Chadwick Ecological Consultants, Inc.
5575 South Sycamore Street, Suite 101, Littleton, Colorado 80120
? (303) 794-5530, Fax: (303) 794-5041, e-mail: Chadeco@aol.com

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TECHNICAL MEMORANDUM

NATIVE AQUATIC INSECT ZINC TOXICITY TESTING

Introduction

Within the currently approved EPA documents used to derive water quality criteria for zinc, there are 36 genera that comprise the acute toxicity database (U.S. EPA 1996). As part of the development of site-specific zinc standards for the West Fork Clear Creek (Clear Creek Segment 5) in the recent South Platte Triennial Review, we recently completed a significant update to this toxicity database - with the acute database now comprised of 51 genera.

In the original and updated databases, toxicity data do exist for many important taxa known to occur in Colorado streams. However, this is often limited to the fish species (e.g., brown trout, brook trout, etc). Zinc toxicity data are limited or lacking for many resident benthic macroinvertebrate genera important in Colorado mountain streams, such as mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) - the so-called EPT taxa. The relative lack of toxicity data on EPT taxa is significant since these groups are often used as being diagnostic of healthy or impaired stream conditions.

In fact, the lack of zinc toxicity data on aquatic insects (especially the lack of data for mayflies) was of particular concern when considering site-specific standards for coldwater trout streams during the recent South Platte water quality hearings.

To provide information for this apparent data gap, Climax Molybdenum Company, Inc., contracted Chadwick Ecological Consultants, Inc. (CEC) to conduct a study meant to acquire acute zinc toxicity data for several macroinvertebrate taxa. These additional data would constitute a substantial improvement to the database and subsequently in the water quality criteria derived from it - especially when developing site-specific criteria.

Methods

Field-Collection of Benthic Macroinvertebrates

Test Organism Issues: Toxicity testing of benthic macroinvertebrates is not common, especially with regard to testing with aquatic insects. This is primarily due to the rarity of known source cultures (i.e., laboratory raised) from which to derive a group of organisms within a known age range. Given the lack of laboratory cultured aquatic insects, field collection of test organisms was conducted. Field collection, as opposed to laboratory culturing, of test organisms for toxicity testing is not EPA's preferred method for deriving toxicity data. Nevertheless, data derived in this manner is frequently used for the derivation of water quality criteria. For example, field-derived test organisms constitute roughly 20% of the data for current acute zinc criteria, U.S. EPA 1996. Field collection of test organisms also has the added benefit of providing toxicity data more representative of the habitats from which the organisms are sampled (i.e., for Colorado's coldwater streams).

Site Selection and Field Sampling: Sampling for test organisms was conducted on three occasions from a site near the mouth of the South Fork Cache la Poudre River (N40°41'139", W105°23'838", 6,750 ft elevation), a tributary of the Cache la Poudre River west of Fort Collins, Colorado. Sampling was conducted September 3, September 10, and October 22, 2004. This site was chosen for a number of reasons, including the use of the Poudre River basin for location of control sites in previous studies on potential impacts of metals on aquatic life (e.g., Kiffney and Clements 1994, 1996). Water quality samples collected concurrent with the insect collection revealed low metals levels (e.g., Cd <0.1 :g/L, Cu < 0.5 :g/L, Pb = 0.1 :g/L, and Zn < 10 :g/L) supporting this location as an appropriate collection site.

For each sampling event, a composite kick net (500 :m mesh) sample was collected from the stream riffle habitats and emptied into a large picking trey. Organisms were very carefully removed from the tray using a plastic pipette with the tip removed (no forceps or invasive handling procedures were used). Individuals were not kept if they appeared injured from the collection process to insure only intact individuals were retained for toxicity testing. Collected organisms were transported to Chadwick & Associates, Inc. in covered plastic tubs kept inside a cooler with an aeration stone to maintain similar ambient temperature and dissolved oxygen levels. To ensure sufficient organisms for the toxicity testing, a minimum of 400-1000 instars of each taxa were collected per sampling trip.

