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Development of a Water-Effect Ratio for Copper, Cadmium, and Lead for the Great Works River in Maine Using *Ceriodaphnia dubia* and *Salvelinus fontinalis*

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The control of chemicals discharged to surface waters of the United States is an important objective of the National Pollutant Discharge Elimination System (NPDES) as specified by the Clean Water Act. The U.S. Environmental Protection Agency (EPA) recommends a combination of chemical-specific and whole-effluent toxicity limits in NPDES permits to accomplish this objective. The requirements for toxicity testing and whole effluent toxicity limitations in NPDES permits are based on the narrative toxicity water quality standards and, in some cases, numerical toxicity standards that are present in EPA regulations (EPA 1991).

The rationale for conducting site-specific studies is to ensure that water quality standards for a body of water are neither over- nor under-protective for the resident species but rather that they adequately protect the structure and function of the aquatic community. Because national water quality criteria were developed to protect the biological integrity of all surface waters in the US, they may require modification for site-specific applications (EPA 1992). As an alternative to national criteria, site-specific criteria can be tailored specifically to the local ecosystem. Site-specific factors like chemical quality of receiving water, flow, temperature and species composition play a major role in developing alternative discharge limits.

The hazard posed by a chemical when released to wastewater and consequently to a receiving system is a function of concentration, bioavailability and influence of site conditions. The site-specific evaluation of toxicity of a chemical combines a knowledge of the properties which influence the behavior of a chemical such as partitioning, chelation, speciation, adsorption to solids, and interaction with other chemicals present in the matrix and an understanding of the toxicity of the chemical and its potential for bioaccumulation.

The primary objective of this study was to develop water effect-ratios for cadmium, copper and lead which can be used to derive site-specific water quality criteria for process water discharged from a metal finishing plant. Process water at this facility is treated in the secodary treatment system and discharged to the Great Works River (Class B watershed). Although many changes and modifications have been made to the wastewater treatment operations to minimize pollutant load

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in process water, the permit issued in 1992 imposed more stringent maximum daily limits for cadmium (4.1 μg/L), copper (25 μg/L) and lead 68 (μg/L) than could be consistently met by the discharger.

MATERIALS AND METHODS

River water was collected upstream from the process-water discharge with a pump (fiberglass-reinforced thermoplastic housing) and a polyvinyl chloride (PVC) pipe. After collection, river water was transported to the laboratory in a 3400 L fiberglass tank. In the laboratory, the river water was recirculated within an epoxy-lined fiberglass reservoir prior to use. The chemical characterization of the dilution waters (reconstituted soft water and river water) is presented in Table 1.

Table 1. Results of the characterization of dilution waters.

Parameter Measured	River Water	Reconstituted Soft Water
Alkalinity as $CaCO_3$ mg/L	6-20	12-26
Hardness as $CaCO_3$ mg/L	16-28	20
Specific conductivity μmhos/cm	70-100	80-130
Temperature °C	14-16	14-16
Dissolved oxygen mg/L	8.7-12.2	9.3-11.4
pH	6.6-7.4	6.3-7.6
Aluminium μg/L	420	<100
Cadmium μg/L	<4	<0.2
Chromium μg/L	<8	<5
Copper μg/L	8.4	<1
Zinc μg/L	11.0	<5
Lead μg/L	<1	<1
Nickel μg/L	<13	<10
Silver μg/L	<0.2	<0.2
Mercury μg/L	<0.1	<0.1

The test organism, *Ceriodaphnia dubia,* was cultured in aged, soft well water. *C. dubia* cultures were fed suspensions of a mixture of the unicellular green alga *Selenastrum capricornutum*, and YCT (yeast, Trout Chow and Cerophyll) once a day. Offspring produced over the first 8-h period were used to initiate the test. Brook trout (*Salvelinus fontinalis*) 8-month old were purchased from a reliable commercial supplier. The fish were held in soft well water which was continuously pumped from an uncontaminated 100-m-deep bedrock well into the fish holding tank. The fish were maintained at 11 to 15 °C under a 16:8 h light:dark photoperiod. Fish were fed live brine shrimp (*Artemia salina*) daily.

Short-term chronic tests with *Ceriodaphnia dubia* were conducted in plastic cups (30 mL), each containing 15 mL of test solution and one daphnid, were covered with a sheet of plastic wrap and placed in a styrofoam (2.5 cm insulation board) rack in a water bath. Test conditions included a temperature of 25 ± 1 °C and 16:8-h light and dark photoperiod. Each treatment and control consisted of ten replicates each containing one daphnid. The renewal of the test solution was conducted daily by transferring the adult organisms to new test solutions. During the renewal process, each cup was examined and the number of offspring produced over a 24-h period was recorded. The *C. dubia* were fed 100 μL of *S. capricornutum*, and 100 μL of YCT suspension.

