Montana Water Resources Research Center
Annual Technical Report
FY 2003

Introduction

Wildfires, drying streams and falling reservoir levels were the norm throughout Montana in FY 2003. But drought is not our only water concern. Whether youre a Musselshell County homeowner worried about losing summer drinking water supplies, a Prairie County sugar beet grower contemplating the possible impacts of coal-bed methane brines on soil and water, or a Madison County fishing guide coping with the effects of whirling disease and fish habitat degradation, chances are that at least one issue of concern is a water issue.

The wet (actually average) winter and spring of 2002-2003 almost persuaded us that our drought was broken. Water experts knew better. Their repeated cautions that multi-year moisture deficits are not made up merely by one healthy precipitation cycle have helped prepare us for this dry summer. This is a good example of how good water science can fuel informed public policy and action.

Generating water knowledge and training water experts are the business of the Montana Water Center. Specifically, it is our charge to bring the brainpower of the Montana University System to bear on our states water issues by sponsoring research, providing continuing education opportunities for water professionals, and educating future water professionals.

Although the Montana Water Center also manages national fish health and drinking water initiatives, the annual base grant from the USGS is the cornerstone of water research and information transfer in Montana. A great deal of our work entails making technical water information accessible to professionals. This year our Non-Point Source Project Database received praise from around the state. This is an Internet-accessible compendium of publicly-funded projects that abate pollution from diffuse sources such as construction and agriculture. If youre seeking to learn how much public money has been expended in, for example, the Sun River watershed, plus who managed the projects, what they did, and where to get copies of project reports, youll appreciate this cyber-destination.

This fiscal year our budget was approximately $1.8 million. The majority of our funding came as grants and cooperative agreements from eight local, state and federal agencies. Private-sector funding from the Cinnabar Foundation, the Steele Water Quality Endowment, and the Whirling Disease Foundation were also essential to Water Center efforts. Early in 2003 we outfitted our media team with a substantial amount of new computer gear to support its development of cutting-edge training tools. At the same time the team moved into refurbished offices near our headquarters building. The tanks and aquaculture system components of the Wild Trout Research Laboratory also underwent a major upgrade and refitting.

We dont try to accomplish everything in-house. This year our staff of a dozen people oversaw 36 research and programming contracts. Our projects supported 24 graduate students and two undergraduates at Montana State University, Montana Tech, the University of Montana, and several out-of-state institutions. Soon these young scientists and engineers will be working water professionals, helping guide us through outbreaks of disease, pollution, and drought. The Montana Water Center is doing its best to equip them for

the considerable challenges of their 21st-century careers.

The Montana University System Water Center, located at MSU-Bozeman, was established by the Water Resources Research Act of 1964. This act created and funded Water Resources Research institutes at land grant universities in 54 states and territories. The mission of the Montana Water Center is to mobilize the resources of Montanas public universities to resolve the states water problems. It does this by sponsoring water-related research, providing training and education for current water professionals, and educating future water professionals.

Research Program

The 104(b) program addresses a spectrum of state water problems through research. Guided annually by our Water Resources Research Advisory Committee, Montana investigators and graduate students study issues like groundwater contamination, post-fire soil erosion, and runoff dynamics. The Advisory Committee identifies research priorities, oversees peer review of proposals, and recommends projects for funding. About $56,443 was awarded to Montana principal invesitgators and their students this year. You can find project reports at: http://water.montana.edu/topics/research/projects/.

Quantitative assessment of the effectiveness of post-fire erosion control techniques

Basic Information

Title:	Quantitative assessment of the effectiveness of post-fire erosion control techniques
Project Number:	2002MT1B
Start Date:	3/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	at large
Research Category:	Climate and Hydrologic Processes
Focus Category:	Geomorphological Processes, Hydrology, Sediments
Descriptors:	
Principal Investigators:	Scott Woods, Thomas DeLuca

Publication

1. Woods S.W. and A. Groen, 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. Wildland Fire Impacts on Watersheds: Understanding, Planning and Response. Denver, Colorado. Oct. 21-23 2003. Geological Society of America.
2. Groen A. and S. W. Woods, 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. American Water Resources Association, Montana State Chapter 2003 Annual Meeting. Butte Montana, October 2003.

Summary of Research

QUANTITATIVE ASSESSMENT OF THE EFFECTIVENESS OF POST-FIRE EROSION CONTROL TECHNIQUES

Dr. Scott Woods and Dr. Thomas DeLuca

University of Montana
Department of Ecosystem and Conservation Sciences

Abstract

A rainfall simulator was used to compare erosion and runoff rates from 0.5 m^2 plots treated with aerial grass seeding or straw mulch to untreated control plots in an area burned by the 2002 Fox Creek Fire in western Montana. The objective was to determine whether these treatments were effective in reducing post-fire runoff and erosion. There were ten replicates of each treatment and control plot. Rainfall was applied to each plot at an intensity of ~80 mm/hr for one hour. Mean values for total runoff from the aerial seeded and straw mulch plots were 30 and 28 mm, respectively, compared to 44 mm for the controls. Peak runoff rates from the aerial seeded and straw mulch plots had mean values of 41 mm/hr and 40 mm/hr, respectively, compared to 59 mm/hr for the controls. The mass of sediment eroded from the aerial seeded and straw mulch plots had means of 0.59 kg/m^2 and 0.10 kg/m^2, respectively, compared to 0.79 kg/m^2 for the controls. The results indicate that while both aerial seeding and straw mulch reduced surface runoff and erosion in the first year after the fire, straw mulch was more than three times as effective in reducing surface erosion rates.

