Development of a Copper Criteria Adjustment Procedure for Michigan's Upper Peninsula Waters

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EXECUTIVE SUMMARY

Results of laboratory tests and water quality monitoring revealed that elevated copper concentrations in several Upper Peninsula (U.P.), Michigan streams, were not associated with toxic effects, or adverse impacts on resident aquatic macroinvertebrate or fish communities. These results suggest that Michigan's current copper standard may be overprotective for streams and rivers in the U.P., and perhaps in other State waters as well. This research program was designed to develop a copper criteria adjustment procedure for U.P. waters using a scientifically defensible approach that accounts for site-specific conditions. The program goal was to identify water quality parameters that mitigate copper toxicity and employ them in the derivation of site-specific copper criteria for U.P. waters. Recent research indicates that dissolved organic carbon may influence copper toxicity more than water hardness. Therefore, we selected U.P. rivers and streams with a wide range of water hardness (range: 15 - 213 mg/L $CaCO_3$) and dissolved organic carbon (DOC) (range: <1 – 30 mg/L). Water was collected from 18 sites in 17 different waterbodies (15 rivers and streams; two lakes) for chemical analysis and water-effect ratio (WER) determination using 48 h static exposures to *Ceriodaphnia dubia*. The copper biotic ligand model (BLM) was also applied to the data to determine the appropriateness of this draft USEPA criteria development model for Michigan U.P. waters. A smaller subset of the 18 original sites spanning the full range of water hardness and DOC concentration in the U.P. were sampled seasonally to examine temporal influence on WERs. The data indicate that: i) a single standard for copper in the U.P. is not appropriate; ii) copper toxicity in U.P. waters is highly dependent on DOC concentration; iii) copper toxicity in U.P. waters is poorly correlated with water hardness (also alkalinity and pH); and iv) the copper BLM consistently overestimates observed LC50 values and WERs in U.P. waters. Modification of Michigan's copper standard at any given U.P. site appears to be best achieved by linear graphic interpolation of the WER from measured DOC concentrations.

INTRODUCTION

The State of Michigan's Water Quality Standards (MWQS) program establishes water quality criteria, which form the basis for wasteload allocations in National Pollutant Discharge Elimination System (NPDES) permitting. Results of laboratory tests and water quality monitoring revealed that elevated copper concentrations in several Upper Peninsula (U.P.), Michigan streams, were not associated with toxic effects, or adverse impacts on resident aquatic macroinvertebrate or fish communities. These results suggest that the State's current acute copper standard (7.285 µg/L at a water hardness of 50 mg/L, expressed as $CaCO_3$) may be overprotective for a number of the streams and rivers in the U.P., and perhaps for other rivers and streams in Michigan as well. From a regulatory viewpoint, the State believes it is reasonable to investigate the acceptability of alternative, water quality-based procedures for modifying the State's copper criteria.

The current water quality standard for copper in Michigan is expressed as a function of water hardness. Historically, the preponderance of laboratory investigations has shown that dissolved metals in clean laboratory water are affected by cationic competition (e.g. calcium and magnesium), reducing metal availability and resultant toxicity to aquatic life (Erickson et al. 1996, Playle et al. 1993, Pagenkopf 1983, Naddy et al. 2003). Throughout the scientific, regulated and regulatory communities, however, there is increasing awareness that the toxicity of copper (and several other divalent metals) is also affected dramatically by other water quality parameters, especially suspended solids and organic ligands (Hyne et al. 2005, Luider et al. 2004, Ryan et al. 2004, De Schamphelaere et al. 2004, Schwartz et al. 2004, Villavicencio et al. 2004). When metals are complexed (bound to suspended solids and organic ligands), their availability and resultant toxicity to aquatic life is greatly reduced (Ma et al. 1999, MacRae et al. 1999, McGeer et al. 2002, Pagenkopf et al. 1974).

Modification of aquatic life criteria values on a site-specific basis to reflect local environmental conditions is permitted under Subrule (r)(ii) of R323.1057 (Part 4 Rules) of the Michigan Administrative Code. Under this subrule, site-specific aquatic life values may be derived using the recalculation, water effect ratio (WER), or resident species procedures. The WER procedure in particular accounts for the effects of site water quality variables (e.g., water hardness, dissolved organic carbon, alkalinity, pH) on metal bioavailability (Niyogi and Wood 2004, Welsh et al. 2000). It has been successfully used to develop site-specific standards at a number of sites in the United States. One of the most visible examples involved the New York/New Jersey Harbor Estuary, where EPA (in cooperation with the states of New York and New Jersey) developed a site-specific copper criterion for the entire Harbor estuary, including the tributaries (SAIC 1993). Similarly, because many U.P. streams and lakes have high organic ligand concentrations and moderate water hardness concentrations, there is good reason to believe that the potential copper WERs for these waterbodies will exceed 1.0.

By using the underlying principles associated with the WER procedure, it may be possible to develop adequate site-specific water quality criteria for copper in the Michigan U.P. Such approach would protect aquatic communities while minimizing unnecessary economic impacts. To date, as many as twenty individual site WERs have been developed or attempted for nine facilities/waterbodies in the U.P. The reported final WER values range anywhere from 2.1 - 19 (personal communication, William Dimond, Michigan Department of Environmental Quality, July 20, 2004).

The objective of this project was to conduct aquatic toxicity testing and water quality sampling/modeling to evaluate the bioavailability of copper in U.P. surface waters, and develop a copper criterion adjustment procedure for the entire region. The availability of a region-specific water quality criteria adjustment procedure for copper would greatly improve efficiency of water pollution protection activities, including: NPDES permits, remedial action plans, 305(b) reports, municipal sewer use ordinances and total maximum daily loads. Without a regional copper criterion modification procedure, U.P. dischargers are forced to develop individual WERs for their receiving waters. This alternative represents a considerable financial burden, especially for small municipalities which discharge to U.P. rivers and streams. Establishment of an environmentally-realistic copper criteria adjustment procedure for the U.P. would also help reduce any unnecessary economic and social impacts caused by NPDES permit limits which do not reflect the true bioavailability of copper in the site water. This research was performed to determine if a cost-effective, reasonable water quality criteria adjustment procedure can be developed for receiving waters in the Michigan U.P.

METHODS

Sampling of Natural Waters

Prior to water collection and sample analysis, historic water chemistry data archived for a wide spectrum of U.P. streams and rivers (via USGS NAWQA, USEPA STORET, and MDEQ) were evaluated to determine regional concentration profiles for organic carbon and water hardness. These data were used to identify low, medium and high concentration ranges for the two parameters to facilitate initial water chemistry sampling and preliminary WER assessment via a modified WER procedure (limited copper analysis). Based on the initial sampling and WER assessment, a set of core sites, representative of the full range of conditions in the U.P. (Figure 1), was subsequently selected for definitive toxicity testing and BLM analysis during low-flow conditions (August 2005). A smaller subset (N=6 sites) was selected for seasonal (temporal) assessment. Table 1 provides site descriptions and other pertinent geographical information for the 15 rivers and two lakes (N=18 sites total) sampled in the study.

Water samples (grab) were collected using "clean-hands" techniques (U.S. EPA 1996). Prior to sample collection, all sample equipment (stainless steel bucket, plastic funnel and ice scoop, 500 mL amber glass bottles, and 4L polyethylene cubitainers) were cleaned, acid washed, and rinsed three times with deionized water. The sample containers were labeled with the sample location name and number immediately prior to sample collection. The sample collection staff used powder free latex gloves while collecting the samples. All sampling equipment was thoroughly rinsed with site water three times prior to sample collection, and then rinsed three times with deionized water before leaving the sample location. The labeled sample cubitainers were placed in coolers with ice (8-16 pounds) immediately after collection and shipped to GLEC in Columbus, Ohio via overnight courier for acute toxicity testing and preparation for chemical analysis.

Water Chemistry Characterization

The total hardness of the laboratory and site waters was determined by ethylene diamine tetra acetic acid titration (U.S. EPA Method 130.2). Alkalinity was determined by electrometric titration (U.S. EPA Method 310.1). The pH was determined using an Orion pH Meter Model 230A, calibrated daily to pH 4, 7, and 10. Temperature was determined using a Dual J-T-E-K thermocouple thermometer, Model 600 (Barnant Co.).

All analyses for anions, cations, copper, organic carbon, sulfide and suspended solids were performed by Underwriters Laboratories, South Bend, IN. Laboratory and site dilution waters were filtered and preserved (as appropriate) then shipped on ice via overnight courier prior to analysis. Water samples for analysis were always shipped within 1-3 days of sampling to ensure adherence to holding times. A summary of the methods and corresponding detection and reporting limits for key water quality parameters is provided in Table 2. All recovery and accuracy values reported herein are presented as percent deviation from true value.

Major cations (calcium, magnesium, potassium and sodium) in laboratory and site dilution waters were determined by inductively coupled plasma atomic emission spectrometry (U.S. EPA 1991, revision 3.3 Method 200.7), with a Varian Vista MPX, and argon as carrier gas. The standard calibration solution and reference material used was from Inorganic Ventures (1000 mg/L). Recovery was within ± 30 percent of reference. Instrument accuracy was 10 percent, with precision estimated as relative standard deviation <20 percent. The relative percent difference of duplicate samples was also <20 percent. Anion (chloride and sulfate) concentrations were determined by ion chromatograph (U.S. EPA 1993, revision 2.1 Method 300.0), using an ion chromatograph (Dionex DX-500) with helium as carrier gas. The standard calibration solution was as above, as were instrument accuracy, precision and duplicate results. The reference material was from Spex (1,000 mg/L). Recovery was within ± 20 percent. Total suspended solids were determined by gravimetric analysis, APHA method 2540D. Reference material used was from Spex (100 mg/L). Recovery was within ± 10 percent. Instrument accuracy was 10 percent, with precision estimated as relative standard deviation <20 percent. The relative difference of duplicate samples was <20 percent.

Test waters were analyzed for total and dissolved copper at test initiation and again for dissolved copper at the end of the experiments. Test waters were acidified to pH 2 to 3 with ultrapure nitric acid (J.T. Baker, Ultrex II ultrapure reagent) before analysis by inductively coupled plasma mass spectrometry (U.S. EPA 1991, revision 4.4 Method 200.8), using a Varian Ultramass and argon gas as carrier. The standard calibration solution and reference material used was from Inorganic Ventures (10 mg/L). Recovery was within ± 30 percent of reference. Instrument accuracy was 10 percent, with precision estimated as relative standard deviation <20 percent. The relative percent difference of duplicate samples was also <20 percent. Dissolved organic carbon was determined by UV persulfate, APHA method 5310C, using a

Tekmar/Dohrmann Phoenix 8000 and nitrogen gas as carrier. The standard calibration solution used was Inorganic Ventures (1000 mg/L). Reference material was from LabChem (1000 mg/L). Recovery was within ± 10 percent of reference. Instrument accuracy was 10 percent with precision estimated as relative standard deviation <20 percent.

Test waters for dissolved copper and organic carbon analyses were filtered in the laboratory prior to shipment to Underwriters Laboratories using Pall sterile Acrodisc 25 mm syringe filters with 0.45 um Supor® membranes fitted to acid-washed and deionized water-rinsed 60 ml siliconized syringes (VWR). To gain a better understanding of the humic substances that comprise the aromatic fraction of the DOC in U.P. waters, specific ultraviolet absorbance (SUVA) at 254 nm wavelength and normalized for DOC concentration was measured following methods described in Weishaar et al. (2003). Briefly, UV-visible absorbance measurements were performed on a Varian Cary 100-Bio UV-Vis spectrophotometer with distilled water as the blank. Measurement of the distilled water was made every 7-8 readings to ensure instrument stability. Duplicate samples were made to ensure precision. A quartz cell with 1.0 cm path length was used. Samples were allowed to warm to room temperature before measurement.

Toxicity Assays and *Ceriodaphnia* Cultures

Acute toxicity tests were conducted using EPA methods (U.S. EPA 2002). *Ceriodaphnia dubia* (< 24-h old) were tested in 48-h static exposures. For each test, 20 animals were exposed to each of several copper concentrations (five or eight for laboratory and site water tests, respectively), and a control. Each assay was carried out using four replicate plastic beakers of 30 ml capacity with 25 ml of assay media under controlled conditions (temperature 25°C ± 1°C; photoperiod 16 h light and 8 h dark: 10-20 $\mu E/m^2/s$). The number of affected daphnids in each test vessel was monitored at 24 and 48 h. Dissolved oxygen, pH, and temperature were monitored in each concentration at 0, 24, and 48 h of exposure. Conductivity was monitored at the beginning and end of the test. Routine water chemistry measurements were taken from a separate replicate to prevent contamination from probes.

Moderately hard reconstituted water (MHW) was used as dilution water for the laboratory water exposures. Laboratory MHW was prepared as in US EPA (2002). The base water used to prepare the reconstituted waters was ultra-pure deionized (Millipore Milli-RO® 20) City of Columbus, Ohio tap water. Reagent grade salts (Mallinckrodt Baker, Inc.) were added in the appropriate amounts to the deionized water and mixed at room temperature (approximately 22°C).

Reagent grade cupric sulfate, five hydrate (A.C.S grade, 98.5% pure, Fisher Scientific; Lot No. 045279) received January 21, 2005 was used to make stock and test solutions in all definitive side-by-side laboratory MHW and site water toxicity tests for WER determinations. Stock solutions were prepared by dissolving cupric sulfate in deionized water the day the WER tests were initiated. A solution of the highest test concentration was prepared by adding an appropriate volume of the cupric sulfate stock solution to a measured volume of laboratory MHW or site water, and mixed. This highest copper-spiked test solution was allowed to equilibrate for 1.5 to 2.5 hours before being serially diluted with un-spiked laboratory MHW or site water using a 0.6X dilution factor. These diluted test solutions were then allowed to equilibrate another approximately 2 hours before initiating the tests.

Stock cultures of *C. dubia* used in the study were originally obtained from Aquatic Bio Systems of Fort Collins, Colorado. *C. dubia* were cultured in 30 ml vessels containing 1 adult and 20 ml of natural source water (The Ohio State University wetland or quarry water), and maintained in environmental chambers under controlled conditions (temperature 25°C ± 1°C; photoperiod 16 h light and 8 h dark: 10-20 μE/m^2/s). Three times a week, cultures were transferred to fresh water containing 2.0 ml of a yeast/trout food/Cerophyl (YTC) food suspension (see U.S. EPA 2002 for preparation), and 2.5 ml of 2.3 x 10^8 cells/ml of the green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), per liter. The days the culture water was not changed, each vessel received 1 drop each of the YTC suspension and algae. Survival and reproduction of culture animals were monitored, and general water chemistry measurements (dissolved oxygen, pH, temperature and specific conductivity) were made and recorded every time culture water was changed. Test animals were obtained from cultures where survival of culture animals exceeded 80 percent, and which produced at least three broods per female. Twenty-four hours prior to test initiation, all young were removed from the culture chambers to ensure that only daphnids $\leq$ 24-h old would be available to initiate the tests.

An LC50 based on the number of dead specimens (as determined by gentle prodding) was determined at 48 h of exposure. Dissolved copper concentrations used for calculating LC50 values were the geometric mean values of test water samples taken at time t=0 and +48h. The LC50 values were calculated using Probit (version 1.5), or the Trimmed Spearman-Karber (TSK) Program, version 1.5 (U.S. EPA-Cincinnati) with automatic trim, or estimated via graphical interpolation following the data analysis hierarchy in U.S. EPA (2002). Tests were considered acceptable if 90 percent or greater of controls survived.

The acute toxicity of copper to *C. dubia* in samples of U.P. site water was measured thirty-nine times over approximately a fourteen month period beginning February 2005 and ending April 2006 (Table 3). Each site water test was run concurrently with tests of the toxicity of copper to the same cohort of *C. dubia* in laboratory MHW. An additional fifteen preliminary acute copper toxicity tests were conducted with *C. dubia* using water sampled from each site prior to the definitive WER testing (sample events initiated October 2004 and again in February and March 2005; data not shown). WER values for a given site and sampling event were calculated in the traditional manner as the dissolved site-water copper LC50 divided by the dissolved laboratory MHW water copper LC50 from tests run concurrently in the two dilution waters. The WER values were calculated with (termed "hardness-adjusted WER" hereafter) and without (termed "measured WER" hereafter) adjusting the Cu LC50 values in laboratory MHW to match the hardness of the site water, as in the Interim WER guidance (EPA 1994). For purpose of comparison, "SMAV WERs" were also calculated as the dissolved site-water copper LC50 divided by the dissolved copper species mean acute value (SMAV) for *C. dubia* (a value of 19.18 µg/L at a water hardness of 86 mg/L as $CaCO_3$), as in the Streamlined WER guidance (USEPA 2001). In addition to the above, the copper BLM (HydroQual, version ap05) was used to predict the toxicity of copper to *C. dubia* in site water and laboratory MHW water to predict WER values (i.e., BLM-predicted WER).

