27th Annual Meeting of the Rocky Mountain Chapter of the Society of Environmental Toxicology and Chemistry

April 17th & 18th, 2014

Thursday workshop 4/17/2014

University of Colorado – Downtown Denver Campus
Science Building (SI), 1151 Arapahoe Ave.
Denver, Colorado 80217

Friday meeting 4/18/2014

US EPA Region 8 – Downtown Denver
1595 Wynkoop Street Denver, CO 80202

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Meeting registration and details are also available on the RMSETAC website www.rmsetac.org

Thursday workshop 17 April 2014

University of Colorado – Downtown Denver Campus
Science Building (SI), 1151 Arapahoe Ave.
Denver, Colorado 80217

8:30-9:00 Registration and Coffee

9-10:30 Workshop on Population Modeling Part 1 – Dr. Dana Winkelman

10:30-10:45 Coffee Break

10:45-12:15 Workshop on Population Modeling Part 2 – Dr. Dana Winkelman

12:30-2:00 Lunch downtown Denver

2014 Short Course

Thursday, 17 April 2014 (8:30am-12:30pm)

Population Modeling for Ecotoxicology

Dr. Dana Winkelman
Colorado Cooperative Fish and Wildlife Research Unit

Rationale:
In a summary report on population-level ecological risk assessment (EPA/100/R-09/006; http://www.epa.gov/raf/files/population_level_era_report_final.pdf) expert panelists concluded that three general assessment approaches could be taken: observational, experimental, and modeling. These approaches are not mutually exclusive, and each approach supports conclusions derived from another. For instance, accurate population modeling will require estimates for parameters and variables likely derived from experiments and field observations. The panel also noted that population models could be important integrators of data acquired through observational and experimental approaches. Population models can also be used heuristically to refine experimental questions and can help guide further research. One of the most important issues identified by the panel was communicating the general usefulness of population modeling to decision makers and project managers. Having a basic understanding of population modeling is essential for decision making, as well as for integrating population assessment information.

Goal:
The workshop will present a primer in basic population dynamics and modeling with the ultimate goal of elucidating key issues relevant to ecotoxicology and population assessment. The workshop is intended for a broad audience and should be easily understandable to anyone with a scientific background. The goal is to have a better understanding of basic population processes and how they relate to toxicology.

Workshop Strategy:
We will build models as we go through the topics; anyone with a basic understanding of spreadsheet calculations will be able to construct and use these models. My strategy will be to briefly present a topic (listed below) and then help you build a model to illustrate and explore the topic.

Overview of topics:
Basic Models
We will consider some basic closed population models
- Density Independent Model with Explicit births and deaths
- Including Age- or Stage-structure
- Density Dependence with Explicit births and deaths
  - We will consider and discuss what is explicitly meant by Density Dependence and why we should model or care about it.
- Including Toxicology
- Including Stochasticity - Why variability is important and what causes it.

Prerequisites:
1. Bring a laptop with Microsoft Excel installed.
2. A basic understanding of spreadsheet calculations.

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RMSETAC Meeting Schedule Friday, 18 April 2014

8:30-9:00 Registration and Coffee

9:00-9:10 Greeting from US EPA and University of Colorado Denver

9:10-10:00 Featured Presentation: **Larry Barber**. US Geological Survey, National Research Program. *Effects of Drugs on Aquatic Organisms.*

10:00-10:15 **Schwindt, Adam**. Colorado State University. *Population failure in fish from estrogen contaminated environments is regulated by direct and transgenerational effects on survival and fecundity*

10:15-10:30 **Vajda, Alan**. University of Colordo Denver. *Integrated assessment of wastewater effluent estrogenicity in the Upper Murray River, Australia, to the Murray Rainbowfish (Melanotaenia fluviatilis)*

10:30-11:00 Break and Poster Social I

11:00-11:15 **Clements, William**, Update on SETAC North America
and Carrie Claytor, update on SETAC 2015 in Salt Lake City

11:15-11:30 ***Montaño, Manuel**. Colorado School of Mines*. Future directions for engineered nanomaterial detection in environmental systems.*

11:30-11:45 ***Wang, Jingjing.** Colorado School of Mines. *Tracking carbon nanotube release from polymer nanocomposites using spICP-MS.*

11:45-12:00 **Hall, Scott.** ENVIRON. *Chronic toxicity of boron to freshwater worms and mussels in a unique test design.*

12:00-1:30 Lunch (on your own) and Board of Directors Meeting

1:30-1:45 **Morris, Jeff**. Stratus. *Acute and olfactory copper toxicity to fish using laboratory and site-collected water from Alaska.*

1:45-2:00 **Smith, Erin.** GEI. *Cyprinids and selenium – a case study.*

2:00-2:15 **Roark, Shaun.** GEI. *Challenges in the application of tolerance values to evaluate effects of fine sediments on aquatic life in Colorado.*

2:15-2:30 **Liu, Yong.** University of Colorado Denver. *Ozone initiated heterogeneous oxidation of squalene and its potential health impact.*

2:30-2:45 **Schmidt, Travis.** US Geological Survey. *The living dead: When aquatic life standards protect aquatic insect larvae but not adults.*

2:45-3:00 **Wesner, Jeff.** University of South Dakota. *Metal exposure increases mortality of aquatic insects during metamorphosis.*

3:00-3:30 Coffee and Poster Social II

3:30-3:45 **Krauss, Johanna**. US Geological Survey. *Metamorphosis in insects alters risk of contaminant exposure in food webs.*

3:45-4:00 ***Kotalik, Christopher.** Colorado State University. *Effects of magnesium chloride road salt on aquatic macroinvertebrate communities in Rocky Mountain streams.*

4:00-4:15 ***Traudt, Elizabeth**. Colorado School of Mines. Toxicity of ternary mixtures of nickel, copper, zinc and cadmium to *Daphnia magna.*

4:15-4:30 ***Williamson, Jacob**. Colorado State University. *Development of in-situ methods for investigating recovery of acid mine drainage contaminated sediments.*

