtox assay results with the other standard whole effluent toxicity tests was evaluated using the nonparametric Spearman's rank correlation analysis, and this procedure was carried out using SPSS (version 7.0, SPSS, Chicago, IL). In addi-

tion, the sensitivity of the Microtox assay was determined by the log rank comparison and by calculating of the relative sensitivity following the methods presented by Bulich (1982) and Toussaint *et al*. (1995), respectively.

RESULTS AND DISCUSSION

The results of the physical–chemical analyses for the metal plating wastewater samples are presented in Table I, and the toxicity test results are summarized in Table II. The Microtox assay detected adverse effects in samples 1, 2, 3, and 9 which increased with exposure time. This enhancement of toxicity with time is usually attributed to the delayed action of various metal species in the samples. Sample 1 contained high concentrations of copper (14.7 mg/L) and zinc (81.6 mg/L) and was the most toxic to the Microtox bacteria. The EC50 value of copper to *V. fischeri* after a 30-min exposure ranged between 0.5 and 2.0 mg/L (Vasseur *et al*., 1984a) and that for zinc was 0.7 mg/L (Miller *et al*., 1985). For samples 3 and 9, hexavalent chromium, copper, and nickel enhanced the toxicity. The hexavalent chromiums' EC50 value after a 30-min exposure of *V. fischeri* was between 16 to 58 mg/L, and 17.7 mg/L for nickel (Vasseur *et al*., 1984a; Smith C. N. C. Thesis, The University of Reading, Whiteknights, Reading, U.K., 1991).

Sample 2 exhibited toxicity to all organisms tested, while samples 6 and 7, which had higher concentrations of most metals than sample 2, were not toxic. Among the notable differences between sample 2 and samples 6 and 7 were a higher level of COD (470 mg/L) and an un-ionized ammonia concentration of 1.12 mg/L in the former sample. These may have contributed to the toxicity to the test organisms. The results of sample 9 showed substantial differences in the median effective values between the acute *Daphnia* and fish tests; however, the 30-min Microtox assay value was similar to that of the fathead minnow. This may be explained by the presence of hexavalent chromium and nickel to which daphnids are much more susceptible than fish and *V. fischeri* (Biesinger and Christensen, 1972; Pickering, 1974; Adelman *et al*., 1976; Broderius and Smith, 1979; Kaiser, 1980; Pickering, 1980; Jop *et al*., 1986; Khangarot and Ray, 1987; Munkittrick *et al*., 1991). The interpretation of toxicity data observed in sample 10 was somewhat perplexing in that acute toxicity was observed with daphnids and fish but not with V. *fischeri*. The calculated un-ionized ammonia concentrations from total ammonia were 0.41 mg/L at 21°C and 0.27 mg/L at 15°C. Although the concentration of 0.41 mg/L is lower than the median effective concentrations to *D. magna* (0.8–2.94 mg/L) and fathead minnows (1.50 mg/L), it was reasonable to assume that these may have exerted some adverse effect to both organisms. In contrast, the exposure concentrations of *V. fischeri* at 15°C contained only 0.27 mg/L of ammonia and hence had less of an effect. The EC50 value of un-ionized ammonia to the Microtox bacteria (5 min of exposure) is 1.50 mg/L (Qureshi *et al*., 1982). Sample 11 contained metals whose concentrations were comparable to those of sample 10, but it elicited no toxicity in the three species tested. The fact that the water hardness of sample 11 was more than 18 times higher than

TABLE I. The physicochemical components of various metal plating wastewater samples[a]

Sample Number	Chemical Oxygen Demand	Cr (total)	Cr^{6+}	Cu	Fe	Ni	Zn	pH	NH_3[b]	Hardness[c]
1	620	14.7	0.18	12.8	17.0	1.86	81.6	6.8	0.087	340
2	470	3.1	2.81	0.32	0.07	0.06	0.35	7.9	1.123	180
3	55	65.0	62.1	1.60	2.66	55.3	0.65	6.5	0.001	NA
4	50	0.29	< 0.01	< 0.05	0.06	0.30	< 0.05	6.4	0.001	1.2
5	< 5	< 0.05	< 0.01	< 0.05	1.34	0.07	< 0.05	7.8	0.008	240
6	< 5	0.31	< 0.01	0.36	8.59	0.70	0.44	6.3	—	288
7	5	0.40	< 0.05	0.23	10.4	1.33	2.19	7.3	—	248
8	< 5	< 0.05	< 0.05	< 0.05	1.48	0.13	< 0.05	7.8	—	232
9	< 5	103.6	99.0	3.15	6.15	10.0	< 0.5	7.1	—	952
10	110	0.21	< 3.0	< 0.05	0.28	0.08	< 0.05	8.3	0.409	12
11	5	0.13	< 0.005	< 0.05	1.31	0.10	< 0.05	8.0	—	220

[a] Concentration in mg/L, unless otherwise noted; Cd, Pb, Hg, and CN were not detected. Detection limit for Cd and Pb, 0.05 mg/L; Hg, 0.0002 mg/L; CN, 0.01 mg/L; metal concentration expressed as total metals unless otherwise noted.