Problem and Research Objectives, Metholodogy, Principal Findings and Significance

Soil erosion rates in undisturbed forested watersheds are typically very low. However, substantial increases in erosion rates have been observed after forest fires due to the loss of the duff layer, and changes in the soil physical characteristics that increase the surface runoff rate (Helvey, 1980; Morris and Moses, 1987; Robichaud, 2000; DeBano, 2000). Post-fire increases in erosion are a concern due to the loss of soil productivity, and the ecological impacts of increased sedimentation in downstream water bodies (Robichaud et al., 2000). Various erosion control techniques are used to reduce the impact of post-fire erosion on soil and water resources, including: 1) hillslope treatments, such as seeding, mulching and straw wattles, 2) in-stream treatments such as straw bales and log check dams, and 3) road rehabilitation treatments such as upgrading of culverts and ditches. Hillslope treatments are regarded as the most beneficial because they control erosion near the point of origin, thus reducing the probability that eroded soil will reach downstream water bodies (Robichaud et al., 2000).

The costs associated with post-fire erosion control are very high; the U.S. Forest Service spent more than $83 million on its Burn Area Emergency Rehabilitation (BAER) program between 1970 and 2000, of which more than 60% was spent in the 1990s (Robichaud et al., 2000). Public concern over the impacts of forest fires, and the increasing likelihood of large fires near urban interfaces, means that expenditure on post-fire erosion control is likely to remain

high. It is therefore essential that erosion control projects employ only the most effective treatments. However, few studies have determined the effectiveness of individual treatments, and most of the studies that have been conducted used only qualitative measures of effectiveness. A recent review concluded that there is an urgent need for quantitative, statistically defensible data on treatment effectiveness (Robichaud et al., 2000). There is a particular need to assess the effectiveness of hillslope treatments, such as aerial seeding and mulching.

The need for research on erosion control treatment effectiveness is particularly great in the northern Rocky Mountain region, where wildfires have burned extensive areas of state and federal land in recent years. Erosion control efforts in the northern Rocky Mountain region have cost millions of dollars at a time when the region is suffering from an economic downturn. An increased understanding of the effectiveness of post-fire erosion control techniques is needed to allow forest managers to achieve more effective post-fire management, and thus protect the region's critical aquatic resources from the effects of future forest fires. We have been using a combined experimental and observational approach to evaluate the effectiveness of aerial seeding and straw mulching in reducing erosion rates from burned hillslopes. Our study sites are in an area that was burned with high severity during the 6000 acre Fox Creek Fire in northern Montana in July 2002.

Our experimental methodology involves comparing runoff and erosion rates from replicated small (0.5 m^2) plots that have been treated with either aerial seeding or mulching to untreated control plots. A replicate comprises three plots, and there are ten replicates. Each plot is bordered by a square steel frame. The plot frames were installed in August 2002, immediately after the fire. Each frame was pushed approximately 5 cm into the ground surface to isolate the plot from the adjacent hillslope. In spring 2003, one plot in each replicate was treated with aerial seeding. The other two plots in each replicate were sheltered from the seeding operation by covering them with tarpaulin sheets. After the seeding operation was completed, we uncovered the plots, inspected them to ensure that none of the seed had been blown in, and hand picked seed from the plot where necessary. We then applied straw mulch to one of the two remaining plots, at the rate specified by USDA (1995). The third plot in each replicate served as a control.

In early August 2003, we measured the runoff and erosion from the plots in response to simulated rainfall that was applied at a nominal intensity of 80 mm/hr for one hour. Plot runoff was collected at 1-minute intervals for the first 10 minutes and then every 2 minutes thereafter. Runoff volumes in each time interval were used to calculate the runoff rate (mm/hr), and the volumes were summed to determine the total runoff (mm). The mass of sediment eroded from the plot (kg/m^2) was determined by filtering the runoff samples through paper filters, and then drying and weighing the filtered sediment.

The antecedent soil moisture content in each plot was measured using a Hydrosense soil moisture probe. Soil texture was determined from samples collected adjacent to each plot in accordance with Gee and Bauder (1986) and USDA (1994). Percent vegetation cover was determined by overlaying a grid of 100 points across the entire plot, and counting the presence or absence of vegetation at each point. Analysis of variance (ANOVA) followed by multiple comparisons was used to identify differences among the treatments and controls.

Mean values for total runoff from the aerial seeded and straw mulch plots were 30 and 28 mm, respectively, compared to 44 mm for the controls. Peak runoff rates from the aerial seeded and straw mulch plots had mean values of 41 mm/hr and 40 mm/hr, respectively, compared to 59 mm/hr for the controls. The mass of sediment eroded from the aerial seeded and straw mulch plots had means of 0.59 kg/m2 and 0.10 kg/m2, respectively, compared to 0.79 kg/m2 for the

controls. The results indicate that while both aerial seeding and straw mulch reduced surface runoff and erosion in the first year after the fire, straw mulch was more than three times as effective in reducing surface erosion rates.

Our observational study, which is also being conducted in the area affected by the 2002 Fox Creek fire, involves using silt fences to measure erosion rates in areas treated with aerial seeding. In August 2002, we installed nine silt fences below hillslopes where seeding was planned, in accordance with Robichaud and Brown (2002). Aerial seeding was subsequently conducted in spring 2003. Since the seeding covered all of the area burned with high severity and steeper slopes, it was not possible to use adjacent untreated hillslopes as a basis for comparison. Thus, we are using a multivariate analysis to determine the effects of variables such as slope angle, soil texture, and percent cover on erosion rates. Erosion rates were determined by collecting the sediment accumulated in the silt fences at the end of snowmelt and following major summer storm events. The accumulated sediment was weighed, and a subsample of the sediment was dried and weighed so that the total dry mass of sediment could be calculated. Rainfall was measured using a tipping bucket rain gage. Percent vegetation cover was determined from quadrat measurements at 4-6 randomly selected locations within each plot area in accordance with Robichaud and Brown (2002).

Laboratory analysis of the samples collected in 2003 is still ongoing. Soil texture is being determined from samples collected from each plot in accordance with Gee and Bauder (1986) and USDA (1994). Preliminary results from this component of the study indicate that percent ground cover is a primary control on erosion rates from the plots.