4:30-4:45 **Anderson, Jordan**. Colorado Parks and Wildlife. *Interactions between temperature and metal toxicity in Colorado trout species.*

4:45-5:00 **Pillard, David**. TRE Environmental Strategies, LLC. *Common Ions and Dissolved Gases as Causes of Acute and Chronic Freshwater Toxicity in Produced Water – Case Studies*

5:00-5:15 Student judging deliberations

(*** indicates consideration for best student poster or presentation)

5:15-Student Awards and Raffle (please volunteer to judge student presentations)

Posters

***Baroffio, Angelina.** University of Colorado Denver. *Endocrine Disruption in the Fathead Minnow (Pimephales promelas) Following a Series of Upgrades to a Wastewater Treatment Facility*

Baker, Stephanie. GEI Consultants, Inc. *An approach for developing updated selenium criteria for the protection of aquatic life.*

***Harrison, Alexandra**. University of Colorado Denver. *Effects of wastewater contaminants on neurotransmitter function and distribution in the fish brain.*

Love, Natalie. GEI Consultants, Inc. *Case study supporting toxicity testing as a tool to support reservoir management decisions.*

Miller, Keith. University of Denver. *Integrating environmental and sustainable chemistry challenges into capstone chemistry courses.*

***Occhipinti, Kendra**. University of Colorado Denver. *Developing a Tool to Compare Water Treatment Technology and Populations Served for Colorado Watersheds.*

Pargee, Suzanne. GEI Consultants, Inc*. Biokinetic food chain modeling of waterborne selenium pulses into lotic waters: implications for acute water quality criteria.*

Keynote Presentation

Effects of Drugs on Aquatic Organisms

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There is global contamination of surface water and groundwater by diverse groups of anthropogenic chemicals including pharmaceutical compounds. Pharmaceutical contaminants can be introduced into the aquatic environment through many human activities including discharge of wastewater treatment plant effluents, application of bio-solids to agricultural lands, and inputs from pharmaceutical manufacturing facilities. Once introduced into a stream, contaminants are transported along with the water and undergo a range of fates determined by environmental conditions and compound physicochemical characteristics. The in-stream fate of complex contaminant mixtures determines the exposure regime for organisms living in the aquatic ecosystem, and can be variable in time and space. This talk will present several environmental exposure scenarios across the United States, describe the specific chemistry of select classes of drugs, explore their modes of action with different biological systems, and analyze the results within the adverse outcome pathways framework.

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Population failure in fish from estrogen contaminated environments is regulated by direct and transgenerational effects on survival and fecundity

Adam R. Schwindt, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO; Dana L. Winkelman, Colorado Cooperative Fish and Wildlife Research Unit - US Geological Survey, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO

Increased demands on freshwater ecosystems throughout the arid western USA are coincident with an increasing human population. For example, in northeastern Colorado flow in the South Platte River is up to 90% wastewater effluent most of the year. The effluent contains a complex mixture of chemicals that can disrupt hormone systems in fishes. One component of the effluent, 17α-ethinylestradiol (EE2) the synthetic estrogen in birth control, is known to disrupt fish reproduction. While the effects of EE2 on individuals are well known, the consequences for populations are just now being assessed. For these studies we evaluated the effects of EE2 exposure on fathead minnow (*Pimephales promelas*) populations over the course of three generations. We constructed 28, 1100L aquatic mesocosms to house the fish populations at the Colorado State University Foothills Fisheries Laboratory. In the F0 generation, each mesocosm received five male and five female fish exposed to measured EE2 concentrations ranging from 0, 3.2, 5.8, and 10.9ng/L for 102 days (*n*=7). Survivorship and the numbers of eggs, embryos and offspring were quantified. Following the mesocosm study the F1 fish were divided into two groups. One group continued to receive the concentration of EE2 in the mesocosm and in the other the exposure was stopped. F1 reproductive output was assessed after 203 days and survival was estimated in the F2 fish. The effects of EE2 exposure differed depending on the life stage and exposure history. In the F0 generation EE2 reduced adult male survival but had little effect on reproduction. In the F1 generation, fish allowed to recover from EE2 exposure displayed reduced reproductive capacity. In F1 fish subjected to lifetime exposure no successful reproduction was evident. More striking were the effects on the F2 generation. Survival of the F2 fish whose parents were exposed early in life was significantly less than the F2 fish whose parents were exposed only as adults. The effects of EE2 on the F2 generation resulted from exposure through the parental germ line. EE2 exposure may cause population failure by several mechanisms and even though fish may no longer be directly exposed to EE2, effects are still evident.

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Integrated assessment of wastewater effluent estrogenicity in the Upper Murray River, Australia, to the Murray Rainbowfish (*Melanotaenia fluviatilis*)

Alan M. Vajda[1*], Anupama Kumar[2], Marianne Woods[2], Mike Williams[2], Hai Doan[2], Peter Tolsher[3], Rai S. Kookana[2], and Larry B. Barber[4]