The BLM is a computational construct that incorporates all aspects of water chemistry that can affect the toxicity of metals in the natural environment, including complexation (DOC) and competition (hardness) reactions (see Di Toro et al. 2001 and U.S. EPA 2003 for more details regarding the technical basis for the model). The model was evaluated in this study as a possible viable, cost-effective alternative to estimate WERs for site-specific adjustment of copper criteria in the U.P. Measurements of water quality parameters for sites (Table 3) were fed into the model to predict LC50 values for the test waters. The means of all test water pH and temperature values were used for BLM LC50 prediction. BLM-predicted WER values were derived as the quotient of the predicted LC50 values in site water and laboratory MHW.

RESULTS

Chemical Characterization of Study Waters

The U.P. site waters selected for this study represent a very broad range of water quality characteristics (Table 3), and are expected to have included nearly the full range of water quality conditions present in the region. The median DOC and hardness concentrations are 7.86 (range: 1.03 - 30.2) mg C/L, and 74.0 (range: 14.7 - 231) mg/L as $CaCO_3$, respectively. Only the DOC values from Cedar Creek (Marquette County) appeared unique in composition compared to the other sites, as determined by specific UV absorbance (SUVA) at 254 nm (Table 4). pH was also relatively homogenous among the sites (range: 7.29 to 8.42 s.u.), with the differences at least partially explained by differences in calcium content and alkalinity (Figure 2, panels A and B). Thus, the U.P. region represents a very wide range of water quality conditions, with both low and high levels of the characteristics which are expected to affect copper toxicity (DOC, hardness). Sulfide and total suspended solids concentrations in these site waters were found to be extremely low, less than the method reporting limit (0.05 mg/L). Ambient total iron concentrations (mean = 0.4, range: 0.1 – 1) mg/L were not measured as part of the study, but were pooled from existing databases and reports (EPA STORET, USGS NAWQA, MDEQ) when possible.

Copper Toxicity in U.P. Waters

Dissolved copper LC50 values ranged from a low of 6.26 µg/L in ambient Cedar Creek water (Marquette county, February 2006 sample; DOC = 0.92 mg C/L), to a high of 210 µg/L in ambient Hudson Creek water (Mackinac county, August 2006; DOC = 30.2 mg C/L) (Table 5). The mean (geometric, N = 18) copper LC50 value in moderately hard reconstituted dilution water (average DOC <0.5 mg C/L) was 7.29 µg/L.

The results of toxicity testing clearly exhibited the expected relationship between copper LC50 and DOC concentrations (Figure 3). As DOC increased, copper toxicity decreased. The relationship held over the broad spectrum of geology represented by the various sample sites, between waterbody types (streams versus lakes), and different land uses (agricultural, logging, mining). The relationship also held within individual sites, when the DOC and toxicity varied temporally with season (low and high water flow). Over the concentration range of 1 to 30 mg C/L, most of the variation in copper LC50 is explained by the following linear regression equation: Cu LC50 = 7.4799[DOC] + 1.9916 ($r^2 = 0.867$, d.f. = 37, $P < 0.001$). Surprisingly, however, copper toxicity did not decrease at higher water hardness levels (Figure 4). In fact, the very poor correlation between water hardness and copper toxicity further substantiates the predominance of DOC in determining copper bioavailability in these waters. Only at the lowest DOC concentrations (<5 mg C/L) does a very weak positive trend between water hardness and copper LC50 become manifest (Figure 4c).

Figure 5 shows the temporal (seasonal) variation in copper LC50 values among select sites. In all cases, the highest LC50 values were associated with the highest DOC concentration. Typically, the DOC concentration measured at a given site was greatest in early spring (two to three weeks after snowmelt), while the opposite was true in mid to late summer. Notable exceptions were McDonald Creek and Hudson Creek (Figure 5). DOC values also varied seasonally within sites by as much as two- to three-fold (Figure 5).

Predictive Performance of Freshwater Copper BLM

A comparison of BLM-predicted and experimentally-determined (measured/observed) LC50 values indicates that the BLM consistently overestimated observed LC50 values (Table 5). The BLM overestimated the actual LC50 value for every site tested. The relationship between BLM-predicted LC50 and DOC is characterized by the following linear regression equation: BLM-predicted LC50 = 13.87[DOC] + 42.41 (r^2 = 0.4501, d.f. = 37, P =<0.001). The equation indicates that both the slope (factor of 2 times) and intercept (factor of 21 times) are substantially higher than what is observed for measured LC50 values. Log-log plots of BLM predicted versus observed LC50 values are shown in Figure 6. The solid line represents the theoretical line of equality, and the parallel (dashed) lines indicate when the BLM predicted values are within 0.5 and two times the observed values (i.e., factor of two in each direction). The BLM overestimated the toxicity of copper to *C. dubia* by an average 2.8 times, and a maximum 7.4 times. It is worth noting here, however, that the mean BLM-predicted LC50 values for *C. dubia* in laboratory MHW (11 µg/l) was much closer to observed values (mean = 7.3 µg/L); and thus, gross overestimation was an issue only in U.P. waters.

Copper WER Estimation Using DOC

The several WER values calculated using the traditional Interim and Streamlined WER guidance, as well as those predicted using the BLM, are provided in Table 5. As expected, measured WER values were also highly correlated with DOC (Figure 7), but not with hardness. The median measured WER value was 8.70 (range: 1.11 to 34.3), whereas the median hardness-adjusted WER value was 7.48 (range: 1.20 to 59.1). Conversely, the median SMAV WER value decreased compared to the median measured WER value by a factor of over two to 3.48 (range: 0.355 to 24.1), while the median BLM-predicted WER value increased by a factor of two to 15.5 (range: 0.969 to 102). The graphical linear relationships between DOC and traditional hardness-adjusted WERs, streamlined SMAV-calculated WERs, and BLM-predicted WERs are presented in figures 8, 9, and 10, respectively. Each of these latter WERs match hardness of the laboratory MHW and site water (traditional hardness adjusted and streamlined SMAV WERs), or functionally incorporate the competitive interactions of calcium and magnesium ratios in the waters to predict toxicity (BLM-predicted WERs).

DISCUSSION

The purpose of this study was to develop a site-specific water quality criterion adjustment procedure for copper to improve the efficiency of water pollution regulations for the Michigan U.P. Historically, divalent metal criteria such as those for copper have been adjusted considering only the relationship between water hardness and toxicity, without consideration of other water chemistry variables (e.g., alkalinity, pH, specific ions), in particular, natural organic matter. In recognition of this fact, EPA began publishing procedures for establishing site-specific water quality criteria for metals and other chemicals based on species composition and water quality characteristics unique to a given site (U.S. EPA 1984, 1992, and 1994). By far the most common procedure developed for establishing site-specific water quality criteria for metals in general, and for copper in particular, is the water effect ratio (WER) procedure. The WER procedure addresses the effects of site water chemistry on the national/state criteria, which is the focus of this work. The WER procedure is also currently the only procedure for obtaining a reasonable site-specific criterion for copper in the presence of elevated dissolved organic matter (Bergman and Dorward-King, 1997). We show in this study that dissolved organic matter measured as DOC is the only water quality characteristic of significance for adjusting the water quality criteria for copper in the U.P.

Acute Copper Toxicity in U.P. Waters with Varying DOC and Hardness

The U.P of Michigan encompasses a mix of substrates from the sandstone ridges of the western mineral (and copper)-rich mountains, to the swampy flats and limestone substrate of the eastern U.P. DOC and hardness values vary widely across the U.P, ranging from <1 to upwards of 30 mg/L DOC, and from 15 to 213 mg/L hardness as $CaCO_3$. In these waters, DOC can vary by more than two-fold within the same site when measured on a seasonal basis. Under this range of conditions, the acute toxicity of copper to the cladoceran *C. dubia* also varies widely between sites and seasonally, within sites, with measured dissolved copper LC50 values ranging from 6.26 to 209 µg/L. Given such a large variation in toxicity both between and within sites, a copper criteria adjustment using a single (average) WER value or even multiple (by region) values may not be practical, or scientifically defensible. Instead, it appears that copper toxicity can be predicted, and therefore regulated, using the very strong predictive relationship between U.P. site water DOC and copper toxicity determined by this study. The very strong relationship between DOC and copper LC50 holds true not only between sites (see Figure 3), but also within sites, where DOC and toxicity vary temporally with season (Figure 5). Moreover, the data indicate the relationship holds for both lentic and lotic waters as well as between sites subject to different land uses.

The competition between copper, and calcium and magnesium ions (major components of hardness) for the biotic ligands in aquatic organisms, as modeled by the BLM, did not adequately predict copper toxicity in U.P. waters, even when the DOC concentration is low (< 5 mg C/L). Similarly, alkalinity was not correlated with acute copper toxicity, indicating very little formation of copper-carbonate complexes.

A strong association between acute copper toxicity and DOC in Australian streams was recently reported by Hyne et al. (2005). They also observed a weak correlation between hardness and lethal concentrations of copper.

Limited analysis using specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) as an indicator of the chemical composition and reactivity of the DOC in U.P. waters indicates that most the site waters tested fall within a similar range of 3 to 5 $L \cdot mg^{-1} \cdot m^{-1}$ (Table 4). The exception was the ambient water from Cedar Creek (average DOC approximately 1 mg C/L), which exhibited a $SUVA_{254}$ value of approximately 2 $L \cdot mg^{-1} \cdot m^{-1}$). The low $SUVA_{254}$ value for Cedar Creek (DOC < 2 mg/L) appears inconsequential with regards to its influence on the overall relationship between DOC and copper toxicity of U.P. waters. Thus, the humic fraction of Michigan U.P. waters appears relatively uniform, and possibly of similar reactivity (given the very strong relationship between Cu toxicity and DOC among many different sites evaluated over time.

Appropriateness of Using the BLM for U.P. Waters

The version of the BLM used in this study was that recently employed by U.S. EPA to develop the draft acute criterion (CMC) for copper (U.S. EPA 2003). The BLM was selected by EPA to update its freshwater acute criterion for copper because of the ability of the model to incorporate copper speciation reactions and predict toxicity to a variety of organisms over a wide range of water quality conditions. In this study however, the BLM did not always accurately predict Cu toxicity in the U.P. waters tested, with several predictions lying well above the prediction performance standard of ± 2-fold of the observed value.

The BLM overestimated Cu LC50 values for *C. dubia* in the U.P. site waters by a factor of 2.8, with a factor of 7.4 as the high. In contrast, the BLM predicted the Cu LC50 values for *C. dubia* in laboratory MHW water to within a factor of 1.5. As a result, the median BLM-predicted WER value is over two times the median measured WER value which reflects the influence of DOC alone.

The reason(s) why the BLM overestimated copper toxicity to *C. dubia* in U.P. waters is not known at this time. One water quality parameter known to affect dissolved copper concentration in natural waters is sulfide, because of its ability to tightly complex divalent metals (Allen et al. 1993, Di Toro et al. 1990). However, the limited sulfide measurements from the ambient water samples tested in this study indicate this is unlikely in the U.P. because of the very low sulfide concentrations measured in these waters. Another possibility is high colloidal iron concentrations, which could bind free copper and occlude it from availability to the organism rendering the water less toxic than predicted (i.e., cause the BLM to overestimate the LC50 or underestimate toxicity). But iron also strongly complexes with organic matter (Davison and DeVitre 1982), which could increase free copper, and in turn, increase the toxicity of copper.

The BLM employed by U.S. EPA to develop the 2003 draft acute criterion (CMC) for copper (U.S. EPA 2003) is currently being optimized for new parameters, particularly for iron and sulfide (Robert Santore, HydroQual, personal communication). While a new BLM may improve predictions to within acceptable performance standards, use of the model does not appear necessary for Michigan U.P. waters, where DOC clearly dominates the manifestation of copper toxicity to *C. dubia*.

Copper Criteria Adjustment Procedure for the U.P.

The results of this research suggest that a WER-based copper criteria adjustment procedure for U.P. waters is both possible and relatively simple. Given that the only water quality characteristic of real significance to acute copper toxicity in U.P. waters is DOC, WERs can be estimated with a simple linear model: WER = 1.1562[DOC] - 0.5710 (Figure 7). First, the relationship given by the DOC-copper toxicity regression can be used for any U.P. site water at any time. The regression appears to function across a wide spectrum of geology, as well as seasonally within sites. The regression also appears to hold for different waterbody types (lotic and lentic) and land uses. Second, the adjustment procedure is cost-effective, requiring only the valid measure of DOC at a given site. As a conservative approach, the lowest DOC value seasonally measured for a site over at least a one year period could be employed to provide copper criteria adjustments that would be protective of that site. Alternatively, a geometric mean DOC value could be used, with or without an appropriate safety factor. In the case of gross seasonal differences in DOC, criteria could be applied seasonally. Based on the data collected in this study, there is no need to adjust copper criteria using the current convention of water hardness, or based on any other water quality characteristic, except possibly in the case of a very soft, acidic (pH <7) ambient surface water. Finally, the approach can be easily validated with relatively minor effort and minimal technical expertise.

Among the various linear relationships, the simplest and most practical copper criteria adjustment procedure for U.P. streams is between DOC and measured WER values, i.e., quotient of site water and laboratory MHW LC50s without hardness matching. The DOC regression based on measured WER values is statistically superior to others, and is not confounded by the relatively inert influence of water hardness on copper toxicity in the region. For regulatory purposes, however, DOC regression relationships based on the traditional hardness matching of laboratory or SMAV values to site water hardness given by the Interim and Streamlined WER guidance may be most practical for meeting the needs of the permitting program.

RECOMMENDATIONS

Based on results obtained in this research program, we offer the following recommendations for MDEQ's consideration:

1. Region-wide copper criteria expressed as a function of DOC (instead of water hardness) is perhaps the best option based on the study findings, although this option may be impractical because of the wholesale change in criteria required for such recommendation. Alternatively, MDEQ should seriously consider projecting copper criteria with one of the WER models reported above. The regression equation based on DOC versus measured WER values is preferred over others because it does not include the confounding influence of water hardness in the regression equation. That said, a less attractive but viable approach would employ the regression based on either the DOC versus traditional hardness-adjusted WER or SMAV WER consistent with the Interim and Streamlined WER EPA guidance. Of course MDEQ would need to consider how to best adopt the procedure to meet the needs of the permitting program, but we believe that the underlying principles of the approach are scientifically defensible.
2. Prior to adopting the procedure, MDEQ should consider evaluating the applicability of the copper criteria adjustment procedure for a secondary test species such as the fathead minnow. If the dominating influence of DOC on copper toxicity is conserved across test organisms, the realism of water quality-based copper regulation could be greatly enhanced, and both state and private sector resources could be conserved.
3. It is prudent that MDEQ validate any DOC-related criteria adjustment procedure for wastewater (municipal and industrial) prior to implementation. The DOC versus copper toxicity relationships in this report may not accurately reflect the possible different binding

characteristics of some wastewater effluents. This may explain at least some of the difference in toxicity prediction given by the BLM.

4. Finally, MDEQ should consider evaluating applicability of the copper criteria adjustment procedure for the remainder of the State of Michigan. Again, if this concept could be applied throughout the state (or in certain additional geographic regions of the state), the realism of water quality-based copper regulation could be greatly enhanced, and both state and private sector resources could be conserved.

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TABLE 1. DESCRIPTION AND GEOGRAPHICAL LOCATION OF RIVERS AND LAKES SELECTED FOR WER DETERMINATION AND DERIVATION OF A COPPER CRITERIA ADJUSTMENT PROCEDURE FOR THE MICHIGAN U.P.