We plan to repeat our rainfall simulation experiment at the same replicated study plots in 2004, using the methods outlined above. We expect that the grass seeding treatment will be more effective in the second year because of the increased ground cover, while the straw mulch may be less effective because much of it will have decomposed. Differences between treatments and controls may decrease in the second year as natural revegetation increases the ground cover in the control plots. We will also continue data collection from our silt fence study plots, using the same methods as outlined above. We expect that erosion rates will decrease over time as natural revegetation occurs.

Publications and Citations

Woods S.W. and A. Groen, 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. *Wildland Fire Impacts on Watersheds: Understanding, Planning and Response*. Denver, Colorado. Oct. 21-23 2003. Geological Society of America.

Groen A. and S. W. Woods, 2003. Quantitative assessment of the effectiveness of post-fire erosion control techniques. American Water Resources Association, Montana State Chapter 2003 Annual Meeting. Butte Montana, October 2003.

Student Support

Name	Level	Field of study
Amy Groen	MS	Hydrology
Mark Flatt	UG	Hydrology

Notable Achievements and Awards

1. Our work has added to the limited literature available on the effectiveness of post-fire burned area erosion control techniques, and complements similar work being conducted by Dr. Peter Robichaud (USDA Forest Service), Dr. Lee MacDonald (Colorado State University) and others.

2. Ms. Groen received a "Best Student Presentation" award for her presentation on this research at the Montana State AWRA Meeting in Butte, Montana in October 2003.

Topography, groundwater dynamics, and soil frost: first-order controls on snowmelt runoff dynamics and plant species distributions across an uplandwetland transition

Basic Information

Title:	Topography, groundwater dynamics, and soil frost: first-order controls on snowmelt runoff dynamics and plant species distributions across an uplandwetland transition
Project Number:	2003MT9B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Not Applicable
Focus Category:	Hydrology, Wetlands, Groundwater
Descriptors:	
Principal Investigators:	Brian Leonard McGlynn, Richard Sojda

Publication

This project is studying the controls on snowmelt flow pathways, frost depth, and plant species distributions across an upland–wetland transition. It is a first step in the development of a conceptual model of snowmelt flowpaths and hydroecologic dynamics at the landscape scale. The hydrologic dynamics and plant species distributions appear tightly linked at Red Rocks Lake and in the Centennial Valley, making this an ideal site for new investigation in the emerging field of hydroecology. A final report of the results of this research will be available in the FY04 Annual Report.

Understanding and predicting changes in the microbial ecology of mine tailings in response to the addition of dissolved organic carbon

Basic Information

Title:	Understanding and predicting changes in the microbial ecology of mine tailings in response to the addition of dissolved organic carbon
Project Number:	2003MT10B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Not Applicable
Focus Category:	Water Quality, Toxic Substances, Treatment
Descriptors:	
Principal Investigators:	Paul Sturman

Publication

1. This work will contribute to the PhD dissertation of the principal investigator as well as the MS Thesis of Mr. Mark McBroom. Publication of this work in a peer reviewed journal is anticipated in calendar year 2005.

Title: Understanding and predicting changes in the microbial ecology of mine tailings in response to the addition of dissolved organic carbon.

Keywords: Acid mine drainage, microbial ecology
Start Date: 6/1/03
End Date: 5/30/04
Principal Investigator: Paul J. Sturman, Industrial Coordinator & Research Engineer
Center for Biofilm Engineering
366 EPS Building, Montana State University – Bozeman
Bozeman, MT 59717
406-994-2102 (Phone), 406-994-6098 (Fax)
paul_stu@erc.montana.edu (email)

1. Abstract

Recent field- and laboratory-scale experimentation at MSU and elsewhere has indicated that microbial populations within acid-producing mine tailings can be influenced by the addition of dissolved organic carbon. Results from this work have shown that heterotrophic bacteria can be stimulated to consume dissolved oxygen from infiltrating water, thus decreasing the oxidation-reduction (redox) potential throughout the tailings pile and promoting the activity of anaerobic sulfate reducing bacteria (SRB). However, an unintended consequence of the addition of organic carbon may be the stimulation of heterotrophic populations within the mine tailings that are also capable of iron and/or sulfur reduction. The stimulation of these populations via organic carbon addition may be detrimental to remediation efforts. Successful implementation of this technology at the field scale requires a more thorough understanding of the presence, activity, and stimulation of these potentially detrimental populations, as well as beneficial populations (e.g. SRB). In particular, it is necessary to understand and predict the response of iron-oxidizing and sulfate-reducing populations to various organic carbon addition strategies. The research proposed herein seeks to determine the specific response of these microbial populations to commonly used organic carbon sources. The proposed experiments will measure the effects of various organic carbon substrates on specific populations of IOB/SOB and SRB. Population effects will be measured through the use of bacterial cell counts, substrate utilization and advanced molecular techniques. The results will be used to help select the most appropriate sources of organic carbon for field application to mine tailings. These experiments will provide engineers and scientists responsible for implementing mine waste remedial schemes with tools for assessing the microbial condition of mine wastes prior to implementing a solution, and after a treatment is applied. Although remedial measures which rely on microbially catalyzed reactions are in common use, we currently lack the tools to predict (and measure) the responses of important microbial populations.

2. Summary of Research Objectives, Methods, and Preliminary Findings

The objectives of this project were accomplished in microcosm experiments using mine tailings from the long-abandoned Mammoth Mine (Boulder River, MT) and recently deposited tailings from the Golden Sunlight Mine (Cardwell, MT). The following objectives (with associated tasks) were set for this project.

Objective 1: Determine the effects of additions of various sources of organic carbon on populations of iron-oxidizing, sulfur-oxidizing, heterotrophic, and sulfate-reducing bacterial populations.