Organism Selection: A total of four taxa were originally proposed for testing: two mayfly taxa (Ephemeroptera), one stonefly taxon (Plecoptera), and one caddisfly taxon (Trichoptera). Recent acute and/or chronic toxicity data exists for some of these insects for some metals. However, none of the species collected are represented in the currently approved EPA acute zinc toxicity database (U.S. EPA 1996). Multiple species of mayflies were proposed for testing to assess the degree of variability in tolerance within this presumed sensitive order. While it is generally assumed that mayflies are sensitive, the U.S. EPA Rapid Bioassessment Protocol tolerance index (U.S. EPA 1999) notes that a number of mayfly genera are not “sensitive,” but rather can be characterized as somewhat tolerant (i.e., *Baetis*, *Stenonema*, etc.).

Over the three sampling periods, a total of two mayfly taxa, a stonefly, and two caddisfly taxa were collected. Species collected during each trip were selected due to their relatively high abundance at the time of collecting. During the first sampling trip, the mayfly *Drunella grandis* and the caddisfly *Dolophilodes aequalis* (a free-dwelling caddisfly - i.e., no case) were collected. During the second trip, the mayfly *Baetis tricaudatus* and and the stonefly *Isoperla* sp. were collected. Given the performance of the test organisms collected on the first two trips (see discussion below), a third collecting trip was made in October. At that time additional individuals of the mayfly *Drunella grandis* were collected, as well a different caddisfly, *Lepidostoma* sp. (a case dwelling caddisfly).

Laboratory Toxicity Test Conditions

Once in the laboratory, organisms were allowed to acclimate (~ 2-3 days) to ASTM-approved testing conditions prior to test initiation. Test conditions included 16/8 hr light/dark schedule, temperature of 12-13°C, feeding with YCT during acclimation and prior to 48 hr renewal. 96 hr acute *D. grandis* tests were conducted September 7 to 11, 2004, and 96 hr acute *Isoperla* tests were conducted September 13 to 17, 2004. The second acute zinc toxicity test conducted on *Drunella grandis* (mayfly) and the test with *Lepidostoma* sp. (caddisfly) were conducted October 25 to 29, 2004. The tests initiated in September using *Dolophilodes aequalis* (caddisfly) and *Baetis atricaudatus* (mayfly), unfortunately, had to be terminated due to excessive organism mortality in all treatments, including the control, indicating poor acclimation of these two species to sampling and laboratory conditions.

Static-renewal toxicity test procedures followed methods described in ASTM E 729-96 and U.S. EPA (2002). Samples of test water from the various dilutions were shipped to ACZ Laboratories for analysis of total zinc. All values presented herein were generated using measured zinc values.

If the number of organisms were sufficient, the tests were performed using three different hardnesses of reconstituted water, and given the numbers of field collected organisms and their general health, tests were also run in duplicate.

Soft = 50 mg/L as $CaCO_3$ nominal hardness
Hard = 150 mg/L as $CaCO_3$ nominal hardness
Very hard = 300 mg/L as $CaCO_3$ nominal hardness

Tested zinc concentrations varied with hardness, as well (Table 1). Over the three hardness tests, the dilution series consisted of the zinc concentrations (in mg/L) ranging from 0 mg/L to 6.4 mg/L for the mayfly tests. Concentrations in the stonefly and caddisfly test varied over a range of 0 mg/L up to 80 mg/L. Test conditions and detailed results are in the original lab reports (C&A 2004a, b, 2005), which are available upon request.

TABLE 1: Measured zinc concentrations (mg/L) used in the native aquatic insect toxicity testing.