Site	Source	Site I.D.	HUC	County	Latitude	Longitude
Big Garlic Creek at county road 550 section 33	MDEQ	520275	4020105	MARQUETTE	46.683	-87.5712
Cedar Creek near Sands	USGS WIS	4044570	4020201	MARQUETTE	46.4517	-87.3693
Cisco Branch Ontonagon River at USFS road 6930 section 05	MDEQ	660101	4020102	ONTONAGON	46.4089	-89.3436
Davenport Creek at US2 Hendricks twp section 2	MDEQ	490059	4060107	MACKINAC	46.0673	-85.2649
Escanaba River at Cornell	USGS WIS	4059000	4030110	DELTA	45.9086	-87.2137
Escanaba R. at Mead bridge, Wells twp. sec. 1	MDEQ	210093	4030110	DELTA	45.8064	-87.0944
Flat Rock Creek section 33	MDEQ	520255	4030110	MARQUETTE	46.2503	-87.8202
Ford River near Hyde	USGS WIS	4059500	4030109	MARQUETTE	45.7555	-87.2015
Hudson Creek at Leveille Rd.	GLEC 2	4060107	4060107	MACKINAC	45.9838	-85.7198
Manistique River above Manistique (Man.)	USGS WIS	4057004	4060106	SCHOOLCRAFT	45.9716	-86.2432
McDonald Creek @ Mosinee grade; Erwin twp., sec 25	MDEQ	270159	4020101	GOGEBIC	46.3602	89.9952
Menominee R. left 1/3 at 26th St in Menominee	MDEQ	550038	4030108	MENOMINEE	45.1063	-87.6356
Monocle Lake just west of Sault Ste. Marie in Mills twp	GLEC 3	4020203	4020203	CHIPPEWA	46.4694	-84.6414
Six Mile Lake	GLEC 4	4030108	4030108	MARQUETTE	46.0223	-87.9074
Sturgeon River near Chassell	USGS	4043004	4020104	HOUGHTON	46.9784	-88.5237
Tahquamenon River near Paradise	USGS WIS	4045500	4020202	LUCE	46.575	-85.2696
Tioga River at US 41 state roadside park section 8	MDEQ	70070	4020104	BARAGA	46.5753	-88.3407
Trap Rock River near intersection of Valley Rd and Wood Bush	GLEC 1	4020103	4020103	HOUGHTON	47.2716	-88.3606

TABLE 2. METHODS AND METHOD DETECTION AND REPORTING LIMITS FOR CHEMICAL ANALYSIS OF SAMPLES SELECTED FOR WER DETERMINATION AND DERIVATION OF A COPPER CRITERIA ADJUSTMENT PROCEDURE FOR THE MICHIGAN U.P

Analyte	Method	Analysis by	Method Detection Limit	Method Reporting Limit
Copper	EPA 200.8	ICAP-MS	0.273 ug/L	1 ug/L
Dissolved Organic Carbon	APHA 5310C -filtered with EPA 200 series	UV Persulfate	0.0498 mg/L	0.25 mg/L
Total Suspended Solids	APHA 2540 D	Gravimetric		10 mg/L
Chloride	EPA 300.0	Ion chromatography	0.0536 mg/L	2 mg/L
Sulfate	EPA 300.0	Ion chromatography	0.166 mg/L	5 mg/L
Calcium	EPA 200.7	ICAP-AES	0.0114 mg/L	0.1 mg/L
Magnesium	EPA 200.7	ICAP-AES	0.00153 mg/L	0.1 mg/L
Sodium	EPA 200.7	ICAP-AES	0.0108 mg/L	0.1 mg/L
Potassium	EPA 200.7	ICAP-AES	0.00960 mg/L	0.2 mg/L
Sulfide	APHA 4500 D	Colorimetric	0.00670 mg/L	0.05 mg/L

TABLE 3. PHYSICOCHEMICAL DATA FOR LABORATORY MWH AND SITE WATERS USED FOR WER DETERMINATION AND DERIVATION OF A COPPER CRITERIA ADJUSTMENT PROCEDURE FOR THE MICHIGAN U.P.

Test Date	Site Name	Test Temp (oC)	Test pH	Total Cu (ug/L)	Dissolved Cu (ug/L)	DOC (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	S04 (mg/L)	Cl (mg/L)	Alkalinity (mg/L as CaCO3)	Hardness (mg/L as CaCO3)	Total Suspended Solids (mg/L)	Sulfide (mg/L)	Total Iron (mg/L)
Multiple	Laboratory MHW	24.5	7.9	2.35	2.02	0.80	14.4	12.6	27.3	2.57	82.5	2.3	61	86	<10	-	
08-03-05	Big Garlic Cr.	24.8	7.8	1.2	1.1	3.25	20	3.4	1.0	0.87	<5	<2	64	62	<10	-	0.075
02-16-05	Cedar Cr	25.0	7.7	<1.0	<1.0	1.03	22	4.7	2.5	0.73	7.6	3.8	72	75	<10	-	0.075
04-20-05	Cedar Cr	24.7	8.1	1.1	<1.0	1.63	22	4.6	2.4	0.72	7.6	4.0	60	79	<10	-	0.075
08-03-05	Cedar Cr	24.9	7.9	<1.0	<1.0	1.15	23	4.7	2.5	0.79	7.4	3.7	68	72	<10	-	0.075
02-22-06	Cedar Cr	24.3	7.8	<1.0	<1.0	0.92	23	4.7	2.4	0.69	7.8	4.2	76	79	<10	-	0.075
04-20-06	Cedar Cr	24.7	7.8	<1.0	<1.0	0.79	23	4.9	2.5	0.67	7.2	3.5	80	76	<10	-	0.075
08-02-05	Cisco Br.	24.6	7.8	<1.2	<1.0	8.75	14	3.8	1.6	0.85	<5	<2	48	49	<10	-	0.210
08-24-05	Davenport Cr.	24.1	8.2	<1.2	1.4	5.09	38	11	1.0	0.73	<5	<2	136	143	11	-	
08-23-05	Escanaba R. at Cornell	24.3	8.3	<1.0	<1.0	4.95	26	11	11	1.00	7.8	5.9	228	112	<10	<0.05	0.807
02-21-06	Escanaba R. at Cornell	24.5	8.0	1.9	<1.0	6.84	25	10	25	1.50	24	10.0	132	108	<10	-	0.807
04-20-06	Escanaba R. at Cornell	24.4	7.9	1	1.8	13.50	23	8.6	4.1	0.68	10	3.8	78	93	<10	-	0.807
08-23-05	Escanaba R. at Mead	24.2	8.4	1.4	1.8	14.20	34	12	75	5.30	74	34.0	180	135	<10	-	0.370
02-21-06	Escanaba R. at Mead	24.5	8.2	2	1.1	17.10	32	11	66	3.90	69	28.0	180	128	<10	<0.05	0.370
04-19-06	Escanaba R. at Mead	24.6	7.7	1.2	1.1	15.30	16	6.1	13	1.20	18	6.8	56	61	<10	-	0.370
08-03-05	Flat Rock Cr.	24.8	8.1	<1.0	<1.0	5.88	26	11	1.1	1.30	7.7	<2	108	115	<10	-	0.620
03-02-05	Ford R.	24.6	7.9	2.2	1.8	9.37	45	20	2.9	0.82	10	5.0	192	213	<10	-	0.299
04-19-05	Ford R.	24.8	8.3	1.2	<1.0	13.00	29	12	1.5	0.64	6.5	2.5	120	134	<10	-	0.299
08-23-05	Ford R.	24.7	8.4	<1.0	<1.0	6.55	37	22	2.4	1.10	7.2	3.3	188	184	<10	-	0.299
02-21-06	Ford R.	24.2	8.1	<1.0	<1.0	11.00	48	19	2.7	0.74	25	4.9	176	204	<10	-	0.299
04-19-06	Ford R.	24.7	8.0	1.1	<1.0	15.70	29	12	1.6	0.56	14	2.2	102	121	<10	<0.05	0.299
08-25-05	Hudson Cr.	24.9	7.6	<1.2	<1.2	30.20	17	5.8	0.4	0.10	6.5	<2	44	68	<10	-	
02-22-06	Hudson Cr.	24.3	7.4	1.1	1.1	20.05	13	4.7	0.4	0.10	<5	<2	44	53	<10	-	
04-20-06	Hudson Cr.	24.6	7.3	<1.0	<1.0	18.20	9.6	3.5	0.6	0.33	<5	<2	36	36	<10	-	
03-02-05	Manistique R. above dam	24.6	7.5	<1.2	<1.0	6.67	28	6.6	2.0	0.84	17	2.6	84	105	<10	-	1.040
04-19-05	Manistique R. above dam	24.5	7.8	1.9	1.6	9.90	16	3.5	1.3	0.49	10	<2	48	56	<10	-	
08-23-05	Manistique R. DS dam	24.6	8.1	<1.0	<1.0	4.87	31	6.9	2.0	0.70	20	2.3	68	103	<10	-	1.040

Test Date	Site Name	Test Temp (oC)	Test pH	Total Cu (ug/L)	Dissolved Cu (ug/L)	DOC (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	S04 (mg/L)	Cl (mg/L)	Alkalinity (mg/L as CaCO3)	Hardness (mg/L as CaCO3)	Total Suspended Solids (mg/L)	Sulfide (mg/L)	Total Iron (mg/L)
02-22-06	Manistique R. DS dam	24.2	7.7	<1.0	<1.0	7.24	28	6.4	2.0	0.63	19	2.4	84	100	<10	<0.05	
04-20-06	Manistique R. DS dam	24.7	7.5	<1.0	<1.0	11.40	16	3.7	1.3	0.45	12	<2	46	55	<10	-	
08-02-05	McDonald Cr.	25.0	7.4	1.7	2	25.20	13	3.2	1.2	0.49	<5	<2	40	55	<10	-	0.500
02-21-06	McDonald Cr.	24.3	7.3	1.7	1.5	11.60	11	2.9	1.3	0.56	<5	<2	40	45	<10	-	0.500
04-19-06	McDonald Cr.	24.8	6.8	1.5	1.3	17.10	5.7	1.5	0.9	0.45	<5	<2	12	20	<10	<0.05	0.500
08-24-05	Menominee R.	25.2	8.3	<1.2	1.3	6.76	28	13	16	2.20	20	9.2	120	125	<10	-	0.271
08-25-05	Monocle Lake	24.9	7.3	<1.2	1.8	3.82	4.9	1.2	1.0	0.38	<5	<2	20	15	<10	-	
08-03-05	Six Mile Lake	24.7	8.3	<1.0	1.4	10.50	36	20	1.3	1.30	6.9	<2	156	164	<10	-	
02-15-05	Sturgeon R.	24.8	7.5	2.0	1.6	6.57	17	4.8	2.6	1.2	<5	2.4	64	67	<10	-	0.898
04-20-05	Sturgeon R.	24.9	7.5	4	2.7	10.80	9.0	2.3	1.5	0.88	<5	<2	28	35	17	-	0.898
08-24-05	Tahquamenon R.	24.4	7.9	<1.2	1.2	10.70	29	10	25	1.40	16	2.5	88	104	<10	-	0.698
08-02-05	Tioga R.	24.9	7.6	<1.0	<1.0	9.64	12	3.9	1.6	0.89	<5	<2	48	48	<10	-	
08-02-05	Trap Rock R.	24.5	7.7	4	3.7	6.97	17	3.9	2.3	0.77	<5	2.6	52	63	<10	-	0.308
	Median	24.6	7.8	1.6	1.5	9.37	23.0	5.8	2.0	0.74	10.0	3.8	72	79	14	NA	0.370
	Minimum	24.1	6.8	1.0	1.1	0.79	4.9	1.2	0.4	0.10	6.5	2.2	12	15	11	NA	0.075
	Maximum	25.2	8.4	4.0	3.7	30.20	48.0	22.0	75.0	5.30	74.0	34.0	228	213	17	NA	1.04

TABLE 4. SPECIFIC ULTRAVIOLET ABSORBANCE AT 254 NM ($SUVA_{254}$) VALUES OF SELECT U.P. SITE WATER SAMPLES USED FOR ESTIMATING DOC AROMATICITY[a].

	February 2006			April 2006		
Site	Absorbance at 254 nm	DOC (mg/L)	$SUVA_{254}$ ($L\ mg^{-1}\ m^{-1}$)	Absorbance at 254 nm	DOC (mg/L)	$SUVA_{254}$ ($L\ mg^{-1}\ m^{-1}$)
Cedar Cr.	-	-	-	0.01615	0.786	2.06
Escanaba R. Cornell	0.2858	6.84	4.18	0.5751	13.5	4.26
Escanaba R. Mead	0.5952	17.1	3.48	0.6440	15.3	4.21
Ford R.	0.3822	11.0	3.47	0.6336	15.7	4.04
Hudson Cr.	0.5865	20.1	2.93	0.8195	18.2	4.50
Manistique R.	0.2998	7.24	4.14	0.5413	11.4	4.75
McDonald Cr.	0.5200	11.6	4.48	0.7526	17.1	4.40
McGunn's Cr.	-	-	-	0.3934	8.28	4.75

[a] The average absorptivity for all the molecules that comprise the DOC in a water sample; an indicator of the humic fraction of the DOC.

TABLE 5. MEASURED, DOC- AND BLM-PREDICTED 48-h ACUTE LC50 AND WER VALUES FOR *C. dubia* EXPOSED TO COPPER IN SITE WATERS USED FOR WER DETERMINATION AND DERIVATION OF A COPPER CRITERIA ADJUSTMENT PROCEDURE FOR THE MICHIGAN U.P.[a,b]

Test Date	Site Name	DOC (mg/L)	Measured Dissolved Cu LC50 (µg/L)	DOC-Predicted Cu LC50	BLM-Predicted Cu LC50 (µg/L)	Measured WER	Hard-Adjusted WER	SMAV WER	DOC-Predicted Measured WER	DOC-Predicted Hard-adj WER	DOC-Predicted SMAV WER	BLM-Predicted WER
08-03-05	Big Garlic Cr.	3.25	41.6	26.3	54.6	4.8	6.7	3.0	3.2	3.1	1.3	3.1
02-16-05	Cedar Cr. Near Sands	1.03	10.2	9.7	14.0	1.5	2.0	0.6	0.6	-0.7	0.0	2.8
04-20-05	Cedar Cr. Near Sands	1.63	12.1	14.2	37.4	1.7	1.9	0.7	1.3	0.4	0.4	2.3
08-03-05	Cedar Cr. Near Sands	1.15	12.1	10.6	22.3	1.4	1.7	0.7	0.8	-0.5	0.1	1.3
02-22-06	Cedar Cr. Near Sands	0.92	6.3	8.9	16.6	1.1	1.2	0.4	0.5	-0.8	0.0	1.0
04-20-06	Cedar Cr. Near Sands	0.79	12.7	7.9	14.7	1.7	1.9	0.7	0.3	-1.1	-0.1	2.8
08-02-05	Cisco Branch	8.75	57.9	67.4	144.2	8.8	15.2	5.1	9.5	12.5	4.6	8.2
08-24-05	Davenport Cr.	5.09	27.5	40.1	140.7	3.8	2.3	0.9	5.3	6.3	2.5	7.5
08-23-05	Escanaba R. at Cornell	4.95	18.6	39.0	142.1	2.6	2.0	0.8	5.2	6.0	2.4	24.0
02-21-06	Escanaba R. at Cornell	6.84	56.5	53.2	168.5	8.8	7.2	2.4	7.3	9.3	3.5	29.9
04-20-06	Escanaba R. at Cornell	13.50	99.7	103.0	263.4	13.3	12.0	4.8	15.0	20.7	7.5	49.8
08-23-05	Escanaba R. at Mead	14.20	84.5	108.2	586.9	11.8	7.5	3.5	15.8	21.9	7.9	99.0
02-21-06	Escanaba R. at Mead	17.10	162.0	129.9	575.9	25.1	17.6	6.6	19.2	26.8	9.7	102.1
04-19-06	Escanaba R. at Mead	15.30	147.8	116.4	257.5	19.4	26.0	10.6	17.1	23.7	8.6	44.9
08-03-05	Flat Rock Cr.	5.88	64.7	46.0	127.3	7.4	5.8	2.6	6.2	7.6	2.9	7.3
03-02-05	Ford River Near Hyde	9.37	78.5	72.1	178.2	7.8	3.2	1.7	10.3	13.6	5.0	16.0
04-19-05	Ford River Near Hyde	13.00	122.2	99.2	368.1	17.5	11.7	4.2	14.5	19.8	7.2	22.9
08-23-05	Ford River Near Hyde	6.55	48.3	51.0	206.1	6.8	3.2	1.2	7.0	8.8	3.3	34.7
02-21-06	Ford River Near Hyde	11.00	80.0	84.3	270.4	12.4	5.6	1.8	12.1	16.4	6.0	47.9
04-19-06	Ford River Near Hyde	15.70	167.7	119.4	319.7	22.0	15.5	6.3	17.6	24.4	8.8	55.8
08-25-05	Hudson Cr.	30.20	210.0	227.9	415.9	34.3	41.9	13.7	34.3	49.2	17.5	22.3
02-22-06	Hudson Cr.	20.05	136.4	152.0	202.4	24.1	37.6	11.2	22.6	31.9	11.4	11.8
04-20-06	Hudson Cr.	18.20	133.9	138.1	165.7	17.8	39.6	15.6	20.5	28.7	10.3	31.3
03-02-05	Manistique R. above Man	6.67	63.8	51.9	76.8	6.4	5.1	3.2	7.1	9.0	3.4	6.9
04-19-05	Manistique R. above Man	9.90	101.9	76.0	170.2	14.6	22.3	8.6	10.9	14.5	5.3	10.6
08-23-05	Manistique River DS Dam	4.87	28.2	38.4	117.9	4.0	3.2	1.2	5.1	5.9	2.3	19.9
02-22-06	Manistique River DS Dam	7.24	48.0	56.1	110.6	8.5	7.3	2.2	7.8	10.0	3.7	6.4
04-20-06	Manistique River DS Dam	11.40	77.5	87.3	130.8	10.3	15.3	6.1	12.6	17.1	6.2	24.7

Test Date	Site Name	DOC (mg/L)	Measured Dissolved Cu LC50 (µg/L)	DOC-Predicted Cu LC50	BLM-Predicted Cu LC50 (µg/L)	Measured WER	Hard-Adjusted WER	SMAV WER	DOC-Predicted Measured WER	DOC-Predicted Hard-adj WER	DOC-Predicted SMAV WER	BLM-Predicted WER
08-02-05	McDonald Cr.	25.20	185.1	190.5	275.4	28.1	44.0	14.8	28.6	40.7	14.5	15.7
02-21-06	McDonald Cr.	11.60	103.4	88.8	101.5	16.0	29.9	9.9	12.8	17.4	6.4	18.0
04-19-06	McDonald Cr.	17.10	116.9	129.9	88.8	15.3	59.1	24.1	19.2	26.8	9.7	15.5
08-24-05	Menominee R.	6.76	29.3	52.6	198.7	4.1	2.8	1.1	7.2	9.1	3.5	10.6
08-25-05	Monocle Lake	3.82	14.4	30.6	35.3	2.3	12.1	4.0	3.8	4.1	1.7	1.9
08-03-05	Six Mile Lake	10.50	41.6	80.5	296.6	4.8	2.7	1.4	11.6	15.5	5.7	16.9
02-15-05	Sturgeon R. Near Chassell	6.57	59.4	51.1	78.2	9.0	12.7	3.9	7.0	8.8	3.3	15.6
04-20-05	Sturgeon R. Near Chassell	10.80	117.1	82.8	135.5	16.7	40.2	14.4	11.9	16.0	5.9	8.4
08-24-05	Tahquamenon R.	10.70	41.1	82.0	240.4	5.7	4.7	1.8	11.8	15.9	5.8	12.9
08-02-05	Tioga R.	9.64	66.9	74.1	132.4	10.2	18.0	6.9	10.6	14.1	5.2	7.5
08-02-05	Trap Rock R.	6.97	65.7	54.1	101.3	10.0	13.7	4.6	7.5	9.5	3.6	5.8
	Median	9.4	64.7	72.1	142.1	8.8	7.5	3.5	10.3	13.6	5.0	15.5
	Minimum	0.79	6.3	7.9	14.0	1.1	1.2	0.4	0.3	-1.1	-0.1	1.0
	Maximum	30	210.0	227.9	586.9	34.3	59.1	24.1	34.3	49.2	17.5	102.1

[a] The various WER values are as defined and described in the Methods section under Toxicity Assays.