> *Task 1a:* Set up tailings slurry microcosms using unamended tailings, nutrient media containing necessary growth factors, solid phase pyrite and one of several organic carbon sources (negative controls containing no added carbon will also be used).

Task 1b: Operate duplicate set of microcosms under both aerobic and anaerobic conditions to mimic environmental conditions in the near-surface tailings and at depth.

Task 1c: Periodically sample tailings slurries and determine the presence of various populations of microorganisms via both conventional enumeration and advanced molecular techniques.

Objective 2: Determine the effects of additions of various sources of organic carbon on the activity of iron-oxidizing, sulfur-oxidizing, heterotrophic, and sulfate-reducing bacterial populations.

Task 2a: Liquid samples from the tailings slurry microcosms used in Objective 1 will be periodically removed to assess the growth of pertinent populations of microorganisms, including iron-oxidizers, sulfur-oxidizers, heterotrophic bacteria and sulfate-reducing bacteria. Population activity will be quantified by periodic measurement of metabolic by-products, such as sulfide (in the case of SRB), ferric iron (in the case of iron oxidizers), etc.

Research Strategies and Methods

Mine tailings from the Mammoth and the Golden Sunlight Mines were air dried and divided into 25 g aliquots. Microcosms were set up using 150 ml Erlenmeyer flasks, which were either stoppered to prevent oxygen influx (in anaerobic experiments) or covered with aluminum foil (to allow atmospheric oxygen influx). In addition to the tailings, each microcosm included 100 ml of a trace nutrient solution which was amended with either whey, molasses, or methanol. Concentrations of the various sources of organic carbon were normalized according to the bioavailable organic matter in the carbon source. Three concentrations of each organic carbon source were used for each experiment, 100 mg/l, 1 g/l and 5 g/l. Microcosms were then incubated on a shaker table for a total of 60 days, during which 4 sampling events occurred (at approximately 1, 15, 30, and 60 days).

Samples removed in performance of Task 1c were analyzed for IOB/SOB, SRB, and HPC. Iron and sulfur oxidizing bacterial populations in liquid samples removed from microcosms were enumerated via plating on selective agar growth media. SRB were enumerated using most probable number (MPN) techniques.

Findings and Significance

The project start date was delayed approximately 4 months due to difficulties in developing the molecular methods for determining the microbial consortium present within the tailings. Existing methods for the removal of genetic material from solids had to be modified for the highly acidic environment of mine tailings. Initial attempts to remove nucleic acids from tailings were not successful, and the project was delayed in the hope of solving these problems with resources from other funding sources. These difficulties notwithstanding, the project was commenced in December 2003, with microcosm set up and operation.

Initial results from aerobic microcosms suggest that heterotrophic bacteria were generally stimulated by all organic carbon treatments, however, at the highest concentration of methanol addition, some growth inhibition was observed among heterotrophic bacteria in the GSM tailings. Interestingly, in both GSM and Mammoth tailings, the unamended controls increased in numbers by almost an order of magnitude over the first month of operation. This may suggest the presence of populations of heterotrophic bacteria that are also capable of chemolithotrophic growth, an ecological niche that has been observed by other mine waste researchers.

Among anaerobically incubated microcosms, heterotrophic growth was not stimulated to the extent of those incubated aerobically. In GSM microcosms, methanol stimulated heterotrophs most consistently, while molasses resulted in the least heterotrophic growth.

At this time, data from the SRB and IOB/SOB enumerations is incomplete. These data will be compiled for the final report, which will offer insights regarding the use of various organic carbon sources (and concentrations thereof) for the stimulation of various populations of bacteria within mine tailings.

3. Publications/Citations

This work will contribute to the PhD dissertation of the principal investigator as well as the MS Thesis of Mr. Mark McBroom. Publication of this work in a peer reviewed journal is anticipated in calendar year 2005.

4. Student Support

This project has provided partial support for the PhD work of the principal investigator and 1 MS student (Mr. Mark McBroom-Civil/Environmental Engineering) and 1 recent BS student (Ms. Judith Hepner-BioResources Engineering).

5. Achievements and Awards

This work was featured on the MSU Homepage in April 2004, with an accompanying write up and press release. In addition, this project funding was instrumental in garnering further funding support from the USEPA Mine Waste Technology Program (through a subcontract from MSE Technology Applications, Inc.) in the form of a $50,000 1 year project to further study the use of organic carbon as a remedial treatment at the Golden Sunlight Mine in Cardwell Montana.

Competitive interactions between the invasive Potamopyrgus antipodarum and Baetid mayflies: temporal variation and community-level consequences

Basic Information

Title:	Competitive interactions between the invasive Potamopyrgus antipodarum and Baetid mayflies: temporal variation and community-level consequences
Project Number:	2003MT11B
Start Date:	5/1/2003
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	At-large
Research Category:	Not Applicable
Focus Category:	Ecology, Surface Water, Conservation
Descriptors:	
Principal Investigators:	Billie Kerans, Billie Kerans

Publication

1. Cada, C., J. Smith, and B.L. Kerans. 2003. "What about the fish?" in 3rd Annual Potamopyrgus antipodarum Conference, Montana State University, Bozeman
2. Cada, C.A.. and B. L. Kerans. 2004. Competitive interactions between the invasive gastropod Potamopyrgus antipodarum and baetid mayflies. In Annual Meeting of the North American Benthological Society. Vancouver, B.C.