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Reproductive disruption in native fish has been observed downstream from wastewater treatment plant (WWTP) outfalls in the United Kingdom, Europe, Asia, and North America. However, endocrine disruption in fishes native to Australia has not been investigated extensively. The contamination of major continental river systems, such as Australia's Murray River, by endocrine-active chemicals (EACs) derived from the discharge of WWTP effluents, can affect the viability of native fish populations and has implications for agricultural and drinking water users downstream of discharge points. This study characterized the occurrence and temporal variability of EACs in a WWTP effluent discharging to the upper Murray River, assessed endocrine disrupting effects of WWTP effluent on the native Australian Murray rainbowfish, (*Melanotaenia fluviatilis*) and to the well-characterized non-native zebrafish (*Danio rerio*), and evaluated the potential impact on water quality and ecosystem health of the Murray River receiving water. An integrated, 28-day, on-site, continuous-flow, exposure experiment was conducted using adult male Murray rainbowfish and zebrafish to assess the *in vivo* estrogenicity of the WWTP effluent relative to Murray River reference water collected upstream from the WWTP outfall. Fish were exposed under conditions of controlled temperature, photoperiod, diet, and flow to dilutions of WWTP effluent (10%, 50%, 75%, and 100% effluent), and were sampled at 7, 14, and 28 days. Biomarkers of endocrine disruption included plasma vitellogenin concentrations, vitellogenin mRNA, and gonadal histology. The WWTP effluent and reference water were analysed for estrogenic EACs (estrone, 17β-estradiol, ethinylestradiol, and 4-nonylphenol) by liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry; estrone and 17β-estradiol also were analyzed by ELISA. (Anti-) estrogenicity and (anti-) androgenicity were evaluated *in vitro* using yeast-based bioassays (YES/YAS). The measured concentrations of EACs were low and temporally variable. Results from the fish biomarkers showed little indication of endocrine disruption following 28 days of exposure to Murray River reference water or treated WWTP effluent and were consistent with the low concentrations of EACs that were detected. The combination of advanced wastewater treatment that removes EACs, and high in-stream dilution (< 1 % WWTP effluent) of the WWTP effluent following discharge on the Upper Murray River contribute to mitigate potential ecosystem impacts.

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Future Directions for Engineered Nanomaterial Detection in Environmental Systems

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Developing accurate life-cycle assessment models for engineered nanomaterials (ENMs) will require knowledge of where these particles are distributed in the environment and at what concentrations. Unfortunately, the ability to detect and characterize ENMs in the environment are impeded by several factors such as environmental transformation processes, low expected release concentrations of ENMs (ng L^{-1}), and much higher concentrations of naturally occurring nanomaterials when compared to ENMs. Despite much greater concentrations of naturally occurring nanomaterials, key properties of ENMs such as particle size distribution, surface coatings, and elemental ratios, can be used to discern ENMs from natural colloids. Of these properties, elemental ratios may be the most recalcitrant to environmental changes. To this end, ICP-MS is an ideal candidate for determining elemental ratios for the purpose of differentiating ENMs from natural particles. Three different analytical approaches can be employed to determine the presence of ENMs in environmental samples. Bulk analysis of an environmental sample can be used, but requires a substantial amount of ENMs in some cases to result in a marked deviation from the background elemental ratio. Coupling ICP-MS to a fractionation technique such as field-flow fractionation can improve upon bulk elemental ratios by monitoring changes in the elemental ratio on a particle size basis. New developments in single-particle ICP-MS have allowed for micro-second dwell time analysis, improving the resolution of particles amidst high particle number concentrations and in samples containing high levels of dissolved analyte. These micro-second dwell times also allow for monitoring two elements at a time, providing elemental ratios on a particle-by-particle basis. As ENMs continue to be enter into everyday commercial use, their release into the environmental is bound to increase. The ability to determine their presence and concentration in the environment is of utmost importance requiring sophisticated analytical approaches to detect them in the environment.

Consideration for Best Student Paper

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Tracking Carbon Nanotube Release from Polymer Nanocomposites Using spICP-MS

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Carbon nanotubes (CNTs) are widely used in polymer nanocomposites. This raises the concern that release of CNTs may occur during the life cycle of materials, and may thus cause human and environmental exposure. This situation calls for development of reliable nanometrology to assess the stability of nano-enabled materials. Single particle ICP-MS (spICP-MS), a promising method to detect particles in the ng/L range, has been previously applied for detection, quantification, and size distribution analysis of metallic nanoparticles. It is not possible to detect carbon directly with ICP-MS, however CNTs be quantified by measuring the embedded metal nanoparticles, which arise from the presence of residual catalysist. CNT–PCL (polycaprolactone) materials, having two different CNT loading, were prepared and subjected to degradation experiments using UV light. Over a 35 day period weight loss was 40 % for a loading of 0.5 % CNT and 15% for a loading of 1.5 % CNT. This represented the decomposition of the polymer. Particle counts, measured using yttrium, increased with time but did not directly correlate with weight loss for the two loadings. Further work to verify the accuracy of spICP-MS for CNT quantification is ongoing, including comparison to Nano Tracking Analysis. Successful development of spICP-MS for CNTs will allow a better understanding of nanocomposite stability.

Consideration for Best Student Paper

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Chronic Toxicity of Boron to Freshwater Worms and Mussels in a Unique Test Design

Hall, S. and Lockwood, R.

The chronic (21 and 28 d) toxicity of boron was determined for the fatmucket mussel *Lampsilis siliquoidea* and aquatic worm *Lumbriculus variegatus*. The rapid depletion of boric acid from spiked sediments in tests utilizing flow-through overlying waters was addressed by constant addition of boric acid to overlying water at concentrations matching those of the targeted porewater exposures. This proved highly successful in maintaining constant whole-sediment and sediment porewater boron concentrations. Boron sub-lethal IC25 values based on porewater concentrations were 25.9 mg B/L (*L. variegatus*) and 38.5 mg B/L (*L. siliquoidea*), indicating similar test organism sensitivity. Expressed as dry whole sediment values, the respective *L. variegatus* and *L. siliquoidea* sub-lethal (growth) IC25 values for whole-sediment exposures were 235.5 mg B/kg sediment dry weight and 310.6 mg B/kg. The worm lethality-based endpoints indicated higher sensitivity than the sub-lethal endpoints, bringing into question the validity of a “lethality” endpoint for *L. variegatus* given its fragmentation mode of reproduction. Water-only mussel exposures were also tested, resulting in an IC25 value of 34.6 mg B/L, within 20 percent of the porewater value, suggesting the primary route of boron exposure was through the aqueous phase. Study results indicated that for readily water soluble materials, standard sediment test designs may be unsuitable, but water-only exposures can provide toxicological data representative of sediment tests.