[b] The various predicted WER values were calculated using the regression equations in Figures 7, 8, 9 and 10, respectively.

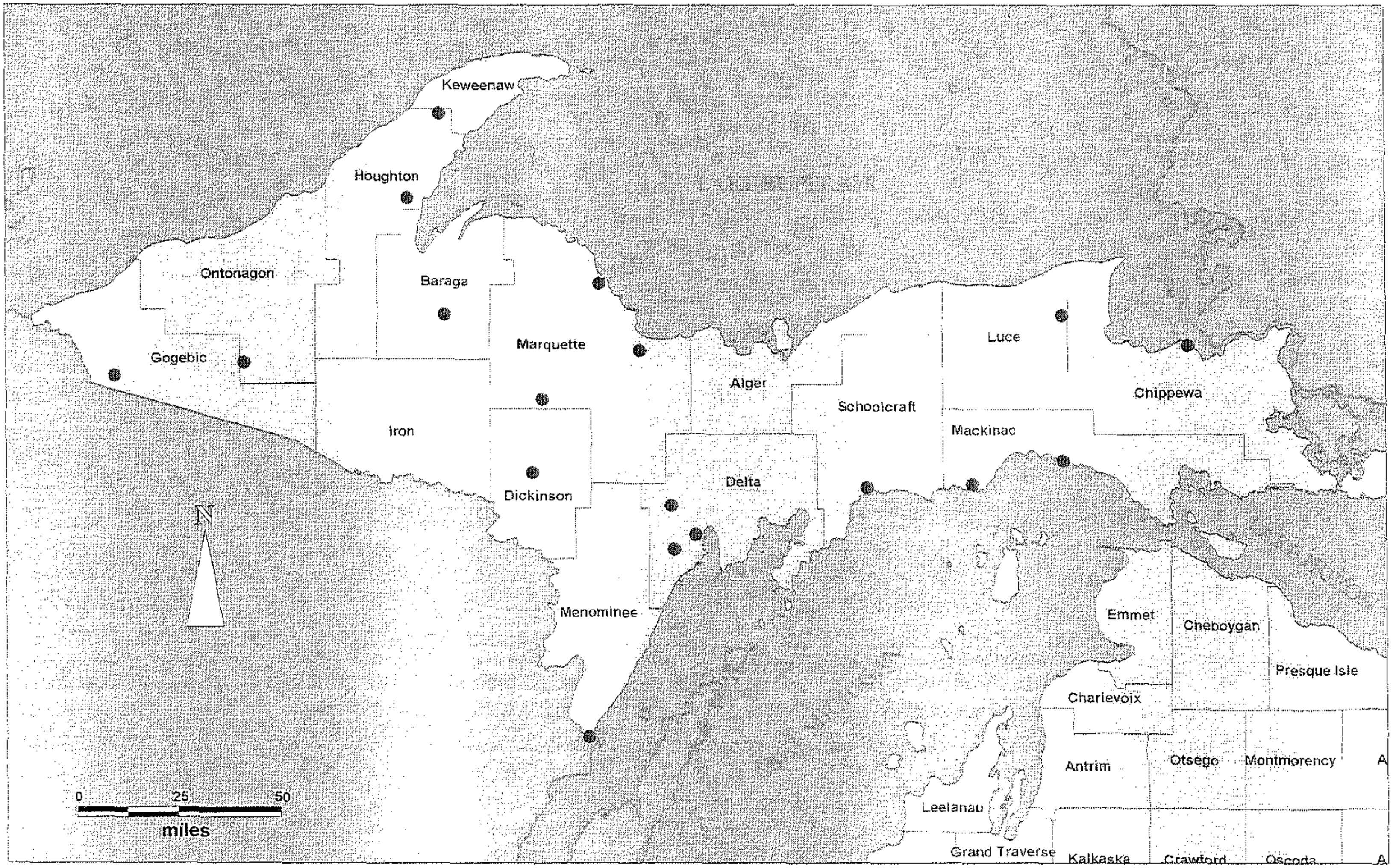
SMAV = Species Mean Acute Value.

BLM = Biotic Ligand Model.

Figure Legends

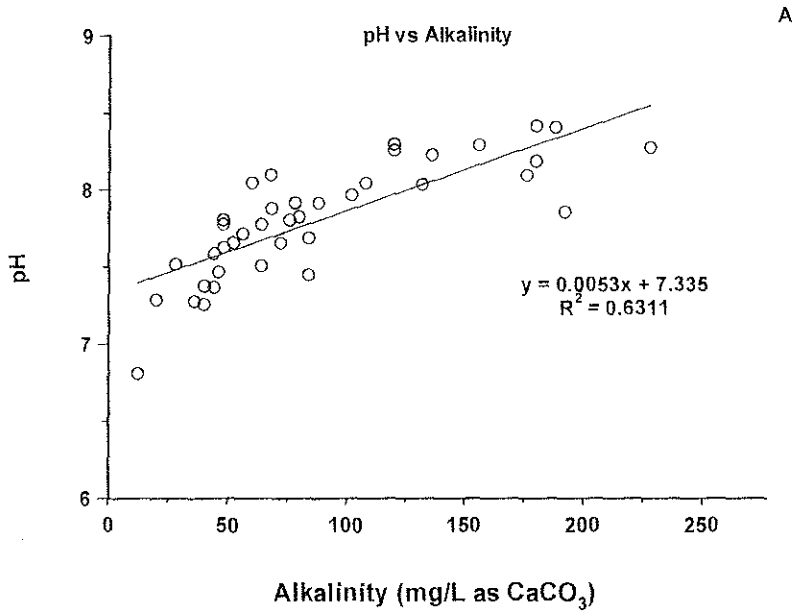
FIGURE 1.	GEOGRAPHICAL LOCATION OF RIVERS AND LAKES USED FOR WER DETERMINATION AND DERIVATION OF A COPPER CRITERIA ADJUSTMENT PROCEDURE FOR THE MICHIGAN U.P.
FIGURE 2.	RELATIONSHIP BETWEEN (A) PH AND ALKALINITY AND (B) PH AND CALCIUM FROM RIVERS AND LAKES IN THE MICHIGAN U.P.
FIGURE 3.	RELATIONSHIP BETWEEN COPPER LC50 AND DOC OF SITE WATERS IN THE MICHIGAN U.P.
FIGURE 4.	RELATIONSHIP BETWEEN CU LC50 AND WATER HARDNESS OF SITE WATERS IN THE MICHIGAN U.P.: (A) DOC RANGE 1-30 MG C/L, (B) DOC RANGE 1-15 MG C/L), AND (C) DOC RANGE 1-5 MG C/L.
FIGURE 5.	COMPARISON OF SEASONAL CU LC50 VALUES FOR SELECT SITES IN THE MICHIGAN U.P. VALUES ABOVE COLUMNS ARE MEASURED DOC CONCENTRATIONS (MG/L) IN THE SITE WATER AT THE TIME OF SAMPLING AND TESTING.
FIGURE 6.	LOG-LOG PLOT OF MEASURED VERSUS BLM-PREDICTED CU LC50 VALUES. SOLID AND DASHED ARE AS EXPLAINED IN THE TEXT.
FIGURE 7.	RELATIONSHIP BETWEEN MEASURED WER AND DOC IN SITE WATERS OF THE MICHIGAN U.P.
FIGURE 8.	RELATIONSHIP BETWEEN TRADITIONAL HARDNESS-ADJUSTED WER AND DOC IN SITE WATERS OF THE MICHIGAN U.P.
FIGURE 9.	RELATIONSHIP BETWEEN STREAMLINED CALCULATED SMAV WER AND DOC IN SITE WATERS OF THE MICHIGAN U.P.
FIGURE 10.	RELATIONSHIP BETWEEN BLM-PREDICTED WER AND DOC IN SITE WATERS OF THE MICHIGAN U.P.

FIGURE 1.



Keweenaw
Houghton
Ontonagon
Baraga
Gogebic
Marquette
Alger
Luce
Chippewa
Schoolcraft
Mackinac
Iron
Dickinson
Delta
Menominee
Emmet
Cheboygan
Presque Isle
Charlevoix
Antrim
Otsego
Montmorency
Leelanau
Grand Traverse
Kalkaska
Crawford
Oscoda
N
0
25
50
miles

FIGURE 2.


A
pH vs Alkalinity
9
8
7
6
pH
y = 0.0053x + 7.335
R² = 0.6311
0
50
100
150
200
250
Alkalinity (mg/L as CaCO3)

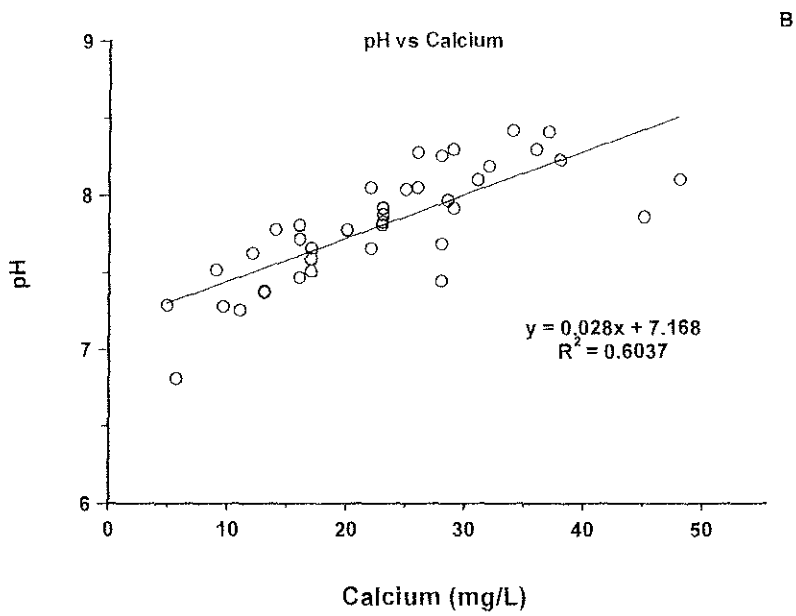

B
pH vs Calcium
9
8
7
6
pH
y = 0.028x + 7.168
R² = 0.6037
0
10
20
30
40
50
Calcium (mg/L)

FIGURE 3.

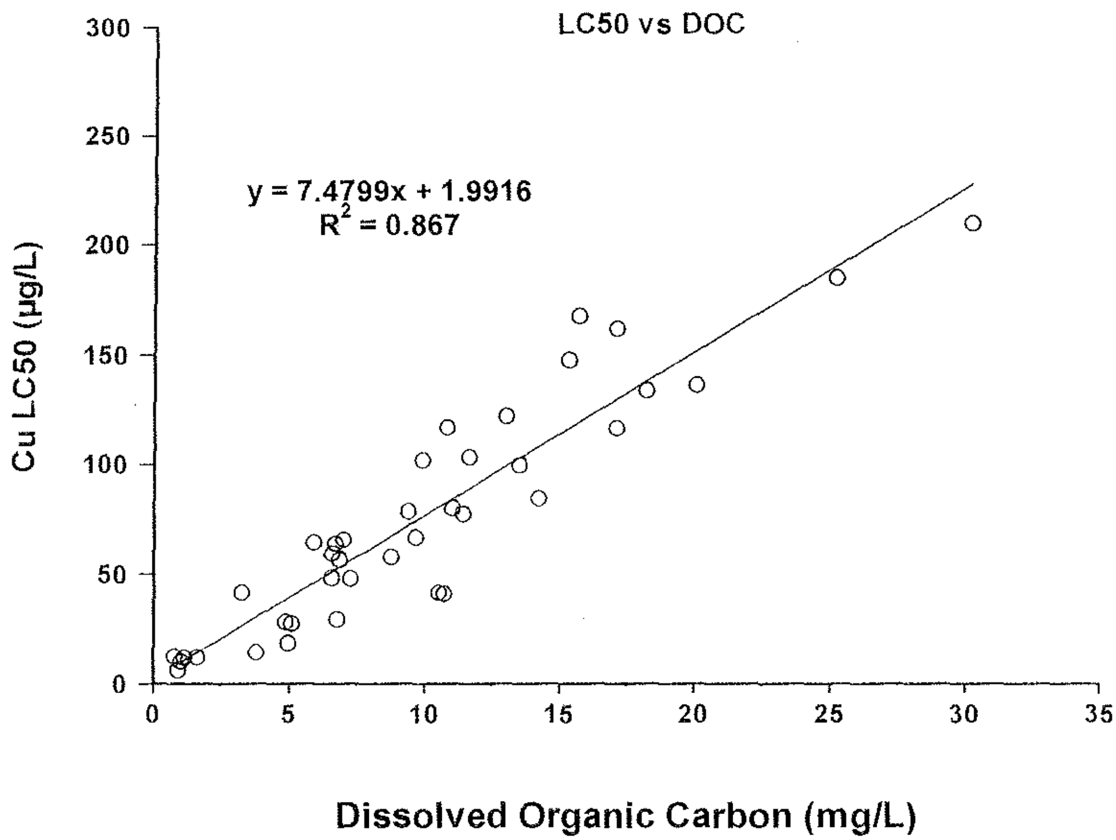

LC50 vs DOC
y = 7.4799x + 1.9916
R² = 0.867
Cu LC50 (µg/L)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
Dissolved Organic Carbon (mg/L)

FIGURE 4.