An Annual Report to the Montana Water Center
US Geological Survey

Competitive interactions between the invasive *Potamopyrgus antipodarum* and baetid mayflies: temporal variation and community-level consequences

Principal Investigator: Billie L. Kerans, Associate Professor, Ecology Department, Montana State University—Bozeman, Bozeman, MT 59717; phone, (406) 994-3725; email:bkerans@montana.edu;

Authored by: Chelsea A. Cada

May 17, 2004

Abstract

We investigated the consequences of the introduction of *Potamopyrgus antipodarum* to Darlinton Spring Creek (Gallatin County, Montana), a popular trout spring-creek fishery where *Potamopyrgus* was recently introduced and their range has expanded. Our overall goal was to examine if and how *Potamopyrgus* changes macroinvertebrate and periphyton assemblages and whether growth of *Salmo trutta* and *Cottus bairdi* differs among areas with varying *Potamopyrgus* abundances. We examined *P. antipodarum* and baetid densities and biomasses, as well as periphyton biomass and fish diet and growth at high and low densities of *P. antipodarum.* We also determined the strength of competitive interactions between *P. antipodarum* and baetid mayflies using two *in situ* competition experiments. Densities of baetid mayflies did not respond as strongly to high-densities of *Potamopyrgus* as we expected, and we observed no statistically significant differences in baetid density between high and low density reaches. *Potamopyrgus* exerted a negative effect on periphyton biomass, the hypothesized resource for which competition between *Potamopyrgus* and baetids occurs, but we did not observe a clear difference between *Potamopyrgus* and *Diphetor* or *Baetis* in their abilities to depress periphyton biomass. In competition experiments, baetid mayflies negatively affected *Potamopyrgus* survivorship but not growth. Similarly, *Potamopyrgus* negatively affected the survivorship but not the growth of the mayflies *Diphetor hageni* and *Baetis tricaudatis.* In the fish growth experiment, *C. bairdi* lost less weight in low densities of *P. antipodarum* compared to high densities of *P. antipodarum.* On the other hand, there was no difference in mean growth for *S. trutta* between low and high densities of *P. antipodarum.* We found only 1 *Potamopyrgus antipodarum* in 1 stomach of *S. trutta.* Additionally, diet composition of trout and *C. bairdi* seemed to change between low- and high density reaches with *Potamopyrgus.* Because *Potamopyrgus* appears to be a strong grazer and competitor, it is likely to affect other macroinvertebrates that rely on periphyton as a food source. As with many invasive species, *Potamopyrgus* is likely to reduce the distribution and abundance of many resident species.

Statement of water problem:

Nonindigenous species pose one of the largest threats to biodiversity and are a major cause of endangerment or extinction of native species (Coblentz 1990, Jenkins 1996). Invasive species seriously threaten the integrity of ecosystems by altering interactions among species (Crooks 2002). For example, invasive predators can change the dynamics among resident predators and their prey, and invasive competitors can displace resident species. Such changes in interactions among species may propagate to other levels of biological scale altering population, community and ecosystem dynamics (e.g., the zebra mussel; Rappaport and Whitford 1999).

The New Zealand Mud Snail, *Potamopyrgus antipodarum*, has recently invaded freshwater ecosystems in the United States including southwestern Montana (Zaranko et al. 1997). The high densities, feeding ecology, and reproductive biology of *Potamopyrgus* suggest that it will compete with other grazing macroinvertebrates potentially causing detrimental effects to other trophic levels including fish populations (e.g., Haynes and Taylor 1984, Dorgelo 1987, Fox et al. 1996,Gangloff et al. 1998). In addition, invasive species cost the American economy about $137 billion per year (Pimentel et al. 2000). *Potamopyrgus antipodarum* might be detrimental to local economies such as the fly-fishing industry in the Bozeman area which generates about $3.5 million annually (The River's Edge, Bozeman).

We investigated the consequences of the introduction of *Potamopyrgus* to Darlinton Spring Creek (Gallatin County, Montana), a popular trout spring-creek fishery. *Potamopyrgus* was recently introduced into the creek where their population has increased and their range has expanded. Darlinton is an ideal location to study the effects of this invader because it supports a simple aquatic community amenable to experimental manipulation and because a section of meanders with similar habitat properties contains varying abundances of *Potamopyrgus*. Thus, we were able to compare aquatic assemblages under varying stages of invasion but where the habitat was similar. Furthermore, our preliminary studies show that macroinvertebrates and grazer food resources decline as *Potamopyrgus* abundances increase (Cada and Kerans, in preparation).

Research Objectives:

Our overall goal was to examine if and how *Potamopyrgus* changes macroinvertebrate and periphyton assemblages and whether fish growth differs among areas with varying *Potamopyrgus* abundances. Our specific objectives were: 1) quantify the differences in the abundances of grazing mayflies as abundances of *Potamopyrgus* varies, 2) quantify the magnitude of inter- and intraspecific competition between grazing mayflies and *Potamopyrgus*, 3) determine how periphyton biomass changes as abundances of *Potamopyrgus* varies, and 4) explore whether growth of insectivorous fishes are lower in areas where the abundances of *Potamopyrgus* are high and the abundances of other macroinvertebrates are low.

Methods:

We conducted this study in Darlinton Spring Creek at the Montana Fish, Wildlife and Parks Cobblestone fishing access site in south-central Montana, USA (45.8638°N, 111.4947°W).

Objective 1—We examined *P. antipodarum* and baetid densities and biomasses at high and low densities of *P. antipodarum.* We expected baetid density and biomass and periphyton biomass to be greater in low-snail than in high-snail reaches. Both macroinvertebrate and periphyton samples were collected monthly (April 2002 to May 2003, plus July, August and October 2003) from two downstream high-snail stream reaches and two upstream low-snail stream reaches. We sampled macroinvertebrates using cobble samples to target the grazing community (Kerans et al. 1995), which we expected to be most influenced by *P. antipodarum*. Thirty-two cobbles, 8 per reach with 2 reaches per snail density, were taken each sampling date. To reduce loss of organisms due to drift when disturbed, we placed a Surber sampler (132-µm-mesh) downstream of the rock and then gently lifted both in unison from the water (Kerans et al. 1995). Cobbles were brushed and rinsed to remove organisms, which were then preserved in Kahle's solution (Pennak 1978). Dimensions of cobbles were measured according to Graham et al. (1988) for subsequent calculation of surface area and macroinvertebrate density.

