The effects of duration and concentration of episodic zinc exposure to the fathead minnow, *P. promelas.*

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Abstract. Section 301 of the Clean Water Act prohibits the discharge of any pollutant into navigable waters unless the discharge complies with the NPDES permits. The NPDES Program was implemented to control water pollution by regulating point sources that discharge pollutants into waters of the United States. These permits have resulted in the removal of billions of pounds of conventional pollutants and millions of pounds of toxic pollutants annually (USEPA 1972).

The need for rapid, cost effective, standardized assessment of effluents led to the development of whole effluent toxicity (WET) testing. WET tests try to mimic, as best as possible, the total effect and actual environmental exposures of aquatic life to toxic pollutants in an effluent without requiring the identification of the specific pollutants. Test methods have been developed for both freshwater and marine systems and recommends running tests using a plant, an invertebrate, and a vertebrate to identify the most sensitive species. Duck weed, *C. dubia*, *D. pulex* or *D. magna*, fathead minnow, rainbow trout, brook trout are all typically used organisms for freshwater testing. Mysid shrimp, sheepshead minnow, and silversides are typical marine species. There are two types of WET test methods. One is an acute (96 hour or less) test that utilizes mortality as the endpoint, the other a chronic test (7 day life-cycle) that uses growth, reproduction, and mortality as endpoints (Diamond and Daley 2000).

A study conducted on 250 dischargers throughout the United States has shown that discrepancies exist between WET tests and actual effects. WET test results were compared to actual impairment of the receiving streams of the effluent. They found that for acute tests 20% of the time there were false positives, meaning that they passed their WET tests but the receiving stream was impaired. 30% of the time they found false negatives, where they failed the WET test but the receiving stream remained unimpaired. Chronic tests showed slightly lower false positives and negatives but still high enough for concern (Diamond and Daley 2000).

While WET tests do provide valuable information they are often limited in their ability to predict what is occurring in the natural environment. Possible reasons for this may include, but are not limited to, no post-exposure observations, strictly aqueous exposure with no diet or dermal exposures, organism level is tested with no consideration for effects on populations and/or communities.

Zinc is commonly used in many industrial processes such as electroplating and galvanizing. It makes its way to our water ways via non point sources such as leaching from galvanized guard rails, or from point sources such as industry effluents. Zinc, as well as other chemicals, have been found to exceed EPA water quality criteria in many US waterways. Yet these waterways still support very robust ecosystems. This illustrates possible discrepancies in the current method of developing water quality criteria and WET testing (Besser and Leib 1999).

Studies examining the zinc concentrations in non-point source runoff show that as flow increases, zinc concentrations can increase to 600 µg/L, five times the ambient water quality criteria for zinc. This is a common phenomenon seen during large storm events (Wilber et al. 1980). Studies have also examined zinc concentrations from point sources such as mine waste. Ambient zinc concentrations reached as high as 1500 µg/L. Storm events cause the opposite effect on point source discharges, decreasing contaminant concentrations (Besser and Leib 1999).

The effect of storm events on contaminant concentrations and the apparent discrepancies between WET testing and actual environmental harm have caused many researchers to examine more environmentally relevant forms of testing such as episodic exposures. Our lab has previously examined the effects of episodic metal exposure to *Daphnia magna*. Mortality was correlated with exposure duration and concentration. There was also evidence that recovery periods between exposures may increase an organism's ability to deal with future exposures (Hoang, Tomasso et al. 2007).

Therefore, we characterized the effects of exposure duration and concentration to the fathead minnow (*P. promelas*), a common species used in aquatic toxicity testing. We then compared reported LC50's for acute continuous exposures to our results to determine if there are discrepancies between the two types of testing.