Short-term (10-day) chronic tests with *S. fontinalis* were conducted in triplicate. Test vessels were glass aquaria (36 L) containing 34 L each of test solution and 10 fish which were placed in a temperature controlled water bath. Approximately 80% of the test solution was renewed every other day. During renewal, unconsumed food and debris were pipetted from the bottom of each aquarium. Test conditions included a temperature of 15 $\pm$ 2 °C and 16:8 h light:dark photoperiod. Fish were then counted and new test solution was gently siphoned into the aquarium. Prior to renewal, the temperature of each new test solution was adjusted to 15 $\pm$ 2 °C. Fish were fed three times a day, with salmon starter. At the termination of the test, fish from each aquarium were collected with a net, rinsed with deionized water and transferred to pre-weighed aluminum pans. Fish were dried for approximately 24-h at 100 °C and weighed.

The total concencentration of each metal was measured in the stock solutions and each test chamber after the introduction of the test solution. Each test concentration was prepared independently in a separate chamber and then delivered to each aquarium. The concentration of total cadmium, copper and lead in test solutions were verified analytically with inductively coupled plasma emission spectroscopy (ICP) according to EPA (1986) methodology using AA-standards. All samples were acidified with concentrated nitric acid to pH of 2 (APHA 1989). The concentration of metals below 10 μg/L were confirmed by furnace spectrophotometery. The detection limit was 1 μg/L for each metal.

Toxicity data were analyzed using the statistical package TOXSTAT, Version 3.3, to define the no-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) according to EPA (1989). The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the limits set by the LOEC and NOEC. Also, a nonparametric technique was used for calculating the median inhibition concentration (IC50).

The water-effect ratio (WER) for each metal and species was calculated by dividing MATC or IC50 value generated from the exposures with river water by MATC or IC50 value obtained from the exposures with reconstituted soft water.

RESULTS AND DISCUSSION

The mean measured concentration of total copper in soft reconstituted water and river water during the toxicity test with *C. dubia* was 81% and 92%, respectively, of the nominal concentrations (Table 2). Analytical results revealed a small coefficient of variation of the copper concentration in both soft reconstituted water (9.5%) and in river water (8%) in test solutions during the 10-d test with brook trout (Table 2). Also, the copper concentration during the tests with brook trout showed good agreement between nominal and measured concentrations, 79% in soft reconstituted water and 78% in river water. Similarly to copper, the mean measured concentration of cadmium in soft reconstituted water and river water during the toxicity test with daphnids was 100% and 83%, respectively, of the nominal concentrations, with a coefficient of variation of 20% in soft reconstituted

Table 2. Nominal and mean measured test concentrations of copper, cadmium and lead in $\mu g/L$ measured during the short-term, static-renewal exposures with *Ceriodaphnia dubia* and *Salvelinus fontinalis*.

Reconstituted Soft Water						River Water					
Nominal			Measured			Nominal			Measured		
Cu	Cd	Pb	Cu	Cd	Pb	Cu	Cd	Pb	Cu	Cd	Pb
Test concentrations from exposures with *Ceriodaphnia dubia*											
1	1	25	1	1	17	5	10	25	10	11	36
2.5	5	50	2	5	51	10	20	50	13	19	49
5	10	100	4	10	99	20	40	100	19	39	120
7.5	20	200	6	19	170	40	80	200	37	65	150
10	40	400	8	41	350	80	160	400	64	124	340
Test concentrations from exposures with *Salvelinus fontinalis*											
50	1	800	36	1	300	100	5	800	79	4	450
100	2	1600	75	2	900	200	10	1600	160	8	900
200	5	3200	158	5	1700	400	20	3200	312	17	2000
300	7.5	5000	242	8	3200	600	40	5000	438	31	3400
400	15		320	18		800	80		646	62	
							160			132	

water and 11% in river water. Also, the cadmium concentration during the 10-day tests with brook trout showed good agreement between nominal and measured concentrations, 90% in soft reconstituted water and 124% in river water. The mean measured concentration of lead in soft reconstituted water and river water during the toxicity test with daphnids was 89% and 90%, respectively, of the nominal concentrations. The measured lead concentrations during the 10-day tests with brook trout were on average 58% and 64% of the nominal concentrations in soft reconstituted water and in river water, respectively.

The results of short-term chronic toxicity tests with *C. dubia* and *S. fontinalis* are summarized in Table 3. Invertebrates were more sensitive to copper, cadmium and lead than brook trout in both reconstituted soft water and river water. The largest gap in response between those two species was noticed with lead, while both species response to cadmium showed the smallest difference. Both species, were equally sensitive to cadmium in soft reconstituted water, while daphnids were two times more sensitive than brook trout to cadmium in river water. In general, reproduction of *C. dubia* was a more sensitive endpoint than survival with the metals tested. The response of brook trout was not so uniform; growth was more sensitive than survival with copper and less sensitive with cadmium and lead.

Table 3. Summary of chronic values calculated from the short-term chronic tests with *Salvelinus fontinalis* and *Ceriodaphnia dubia*. All values are in $\mu g/L$.