We identified and enumerated invertebrates to species using a dissecting scope at 6.3X to 40X magnifications (Merritt and Cummins 1996). We calculated densities for each sample by dividing the taxa abundance by surface area of the corresponding cobble. For baetids, we measured head capsule width to 0.01mm using an ocular micrometer at 40X magnification of randomly chosen individuals (n=20 per species per reach and sampling date), categorized individuals into developmental stages based on wing-pad size (I, II, III, or IV) as defined by Deluchi and Peckarsky (1989), and recorded sex of stage III-IV individuals based on the presence of the enlarged second pair of compound eyes of males (Peckarsky 1993). We measured shell length similarly to baetid head widths and determined both reproductive status and fecundity by dissecting randomly chosen *P. antipodarum* (n=40 per reach and sampling date). Reproductive status was defined as the presence or absence of embryos in a brood pouch whereas fecundity defined as a count of embryos present in the brood pouch.

To satisfy assumptions of normality and equality of variance, density data for all species were transformed using the natural log of x + 1, where x represents any datum point. All statistical analyses were performed using SAS 9.0 for Windows (SAS Institute Inc., Cary, North Carolina, USA). To evaluate density and biomass of *P. antipodarum* and the three baetid species, we used repeated measures nested 2-way ANOVA (PROC GLM) with the response variable repeated over time. The 2 main factors (levels listed in parentheses) included snail (low or high density), reach (A or B) nested within snail. We were particularly interested in the time*snail interaction to determine whether the snail effect differed across time for any of the response variables.

Objective 2—We compared periphyton biomass between high and low-snail reaches using chlorophyll *a* from small cobbles as a surrogate measure. We collected 8 additional cobbles per reach per sampling date, which were frozen and stored in the dark until chlorophyll extraction. We extracted chlorophyll *a* in 90% ethanol by submerging each cobble and using spectrophotometric analysis to measure concentration (Cada and Kerans in preparation). Direct extraction of chlorophyll *a* was chosen over other periphyton sampling methods such as scraping or brushing of the cobbles primarily because these methods can underestimate biomass through loss of tightly adhered diatoms (Aloi 1990, Cattaneo and Roberge 1991). Biomass was calculated as the product

of the extract's concentration and volume divided by the estimated surface area. We estimated surface area of the cobbles as noted for macroinvertebrates. Chlorophyll *a* biomass was analyzed in the same manner as macroinvertebrate densities using repeated measures nested 2-way ANOV*A*.

Objective 3: Competition experiments—To determine the strength of competitive interactions between *P. antipodarum* and baetid mayflies, we conducted two *in situ* experiments in artificial chambers stocked with various density combinations in late summer (28 July – 13 August 2003, Exp1) and early winter (23 October – 11 November 2003, Exp2). We choose the timing of the experiments to occur in different seasons—summer and winter in an attempt to compare the magnitude of competition between seasons. Experiment 1 was several days shorter than Exp2 because invertebrate growth is temperature dependent and body growth of individuals should've accumulated more quickly in Exp1. In addition, emergence increased over time in Exp1, and we wanted to limit the loss of mayflies from the replicates before sample size became too small.

The circular chambers were 11 cm diameter x 14 cm depth with two 4 x 7 cm opposing holes covered by 500-µm-nytex-mesh to allow water exchange. Chambers were mounted in polystyrene floats (1.2 m x 0.6 m x 0.05 m, 4 chambers per float) which were secured in the stream channel with rebar and protected from debris by 0.64 cm wire-mesh. Each chamber received 3 similarly sized pebbles (surface area of about 125 cm^2) prior to invertebrate stocking. We collected the pebbles from the stream channel and carefully removed visible invertebrates to minimize disturbance of periphyton. An extra 18 pebbles were collected and frozen for chlorophyll analysis so that standing crop at the beginning of the experiment was known (Exp2 only).

We chose stocking abundances that reflected the range of densities observed in the field (10,000-20,000 m^{-2}). In Exp1, we compared *Diphetor* and *Potamopyrgus*, whereas in Exp2 we compared *Baetis* and *Potamopyrgus*. Experiment 1 consisted of 3 treatments in an additive design where the total number of individuals in a replicate was constant at 250: *Diphetor* alone (D), *Potamopyrgus* alone (P), and *Diphetor* plus *Potamopyrgus* together (D+P). In contrast, Exp2 comprised 7 treatments using a response-surface design. Assignment of treatments to chambers was completely randomized across floats.

Invertebrate stocking of the experimental chambers occurred within a 30 hr time-frame. We collected invertebrates using kick nets and pipetted a known number of individuals into temporary containers. To reduce mortality from picking-to-stocking, we stocked chambers as the lots of individuals accumulated. We chose *P. antipodarum* ~2 mm length and young baetid nymphs (wing-pads present but not darkened or thickened) for stocking. These sizes precluded prior embryo development by *P. antipodarum* (Richards 2001) in addition to allowing growth by both species and field identification.

Maintenance of chambers and floats occurred every ~3 days. This included cleaning the nytex and wire meshes of debris to aid water exchange and removing dead invertebrates by pipetting to prevent deterioration of water quality. For Exp2, maintenance included removal of snow and ice from the surfaces of chambers and floats. At the end of each experiment we enumerated and preserved live individuals in Kahle's. Additionally, pebbles from the experimental chambers (n=12 and n=120 for Exp1 and

Exp2, respectively) were frozen for chlorophyll analysis and calculation of periphyton biomass (see methods in field surveys).