[b] As the un-ionized form at 21°C and calculated from total ammonia concentrations according to Emerson *et al*. (1975).

[c] Unit - mg/L as $CaCO_3$.

TABLE II. The E(L)C50, and IC25 values with corresponding confidence intervals derived from various biochemical and biological toxicity tests[a]

Sample Number	Microtox EC50			AD EC50		AF LC50		CC IC25	CF IC25
	5 min	15 min	30 min	24 hr	48 hr	48 hr	96 hr	7 d	7 d
1	20.01	3.68	0.93	0.11	0.06	3.79	2.99	0.16	0.24
	18.98–21.10	3.22–4.19	0.78–1.10	0.07–0.15	0.04–0.09	3.01–4.78	2.38–3.75	0.16–0.17	0.12–0.92
2	70.04	62.07	51.83	> 10	7.21	63.00	56.76	3.16	13.71
	62.30–78.73	55.69–69.19	48.68–55.18		5.98–8.70	57.25–69.31	49.39–65.23	2.90–3.91	4.06–32.26
3	> 90	74.74	30.96	0.12	0.015	21.76	12.79	0.007	> 5
		56.32–99.19	28.68–33.43	0.09–0.17	0.011–0.020	19.43–24.38	11.27–14.51	0.006–0.009	
4	> 90	> 90	> 90	> 100	> 100	> 100	> 100	1.49	> 100
								0.72–2.97	
5	> 90	> 90	> 90	> 100	> 100	> 100	> 100	> 100	> 100
6	> 90	> 90	> 90	> 100	> 100	> 100	> 100	NP	NP
7	> 90	> 90	> 90	> 100	> 100	> 100	> 100	NP	NP
8	> 90	> 90	> 90	> 100	> 100	> 100	> 100	NP	NP
9	> 90	> 90	77.19	0.04	0.013	88.60	88.86	NP	NP
			62.57–95.22	0.03–0.05	0.010–0.016	77.05–102.49	77.05–102.49		
10	> 90	> 90	> 90	59.46	43.00	91.84	75.16	NP	NP
				52.13–67.82	35.90–51.50	78.62–107.28	70.88–79.71		
11	> 90	> 90	> 90	> 100	> 100	> 100	> 100	NP	NP

AD EC50 = EC50 of acute *D. magna* toxicity test; AF LC50 = LC50 of acute fathead minnow toxicity test; CC IC25 = IC25 of chronic *C. dubia* toxicity test; CF IC25 = IC25 of chronic fathead minnow toxicity test; NP = not performed.

[a] In % of metal plating wastewater.

that of sample 10 may have partially contributed to this observation. The effect of hardness in altering metal toxicities is well known. The reduction in toxicity related to hardness, as derived from a 40-hr LC50 value for rainbow trout, was estimated to be around 4 times that for copper and zinc when the hardness was increased from 10 to 100 mg/L (U.S. EPA, 1986). Also, Pickering and Henderson (1966) reported almost a 10-fold reduction in the LC50 values obtained from 96-hr fathead minnow tests with nickel when water hardness was increased from 20 to 360 mg/L.

Based on all these comparisons, the Microtox assay was not as sensitive as the acute and chronic cladoceran tests but was similar to the acute and chronic fathead minnow test results (Table II). In their review, Munkittrick *et al.* (1991) indicated that *V. fischeri* was not as sensitive to inorganic chemicals as *Daphnia* or the rainbow trout but was more comparable to the fathead minnows for some metals.

Spearman's Rank Correlation Analysis

Regression analysis could not be performed since a small residual number of data points remained after the exclusion of nontoxic samples. Therefore, the nonparametric Spearman's rank correlation analysis was applied. This procedure utilized ranks converted from the E(L)C50 and IC25 values of each toxicity test to evaluate a correlation between the Microtox assay and the other toxicity tests. The results of this analysis are shown in Table III. Regardless of exposure time, the Microtox assay exhibited a significant correlation with the acute fathead minnow toxicity tests ($p < 0.05$) and as exposure time increased from 5 to 30 min, more highly significant correlations were observed. With acute *Daphnia* and chronic fish tests, the Microtox assay showed significant correlation at 15 and 30 min of exposure ($p < 0.05$).

This result indicated that there was a low probability that the similarity in ranking observed between the data from the Microtox assay and the acute fish test could have occurred by chance ($p < 0.05$). In a study conducted with metal-rich industrial wastewater (Wängberg *et al.*, 1995), the Microtox assay (exposed for 5 and 15 min) gave a poor correlation with both *C. dubia* and rainbow trout ($p > 0.05$, based on recalculation). This, however, appeared to be attributed to the different sensitivities of test organisms to metals.