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Acute and Olfactory Copper Toxicity to Fish Using Laboratory and Site-Collected Water from Alaska

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The Biotic Ligand Model (BLM) was used to predict the toxicity (LC_{50}) of copper added to low-hardness (~30 mg/L as $CaCO_3$) laboratory and site-collected (Upper Talarik Creek, Alaska) waters and these predictions were compared to the results of 96-hour bioassays conducted using rainbow trout (laboratory water) and fathead minnows (laboratory and site waters). The BLM under-predicted the toxicity of copper in the laboratory water for both rainbow trout and fathead minnows by 1.5 to 2.1 fold, respectively. The BLM's prediction of copper toxicity to fathead minnows in site-collected water was closer to the actual bioassay result and only under-predicted the toxicity by 1.2 fold. Factors potentially influencing these experimental results will be discussed. Additionally, behavioral assays were conducted using rainbow trout in low-hardness laboratory water to determine the effects of copper on olfaction using an alarm cue. This bioassay was designed as a 96-h flow-through exposure to a range of copper concentrations (0–8 µg/L) with two fish per aquarium. An alarm cue was added to each aquarium after 3, 24, 48, and 96 hours of copper exposure, and fish behavior was recorded using cameras mounted above each aquarium. Following 96 hours of copper exposure, all remaining fish were held in clean water for 24 hours and the alarm cue was added to determine if any effects of copper appeared to be reversible within 24 hours. Analysis of these videos and data are ongoing and the preliminary results will be discussed. Preliminary results from these tests indicate that site-specific testing is warranted whenever possible. We will discuss certain anomalous results that are generated when modeling the toxicity of copper to rainbow trout in low-hardness waters such as those in many headwater streams in Alaska, which suggest that the use of the BLM at those sites should proceed with caution.

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Cyprinids and selenium – A case study

*Smith, E.R., Baker, S.D., and Canton, S.P., GEI Consultants, Denver, Colorado.
Selenium (Se) is an essential micronutrient that occurs in virtually all environmental media at trace concentrations. While anthropogenic activities such as irrigation are known to increase Se beyond background concentrations in many aquatic ecosystems, the Saint Charles River basin in south-central Colorado is known to have naturally elevated Se concentrations originating from geological sources. In 2011 and 2012, we collected fish population and water chemistry data from multiple sites in the Saint Charles River to determine potential sources of Se and evaluate whether Se is impairing aquatic life in this stream. Water column Se concentrations, measured monthly, varied both spatially and temporally and ranged from less than detection up to 650 µg/L. Despite this wide range in Se concentrations, all study sites supported similarly healthy and diverse fish populations primarily composed of minnows, stonerollers, and dace (Family Cyprinidae). Multiple age classes (young-of-year, juveniles, and adults) for many species were observed throughout the study area, indicating reproduction is occurring despite often extremely high Se levels. The results of this study supported adoption of ambient-based site-specific Se standards for the Saint Charles River.

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Challenges in the application of tolerance values to evaluate effects of fine sediments on aquatic life in Colorado.

Roark, S.A., Lynch, J.S., Smith, E.R., Kovach, A.K., De Jong, G.D., Canton, S.P.
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As the State of Colorado updates its Sediments Policy, Tolerance Values (TVs) are one of the tools being considered to evaluate whether fine sediments are having a detrimental effect on aquatic life use. TVs have been widely used in benthic invertebrate bioassessments throughout the United States as generalized indicators of stress but are usually not designed to distinguish among stressors. A number of stressor-specific TVs have recently been developed, including TVs specific to fine sediments. As a test of the approach, we applied published TVs for fine sediments at sites across Colorado to determine if TVs could be used to identify sites potentially affected by fine sediments, or if confounding effects of other stressors or variables (e.g., elevation, slope, ecoregion) significantly confounded the assessments. We found that site scores for fine sediment TVs and generalized TVs (i.e., as used in an abundance-weighted approach) were tightly correlated, limiting the utility of TVs to distinguish among stressors, and that environmental and habitat variables represent confounding factors that need to be considered in the implementation of fine sediment TVs.

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Ozone initiated heterogeneous oxidation of squalene and its potential health impact

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Indoor air pollution has emerged as major environmental risks and health concern. In this work, we employed ATR-IR, mass spec and DTT assay to investigate ozone interaction with squalene, major composition of skin oil and the roles of environmental conditions including temperature and relative humidity in the interfacial ozone chemistry. Results show heterogeneous oxidation is very rapid and proceeds via ozonolysis mechanism. Overall ozone reactive uptake is nearly independent of ambient temperature and relativity humidity. In contrast, temperature seemingly affects reaction product formation. DTT assay reveals that redox activity of resulting products is significantly enhanced upon ozone exposure and increases with increasing exposure time. Further water uptake study reveals that ozone exposure enhances hydrophilicity of reaction products. The changes in redox activity and hydrophilicity pose higher risk for public health.

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The living dead: When aquatic life standards protect aquatic insect larvae but not adults.

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Abstract: Field, mesocosm, and laboratory tests suggest aquatic insect larvae are reasonably protected by aquatic life standards for metals, however, metal concentrations below aquatic life standards can eliminate aquatic insect emergence. An ecological consequence of this phenomenon is that the effects of aqueous contamination are propagating to adjacent ecosystems by severing the exchange of carbon and nutrients between the aquatic and riparian ecosystems. This also limits the opportunity for the reproduction necessary to sustain local populations and communities. Despite the seemingly living dead (aquatic insect larvae in contaminated habitats), field data do not suggest a limitation in recruitment. It is possible that other processes (drift, oviposition by adults from elsewhere) could deliver enough individuals to sustain a local population, compensating for the loss in recruitment via local adult oviposition. We investigate these processes as explanations for this phenomenon and discuss their ecological and evolutionary implications.