Test Date/Species		Zinc Concentration (mg/L)					
9/7/04 *Drunella grandis*	soft	0	0.1*	0.2*	0.4*	0.8*	1.56
	hard	0	0.1*	0.2*	0.4*	0.8*	2.19
	very hard	0	0.2*	0.4*	0.8*	1.6*	3.27
9/13/04 - *Isoperla* sp.	Hard	0	1.82	3.55	7.21	13.80	27.00
10/25/04 *Drunella grandis*	soft	0.06	0.21	0.40	0.79	1.60	3.05
	hard	0	0.20	0.39	0.76	1.55	3.05
	very hard	0	0.4	0.82	1.59	3.19	6.29
10/25/04 *Lepidostoma* sp.	soft	0	2.49	4.97	9.69	19.10	38.80
	hard	0	2.5*	5.0*	10.0*	20.0*	38.80
	very hard	0	5.0*	10.0*	20.0*	40.0*	81.70

* nominal values

Results

Organism Survival: Adapting to Laboratory Conditions

One unavoidable obstacle with conducting toxicity tests with field-collected organisms from Colorado mountain streams is selecting taxa that can adapt to laboratory conditions (i.e., static test chambers). Sensitivity to change in physical conditions (e.g temperature, dissolved oxygen, flowing vs. non-flowing) as well as sensitivity to handling stress is basically unknown for many of the benthic macroinvertebrates common to Colorado mountain streams.

The mayfly *D. grandis* performed very well during both the field collection phase (including transport to the lab) and under laboratory conditions for this study. Mean control survival of *D. grandis* was >80% in all waters tested during the both series of tests. In contrast, the other mayfly, *B. tricaudatus*, performed poorly, exhibiting mortality prior to and during the test (which had to be terminated very early due to excessive mortality in all treatments, including the controls). Although *Baetis* is a ubiquitous mayfly in Colorado streams, it is somewhat fragile (a trait even noticeable in our processing of typical stream population samples). It appears that the mortality observed for this mayfly was a response to handling stress - basically a result of the field collection and transport efforts - stressors that did not appear to affect *Drunella*.

Similar divergent responses to the transition from field to laboratory conditions were observed for the two field-collected caddisflies. Control survival of *Lepidostoma sp*. was >95% in test waters, whereas the *D. aequalis* test also had to be terminated early due to excessive mortality in all treatments, including the control. In this case, however, we do not believe the problems with *Dolophilodes* was due to "handling stress." Rather, for this caddisfly it may be that laboratory conditions were too different from their preferred stream habitat (especially static vs flowing water).

There were enough organisms to conduct successful replicate tests at each hardness for both sets of the mayfly *D. grandis* tests. For the *Isoperla* tests it was determined after test initiation that multiple stonefly species were present in the tests. Test organisms were examined and other genera were removed, resulting in the same stonefly genus for all tests. As a result, the *Isoperla* soft and very hard tests could not be completed due to insufficient number of remaining test organisms. In addition, the data from the duplicate tests of the hard reconstituted water had to be pooled and reported as one test. Given the lesser abundance in the field, the *Lepidostoma* tests were run in duplicate only with hard reconstituted water test (although soft and very hard tests were also run).

Zinc Toxicity Tests Results

The results of the two series of completed zinc toxicity tests are summarized below in Tables 2 and 3. The key result of the toxicity testing was that no acute toxicity was recorded for the mayfly *Drunella grandis* at zinc concentrations up to 6.3 mg/L (depending on hardness). For the stonefly *Isoperla* there was no acute toxicity at zinc concentrations up to 27 mg/L in hard water. We did find measurable acute toxicity for the caddisfly *Lepidostoma* in soft water, with an LC_{50} of 26.2 mg/L zinc. However, in hard and very hard water, there was no acute toxicity for *Lepidostoma* at concentrations of 38.8 mg/L and 81.7 mg/L, respectively.

TABLE 2: Acute total zinc toxicity for the mayfly *Drunella grandis* (Tested 7-11 September 2004) and the stonefly *Isoperla* sp. (Tested 13-17 September 2004).