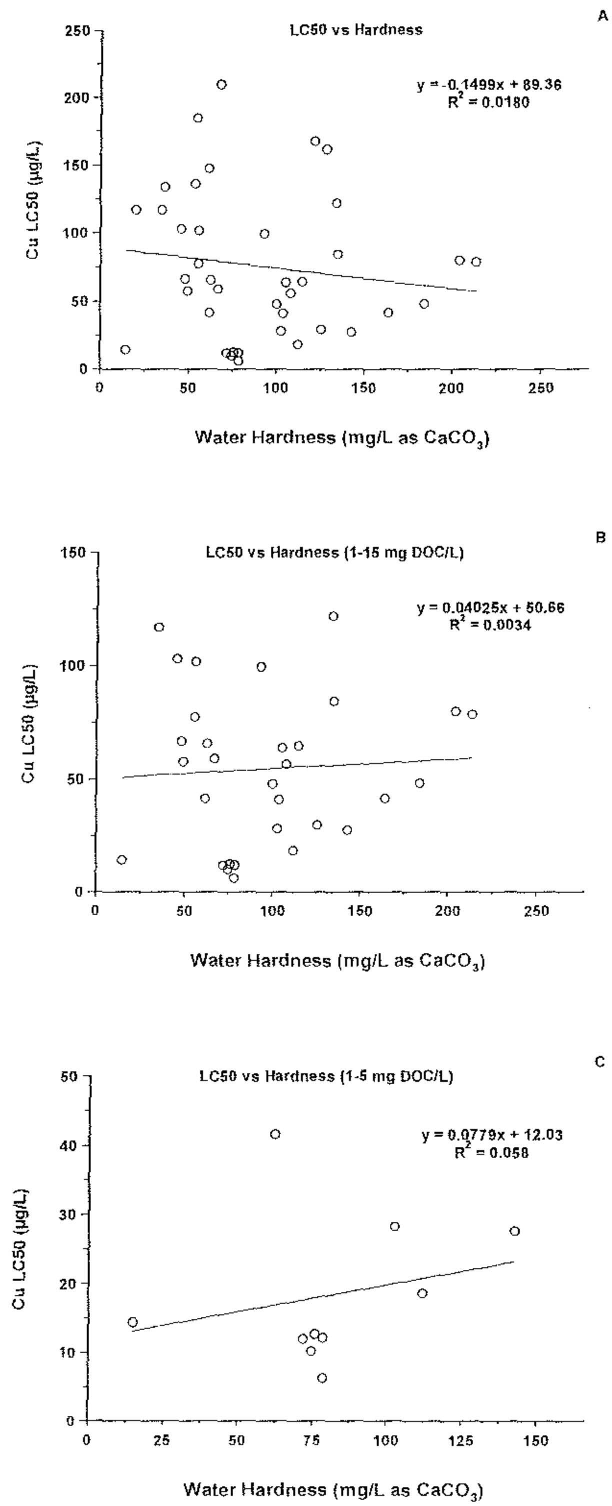

A
LC50 vs Hardness
y = -0.1499x + 89.36
R² = 0.0180
Cu LC50 (µg/L)
0
50
100
150
200
250
0
50
100
150
200
250
Water Hardness (mg/L as CaCO3)
B
LC50 vs Hardness (1-15 mg DOC/L)
y = 0.04025x + 50.66
R² = 0.0034
Cu LC50 (µg/L)
0
50
100
150
0
50
100
150
200
250
Water Hardness (mg/L as CaCO3)
C
LC50 vs Hardness (1-5 mg DOC/L)
y = 0.0779x + 12.03
R² = 0.058
Cu LC50 (µg/L)
0
10
20
30
40
50
0
25
50
75
100
125
150
Water Hardness (mg/L as CaCO3)

FIGURE 5.

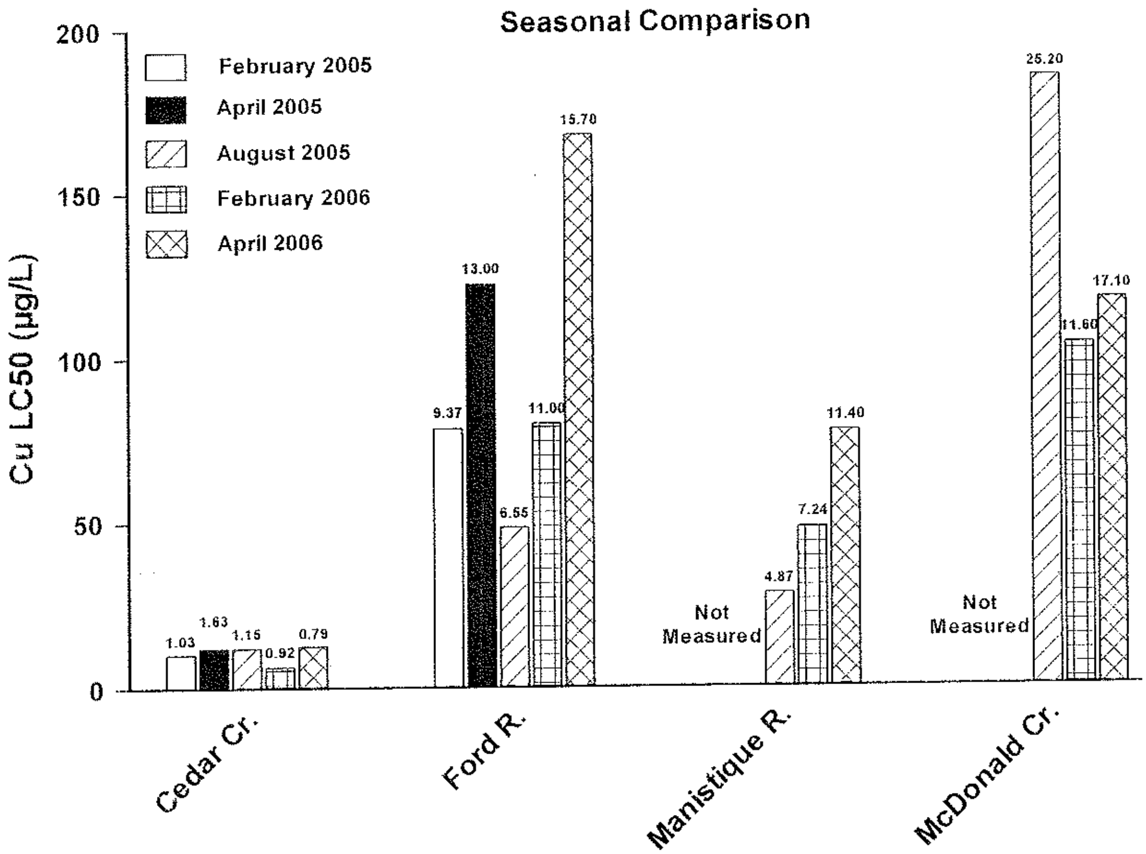
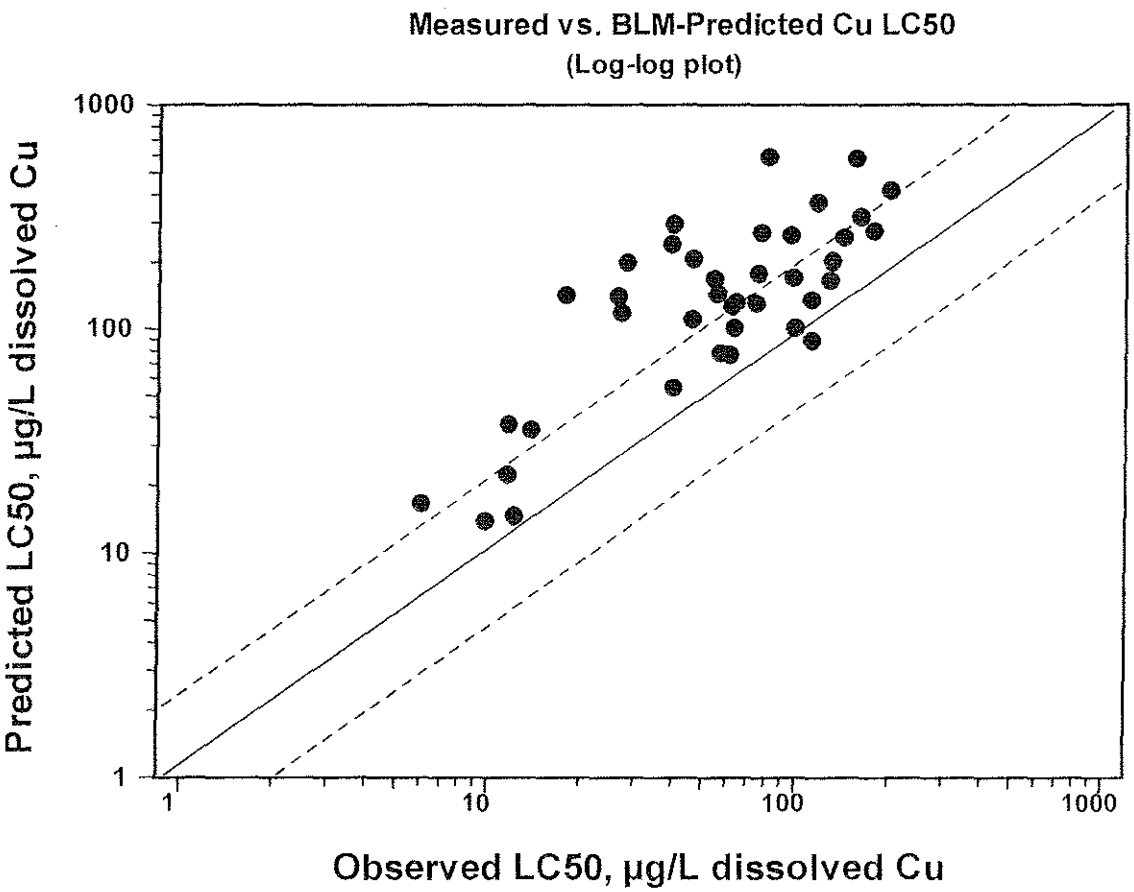

Seasonal Comparison
February 2005
April 2005
August 2005
February 2006
April 2006
Cu LC50 (µg/L)
200
150
100
50
0
1.03
1.63
1.15
0.92
0.79
9.37
13.00
6.55
11.00
15.70
Not Measured
4.87
7.24
11.40
Not Measured
25.20
11.60
17.10
Cedar Cr.
Ford R.
Manistique R.
McDonald Cr.

FIGURE 6.



Measured vs. BLM-Predicted Cu LC50
(Log-log plot)
1000
100
10
1
1
10
100
1000
Predicted LC50, µg/L dissolved Cu
Observed LC50, µg/L dissolved Cu

FIGURE 7.

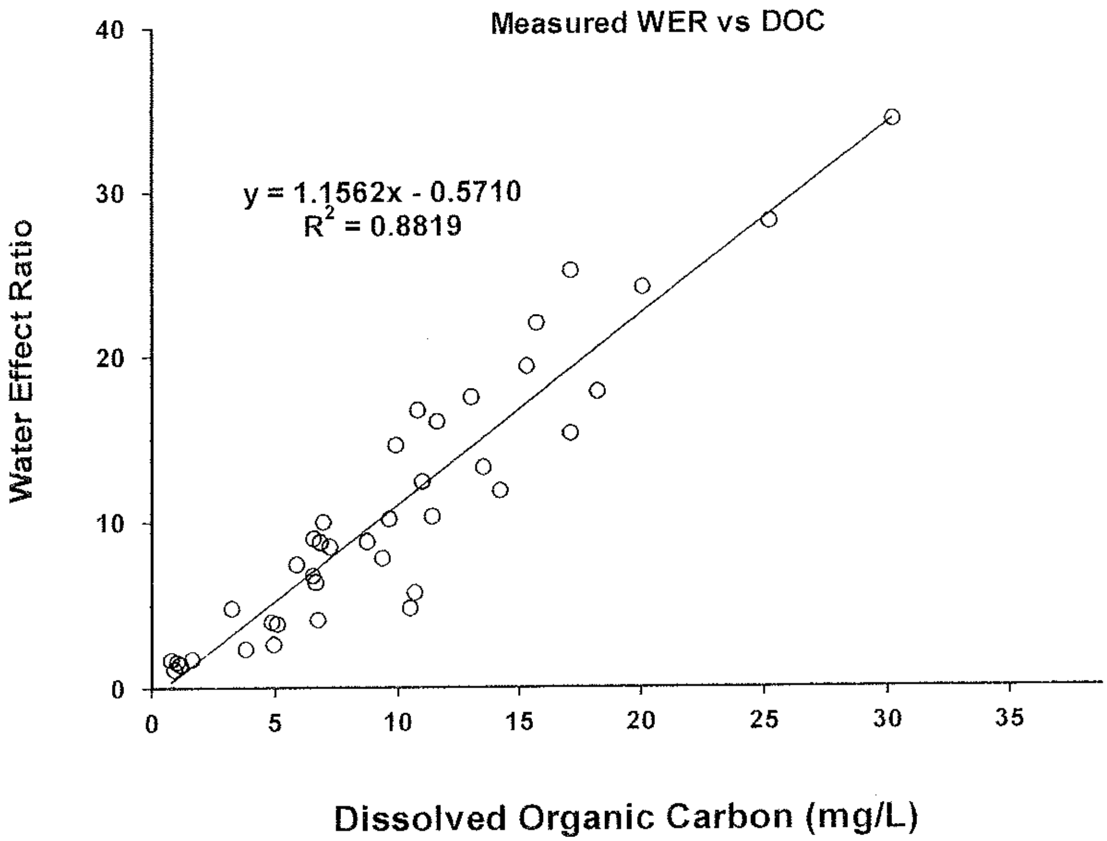
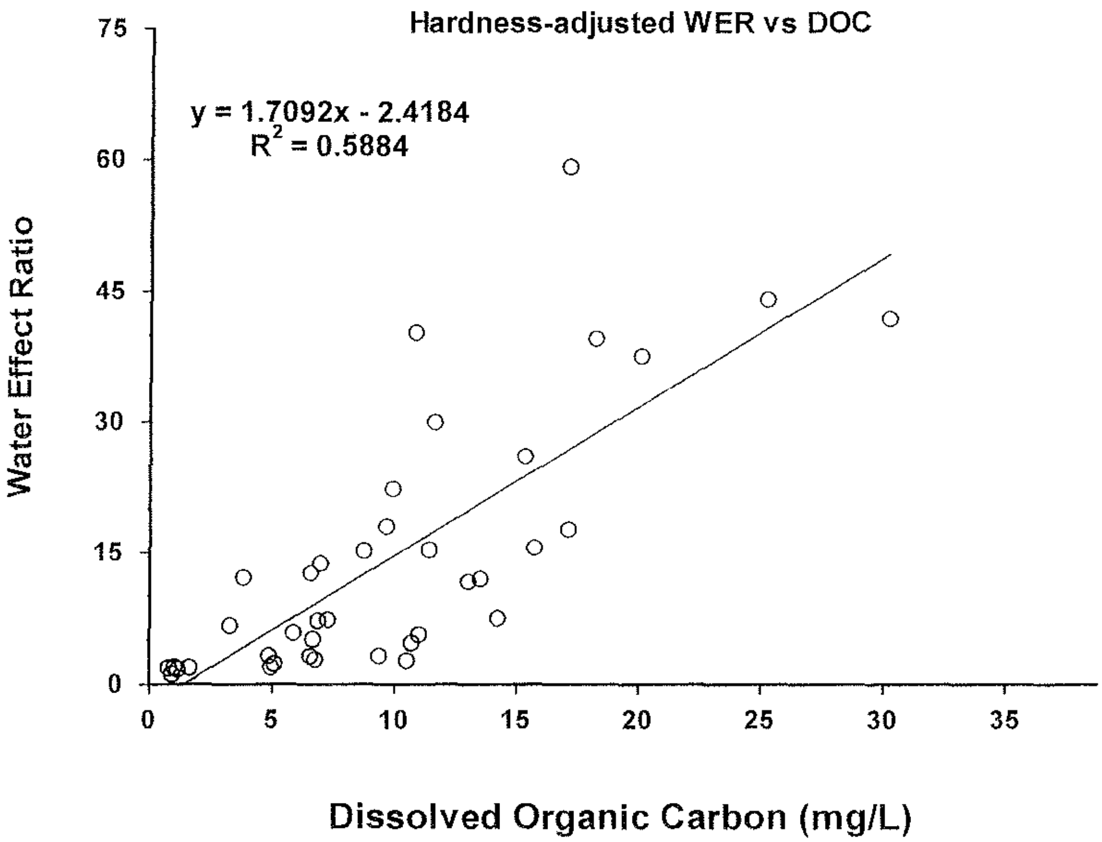
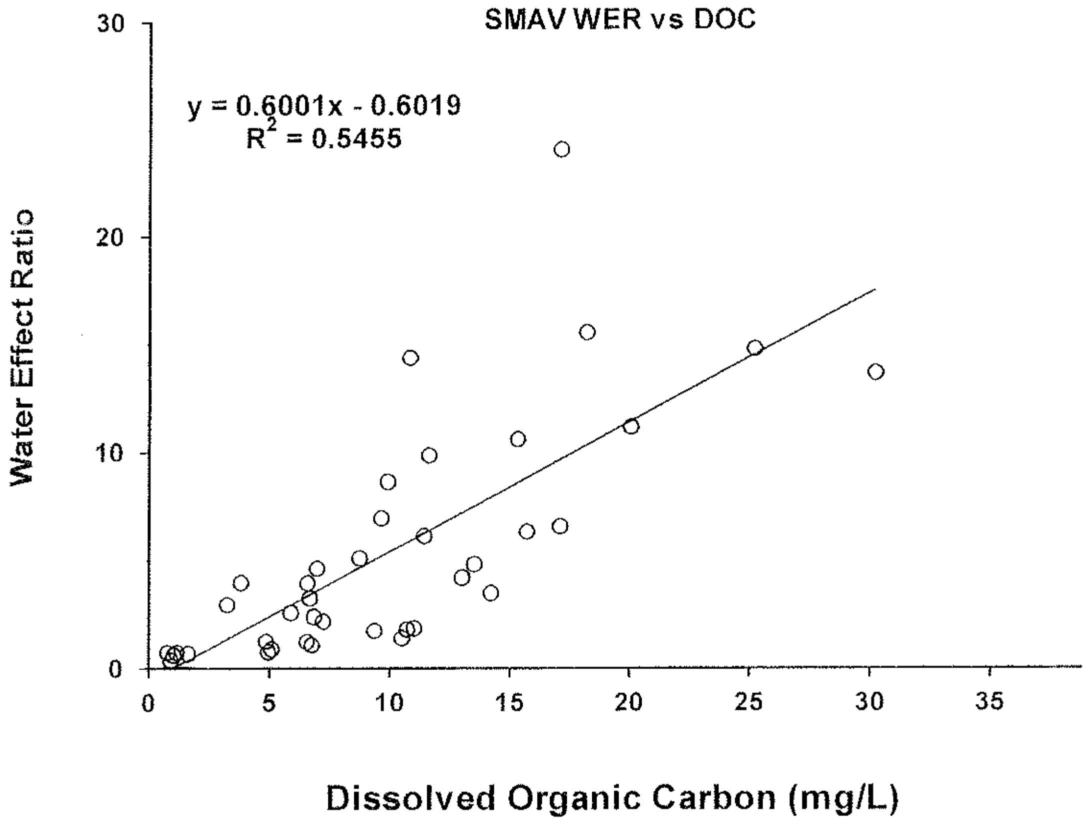

Measured WER vs DOC
y = 1.1562x - 0.5710
R2 = 0.8819
Water Effect Ratio
Dissolved Organic Carbon (mg/L)
0
10
20
30
40
0
5
10
15
20
25
30
35

FIGURE 8.