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Metal exposure increases mortality of aquatic insects during metamorphosis

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The response of larval aquatic insects to stream stressors like metals is a common biomonitoring tool worldwide. However, nearly all larval insects emerge as winged adults, and recent surveys indicate that emergence may be a more sensitive indicator of stream metal toxicity than larval survival. One hypothesis to explain this pattern is that insects exposed to elevated metal in their larval stages have a reduced ability to successfully complete metamorphosis. To test this hypothesis we exposed late-instar larvae of the mayfly, Centroptilum triangulifer, to an aqueous Zn gradient (32 - 476 µg/L) in the lab. Larval survival on day six, when emergence began, was unaffected by Zn. Zinc reduced wingpad development at concentrations above 139 µg/L. In contrast, subimago and adult emergence declined with any increase in Zn. For example, at Zn concentrations of 105 µg/L, the hardness-adjusted aquatic life criterion, 89% of larvae developed wingpads, but only 75% emerged from the water and only 28% completed the completed the last molt to become sexually mature adults. Subimago dry mass declined with increasing Zn exposure. These results support the hypothesis that metamorphosis may act as a survival bottleneck, enhancing the effects of stream metal exposure beyond the aquatic-terrestrial boundary.

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Metamorphosis in insects alters risk of contaminant exposure in food webs

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Abstract: Metamorphosing insects are integral to most freshwater and terrestrial food webs. Metamorphosis can cause large chemical changes in insects, altering contaminant burden and stable isotope signatures used to trace diet. These changes influence predator exposure to contaminants, contaminant fluxes between ecosystems, and our understanding of energy flow through food webs. To develop a framework for predicting and managing these effects, we analyzed data from 39 studies of 66 contaminants and stable isotopes. Metamorphosis effects varied greatly among contaminants. Metals and polycyclic aromatic hydrocarbons were predominantly lost during metamorphosis leading to ~2 to 125 times higher larval concentrations and higher risk for predators of larvae. In contrast, manufactured organic contaminants (such as polychlorinated biphenyls) were retained, posing ~3 times higher risk to predators of adult insects. Furthermore, $\delta^{15}N$ is enriched in adults during metamorphosis. This indicates that the current common practice of using larval $\delta^{15}N$ to estimate trophic level and biomagnification in adult food webs, and vice versa, is incorrect. Thus, metamorphosis alters the spatial and temporal propagation of contaminant effects, altering management decisions about potential exposure risk to food webs.

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Effects of magnesium chloride road salt on aquatic macroinvertebrate communities in Rocky Mountain streams

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Streams and rivers often intercept and run parallel to roads and highways where road salts are seasonally applied. This research investigates $MgCl_2$ road deicer exposure to aquatic benthic macroinvertebrate communities in the Southern Rocky Mountain ecoregion in Colorado, USA, by using stream microcosms. Exposure concentrations of $MgCl_2$ bracketed the U.S. Environmental Protection Agency (U.S. EPA's) chronic chloride criteria for protection of aquatic life (230 mg/L) at 75, 200, and 400 (low, medium, and high) mg/L, respectively. The 10-day exposure yielded no drift effect, but significantly reduced total taxa abundance and richness across treatments, particularly reducing the number of individuals of Ephemeroptera and Plecoptera taxa. Mortality was observed well below the U.S. EPA's recommendation for chronic chloride exposure in the low treatment of 75 mg/L, with dominant taxa groups of Capniidae and Heptagenidae heavily impacted. However, Trichoptera, Chironomidae, and Diptera, showed no mortality effect and were tolerant to all exposure concentrations. Our research suggests that certain taxa found in aquatic benthic macroinvertebrate communities in the Southern Rocky Mountain ecoregion are highly sensitive to road deicer $MgCl_2$, even at comparatively low concentrations. Many states in the west, such as Colorado, have not adopted chloride values into state water quality standards for protection of aquatic life. Research that identifies sensitive and tolerant Rocky Mountain benthic macroinvertebrate taxa will be necessary in order to effectively develop water quality criteria for chloride.

Consideration for Best Student Paper

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Toxicity of ternary mixtures of nickel, copper, zinc and cadmium to *Daphnia magna*

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Potentially toxic metals including nickel, zinc and copper are often present in the natural water systems due to a variety of point and non-point sources, leading to mixture toxicity that is difficult to predict. To further develop multi-metal toxicity models, the toxicity of Ni and Cd were tested in ternary combinations with Cu and Zn. Previously we performed toxicity tests with binary metal mixtures of Ni and (1) Cd, which is considerably more toxic than Ni, (2) Cu, which is less toxic than Cd but more toxic than Ni, and (3) Zn, which has a toxicity threshold similar to Ni. Although sub-lethal concentrations of Ni protected against the toxicity of Cd, greater-than-additive toxicity occurred in binary mixtures of Cu and Ni; and response-additive toxicity occurred in binary combinations of Ni and Zn. We hypothesize that these results provide evidence for the competition among the metals for binding to biological ligands and/or dissolved organic matter; however, the effectiveness of each mechanism is unknown when a mixture contains more than two metals. To analyze the interactions, we exposed *Daphnia magna* neonates to the individual metals (Cd, Cu, Ni, Zn) and in ternary combinations in standard 48-h toxicity tests conducted in USEPA moderately hard reconstituted water with 3 mg DOC/L added as Suwannee River fulvic acid. For these ternary mixtures, two metals were held constant at specified concentrations while the third metal was varied through a series that ranged from nonlethal to lethal concentrations. In ternary mixtures with Ni, Cd and Cu, greater-than-additive effects 'overshadowed' the protective effect that had previously been observed between Ni and Cd. Similarly, Cd-Ni-Zn mixtures produced response-additive toxicity that once again overshadowed the protective mechanisms evident in Cd-Ni and Cd-Zn mixtures. These findings illustrate potential competition of toxic mechanisms between various metal mixtures.