Test Species	Common Name	Hardness (mg/L CaCO3)	LC_{50} (mg/L Zn)
Drunella grandis	Mayfly	50.6	>1.56
Drunella grandis	Mayfly	50.6	>1.56
Drunella grandis	Mayfly	172.0	>2.19
Drunella grandis	Mayfly	172.0	>2.19
Drunella grandis	Mayfly	260.7	>3.27
Drunella grandis	Mayfly	260.7	>3.27
Isoperla sp.	Stonefly	182.2	>27.0

TABLE 3: Acute total zinc toxicity for the mayfly *Drunella grandis* (Tested 25-29 October 2004) and the caddisfly *Lepidostoma* sp. (Tested 25-29 October 2004).

Test Species	Common Name	Hardness (mg/L CaCO3)	LC_{50} (mg/L Zn)
Drunella grandis	Mayfly	54.2	>3.05
Drunella grandis	Mayfly	54.2	>3.05
Drunella grandis	Mayfly	175.0	>3.05
Drunella grandis	Mayfly	175.0	>3.05
Drunella grandis	Mayfly	277.7	>6.29
Drunella grandis	Mayfly	277.7	>6.29
Lepidostoma sp.	Caddisfly	62.1	26.2
Lepidostoma sp.	Caddisfly	189.4	>38.8
Lepidostoma sp.	Caddisfly	189.4	>38.8
Lepidostoma sp.	Caddisfly	308.8	>81.7

Comparative Zinc Toxicity for Aquatic Insects

These data increase the existing toxicity information for aquatic insects considerably. Using the summarized zinc toxicity data from our West Fork Clear Creek report (CEC 2004), we can compare the results of this study with other reported toxicity data for aquatic insects (Table 4).

Based on available data, including these new test data, aquatic insects are relatively tolerant to zinc toxicity - even the EPT taxa used in the present study. Most surprising, perhaps, is the apparent tolerance of the mayfly *Drunella* to zinc in this test. Based on discussions with U.S. EPA, the Division, and CDOW with regard to the site specific zinc standards for West Fork Clear Creek, it was apparent that the agencies assumed an acute value for mayflies would be lower than 0.4 mg/L. This assumption by the agencies was the basis for re-inclusion of toxicity data for the non-resident amphipod *Hyalella*, with an acute value of 0.24 mg/L. However, in our study, we did not have acute zinc toxicity in soft water even at 3.2 mg/L zinc for the mayfly.

TABLE 4: Comparative acute toxicity of zinc to aquatic insects.

Test Species	Common Name	Hardness (mg/L CaCO3)	LC_{50} (mg/L Zn)	Reference
Argia	Damselfly	50	88.4	(cited in EPA 1996)
Zygoptera	Damselfly	50	26.2	Rehwoldt *et al*. 1973
Trichoptera	Caddisfly	50	58.1	Rehwoldt *et al*. 1973
Lepidostoma	Caddisfly	62.1	26.2	Present Study
Lepidostoma	Caddisfly	189.4	>38.8	Present Study
Lepidostoma	Caddisfly	308.8	>81.7	Present Study
Isoperla sp.	Stonefly	182.2	>27.0	Present Study
Chironomus	Midge	50	18.2	Rehwoldt *et al*. 1973
Ranatra elongata	Water Scorpion	112.4	1.66	Shukla *et al*. 1983
Drunella grandis	Mayfly	50.6	>1.56	Present Study
Drunella grandis	Mayfly	54.2	>3.05	Present Study
Drunella grandis	Mayfly	172.0	>2.19	Present Study
Drunella grandis	Mayfly	175.0	>3.05	Present Study
Drunella grandis	Mayfly	260.7	>3.27	Present Study
Drunella grandis	Mayfly	277.7	>6.29	Present Study

Summary and Discussion

There were two unknowns being addressed by this study. The first was related to unknown zinc toxicity to aquatic insects common to Colorado mountain streams. The second was a more fundamental unknown - can organisms sampled from Colorado mountain streams acclimate to laboratory conditions for standardized toxicity testing?

This study provided valuable information on both issues. We were able to successfully collect, transport, acclimate, and test under laboratory conditions a mayfly, stonefly, and caddisfly from the South Fork Cache la Poudre River. Problems were identified with other taxa that should improve future test success through selective choice of test species.