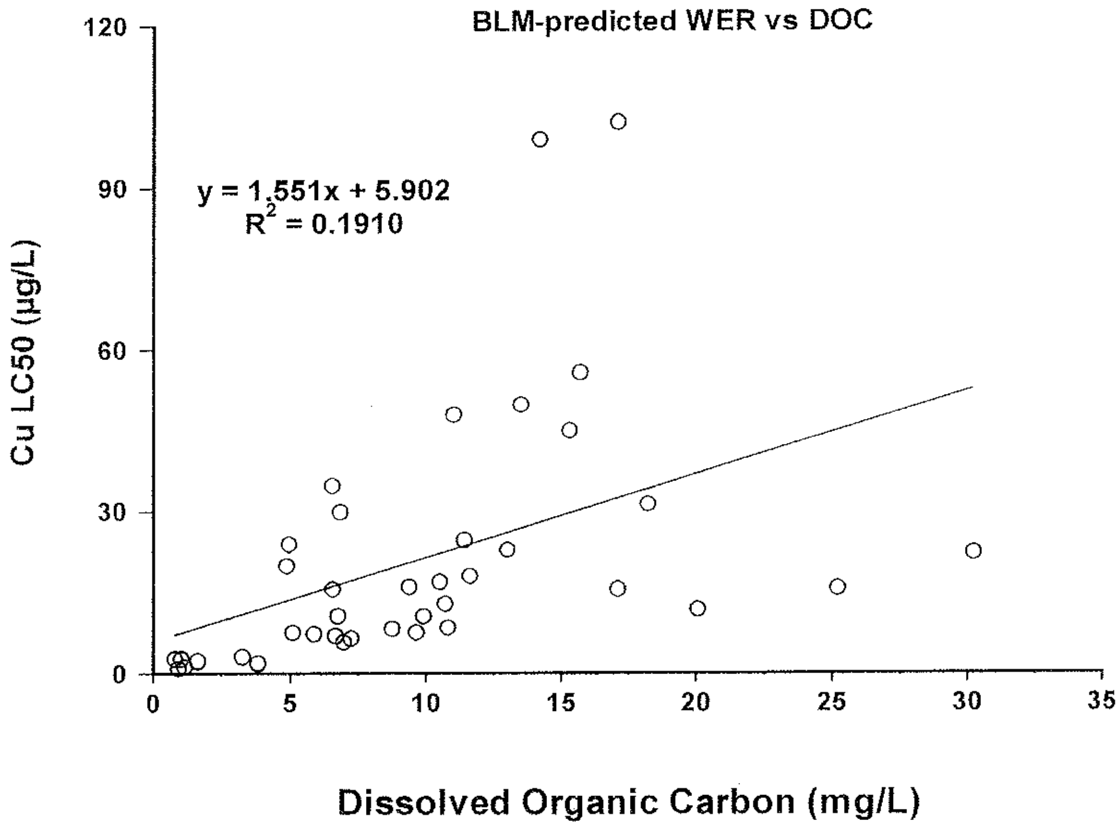
Hardness-adjusted WER vs DOC
y = 1.7092x - 2.4184
R² = 0.5884
Water Effect Ratio
75
60
45
30
15
0
0
5
10
15
20
25
30
35
Dissolved Organic Carbon (mg/L)

FIGURE 9.



SMAV WER vs DOC
y = 0.6001x - 0.6019
R2 = 0.5455
Water Effect Ratio
Dissolved Organic Carbon (mg/L)
30
20
10
0
0
5
10
15
20
25
30
35

FIGURE 10.



BLM-predicted WER vs DOC
y = 1.551x + 5.902
R² = 0.1910
Cu LC50 (µg/L)
120
90
60
30
0
0
5
10
15
20
25
30
35
Dissolved Organic Carbon (mg/L)

McGunn's Creek

MEMORANDUM

September 18, 2006

To: William Dimond

From: Tyler Linton

Cc: Dennis Bush
Gerald Saalfeld
Bill Taft
Mick DeGraeve

RE: DEVIATION FROM TOXICITY TEST PROTOCOL FOR PROJECT #04-12, CONTRACT #: 071B1001643

This memorandum was prepared to document a deviation in our test acceptability criteria, as per section 7.1.1 in the Quality Assurance Project Plan (QAPP) entitled: *Quality Assurance Project Plan for the Development of a Copper Standard for Michigan's Upper Peninsula Waters*, Contract Number: 071B1001643, Project Number: 04-12. The deviation is the result of observed excessive mortality in the moderately hard reconstituted water (MHW) control for two 48-h static acute toxicity tests using *Ceriodaphnia dubia* as the test organism. The tests were conducted in support of the water-effect ratio (WER) evaluation initiated on August 17, 2006, and again on August 29, 2006 for site water samples from McGunn's Creek, MI. Regarding this particular deviation from the test acceptability criteria, the approved quality assurance project plan (QAPP) for this project states the following:

"The acceptability of the aquatic toxicity testing phase of the study will be determined after consideration of the following.....

- More than 10 percent of the organisms in any required acute toxicity test control treatment died or showed signs of disease or stress (such as discoloration, unusual behavior, immobilization or loss of equilibrium), during the tests."...

Section 9.0 of the QAPP (Corrective Action) also states that:

"Corrective action is the process of identifying, recommending, approving, and implementing measures to manage circumstances requiring a deviation from the QAPP. Corrective action will be approved by the MDEQ prior to being implemented. The GLEC PM (Program Manager) and DPM (Deputy Program Manager) will be responsible for identifying and requesting corrective action pertaining to any aspect of the field sampling activities, the laboratory analysis or the data analysis. All field and laboratory personnel will assist in identifying the need for corrective action. Any corrective action taken will be documented in a record book."

And in Section 10 of the QAPP (Data Management, Analysis and Reporting Procedure), the text states:

"The following data deliverables will be included in the report:

...Anything unusual about the tests, including deviations from specific protocols and any other relevant information."

To partially fulfill the above requirement, we are using this memorandum to first: inform MDEQ of the deviation from the specific test acceptability criterion identified above; second, to justify our decision to not take any corrective action in this particular case; and finally, to seek approval from MDEQ of this decision (i.e., to consider the results from the two 48-h static acute toxicity tests in question as valid estimates of toxicity).

As a summary of the events leading to this memorandum, we provide the following information relating to the subject:

The deviation from the test acceptability criterion (25 percent mortality) was first observed in the 48-h static acute toxicity test with *C. dubia* in moderately hard water (MHW), which was initiated concurrent with a test using copper-spiked ambient water from McGunn's Creek, MI tests initiated on August 17, 2006. In the MHW test, survival in the control water after 24 hours was 100%, with no organisms exhibiting erratic behavior (non-swimming or labored and erratic swimming pattern; inability to respond to gentle prodding). The laboratory control consisted of four replicates of five animals each at a nominal test copper concentration operationally equivalent to approximately 2 μg/L. At test termination (48-hr), there was one death (out of 5) in 3 of the replicates, and 2 deaths in the remaining replicate (25% overall control mortality). Conversely, there was only one death (out of 20) in the lowest treatment concentration at a nominal copper concentration of approximately 2.3 μg/L. In addition, in the site water test control concentration, (McGunn's Creek water without any additional copper added), survival was 100 percent after 48-hr. The estimated LC50 values based on the nominal test concentrations in the two WER tests for this sampling event were 7.73 and 174 μg Cu/L for the MHW and site water tests, respectively (see Attachment A for raw data sheets and nominal LC50 calculations using Spearman-Karber).

MDEQ was immediately informed of the failure of the MHW test control to meet the test acceptability criterion, and a second set of WER tests was initiated the following week with a second site water sample collected from McGunn's Creek, MI on August 28, 2006 (tests initiated August 29, 2006). Similar to the test results from the previous week, in the MHW test, there was one death (out of five) in two of the control replicates, and three deaths in one other replicate (25% overall control mortality). There was again one death (out of 20) in the lowest MHW test concentration (2.3 μg/L). After 48-hr in the site water test control concentration (i.e., McGunn's Creek water without any additional copper added), survival was 100 percent. The estimated LC50 value based on the nominal test concentrations in the two WER tests for this sampling event were 7.42 and 149 μg Cu/L for the MHW and site water tests, respectively (see Attachment B for raw data sheets and nominal LC50 calculations using Spearman-Karber).

Given: (1)the similarity of the two nominal LC50 values estimated for the MHW tests to the average LC50 historically obtained for previous tests in our laboratory (approximately 7.3 μg Cu/L); (2) that excessive mortality was not pervasive throughout the rest of the test treatments, and (3) that there were no deaths exhibited by this cohort of *C. dubia* in the site water controls (including no mortality in the next several site water test treatments), we do not believe the organisms were "especially susceptible" to copper, or were in a weakened state. In addition, a review of our culture records does not suggest an unhealthy stock *C. dubia* population as a reason for the failure of the MHW test (see Attachment C for a photocopy of our stock reproduction and health records). Also, in both tests, we observed a meaningful dose-response relationship that allowed us to calculate a meaningful LC50 estimate. Therefore, in this instance, we do not consider the control mortality in the MHW tests just cause for invalidating the McGunn's Creek WER tests, nor do we believe there is a need for further corrective action. Instead, we recommend using the "measured" LC50 values for the latest McGunn's Creek site water tests with the MHW test results. Alternately, the Streamlined WER Guidance SMAV for *C. dubia*, pending MDEQ technical direction, could be used to interpret the test results.

Note: Test water samples from the August 29-31, 2006 WER tests are preserved and currently being held at GLEC. The samples will be sent for copper analysis pending MDEQ's response to this memorandum.

Attachments (forwarded via overnight carrier)

Attachment A

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: 8/17/06 TEST NUMBER: 4412-00 DURATION: 48 h
TOXICANT : 8197
SPECIES: C.dubia

RAW DATA:	Concentration (yg/l)	Number Exposed	Mortalities
--- ----			
	.00	20	5
	2.30	20	1
	3.90	20	5
	6.50	20	7
	10.80	18	15
	18.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 7.73
95% LOWER CONFIDENCE: 6.62
95% UPPER CONFIDENCE: 9.02

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

GLEC
Great Lakes Environmental Center

MH

DAPHNID 48-HOUR STATIC-ACUTE TOXICITY TEST FOR WATER EFFECT RATIO STUDIES

TEST MATERIAL: 8195* 1909

TYPE OF TEST: WER

DILUTION WATER: MH

PROJECT NUMBER: 4412-00

NO. ANIMALS/CHAMBER: 5

PHOTOPERIOD (L:D): 16:8

TEST SPECIES: C. dubia

NO. CHAMBERS: 4

LIGHT INTENSITY (lux): 500-1000

INVESTIGATORS:

AGE/SOURCE OF ANIMALS: <24hrs / DC 8/9 SR 8/11 MH

TEST TEMPERATURE (°C): 25±1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	Control				2.3 mg/L				3.9 mg/L				6.5 mg/L				10.8 mg/L				18 mg/L			
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/17/06 16:30	0	CRP	DO (mg/L)	7.9				8.0				8.0				8.0				8.1				8.1			
			Temperature (°C)	24.7				24.2				24.6				24.5				24.5				24.5			
			pH	7.5				7.4				7.4				7.4				7.5				7.4			
			Sp. Cond. (µmhos/cm)	287				301				303				308				309				309			
8-18-06 16:00	1	YBK	No. Live	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	3*	4	0	0	0	0
			Observations	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	2ERR	3ERR	—	—	—	—
			DO (mg/L)																								
			Temperature (°C)	24.2				24.2				24.2				24.2				24.2				24.2			
			pH																								
			Sp. Cond. (µmhos/cm)																								
8-19-06 10:00	2	CRP	No. Live	3	4	4	4	4	5	5	5	5	2	4	4	4	2	3	4	0	2	0	1	0	0	0	0
			Observations	1ERR	1ERR	1ERR	N	N	2ERR	1ERR	N	1ERR	ERR	1ERR	2ERR	1ERR	1ERR	2ERR	2ERR	—	ERR	—	ERR	—	—	—	—
			DO (mg/L)	7.9				7.6				7.6				7.7				7.9				7.9			
			Temperature (°C)	24.7				24.7				24.7				24.7				24.7				24.7			
			pH	7.4				7.3				7.3				7.3				7.4				7.4			
			Sp. Cond. (µmhos/cm)	293				305				308				310				310				307			

*found only 3 cerios 8-18-06 YBK

Observation Key:
N - Normal
ERR - Erratic Swimming
DOB - Dried Out on Beaker
PM - Particulate Matter
FS - Film on Surface
F - Floater

REVIEWED BY: [signature]

DATE: 9-12-06

*entry error ACS 8/17/06

GLEC Great Lakes Environmental Center

MH

DAPHNID 48-HOUR STATIC-ACUTE TOXICITY TEST FOR WATER EFFECT RATIO STUDIES

TEST MATERIAL: 1909

PROJECT NUMBER: 4412-00

TEST SPECIES: C. dubia

INVESTIGATORS:

TYPE OF TEST:

NO. ANIMALS/CHAMBER: 5

NO. CHAMBERS: 4

AGE/SOURCE OF ANIMALS:

DILUTION WATER:

PHOTOPERIOD (L:D): 16:8

LIGHT INTENSITY (lux): 500-1000

TEST TEMPERATURE (°C): 25±1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	30 mg/L																							
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/17/06 1630	0	CRP	DO (mg/L)	8.1																							
			Temperature (°C)	24.5																							
			pH	7.4																							
			Sp. Cond. (μmhos/cm)	308																							
8-18-06 16:00	1	YBK	No. Live	*50	0	0	0																				
			Observations	*—	—	—	—																				
			DO (mg/L)																								
			Temperature (°C)	24.2																							
			pH																								
			Sp. Cond. (μmhos/cm)																								
8/19/06 1600	2	CRP	No. Live	0	0	0	0																				
			Observations	—	—	—	—																				
			DO (mg/L)																								
			Temperature (°C)	24.7																							
			pH																								
			Sp. Cond. (μmhos/cm)																								

*entry error 8-18-06 YBK

Observation Key:
N - Normal
ERR - Erratic Swimming
DOB - Dried Out on Beaker
PM - Particulate Matter
FS - Film on Surface
F - Floater

REVIEWED BY: [signature]

DATE: 9-12-06

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: 8/17/06 TEST NUMBER: 4412-00 DURATION: 48 h
TOXICANT : 8196
SPECIES: C.dubia

RAW DATA:	Concentration (yg/1)	Number Exposed	Mortalities
--- ----	.00	20	0
	5.00	20	1
	8.30	20	0
	13.90	20	0
	23.10	20	0
	38.60	20	0
	64.30	20	0
	107.00	20	0
	178.60	20	11
	298.00	20	20

SPEARMAN-KARBER TRIM: .71%

SPEARMAN-KARBER ESTIMATES: LC50: 173.69
95% LOWER CONFIDENCE: 154.66
95% UPPER CONFIDENCE: 195.05

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

MCG

DAPHNID 48-HOUR STATIC-ACUTE TOXICITY TEST FOR WATER EFFECT RATIO STUDIES

GLEC
Great Lakes Environmental Center

TEST MATERIAL: 8196
PROJECT NUMBER: 4412-00
TEST SPECIES: C. dubia
INVESTIGATORS: MCG

TYPE OF TEST: WER
NO. ANIMALS/CHAMBER: 5
NO. CHAMBERS: 4
AGE/SOURCE OF ANIMALS: <24hr DC 8/9 SR 8/11

DILUTION WATER: MCG
PHOTOPERIOD (L:D): 16:8
LIGHT INTENSITY (lux): 500-1000
TEST TEMPERATURE (°C): 25±1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	Control				5.0 ug/L				8.3 ug/L				13.9 ug/L				23.1 ug/L				38.6 ug/L			
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/17/06 15:45	0	ACS	DO (mg/L)	8.5				9.0				9.2				9.2				9.2				9.1			
			Temperature (°C)	24.6				24.2				25.3				24.8				24.7				24.7			
			pH	7.1				7.2				7.3				7.3				7.3				7.3			
			Sp. Cond. (µmhos/cm)	523				534				547				550				553				556			
8-18-06 1645	1	CRD	No. Live	5	5	5	5	5	5	5	6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
			Observations	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			DO (mg/L)																								
			Temperature (°C)	24.4				24.4				24.4				24.4				24.4				24.4			
			pH																								
			Sp. Cond. (µmhos/cm)																								
8-19-06 1630	2	CRD	No. Live	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
			Observations	N	N	N	N	N	N	N	N	N	N	N	N	1ERR	N	N	N	N	N	N	N	N	N	N	N
			DO (mg/L)	8.0				8.0				8.0				8.0				8.0				8.0			
			Temperature (°C)	25.2				25.2				25.2				25.2				25.2				25.2			
			pH	7.8				7.8				7.8				7.9				7.9				7.9			
			Sp. Cond. (µmhos/cm)	507				548				552				554				554				557			