Consideration for Best Student Paper

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Development of *in-situ* methods for investigating recovery of acid mine drainage contaminated sediments

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Acid mine drainage (AMD) can impact the water column and sediment of receiving waters, in part due to low pH and elevated dissolved-metal concentrations in the water and deposition of metal-oxide particules on the sediments. An example is the North Fork of Clear Creek (NFCC) at Blackhawk/Central City, in central Colorado. The NFCC has poor water quality, contaminated sediment, and a minimal population of benthic invertebrates downstream of the two major AMD inputs. To help predict recovery of this stream system after planned remediation, we developed a method to determine the kinetics of the release of metal-oxides from NFCC sediment. This method can indicate an approximate time scale for stream recovery after an incoming point source has been remediated. It can also be used in conjunction with analysis of impacts to aquatic invertebrates, because our method uses the same samplers that have been used to investigate benthic recovery in contaminated sediments. For this demonstration study, we collected field-contaminated sediments in spring of 2012 and 2013by placing uncontaminated rock trays in the NFCC downstream of the AMD inputs. After allowing a period of in-stream exposure for metal-oxides to accumulate on the trays, we removed the trays from the stream and relocated them upstream to a non-contaminated reference site. Samples were then removed at several time points and extracted with an acid solution, which was analyzed for metal concentrations. In 2012, after one week upstream, the average iron concentration in the trays decreased ≈30%. However in 2013, after 10 days upstream the average iron concentration in the trays decreased ≈10%. This decreased percent removal reflects an improved method that more effectively retained iron-oxide floc that was previously lost during transport and sampling of the trays. We will continue to optimize field and laboratory methods to more accurately represent a natural system.

Consideration for Best Student Paper

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Interactions between temperature and metal toxicity in Colorado trout species

Anderson, J.R. and Brinkman, S.F.

Traditional toxicity tests expose aquatic organisms to contaminants in the water while trying to maintain constant conditions. However, in a natural setting conditions are never constant, flows change from season to season and even from hour to hour, water quality can fluctuate due to many factors, and temperature is constantly either ramping up or ramping down. Few studies have examined the interactions between temperature and heavy metal toxicity on fish. Considering that temperature is an extremely important aspect to almost all aspects of a fish's metabolism, we were very interested in the effects that heavy metals might have on a fish's ability to resist high temperatures and also on the effects that high temperatures might have on a fishes ability to resist metal toxicity. In order to evaluate interactions of temperature and metal toxicity, 96 hour copper and zinc toxicity tests were conducted with brook trout at low (10ºC)and high temperature (20ºC). At the conclusion of the test, fry from the four lowest exposure concentrations were subjected to the critical thermal maximum (CTM) methodology, a measure of upper thermal tolerance. Fry from copper treatments exhibited lower CTMs than those from the controls. The CTMs of fry from Zinc treatments were unaffected. In order to further explore how temperature might affect copper toxicity we conducted three more copper toxicity tests, this time with Colorado River Cutthroat Trout. Toxicity tests were conducted at constant 12°C, constant 20°C, and where the mean temperature was 20ºC but fluctuated on a diel cycle from 15°C to 25°C. When compared to the two static toxicity tests, the fluctuating toxicity test caused the mortality to happen more quickly in the same nominal copper concentrations and exhibited more mortality in treatments with lower copper concentrations. This resulted in a lower LC50 for the fluctuating temperature test than was observed in the static tests. The results of these experiments indicate that dissolved copper at sub-lethal concentrations can limit an organism's ability to tolerate high temperatures and also that high temperatures can limit an organism’s ability to withstand copper concentrations that would otherwise be sub-lethal.

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Common Ions and Dissolved Gases as Causes of Acute and Chronic Freshwater Toxicity in Produced Water – Case Studies

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Produced water, generated concurrently with coal bed natural gas (CBNG), may have elevated concentrations of dissolved gases (e.g., methane) as well as certain freshwater ions. Freshwater ions that tend to be elevated in these waters include bicarbonate, chloride, ammonia, and sodium. Given that the freshwater ions are not equally toxic, the relative composition of the ions comprising the total dissolved solids (TDS) is important. Per NPDES/State PDES permits, acute or chronic whole effluent toxicity (WET) tests are typically required on discharges from these CBNG outfalls. When WET is observed, additional testing is performed in order to determine the cause of toxicity (via toxicity identification evaluations [TIE]) so that treatment options can be implemented. Case studies will be presented demonstrating the results of various WET and TIE investigations. Fidelity Exploration and Production's operations in the Tongue River represent one specific example. Acute WET studies conducted with *Ceriodaphnia dubia* sometimes failed the permit-required limits (LC_{50}< 100% PW). Numerous TIE rounds showed fluctuating results under similar water quality conditions, due to a large degree to variability in an organism's response and simple binary ("live or dead") data collected in acute tests. Mock effluents, prepared to match the concentrations of seven major ions, were prepared and tested side-by-side with produced water. Toxicity in the mock effluents was usually the same or greater than that observed in the effluent toxicity, suggesting that TDS alone, and in particular bicarbonate, was responsible for the observed toxicity. The results of this and other work will be presented to highlight the results of several toxicity reduction evaluation efforts with CBNG effluents.

POSTER

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An approach for developing updated selenium criteria for the protection of aquatic life

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While the U.S. EPA is in the process of revising the national ambient water quality criteria (AWQC) for selenium (Se), it is unknown when a new draft will be available. However, derivation of updated Se criteria is currently scientifically defensible due to new acute water-column toxicity and tissue-based chronic toxicity data made available since the current criteria (U.S. EPA 1987) and the last draft criteria (U.S. EPA 2004) were released. Therefore, interested states may develop their own updated criteria instead of relying on the outdated Se criteria from 1987. Such an effort to develop updated state-wide Se criteria was recently conducted in Kentucky. The resulting proposed water-column based acute criterion takes into account the fraction of selenite and selenate and considers sulfate's toxicity-reducing effect on selenate. Tissue-based chronic criteria were developed, as it is expected that U.S. EPA's updated chronic criterion will include a component based on Se concentrations in fish tissues. To provide stakeholders with options for assessment evaluations, we developed chronic criteria based on either whole-body or egg/ovary concentrations. However, given the potential expense and difficulty that required tissue collection will pose for the regulated community, we proposed a multi-step criterion that begins with screening of water-column data to weed out locations with low Se and limited risk to aquatic life. The current national chronic criterion of 5.0 µg/L will be used as a threshold, and if exceeded, will trigger the requirement to collect fish tissues (whole-body or egg/ovary) to assess attainment. These proposed criteria present a significant improvement over the outdated 1987 U.S. EPA Se criteria currently in use by most, if not all, states. In addition, it is expected that the Se criteria being developed by U.S. EPA will follow a similar approach.