Log Rank Comparison

To evaluate the agreement of the Microtox assay with other tests in terms of toxic responses, the E(L)C50 values were separated into six classes based on one half log intervals (Bulich, 1982), and then the number of E(L)C50 values that fell within a certain range of agreement (i.e., within 0.5 order difference) were counted (Table IV). Based upon this comparison, the

TABLE III. The Spearman's rank correlation coefficients between the Microtox assay and other tests[a]

Microtox	AD 24 hr	AD 48 hr	AF 48 hr	AF 96 hr	CC 7 d	CF 7 d
5 min	0.458	0.412	0.566[b]	0.662[b]	0.112	0.688
15 min	0.594[b]	0.612[b]	0.691[c]	0.845[c]	0.410	0.895[b]
30 min	0.865[b]	0.861[c]	0.740[c]	0.896[c]	0.718	1.000[c]

AD = acute *D. magna* toxicity test; AF = acute fathead minnow toxicity test; CC = chronic *C. dubia* toxicity test; CF = chronic fathead minnow toxicity test.

[a] $n = 11$ except for 24-hr acute *D. magna* test ($n = 10$) and chronic *C. dubia* and fathead minnow tests ($n = 5$).

[b] $p < 0.05$ (one tailed).

[c] $p < 0.01$ (one tailed).

TABLE IV. Log rank comparison between the Microtox assay and other tests[a]

Results within	AD 24 hr vs Microtox			AD 48 hr vs Microtox			AF 48 hr vs Microtox			AF 96 hr vs Microtox			CC 7 d vs Microtox			CF 7 d vs Microtox		
	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min
0.5 log	8	8	9	7	7	8	10	11	10	9	11	11	1	1	2	3	3	5
1.0 log	8	9	9	8	9	9	11		11	11			1	2	2	3	5	
1.5 log	9	9	10	9	9	10							3	3	4	5		
2.0 log	9	10	11	9	10	11							4	5	5			
2.5 log	11	11		11	11								5					
n	11	11	11	11	11	11	11	11	11	11	11	11	5	5	5	5	5	5

AD = acute *D. magna* toxicity test; AF = acute fathead minnow toxicity test; CC = chronic *C. dubia* toxicity test; CF = chronic fathead minnow toxicity test.

[a] Values presented—number of data pairs.

agreement between the Microtox and acute fish test results became more evident. On average, greater than 90% of the Microtox and the acute fish data were within a 0.5 order of magnitude difference. Acute *D. magna* toxicity tests displayed a wider range of differences from the Microtox assay; however, the observed sample proportion of 60–80% fell within the range of 0.5 log. This was, however, a better agreement than the one reported by Bulich (1982). The fact that in this study a more homogeneous set of test samples (specifically metal plating wastewater) were employed could have contributed to a better agreement. Improved correlations between the bacterial assay and other acute/chronic tests were observed as the Microtox exposure increased from 5 to 30 min.

Relative Sensitivity of the Microtox Assay

The relative sensitivity of the Microtox assay was compared with the other acute toxicity tests. The mean sensitivity rank of each test result was calculated according to Toussaint *et al*. (1995). The mean sensitivity ranks with *D. magna* and fathead minnows were 1.0 and 2.6, respectively, and the value of 2.4 obtained from the Microtox assay fell within that range. The Toussaint *et al*. (1995) results showed that the relative sensitivity of the Microtox assay (5 min of exposure) fell just outside of the range. It should be noted, however, that they used various inorganic and organic chemicals ($n = 11$) including metals and pesticides, whereas a single type of wastewater was utilized for this comparison. Based on these observations with metal plating wastes, the Microtox assay was considered to have an acceptable overall sensitivity when compared to the standard acute tests.

CONCLUSION

The Microtox assay, which employs the marine bacterium *V. fischeri*, was evaluated for its applicability in monitoring metal plating wastewater. The results of the Microtox assay measured after 5, 15, and 30 min of exposure, were compared with data derived from con-

ventional whole effluent toxicity testing methods with *D. magna*, *C. dubia*, and the fathead minnows. The Spearman's rank correlation analyses showed that the Microtox assay correlated better with the acute fish and the daphnid toxicity tests with metal-rich wastewater samples ($p < 0.05$). The Microtox assay also produced good comparable results to that of acute fathead minnow toxicity test in log rank comparison (> 90% within 0.5 order of difference). These observations were consistent to other studies conducted with inorganic contaminants. The relative sensitivity of the 30-min Microtox assay was within the range of sensitivities obtained with the two frequently used acute *Daphnia*/fish toxicity tests. In conclusion, the Microtox assay correlated well especially with the acute fathead minnow tests in metal plating wastewater samples, and it appears to have utility in monitoring this type of wastewater.

The authors thank L. I. Sweet for valuable comments.

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