With regard to zinc toxicity, we were partially successful. We were able to develop an actual, calculated acute (LC_{50}) level only for the caddisfly *Lepidostoma* in soft water. For the other tests, the zinc concentrations tested were not high enough to elicit an acute response, including the second test with the mayfly *Drunella* when the zinc concentrations were doubled. Regardless, the values obtained are similar to other non-EPT aquatic insect zinc toxicity data.

The lack of response in soft water from *Drunella* at concentration of 3.2 mg/L and the stonefly *Isoperla* at concentration of 28 mg/L, as well as the relatively high acute value of 27.2 mg/L for the caddisfly *Lepidostoma*, are expected given reported zinc sensitivities of these organisms (or related taxa) in Colorado streams (Kiffney and Clements 1994, 1996, Clements *et al*. 2000). In a study with a field collected community of stream organisms (i.e., rock baskets) and a mixture of zinc, cadmium, and copper, *Drunella* exhibited a significant response in waters containing 0.1 mg/L zinc (Kiffney and Clements 1994). In this same study, caddisflies (primarily *Lepidostoma*) also exhibited a 54% decline in abundance in treatments containing 11 mg/L zinc (although this decline was not statistically significant). These zinc values are considerably lower than those used in our study without effect. It appears possible that the other metals present in that study stream, i.e., cadmium and copper, may have had a greater toxicity influence, either combined or separately, than the zinc alone.

Recommendations

This initial study was "exploratory" in nature. Laboratory toxicity testing with aquatic insects native to Colorado streams has rarely been conducted. As such, we necessarily had to make decisions on test conditions and tested zinc concentrations that fit with existing knowledge.

Test Conditions: It appears that standard, static-renewal test conditions are adequate for a number of native aquatic insect taxa in Colorado. However, flow-through testing may be necessary for other taxa that are more dependant on more stream-like conditions.

Test Species: It appears that the mayfly *Drunella* is well suited for field collection and standard laboratory acute static-renewal toxicity testing. Small, case-dwelling caddisflies, like *Lepidostoma*, also appear to be suitable for such lab testing. The overall performance of these species during this initial testing would suggest that these taxa could also be used successfully for longer term, chronic (28 day) testing.

Species used to-date were those that were abundant enough at time of collection to provide the numbers of test organisms required for the testing conducted. There are other species that may also be useful in assessing zinc toxicity. For example, given the scientific literature on Colorado streams with historic mining (e.g., Clements, *et al*. 2000), it would be beneficial to try additional testing with a mayfly in the Family Heptageniidae, such as *Rhithrogena*. Given the life-habits of this mayfly, however, we would recommend use of a flow-through system to test this organism.

Zinc Toxicity: The results of the zinc toxicity studies herein provide useful information on the sensitivity (or tolerance) of these insects to zinc. However, these became basically "range-finding" tests, given the lack of acute toxicity in most of the study. Additional acute testing on these species at even higher concentrations would be useful to allow derivation of "true" acute values. Acute testing of additional species would also provide zinc toxicity data on a broader array of aquatic insects.

Recommendations: This "pilot study" showed that field collected aquatic insects from Colorado mountain streams can successfully be used under laboratory conditions for conducting acute toxicity tests. We would recommend an expansion of this study, as follows:

1) An additional acute static-renewal toxicity test with the mayfly *Drunella* at higher zinc concentrations in soft water only for development of actual measured acute levels for this organism.
2) An acute flow-through toxicity test with the mayfly *Rhithrogena* to determine the zinc sensitivity of this organism in soft water.
3) Another acute static-renewal zinc toxicity test with *Isoperla* at higher concentrations in soft water for development of actual measured acute levels for this organism.
4) Chronic static-renewal zinc toxicity testing with *Drunella* and a caddisfly (*Lepidostoma* or perhaps *Brachycentrus*, another case-dwelling caddisfly also abundant in Colorado streams) to provide currently unknown chronic zinc toxicity data for native aquatic insects in Colorado.

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