POSTER

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Endocrine Disruption in the Fathead Minnow (*Pimephales promelas*) Following a Series of Upgrades to a Wastewater Treatment Facility

Baroffio, A., Vajda, A.M.

Our study is part of a continued characterization of temporal variation and occurrence of EDCs (endocrine disrupting compounds) in a wastewater treatment plant (WWTP) effluent discharging to Boulder Creek. This site has been extensively experimentally evaluated in previous years by Dr. Alan Vajda's laboratory, before and after a major upgrade in 2007. This study aims to provide the same pre- and post-upgrade evaluations for a second upgrade, which occurred in 2012 and implemented a UV purification processes as a replacement for procedures utilizing chlorine. We evaluated endocrine disrupting effects of this WWTP effluent on the native Fathead Minnow (Pimephales promelas) and the potential impact on the Boulder Creek receiving water. We conducted an integrative, 8-week, on-site, continuous-flow, exposure experiment using adult male (and a subset of adult female) Fathead Minnows to assess in vivo estrogenicity of the WWTP effluent water, relative to reference water collected upstream from the outfall. Fish were exposed under controlled temperature, photoperiod, diet, and flow, and were sampled at baseline, four weeks, six weeks, and eight weeks. A major flood event occurred just before the sampling for week six. We selected a number of biomarkers for endocrine disruption, including plasma vitellogenin concentrations, spermatozoa abundance, and gonadal and liver cell apoptosis. The WWTP effluent and reference water was analyzed for EDCs by liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry. We analyzed concentrations of plasma vitellogenin protein by ELISA. Measured concentrations of EDCs were shown to be low and temporally variable.

Consideration for Best Student Poster

POSTER

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Effects of wastewater contaminants on neurotransmitter function and distribution in the fish brain

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A series of integrated fish exposure experiments were conducted from 2006-2010 in collaboration with St. Cloud State University, University of South Dakota, CU Boulder, and the USGS. These experiments evaluated the effects of a range of dosages of individual wastewater chemicals from diverse classes, and of mixtures of chemicals on fish courtship behavior, reproductive output, gonadal histology, and plasma biomarkers of estrogen exposure. In addition, brains were collected and fixed at dissection, and have since been sectioned, mounted and stained for markers of neurotransmitter function (tyrosine hydroxylase, and dopamine-beta hydroxylase). This study was designed to provide a comprehensive and integrated evaluation of the morphological, physiological, and behavioral impacts of environmental estrogens in environmentally relevant mixtures on reproductive competence in male fathead minnows (*Pimephales promelas*). The consequences of estrogen exposure for reproductive fitness were determined through evaluation of secondary sex characters, vitellogenesis, testicular morphology, and nest-holding ability in a post-exposure reproductive challenge assay. Initial study results suggest complex, often paradoxical, relationship between contaminant exposure and behavioral outcome (Vajda et al., in prep). For example, fish exposed to the lowest concentrations of weak estrogens (E1) showed a greater degree of disruption of reproductive behavior than fish exposed to higher concentrations of strong estrogens (EE2). The goal of the present study is to evaluate key neurotransmitter systems involved in the regulation of behavior and gametogenesis in fish in relation to the previously measured whole-organism reproductive response.

Consideration for Best Student Poster

POSTER

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Case study supporting toxicity testing as a tool to support reservoir management decisions

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Canyon Lake is located in Riverside County, California and exhibits hyper-eutrophic conditions mainly due to substantial internal nutrient loading. As part of the TMDL and Comprehensive Nutrient Reduction Plan, GEI Consultants, Inc. assisted stakeholders in conducting whole effluent toxicity (WET) tests at two sites on the lake to evaluate potential toxicity to aquatic life during in-lake treatment using aluminum sulfate (alum). The addition of alum is designed to help achieve phosphorus reduction goals and the chlorophyll-a goal of the TMDL. WET tests followed water effects ratio protocol with some modifications to account for the flocculent produced during laboratory treatments using nominal concentrations of 1 to 40 mg/L of alum. No toxicity was detected for *Ceriodaphnia dubia* or *Pimephales promelas* in either of the site waters. Toxicity was detected in the moderately hard reconstituted water test for both species, primarily due to the fact that there was no phosphorus or organic carbon to bind with the aluminum, resulting in freely available aluminum for organism uptake. The measured aluminum concentrations support this theory. This toxicity study showed alum treatment would not cause lethal or sub-lethal effects on aquatic life even at concentrations double the proposed dosage. These results reassured nearby residents, users of the lake, and the stakeholders that alum treatment is a practical approach to manage internal nutrient loading and nuisance algae growth. The initial alum treatment has proved successful at reducing algae growth. This case study is a strong example that toxicity testing can be a useful tool to aid in reservoir management decisions.

POSTER

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Integrating environmental and sustainable chemistry challenges into capstone chemistry courses

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Chemistry Frontiers, a capstone course for chemistry and biochemistry majors, is a research-based course focused on applying the scientific method to real-world challenges and timely scientific questions. Student teams develop experiments based on a student-derived hypothesis related to an instructor challenge. In recent years, the course has been designed around environmental chemistry challenges related to biofuels from algae. Student teams implement the experiments, interpret the results, repeat the process (if necessary), and report their results in a public presentation. A written summary of the research is prepared in the format of a journal article. Examples of recent student work, as well as lessons learned from the instructor, will be provided, Student feedback is extremely positive, with many students commenting that while the course was demanding, it was one of their most valuable courses.

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Developing a Tool to Compare Water Treatment Technology and Populations Served for Colorado Watersheds

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Previous assessments for water treatment have consisted in measuring permit parameters and populations served. It would be beneficial to compare this data so that we have a better understanding of the impact of lower grade, e.g. trickling filters on populations within the state and questions as to whether a technical plant upgrade is feasible and necessary based on such impacts. There does not seem to be one singular tool to compare the technology for water treatment being used compared to the populations served, and the discharge volumes of the water treatment facility. We aimed to develop a strategy for compiling this information and organize this information such that a future geospatial tool could be developed to illustrate these data.