Species	Type of Water	Metal	Endpoint	NOEC	LOEC	MATC	IC50
C. dubia	Soft Water	copper	survival	4	6		
			reproduction	4	6	5	5
C. dubia	River Water	copper	survival	19	37		
			reproduction	10	14	11	15
S. fontinalis	Soft Water	copper	survival	75	158		
			growth	75	158	109	187
S. fontinalis	River Water	copper	survival	312	438		
			growth	79	160	112	292
C. dubia	Soft Water	cadmium	survival	19	41		
			reproduction	10	19	14	23
C. dubia	River Water	cadmium	survival	19	39		
			reproduction	11	19	15	19
S. fontinalis	Soft Water	cadmium	survival	8	18	12	14
			growth	18			
S. fontinalis	River Water	cadmium	survival	62	132	91	110
			growth	132			
C. dubia	Soft Water	lead	survival	99	170		
			reproduction	51	99	71	107
C. dubia	River Water	lead	survival	150	340	226	200
			reproduction	150	340		
S. fontinalis	Soft Water	lead	survival	1700	3200	2332	>3200
			growth	3200			
S. fontinalis	River Water	lead	survival	2000	3400	2608	1200
			growth	3400			

The mean WER for copper, cadmium and lead was 2, 4 and 3, respectively. The MATC or IC50 values for these metals calculated from the short-term chronic tests with both species did not demonstrate a significant difference between the soft reconstituted water and river water, with the exception of the MATC and IC50 values calculated for cadmium from the short-term chronic tests with *S. fontinalis.* The WER for each metal was not large enough to make an adjustment to criteria based on current Indicator Species Procedure (EPA 1984).

The toxicity tests conducted as a part of the NPDES biomonitoring prior to this study indicated that process water was slightly toxic to *C. dubia* and *S. fontinalis.* Survival of daphnids and brook trout was not affected at any test concentration, while daphnid reproduction and brook trout growth were statistically lower (α 0.01) in the whole (undiluted) sample compared to daphnid reproduction and brook trout growth in the controls. This residual toxicity was associated with cadmium and copper (Jop et al. 1992). Although process water was slightly toxic to daphnids and brook trout, the NOEC concentration exceeded over 5 times the chronic value and chronic toxicity was not present within the initial mixing zone.

Therefore, the emphasis in the ecotoxicological program was shifted from compliance with toxicity limit to compliance with permit limitation for copper, cadmium and lead.

The results generated during this testing program did not allow a change in the current limits for copper, cadmium and lead due to limitations in current EPA guidelines (EPA 1984; EPA 1992), but allowed an estimate of safe limits for these metals in river water. For example, the MATC value for cadmium in river water was 15 μg/L for daphnids and 91 μg/L for brook trout, which is 3 times (daphnia) and 14 times (brook trout) the proposed daily limit for cadmium discharge. The proposed daily limit for lead is only 8.7 μg/L, while the MATC for daphnia and brook trout in river water was 226 μg/L and 2600 μg/L, respectively.

The application and usefulness of the single-species acute or chronic battery of testing proposed for predicting the impact of the wastewaters on ecosystem structure and function is severely limited. For New England surface waters which are typically low in hardness the "Water-Effect" ratio is not expected to provide significant relief from the National Criteria. More importantly, development of local criteria must integrate an understanding of the biological conditions in the receiving water and discharge criteria which minimize the potential for chronic effects. The absence of any consideration for an ecological census of receiving water conditions, e.g., EPA's rapid bioassessment procedures, or acknowledgement of the importance of demonstrating chronic no effect levels certainly restricts the utility of the site-specific water quality criteria development.

The definition of an acceptable level of metals for an ecosystem depends on the criteria used for determining the ecological significance of changes that may be created from the discharge conditions. If the magnitude and extent of the ecological effects increase gradually over time it could be very difficult to specify a point where the effects become large enough to warrant protective measures. For most metals the most sensitive species are within one order of magnitude more sensitive than other species. Review of the literature for copper effects indicates that acute toxicity values for 45 freshwater species spanned a 200-fold range for copper concentrations, but values for more than half of the species were within a factor of 25 of the lowest extreme. The highest chronic value was only five times the lowest (EPA 1985). This is an important distinction not recognized by using simply toxicity test results for the proposed new criteria (EPA 1994). The focus on the differences observed in short-term effects appears to place too great a weight on hypotheses and insufficient weight on the value of empirical evidence from the receiving water. Often, it can be demonstrated that despite exceeding water quality criteria values, a discharge has no measurable impact on the organisms inhabiting the receiving water. The results derived from this testing program indicated that the concentrations of metals present in process water were substantially below the toxic threshold level for daphnia and brook trout. It can be assumed, therefore, that organisms living in the water column are not affected by the discharge due to a sufficient safety margin. Bioassessment of the resident bottom fauna and sediment toxicity could define long-range changes due to process-water discharge and assessed whether accumulation of metals in sediment has a biologically measurable affect.

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