We quantified the strength of competition using two characters of fitness including survivorship and per capita growth. We calculated survivorship in each chamber for Exp2 as the number of individuals alive at the experiment end divided by the initial abundance of its respective treatment. Survivorship per chamber in Exp1 was calculated similarly to Exp2 except that the final abundance was corrected for loss due to emergence (i.e., estimated mean daily emergence was added to each final abundance). In addition, per capita growth rates for both species were calculated based on the difference between final and initial biomass divided by the number of days in the experiment. To estimate initial and final biomasses, we measured shell length or head-capsule width and converted these measurements to biomass using regressions from Benke et. al (1999) and Cada and Kerans (in preparation). For initial biomass, we measured 40 individuals per species, which we subsampled from the individuals available for stocking. For final biomass, we measured up to 40 individuals per species per replicate, depending on survivorship of the invertebrates.

To satisfy assumptions of normality and equality of variance, survivorship and per capita growth data required natural log transformation for both species and both experiments. To evaluate competition Exp1, we used 1-way ANOVA for each competitor with treatment as the factor to determine whether density influenced mean survivorship and per capita growth rates. Factor levels were *Diphetor* alone (D), *Potamopyrgus* alone (P), or *Diphetor* plus *Potamopyrgus* (D+P). Additionally, we used 1-way ANOVA to determine whether treatments affected chlorophyll *a* biomass through differential grazing pressure. This analysis included an "initial" factor level that represented chlorophyll a biomass from the stream channel at the start of the experiment and a "control" factor level that represented chlorophyll *a* biomass from experimental chambers with zero invertebrates. To evaluate competition Exp2, we used 2-way ANOVA for each competitor with species ("alone" or "B+P") and density ("low" or "high") as the factors.

We also used 1-way ANOVA to determine whether the species and density factors affected chlorophyll *a* biomass. This analysis included additional levels: 'initial' and "final" that represented chlorophyll a biomass from the stream channel at the start and end of the experiment as well as a "control" level that represented chlorophyll a biomass from experimental chambers with zero invertebrates.

Objective 4: fish growth— We estimated the effects *P. antipodarum* density (referred to as "low snail" or "high snail") on the growth rates and *body condition* of *Salmo trutta* and *Cottus bairdi* using an *in situ* enclosure experiment. Enclosures were constructed from 2.5 x 2.5 cm pine frames to dimensions of 61 x 61 x 30.5 cm for *C. bairdi* and 61 x 91.5 x 91.5 cm for *S. trutta* and were wrapped with 0.85 cm nylon-netting or 0.64 cm hard-wire cloth, respectively. Bottoms and tops of enclosures were covered with nylon window-screening rather than netting or hard-wire cloth. All mesh was secured with staples. A total of six trout enclosures and six sculpin enclosures were placed in high-snail and low-snail reaches of Darlinton Spring Creek. We placed trout enclosures near the thalweg and added several large cobbles to provide a flow-refuge (Wilzbach et al. 1986), whereas we placed sculpin enclosures in riffles and covered the

bottom with pebbles to simulate their habitat preference. Both enclosure types were secured to rebar posts driven into the stream bed. The rebar posts, about 30 cm upstream of each enclosure, also supported chicken-wire that served to reduce clogging of the enclosures' mesh and improve water flow within enclosures. All mesh was cleaned of debris every 2-3 days throughout the duration of the experiment. We measured water flow at the front and rear of each enclosure using a Swoffer 3000 and measured physicochemical water conditions at each enclosure using a Yellow Springs Instrument (YSI).

We collected one-year old *S. trutta* (*Salmo trutta*, ~7 cm length) and sculpin (*Cottus bairdi*, 7-12 cm length) by electrofishing 1 July 2003. Fishes were anesthetized using MS-222 for handling. For each individual, we measured fork length (nearest mm) and wet mass (nearest 0.1g) at the beginning and the end of the experiment (Wilzbach et al. 1986). Three sculpin per enclosure were stocked 1 July 2003, and 5 *S. trutta* per enclosure were stocked on 2 July 2003, after being held overnight within Darlinton Spring Creek. Because high flow events (regulation of water level for irrigation) between 9 July and 14 July washed-out two trout enclosures (one high-snail and one low-snail), individuals were redistributed within their snail-treatment and enclosures thereafter contained only 3 trout. We terminated the sculpin experiment 31 July 2003 and the trout experiment 6 August 2003.

We estimated daily growth of *S. trutta and C. bairdi* as the difference in weight from the start and end of the experiment divided by the number of days in the experiment. Growth was transformed by ln (x + 1). We used 2-way ANOVA to compare the difference in growth between species (levels: sculpin and brown) and between snail-treatments (low and high density).

To compare fish diet with food availability, we will use a selectivity index to indicate whether *C. bairdi* and *S. trutta* prefer to prey on some taxa at a rate greater than they are available in the environment (Chesson 1978). To estimate food availability, we sampled macroinvertebrates from low-snail and high-snail reaches on 9 July and 6 August 2003 (see Chapter 1 for further detail). Additionally, macroinvertebrate densities within sculpin enclosures were sampled using cobble samples (n=3 per enclosure) according to the same procedures in Chapter 1.

Principal Findings:

Objective 1.—*Potamopyrgus* densities peaked during summer months of 2002 (24,750 m^{-2}) but reached their lowest levels in spring 2002 and 2003 (< 1000 m^{-2}). In general, densities were lower in 2003 than in 2002. *Potamopyrgus* reproduced year-round and did not exhibit clear cohorts, which is consistent with other findings on *P. antipodarum* reproduction (Winterbourn 1970b). *Potamopyrgus* densities were smaller in 2003 than 2002, perhaps because of its biology and life history or as a consequence of invasion dynamics. *Potamopyrgus* may be sensitive to cold temperatures (Hylleberg and Siegismund 1987) and an early, particularly low-temperature event may have decreased survival of individuals in late winter and early spring 2003. In support of this hypothesis, minimum and maximum temperatures in October were nearly three degrees cooler in 2002 than in 2003 (2.76-14.11 ºC and 5.42-17.59 ºC, respectively). Alternatively, many invasive species exhibit dynamic population behavior with large cycles or experience a

"boom and bust" where populations decline markedly after initial high abundances (Williamson 1996). However, large intra-annual changes in *Potamopyrgus* densities have been observed for this species (Dorgelo 1987, Schreiber et al. 1998), suggesting population density variation for this species is not part of a boom and bust cycle. For example, densities in Darlinton Spring Creek dropped from nearly 28,000 m^{-2} in November 2000 to almost 9,000 m^{-2} in June 2001 (Cada and Kerans, in preparation). Thus it seems more likely that this population fluctuates temporally as some function of the winter environment (e.g., low temperature, low productivity).