Consideration for Best Student Poster

POSTER

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Biokinetic food chain modeling of waterborne selenium pulses into lotic waters: implications for acute water quality criteria

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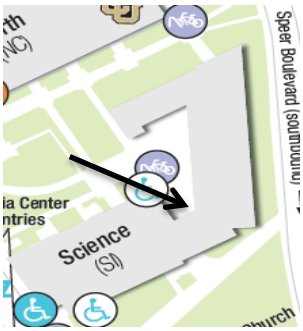
The USEPA's current acute selenium (Se) criterion for aquatic life is based on the concentration of Se in the water column. However, it is possible that this criterion may not protect aquatic life from dietborne chronic Se toxicity that could result from high-Se pulse events. A biokinetic Se bioaccumulation model based on food chain modeling was previously developed by Brix and DeForest (2008) to evaluate the water column concentrations and durations of Se pulses that would result in a given whole-body fish tissue Se concentration. Our objective was to use data from new Se biokinetic studies and data for additional species to develop an updated biokinetic food chain Se model that could be used to predict fish tissue Se concentrations resulting from Se pulses. In addition, we assessed whether a model could be used to derive an acute water column-based Se criterion that is protective of a chronic fish tissue-based criterion. The USEPA recommended a draft chronic whole-body fish tissue Se criterion of 7.9 µg/g dry weight (dw) in 2004, but for the past ten years has been in the process of revising this draft criterion. Most recently, the State of Kentucky adopted a chronic whole-body fish tissue Se criterion of 8.6 µg/g dw. This criterion was developed following USEPA guidelines for criteria development and sets a precedent for a whole-body fish tissue Se criterion that may be adopted in other states; therefore, this threshold was used in the current evaluation. Inputs to the model included background water column Se concentrations, water column Se concentrations during pulse events, and the duration of each pulse event. The model was then used to evaluate pulse Se concentrations predicted to result in the whole-body fish Se threshold over various pulse durations (1-hr, 24-hr, and 96-hr).

LOGISTICS INFORMATION

Thursday workshop 8:30am-1pm 4/17/2014
University of Colorado – Downtown Denver Campus
Science Building (SI1086), 1151 Arapahoe Ave.
Denver, Colorado 80217



Workshop location: University of Colorado Denver downtown campus, in the Science Building (SI, room **SI 1086**), located along the south side of Speer Boulevard at 1151 Arapahoe Ave., in between Lawrence Ave. and Arapahoe Ave., across the street from the Denver Center for Performing Arts. The workshop on Thursday 4/17/2014 (8:30am – 1pm) is in room SI 1086.

Getting there: The Science Building is a 10-minute walk from the Market Street Station, and the Auraria Campus is served by RTD bus and light rail. If driving, from Interstate Hwy 25, take the Speer Boulevard exit, East. The CU Auraria Campus is about a mile from I25. There is limited parking on campus, but lots and rates can be found at the following link: (http://www.ahec.edu/parking/2011%20Parking%20Map.pdf)

LOGISTICS INFORMATION (continued)

Friday Meeting 4/18/2014
US EPA Region 8 Headquarters Building
1595 Wynkoop Street Denver, CO 80202

Meeting location: US EPA Region 8 Headquarters Building in Denver, CO. The U.S. EPA Region 8 Headquarters Building is located in lower Downtown Denver, adjacent to Union Station. The hassle-free way for Denver SETAC members to get to this conference is to take the bus. Schedules and maps are available at www.rtd-denver.com. For those of you who have to drive, this website will also have the locations of close-by Park-and-Ride locations where you can park your ride to avoid the parkingcosts downtown. If you are flying in from out-of-town, Denver International Airport is the place. Shuttles are available to take you downtown.

Parking: Downtown parking options are plentiful, but costly (www.experiencedowntowndenver.com). The closest parking lot is the Union Station parking lot located on the NW corner of 16th St. and Wynkoop. Daily rates are typically $10/day, but vary daily.

Lodging: The Jet Hotel is the hippest and closest hotel to the meeting (www.thejethotel.com) and the historic Oxford Hotel is the closest hotel that will offer a government rate room (http://www.theoxfordhotel.com/). However, many other hotels are a short, free bus ride away from the meeting. The “Experience Downtown Denver” website (www.experiencedowntowndenver.com) is a great resource for finding a place to stay along the16th-Street Mall, which is where the shuttle runs.

Building Security: The EPA office is a secure building. All visitors must have a valid photo ID, and will be subject to security screening which will consist of a x-ray of personal items and passing through a metal detector. This is similar to security screening found at an airport. Firearms and dangerous weapons are not permitted in federal buildings. Visitors will log in at the reception desk located on the 2nd floor in the conference center, and a visitor badge will be issued and worn on the front of the individual’s clothing at all times. Cameras and the taking of photographs of the Region 8 Regional HQ facility are not authorized.

27th Annual Meeting of the
Rocky Mountain Chapter of the
Society of Environmental Toxicology and Chemistry
April 17 & 18, 2014

Register by April 11th, 2014 to receive a discount on registration

New! RMSETAC will be accepting payment via credit cards the day of the event. If you plan to pay with a credit card, please check the box by the total and email or mail the complete form to the address below.

Name: _____ Affiliation: _____
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Register for one day:

April 17th – Population Modeling Workshop (half day)
$____ - Non-student ($35)
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April 18th -- Scientific Presentations and Business Meeting (full day)
$____ - Non-student ($40)
$____ - Student ($25); must be currently enrolled as an undergraduate or graduate student

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Register for both days:

April 17th & 18th -- Workshop and Scientific Conference
$____ - Non-student ($65)
$____ - Student ($35); must be currently enrolled as an undergraduate or graduate student

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Need to renew your RMSETAC membership? Are you currently a member?

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+ $10 (if registering after 4/11/14)

$_____ TOTAL

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Mail registration form and check to:
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Or email registration form to:
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