Observation Key:

N	-	Normal
ERR	-	Erratic Swimming
DOB	-	Dried Out on Beaker
PM	-	Particulate Matter
FS	-	Film on Surface
F	-	Floater

REVIEWED BY: [signature]

DATE: 9-12-06

GLEC
Great Lakes Environmental Center

MCG

DAPHNID 48-HOUR STATIC-ACUTE TOXICITY TEST FOR WATER EFFECT RATIO STUDIES

TEST MATERIAL: 8196
PROJECT NUMBER: 4412-00
TEST SPECIES: C. dubia
INVESTIGATORS:

TYPE OF TEST: WER
NO. ANIMALS/CHAMBER: 5
NO. CHAMBERS: 4
AGE/SOURCE OF ANIMALS: <24hr DC 8/9 SRB/11

DILUTION WATER: MGC
PHOTOPERIOD (L:D): 16:8
LIGHT INTENSITY (lux): 500-1000
TEST TEMPERATURE (°C): 25±1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	64.3 mg/L				107 mg/L				178.6 mg/L				298 mg/L				496 mg/L							
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/17/06	0	ACS	DO (mg/L)	9.0				8.9				8.9				8.8				8.8							
15:15			Temperature (°C)	25.0				25.5				25.0				25.2				25.5							
			pH	7.3				7.4				7.4				7.3				7.3							
			Sp. Cond. (µmhos/cm)	552				563				565				567				568							
8-18-06	1	CAP	No. Live	5	5	5	5	5	5	5	5	5	3	1	2	0	0	0	0	0	0	0	0				
1646			Observations	N	N	N	N	N	N	N	1ERR	N	N	N	1ERR	–	–	–	–	–	–	–	–				
			DO (mg/L)																								
			Temperature (°C)	24.4				24.4				24.4				24.4				24.4							
			pH																								
			Sp. Cond. (µmhos/cm)																								
8-19-06	2	CAP	No. Live	5	5	5	5	5	3	5	5	4	2	1	2	0	0	0	0	0	0	0	0				
1630			Observations	N	N	1ERR	N	N	N	N	1ERR	1ERR	N	CAF	1ERR	–	–	–	–	–	–	–	–				
			DO (mg/L)	8.0				7.5				7.5				7.6											
			Temperature (°C)	25.2				25.2				25.2				25.2											
			pH	7.8				7.8				7.8				7.9											
			Sp. Cond. (µmhos/cm)	559				558				557				557											

Observation Key:
N - Normal
ERR - Erratic Swimming
DOB - Dried Out on Beaker
PM - Particulate Matter
FS - Film on Surface
F - Floater

REVIEWED BY: [signature]
DATE: 9-12-06

Attachment B

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: 8/29/06 TEST NUMBER: 4412 DURATION: 48 h
TOXICANT : 8197 MH
SPECIES: C.dubia

RAW DATA:	Concentration (yg/l)	Number Exposed	Mortalities
	.00	20	5
	2.30	20	1
	3.90	20	6
	6.50	20	4
	10.80	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 7.42
95% LOWER CONFIDENCE: 6.68
95% UPPER CONFIDENCE: 8.24

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

MH

DAPHNID 48-HOUR STATIC ACUTE TOXICITY TEST

GLEC

TEST MATERIAL: EEC 8197

PROJECT NUMBER: 4912-00

TEST SPECIES: C.dubia

INVESTIGATORS:

TYPE OF TEST: WER

NUMBER OF DAPHNIDS/CHAMBER: 5

NUMBER OF CHAMBERS: 4

AGE OF DAPHNIDS: <24 hrs. DC 8/18 → PC 8/23

DILUTION WATER:

PHOTOPERIOD (L:D): 16 : 8

LIGHT INTENSITY (lux): 500-1000

TEST TEMPERATURE (°C): 25 +/- 1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	CONTROL				2.3 mg/L				3.9 mg/L				6.5 mg/L				10.8 mg/L				18 mg/L			
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8-29-06	0	CAF	DO (mg/L)	8.0				8.0				8.1				7.9				8.0				8.1			
			Temperature (°C)	~~*24.6~~ 24.1				~~*24.4~~ 24.1				~~*25.2~~ 24.1				24.1				24.1				24.1			
1615			pH	7.6				7.6				7.6				7.6				7.6				7.6			
			Sp.Cond. (µmhos/cm)	293				296				299				299				300				300			
8-30-06	1	YBK	No. Live	5	4	5	~~*3~~ 4	5	5	5	5	5	5	5	5	5	5	4	5	2	1	0	0	0	0	0	0
14:45			Observations	N	N	N	N	N	N	N	N	N	ERR	N	N	N	N	N	N	N	N	—	—	—	—	—	—
			Temperature (°C)	25.1																							
8-31-06	2	YBK	No. Live	5	4	2	4	5	4 ~~5~~ ⓧ	5	5	5	4	4	1	5	4 ~~5~~ ⓧ	ⓧ ~~4~~ 2	5	0	0	0	0	0	0	0	0
			Observations	N	N	ERR ⓧ	N	N	N	N	N	N	N	N	N	N	N	N	N	—	—	—	—	—	—	—	—
8:30			DO (mg/L)	7.6				7.8				7.8				7.5				7.6				7.5			
			Temperature (°C)	25.0				25.0				25.0				25.0				25.0				25.0			
			pH	7.3				7.4				7.5				7.5				7.5				7.5			

*entry error ACS 8/29/06

*entry error YBK 8-30-06

ⓧ entry err YBK 8-31-06

Observation Key:

DOB - Dried Out on Beaker
ERR - Erratic Swimming
F - Floater
N - Normal
PM - Particulate Matter
FS - Film on Surface
IMM - Immobile
NA - Not Applicable

REVIEWED BY: [signature]

DATE: 9-12-06

MH

DAPHNID 48-HOUR STATIC ACUTE TOXICITY TEST

GLEC

TEST MATERIAL: EEC 8/97
PROJECT NUMBER: 9912-00
TEST SPECIES: C.dubia
INVESTIGATORS:

TYPE OF TEST: WER
NUMBER OF DAPHNIDS/CHAMBER: 5
NUMBER OF CHAMBERS: 4
AGE OF DAPHNIDS: <24 hrs.

DILUTION WATER:
PHOTOPERIOD (L:D): 16 : 8
LIGHT INTENSITY (lux): 500-1000
TEST TEMPERATURE (°C): 25 +/- 1°C

Date time	Test Day	Tech. Initials	Treatment Level	30 mg/L (~~CONTROL~~)																							
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8-29-06 1615	0	CRP	DO (mg/L)	7.9																							
			Temperature (°C)	24.1																							
			pH	7.6																							
			Sp.Cond. (μmhos/cm)	300																							
8-30-06 14:45	1	YBK	No. Live	0	0	0	0																				
			Observations	—	—	—	—																				
			Temperature (°C)	25.1																							
8-31-06 3:30	2	YBK	No. Live	0	0	0	0																				
			Observations	—	—	—	—																				
			DO (mg/L)	7.5																							
			Temperature (°C)	25.0																							
			pH	7.5																							

Observation Key:

DOB - Dried Out on Beaker
ERR - Erratic Swimming
F - Floater
N - Normal
PM - Particulate Matter
FS - Film on Surface
IMM - Immobile
NA - Not Applicable

REVIEWED BY: [signature]
DATE: 9-12-06

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

DATE: 8/29/06 TEST NUMBER: 4412-00 DURATION: 48 h
TOXICANT : 8197
SPECIES: C.dubia

RAW DATA:

Concentration (yg/l)	Number Exposed	Mortalities
.00	20	0
5.00	20	0
8.30	20	0
13.90	20	0
23.10	20	0
38.60	20	0
64.30	20	0
107.00	19	1
178.60	20	16
298.00	20	20

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 149.09
95% LOWER CONFIDENCE: 134.16
95% UPPER CONFIDENCE: 165.68

MGC Site

GLEC

DAPHNID 48-HOUR STATIC ACUTE TOXICITY TEST

TEST MATERIAL: EEC 8197
TYPE OF TEST: WER
DILUTION WATER: MGC
PROJECT NUMBER: 4912-00
NUMBER OF DAPHNIDS/CHAMBER: 5
PHOTOPERIOD (L:D): 16 : 8
TEST SPECIES: C.dubia
NUMBER OF CHAMBERS: 4
LIGHT INTENSITY (lux): 500-1000
INVESTIGATORS:
AGE OF DAPHNIDS: <24 hrs. DC 8/18 + DC 8/23
TEST TEMPERATURE (°C): 25 +/- 1°C

Date / Time	Test Day	Tech. Initials	Treatment Level	CONTROL				5.0 mg/L				8.3 mg/L				13.9 mg/L				23.1 mg/L				38.6 mg/L			
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/29/06 15:15	0	AI S	DO (mg/L)	8.1				8.2				8.5				8.6				8.5				8.6			
			Temperature (°C)	24.6				24.4				25.2				24.7				24.7				24.8			
			pH	7.4				7.6				7.6				7.6				7.6				7.6			
			Sp.Cond. (µmhos/cm)	499				532				547				551				553				556			
8-30-06 10:00	1	YBK	No. Live	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
			Observations	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Temperature (°C)	25.1																							
8-31-06 14:45	2	YBK	No. Live	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
			Observations	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			DO (mg/L)	7.6				8.0				7.8				8.0				8.0				7.7			
			Temperature (°C)	24.5				24.5				24.5				24.5				24.5				24.5			
			pH	7.9				7.9				8.0				8.0				8.0				7.9			

Observation Key:

DOB - Dried Out on Beaker
PM - Particulate Matter
ERR - Erratic Swimming
FS - Film on Surface
F - Floater
IMM - Immobile
N - Normal
NA - Not Applicable

REVIEWED BY: Chr Tr

DATE: 9-12-06

MGC Site

GLEC

DAPHNID 48-HOUR STATIC ACUTE TOXICITY TEST

TEST MATERIAL: EEC 8197

PROJECT NUMBER: 4912-00

TEST SPECIES: C.dubia

INVESTIGATORS:

TYPE OF TEST: WER

NUMBER OF DAPHNIDS/CHAMBER: 5

NUMBER OF CHAMBERS: 4

AGE OF DAPHNIDS: <24 hrs. DC 8/18 & DC 8/23

DILUTION WATER: MGC

PHOTOPERIOD (L:D): 16 : 8

LIGHT INTENSITY (lux): 500-1000

TEST TEMPERATURE (°C): 25 +/- 1°C

Date Time	Test Day	Tech. Initials	Treatment Level	64.3 mg/L (~~CONTROL~~)				107 mg/L				148.6 mg/L				298 mg/L				496 mg/L							
			Replicate Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
8/29/06 15:15	0	ACS	DO (mg/L)	8.4				8.3				8.4				8.4				8.5							
			Temperature (°C)	24.8				24.7				24.9				24.9				24.9							
			pH	7.6				7.6				7.6				7.6				7.5							
			Sp.Cond. (µmhos/cm)	546				543				554				529				558							
8-30-06 10:00	1	YBK	No. Live	5	5	5	5	5	5	4*	4	4	3	2	1	0	0	0	0	0	0	0	0				
			Observations	N	N	N	N	N	N	N	N	2ERR	N	1ERR	ERR	—	—	—	—	—	—	—	—				
			Temperature (°C)	25.1																							
8-31-06 14:45	2	YBK	No. Live	5	5	5	5	5	5	4	4	2	1	1	0	0	0	0	0	0	0	0	0				
			Observations	N	N	N	N	N	N	N	N	N	N	N	—	—	—	—	—	—	—	—	—				
			DO (mg/L)	7.6				7.5				7.6				7.6				7.6							
			Temperature (°C)	24.5				24.5				24.5				24.5				24.5							
			pH	7.9				7.9				8.0				8.0				8.0							

* only 4 cerios found 8-30-06 YBK

Observation Key:

DOB - Dried Out on Beaker
ERR - Erratic Swimming
F - Floater
N - Normal
PM - Particulate Matter
FS - Film on Surface
IMM - Immobile
NA - Not Applicable

REVIEWED BY: [signature]

DATE: 9-12-06

Attachment C

GLEC

Ceriodaphnia dubia ACCLIMATION CULTURES

Initial Chemistries (New)

DATE	WATER		DO (mg/L)	pH	Temp. (°C)	Spec. Cond. (µmhos/cm)	Initials
	TYPE	BATCH #					
8/9/06	DC	7	7.8	8.3	24.9	645	ACS
8/10/06	DC	7	7.8	8.3	24.7	645	ACS
8/11/06	DC	7	7.9	8.3	24.7	641	ACS
8/14/06	DC	7	7.9	8.3	24.6	635	ACS
8/16/06	DC	7	7.9	8.2	25.9	636	ACS
		D.C.	due to	age	8/18/06	ACS	

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # OC 8/9 #1 | Young from Vessel # SR 7/28 & OC 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/9/06	0	+ →										ACS	—	—	BF
8/10/06	1	+ →										ACS	—	—	CF
8/11	2	+e →										ACS	8.2	25.1	Reduced to 1
8/12	3	A →										ACS	—	—	
8/13	4	+e	5	+e →		6	6	+e	5	5	5	ACS	—	—	
8/14	5	6	+e	6	6	+e	7	4	+e →		1[⊕]	ACS	—	—	
8/15	6	8	6	7	6	8	+e	8	8	8	+e	ACS	—	—	
8/16	7	B →							B	B	9	ACS	—	—	
8/17	8	4	8	8	8	9	10	+e	8	9	11	ACS	—	—	
8/18	9	A	B	B	B	B	C	B	B	B	B	SAC	—	—	

Legend:

e = Eggs Present
+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

⊕ from previous brood ACS 8/14/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # OC 8/9 #2 | Young from Vessel # SR 7/28 & OC 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/9/06	0	+ →										ACS	—	—	BF
8/10/06	1	+ →										ACS	—	—	CF
8/11	2	+e →										ACS	—	—	Reduced to 1
8/12	3	A →										ACS	—	—	
8/13	4	+e →		6	7	5	8	6	6	6	6	ACS	—	—	
8/14	5	7	8	+e →			6	+e	5	+e →		ACS	—	—	
8/15	6	8	8	8	8	7	1*	7	+e	7	9	ACS	—	—	
8/16	7	B →										ACS	—	—	
8/17	8	+e	A	C	B	B	10	8	9	B	10	ACS	—	—	
8/18	9	B	A	B	C	B	C	B	B	B	B	SAC	-	-	

Legend:

e = Eggs Present
+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

*from previous brood ACS 8/15/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/9 #3

Young from Vessel # SR 7/28 & DC 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/9/06	0	+ →									→	ACS	—	—	BF
8/10/06	1	+ →									→	ACS	—	—	CF
8/11	2	+e →									→	ACS	—	—	Reduced to 1
8/12	3	A →									→	ACS	—	—	
8/13	4	5	4	4	+e	6	+e	4	6	7	7	ACS	—	—	
8/14	5	+e →	→	6	6	—	7	8	+e	8	+e	ACS	—	—	
8/15	6	7	7	+e	6		6	1⊕	7	1⊕	9	ACS	—	—	
8/16	7	B →			→		B →				→	ACS	—	—	
8/17	8	B	C	B	B		+e	9	B	11	B	ACS	—	—	
8/18	9	C	B	B	+e		B	B	B	B	B	JAC	—	—	

Legend:
e = Eggs Present
+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/9 #4 | Young from Vessel # SR 7/28 & DC 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/9/06	0	+ →									→	ACS	—	—	BF
8/10/06	1	+ →									→	ACS	—	—	CF
8/11	2	+e →									→	ACS	—	—	Reduced to 1
8/12	3	A →									→	ACS	—	—	
8/13	4	6	7	6	7	~~7~~* +e	7	+e	5	6	6	ACS	—	—	
8/14	5	1⊕	+e	7	8	6	+e	4	+e →		→	ACS	—	—	
8/15	6	8	7	+e	+e	7	8	6	7	9	7	ACS	—	—	
8/16	7	B →									→	ACS	—	—	
8/17	8	C →	→	10	11	B	C	+e	9	B	C	ACS	—	—	
8/18/06	9	B	B	B	+e	C	B	B	B	—	B	SAC	—	—	