An experimental matrix was designed to testing both exposure duration and exposure concentration. Six concentrations between 100 and 1600 µg/L were examined with six different exposure durations, between one hour and twenty-four hours, for each concentration as well as a continuous exposure, for 21 days. Each exposure concentrations and duration had four replicates with 10 fish per replicate. Endpoints were survival (monitored daily), and growth (final weight). All exposures were performed in 600mL polypropylene cups. Water was prepared by adding equivalent amounts of zinc chloride to moderately hard water to obtain the nominal concentrations. Less than 24 hour old fathead minnows were placed into their respective exposure cups for the designated exposure period, then transferred to cups containing moderately hard water, and monitored for 21 days. Organisms were fed brine shrimp daily. After a feeding period of approximately two hours, cups were renewed.

Water quality was monitored on days 0, 7, 14, and 21 during each test. Survival was recorded during the daily renewals, and final weight was measured at the experiments conclusion. Zinc concentrations were confirmed using atomic absorption spectrometry or inductively coupled plasma spectrometry. Statistical significance was determined using one way ANOVA's

The results of the survival tests showed that exposure duration had a significant effect for many of our higher concentrations. Two hour exposures had significant decreases in survival after 21 days at 1428 µg/L. The four hour and eight hour exposures had significant decreases in the 726 µg/L and the 1428 µg/L treatments. Twelve and twenty-four hour exposures at 725 and 1426 µg/L cause 100% mortality within one day and were statistically different than all other treatments. Continuous exposures caused significant decreases in 365, 726 and 1428 µg/L treatments.

When comparing the survival data, there is a correlation of exposure duration and exposure concentration. Survival was decreased in an exposure time dependent manner as well as a dose dependent manner. When comparing LC50's, there was an exposure duration dependent effect with the exception of the 24 hour exposure which was slightly higher than the 12 hour exposure. This may be due to the uncertainty with this data point as Spearman-Karber was not able to accurately predict the confidence limits for this duration. When comparing LT50's there was an exponential decrease with increasing concentrations.

There was an increase in average final individual weight as exposure concentration increased. Because organism weight was not characterized throughout the study, feed ratio was kept the same during the study. Therefore, fish in cups with increased mortality in the higher concentrations had the same amount of food as cups with lower mortality and had more opportunity to feed and grow. There may have been a slight trend in final weight for exposure duration at the lower concentrations but this cannot be confirmed statistically because replicates needed to be pooled due to the low weight of these animals. This was also negated by the continuous exposure having the highest average final weight of all the treatments. Analysis based on total weight per cup may yield data more comparable to the survival data.

The highest LC50 for this study was 1384 µg/L for the 2 hour exposure. EPA's ecotox database was used to compare our LC50's to reported 96 hour LC50's. Studies with similar hardness to this study reported 96 hour LC50's between 9,900 and 25,000 µg/L. This means there is a 7-18 fold difference between the current study and reported 96 hour LC50's. The large difference between traditional testing methods (96 hour LC50) and more environmentally relevant tests (21 day episodic exposures) indicates that current testing methods may be inadequate to predict real world exposure. Results from this study also indicate that zinc may cause latent mortality that is not captured with traditional toxicity testing which is also consistent with similar studies conducted on *D. magna* (Hoang, Tomasso et al. 2007).

References:

Besser, J. M. and K. J. Leib. 1999. Modeling frequency of occurrence of toxic concentrations of zinc and copper in the upper Animas River. U.S. Geological Survey Water-Resources Investigations Report 99-4018A: 7.

Diamond, J. and C. Daley. 2000. What is the relationship between whole effluent toxicity and instream biological condition? Environmental Toxicology and Chemistry 19(1): 158-168.

Hoang, T., J. Tomasso, et al. 2007. An integrated model describing the toxic responses of Daphnia magna to pulsed exposures of three metals. Environmental Toxicology and Chemistry 26(10): 2247-2247.

USEPA. 1972. Federal Water Pollution Control Amendments of 1972. http://www.epa.gov/lawsregs/laws/index.html#env [Accessed 9/15/2008]
Wilber, W.G., Hunter, J.V., Balmat, J. 1980. Zinc in urban storm and wastewaters. In Nriagu, J.O. Zinc in the Environment. Wiley and Sons, New York, NY, USA. pp 230-263.