Legend:
e = Eggs Present
+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed
NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

*entry error ACS 8/13/06
⊕ from previous brood ACS 8/14/06 ACS

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/9 #5

Young from Vessel # SR 7/28 a DC 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/9/06	0	+	→	→	→	→	→	→	→	→	→	ACS	—	—	BF
8/10/06	1	+	→	→	→	→	→	→	→	→	→	ACS	—	—	CF
8/11	2	+e	→	→	→	→	→	→	→	→	→	ACS	—	—	Reduced to 1
8/12	3	+e	A	→	→	→	→	→	→	→	→	ACS	—	—	
8/13	4	+e	6	+e	→	6	6	7	8	+e	6	ACS	—	—	
8/14	5	6	+e	6	6	7	10	7	6	9	8	ACS	—	—	
8/15	6	4	7	7	9	+e	9	+e	→	7	+e	ACS	—	—	
8/16	7	B	→	→	→	→	→	→	→	→	→	ACS	—	—	
8/17	8	+e	C	B	→	13	4	9	9	11	B	ACS	—	—	
8/18/06	9	B	B	B	B	+e	B	B	C	B	B	SAL	—	—	

Legend:

e = Eggs Present
\+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

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Ceriodaphnia dubia ACCLIMATION CULTURES

Initial Chemistries (New)

DATE	WATER		DO (mg/L)	pH	Temp. (°C)	Spec. Cond. (µmhos/cm)	Initials
	TYPE	BATCH #					
8/11/06	SR	13	7.8	7.9	24.7	483	ACS
8/13/06	SR	13	8.0	8.1	24.4	489	ACS
8/14*/06	SR	13	8.0	8.0	24.6	485	ACS
8/16/06	SR	13	7.9	8.1	26.9	476	ACS
8/18/06	SR	13	7.6	8.0	25.3	489	SAC
8/21	SR	13	8.0	8.1	25.1	476	ACS
	D.C. due	to lack	of need		8-23-06		[illegible]

*entry error ACS 8/16/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # SR 8/11 #1

Young from Vessel # DC 8/1 + SR/DC 8/4

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/11/06	0	+	→	→	→	→	→	→	→	→	→	ACS	—	—	BF
8/12/06	1	+	→	→	→	→	→	→	→	→	→	ACS	—	—	CF
8/13/06	2	+e	→	→	→	→	→	→	→	→	→	ACS	—	—	Reduced to 1
8/14/06	3	+e	A	+e	A	→	+e	→	A	+e	→	ACS	—	—	
8/15/06	4	A	+e	A	+e	→	A	→	+e	A	+e	ACS	—	—	
8/16/06	5	5	7	6	6	6	6	6	4	3	5	ACS	—	—	
8/17/06	6	4	8	7	8	3	4	5	5	5	5	ACS	—	—	
8/18/06	7	+e	B	→	→	A	B	+e	B	B	B	SAE	—	—	
8/19/06	8	B	+e	+e	+e	+e	+e	C	+e	+e	+e	SAE	—	—	
8/20/06	9	C	C	C	C	C	C	C	B	C	B	SAE	—	—	
8/21/06	10	+e	→	B	→	→	→	+e	B	→	→	ACS	—	—	
8-23-06	12	C	→	→	→	→	→	→	→	→	→	CMJ			

Legend:

- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # SR 8/11 #2 | Young from Vessel # DC 8/1 + SR/DC 8/4

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/11/06	0	+ →									→	ACS	—	—	BF
8/12/06	1	+ →									→	ACS	—	—	CF
8/13/06	2	+e →									→	ACS	—	—	Reduced to 1
8/14/06	3	+e	A	+e →						→	A	ACS	—	—	
8/15/06	4	A	+e	A →						→	+e	ACS	—	—	
8/16/06	5	4	6	4	4	6	4	4	6	6	5	ACS	—	—	
8/17	6	4	8	7	5	~~4~~ 5*	6	6	6	10⊕ →	4	ACS	—	—	
8/18/06	7	+e	C	B	A	+e	B →		→	A	A	SAC	—	—	
8/19/06	8	+e	+e	+e	+e	C	+e →				→	SAC	—	—	
8/20/06	9	A	C	C	C	C	C	C	C	C	C	SAC	—	—	
8/21/06	10	+e	B →		→	+e	B →				→	ACS	—	—	
8-23-06	12	C →									→	CM			

Legend:
e = Eggs Present
\+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

⊕ from previous brood ACS 8/17
* entry error ACS 8/17/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # SR 8/11 #3 | Young from Vessel # DC 8/1, SR01 8/1

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/11/06	0	+ →										ACS	—	—	BF
8/12/06	1	+ →										ACS	—	—	CF
8/13/06	2	+e →										ACS	—	—	Reduced to 1
8/14/06	3	+e →						A →		+e →		ACS	—	—	
8/15/06	4	A →					+e →			A →		ACS	—	—	
8/16/06	5	6	6	5	5	5	5	7	5	+e	5	ACS	—	—	
8/17	6	6	7	6	2	1	6	4	4	5	5	ACS	—	—	
8/18/06	7	A	B	B	A	A	B	B →				SAe	—	—	
8/19/06	8	B	+e	+e	1	+e	B	C	C	+e	+e	SAe	-	—	
8/20/06	9	C	B	B	B	C	+e	+e	+e	B	C	SAe	-	—	
8/21/06	10	B →										ACS	—	—	
8-23-06	12	C →										CDT			

Legend:
- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

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Ceriodaphnia dubia ACCLIMATION CULTURES

Initial Chemistries (New)

DATE	WATER		DO (mg/L)	pH	Temp. (°C)	Spec. Cond. (µmhos/cm)	Initials
	TYPE	BATCH #					
8/18/06	DC	7	7.6	8.3	25.2	640	SAC
8/19/06	DC	7	7.8	8.3	25.1	631	SAC
8/21/06	DC	7	8.1	8.3	25.1	624	ACS
8/23/06	DC	7	8.0	8.3	24.8	623	JLT
8/25/06	DC	7	7.8 ~~7.0~~*	8.4	24.7	630	ACS
8/28/06	DC	8	7.9	8.3	24.7	683	ACS

D.C. due to lack of need
ACS 8/30/06

*entry error ACS 8/25/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/18 II | Young from Vessel # DC 8/9

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/18/06	0	+ →										SAC	—	—	BF
8/19/06	1	+ →										SAC	—	—	CF
8/20	2	+e →										SAC	—	—	Reduced to 1
8/21	3	A	A	A	A	A	A	+e	+e	+e	+e	JLT	—	—	
8/22	4	6	5	6	6	7	7	A	A	A	A	JLT	—	—	
8/23	5	9	+e	7	7	6	7	8	6	10	7	JLT	—	—	
8/24	6	9	10	9	10\9	8	10	+e →		10	+e	ACS	—	—	
8/25	7	11	8	8	7	10	7	9	8	+e	7	ACS	9.5	25.2	
8/28	10	C →										ACS	—	—	
8/29	11	+e 10	11	+e →		9	8	11	12	12	11	ACS	—	—	
8/30	12	+e →		B →		+e →		B →			+e	ACS	—	—	

Legend:
e = Eggs Present
\+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

⊕ probably from previous board. ACS 8/24/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/18 # 2 | Young from Vessel # DC 8/9

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/18/06	0	+	→	→	→	→	→	→	→	→	→	SAC	—	—	BF
8/19/06	1	+	→	→	→	→	→	→	→	→	→	SAC	—	—	CF
8/20	2	+e	→	→	→	→	→	→	→	→	→	SAC	—	—	Reduced to 1
8/21	3	+e	→	→	→	A	A	+e	A	A	+e	JLT	—	—	
8/22	4	A	A	B	B	7	7	A	6	4	A	JLT	—	—	
8/23	5	8	7	6	6	10	6	9	e+	→	7	JLT	—	—	
8/24	6	+e	→	→	→	9	9	+e	7	9	1⊕	ACS	—	—	
8/25	7	7	9	8	9	10	7	10	14	8	8	ACS	—	—	
8/28	10	C	→	→	→	→	→	→	→	→	→	ACS	—	—	
8/29	11	12	12	9	6	13	9	13	8	10	11	ACS	—	—	
8/30	12	+e	B	→	→	+e	→	B	+e	→	B	ACS	—	—	

Legend:

- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

⊕ probably from previous board ACS 8/24/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/18 #3 | Young from Vessel # DC 8/9

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/18/06	0	+	—	—	—	—	—	—	—	—	→	SAC	—	—	BF
8/19/06	1	+	—	—	—	—	—	—	—	—	→	SAC	—	—	CF
8/20	2	+e	—	—	—	—	—	—	—	—	→	SAC	—	—	Reduced to 1
8/21	3	+e	A	A	+e	A	+e	+e	A	+e	+e	JLT	←	—	
8/22	4	A	4	3	A	5	A	B	8	A	A	JLT	—	—	Debris in #9
8/23	5	8	7	6	7	6	7	8	+e	7	8	JLT	—	—	
8/24	6	+e	8	8	+e	8*	+e	→	8	+e	→	ACS	—	—	
8/25	7	8	9	8	9	8	6	7	8	8	9	ACS	—	—	#3 erratic
8/28	10	C	—	—	—	—	—	—	—	—	→	ACS	—	—	
8/29	11	10	7	5	9	9	9	10	8	9	10	ACS	—	—	
8/30	12	B	+e	B	→	+e	B	→	+e	B	→	ACS	—	—	

Legend:

e = Eggs Present
\+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

*mom erratic due to tech error ACS 8/24/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8/18 #4 — Young from Vessel # DC 8/9

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8/18/06	0	+ ——→										SAL	—	—	BF
8/19/06	1	+ ——→										SAL	—	—	CF
8/20	2	+e ——→										SAL	—	—	Reduced to 1
8/21	3	+e	A	+e	——→			A	+e ⊛	A	+e	JLT	—	—	
8/22	4	B	9	A	A	A	A	8	A	6	A	JLT	—	—	Debris in #8
8/23/06	5	6	+e	6	7	10	7	+e	8	7	7	JLT	—	—	
8/24	6	1⊕	8	1⊕	10	+e	1⊕	8	1⊕	8	1⊕ 9	ACS	—	—	#3 erratic
8/25	7	9	8	8	+e	9	8	7	8	6	+e	ACS	—	—	
8/28	10	C ——→										ACS	—	—	
8/29	11	11	8	10	+e	9	+e	9	8	8	+e	ACS	—	—	#9 erratic
8/30	12	B	+e	B	——→					↓	B	ACS	—	—	

Legend:
- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

⊛ entry error 8/21/06 JLT

⊕ from previous board ACS 8/24/06

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Ceriodaphnia dubia ACCLIMATION CULTURES

Initial Chemistries (New)

DATE	WATER		DO (mg/L)	pH	Temp. (°C)	Spec. Cond. (μmhos/cm)	Initials
	TYPE	BATCH #					
8-23-06	DC	7	7.8	8.3	25.7	657	JLT
8/24/06	DC	7	8.0	8.3	24.7	613	ACS
8/25/06	DC	7	7.8	8.4	24.7	630	ACS
8/28/06	DC	8	7.9	8.3	24.7	683	ACS
8/30/06	DC	7	7.7	8.4	25.1	632	ACS
9/1/06	DC	7/8	7.8	~~7.8~~* 8.4	25.1	641	ACS

D.C. due to age & lack of need

ACS 9/4/06

*entry error ACS 9/1/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8-23-06 # 1 | Young from Vessel # SR 8-11-06

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8-23-06	0	+										JLT	—	—	BF
8-24-06	1	+										ACS	—	—	CF
8-25-06	2	+e										ACS	—	—	Reduced from 2 or + per cup to 1 per
8-26-06	3	A										cap	—	—	
8-27-06	4	+e										~~CBT~~	—	—	
8/28/06	5	+e										ACS	—	—	#1 erratic on debris
8/29/06	6	—	10	10	4	8	8	10	9	8	10	ACS	—	—	
8/30/06	7		8	10	9	9	8	8	7	8	9	ACS	—	—	
8/31/06	8		+e		9	B	A	+e				ACS	—	—	
9/1/06	9		B		+e		B					ACS	9.5	25.6	
9/4/06	12		C									ACS	—	—	

Legend:
e = Eggs Present
\+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

Fanditizer CAR 8-27-06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8-23-06 # 2														Young from Vessel # SR 8-11-06	
Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8-23-06	0	+ →									→	JLT	—	—	BF
8-24-06	1	+ →									→	ACS	—	—	CF
8-25-06	2	+e →									→	ACS	—	—	Reduced from 2 oz per cup to 1 per
8-26-06	3	A →									→	CRD	—	—	
8-27-06	4	+e	→								→	cr	—	—	
8/28/06	5	8	8	+e	→	1⊕	+e	→	1	+e	→	ACS	—	—	
8/29/06	6	8	10	9	7	11	9	9	9	10	10	ACS	—	—	
8/30/06	7	+e	+e	10	+e	10	6	8	+e	9	8	ACS	—	—	
8/31/06	8	8	C	+e	6	+e →		→	9	+e	→	ACS	—	—	
9/1/06	9	B →									→	ACS	—	—	
9/4/06	12	C →						T			→	ACS	—	—	

Legend:

e = Eggs Present
+ = alive
A = 0-5 Young
B = 6-10 Young
C = > 10 Young
m = Males Observed

NY = No Young Present
DC = Discontinued Culture
YR = Young Removed for Testing
BF = Beaker Form (15 neonates per beaker)
CF = Cup Form (10 healthiest females placed into individual cups)

⊕ from previous brood ACS 8/28/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8-23-06 # 3 | Young from Vessel # 5R 8-11-06

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8-23-06	0	+									→	JLT	—	—	BF
8-24-06	1	+									→	ACS	—	—	CF
8-25-06	2	+e									→	ACS	—	—	Reduced from 2 oz per cup to 1 per
8-26-06	3	A									→	CRD	—	—	
8-27-06	4	+e									→	CM	—	—	
8/28/06	5	—	+e	2	+e	→	1 ⊕	+e			→	ACS	—	—	
8/29/06	6		9	9	8	6	9	9	9	9	8	ACS	—	—	
8/30/06	7		7	10	8	9	9	8	8	6	8	ACS	—	—	
8/31/06	8		+e			→	+e				→	ACS	—	—	
9/1/06	9		B								→	ACS	—	—	
9/4/06	12		C								→	ACS	—	—	

Legend:
- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

⊕ ~~entry~~ from previous brood ACS 8/28/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID #: DC 8-23-06 # 4 | Young from Vessel # 5R 8-11-06

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8-23-06	0	+									→	JLT	—	—	BF
8-24-06	1	+									→	ACS	—	—	CF
8-25-06	2	+e									→	ACS	—	—	Reduced from 2 oz per cup to 1 per
8-26-06	3	A									→	CRD	—	—	
8-27-06	4	+e									→	CAr	—	—	
8/28/06	5	+e			→	1⊕	2	+e			→	ACS	—	—	
8/29/06	6	8	9	8	8	9	7	8	8	10	8	ACS	—	—	#2 erratic on bubble
8/30/06	7	6	7	7	8	7	+e	8	5	9	7	ACS	—	—	
8/31/06	8	1⊕	+e	→	B	4e	+e 8	B	+e	1⊕\B	A	ACS	—	—	
9/1/06	9	B					→	+e	B	+e	A	ACS	—	—	
9/4/06	12	C									→	ACS	—	—	

Legend:
- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

⊕ from previous brood ACS 8/28/06

CERIODAPHNIA DUBIA ACCLIMATION CULTURES

Culture Vessel ID # DC 8-23-06 # 5 | Young from Vessel # 52 8-11-06

Date	Age in Days	Test Chamber Number 1	2	3	4	5	6	7	8	9	10	Initials	Old D.O.	Old Temp	Observation
8-23-06	0	+ →									→	JLT	—	—	BF
8-24-06	1	+ →									→	ACS	—	—	CF
8-25-06	2	+e →									→	ACS	—	—	Reduced from 2.0+ per cup to 1 per.
8-26-06	3	A →									→	CRW	—	—	
8-27-06	4	+e →									→	CAR	—	—	
8/28/06	5	+e →									→	ACS	—	—	
8/29/06	6	9	8	8	9	9	9	10	10	9	8	ACS	—	—	
8/30/06	7	9	7	8	8	8	+e	9	9	7	9	ACS	—	—	
8/31/06	8	B	+el	4	+e →	→	6	4e →	→	→	B	ACS	—	—	

Legend:
- e = Eggs Present
- + = alive
- A = 0-5 Young
- B = 6-10 Young
- C = > 10 Young
- m = Males Observed
- NY = No Young Present
- DC = Discontinued Culture
- YR = Young Removed for Testing
- BF = Beaker Form (15 neonates per beaker)
- CF = Cup Form (10 healthiest females placed into individual cups)

D.C. due to lack of need ACS 8/31/06