All three mayfly species exhibited patterns of abundance and size-class distributions consistent with univoltine life history strategies. Young *Baetis* individuals (stage I) formed a large proportion of the population as early as July and were the dominant life stage in fall and early winter. *Baetis* individuals close to emergence and maturity (stage IV) were present over a wide range of months from late-winter through mid-summer suggesting that emergence occurred throughout these months and was not tightly synchronized. In contrast with *Baetis*, young *Diphetor* and *Acerpenna* individuals did not comprise a large proportion of the population until September and consisted of more than 90% of the population through February. This indicates eggs began hatching in late summer and may have continued throughout winter. In addition, little if any individual growth occurred during winter months as mean head width did not change during that time period. Stage IV individuals of *Diphetor* and *Acerpenna* occurred from late spring throughout the summer, indicating emergence occurred primarily in summer months, although *Diphetor* emergence may have begun slightly before *Acerpenna.* Differential timing of emergence between *Diphetor* and *Acerpenna* may be caused by different developmental requirements such as degree days (add citation) or could be a result of past competitive interactions and temporal habitat partitioning (Connell 1980).

Densities of baetid mayflies did not respond as strongly to high-densities of *Potamopyrgus* as we expected; i.e., we expected mayfly densities to be higher in low-snail reaches than in high-snail reaches at least during fall months as we observed in November 2000 for a similar magnitude of snail densities (Cada and Kerans, in preparation). High variability undoubtedly decreased our ability to detect statistical differences between mean mayfly densities in high-snail and low-snail reaches. It may be that variability is an indicator of an effect of *Potamopyrgus* on baetid mayflies. Variability seems to be greater in those cases where possible interactions between *Potamopyrgus* and baetids occurred. While there were no statistical differences in mayfly densities between high and low snail reaches, I think it worthwhile to explore the trends observed because they may be biologically significant. *Baetis* densities appear greater in low-snail reaches than in high-snail reaches during late winter and relatively late within larval development. *Diphetor* densities tended to be greater in low-snail reaches than in high-snail reaches in late fall and early winter, before larvae began to develop wing pads. In contrast to *Baetis* and *Diphetor*, *Acerpenna* seemed to be positively affected (densities greater in high-snail reaches than in low-snail reaches) beginning in late fall and continuing through early spring. These trends suggest that the interaction between *Potamopyrgus* and baetids can vary but is biologically significant at certain time periods. Additionally, these trends agree with previous field research that showed a strong effect of *Potamopyrgus* on the density and biomass of baetid mayflies in November 2000.

It is important to point out that "high" *Potamopyrgus* densities within our field study do not represent the range of densities that *Potamopyrgus* reaches in other locations (Kerans et al. in press, Hall et al. 2003). In a broader perspective, the densities observed in Darlinton Spring Creek would more correctly be considered "moderate". As a result, the effect of *Potamopyrgus* on baetid mayflies in locations of "high" (i.e., > 50,000) and extremely high (i.e., > 150,000) densities could be much stronger and more apparent than we observed in this study.

Objective 2.—In the field survey, *Potamopyrgus* exerted a negative effect on periphyton biomass (both chlorophyll a and phaeophytin a biomass), the hypothesized resource for which competition between *Potamopyrgus* and baetids occurs. Since we did not observe a clear effect of *Potamopyrgus* on baetid mayflies in the field study, it seems likely that *Potamopyrgus* did not depress resources sufficiently to limit resources and strongly influence baetid densities. Periphyton is probably not the only resource for which *Potamopyrgus* may compete with baetid mayflies. Space is likely to be an important factor because high densities of *Potamopyrgus* should limit habitat availability.

We did not observe a clear difference between *Potamopyrgus* and *Diphetor* or *Baetis* in ability to depress periphyton biomass. In Exp1, *Potamopyrgus* tended to reduce chlorophyll a biomass in comparison with *Diphetor*, whereas in Exp2 *Baetis* depressed periphyton biomass slightly more than *Potamopyrgus*. A better ability by *Baetis* to consume periphyton at low biomass levels agrees with data from a previous behavioral experiment where *Baetis* decreased the ability of *Potamopyrgus* to depress periphyton biomass (Cada and Kerans, unpublished data).

Although *Baetis* may be better able to graze periphyton at low levels of biomass relative to *Potamopyrgus*, *Baetis*' behavioral decisions may change the interaction in the natural environment. That is, *Baetis* is thought to actively enter the drift when food levels reach a certain threshold (Kohler 1989), and rather than remaining in an area of decreased periphyton biomass that results from the presence of *Potamopyrgus*, *Baetis* may choose to drift and seek areas of higher food availability. By choosing to drift, *Baetis* increases its probability of death by predation, decreases the relative amount of time spent foraging, and runs the risk of drifting to an unsuitable habitat, all of which may ultimately decrease fitness.

Objective 3.— For *Potamopyrgus*, we generally observed negative effects of baetid mayflies on survivorship but not on growth. For survivorship, we did not observe differences between low and high densities when *Potamopyrgus* was alone, indicating a lack of intraspecific competition at the densities investigated in this study. In contrast, *Potamopyrgus* survivorship was greater in treatments without *Baetis* or *Diphetor* than treatments with the competitors, which indicates interspecific competition. In the first experiment, *Potamopyrgus* growth tended to be greater in the presence of *Diphetor* than when alone, which may indicate either a release from intraspecific competition or facilitation. In contrast to the first experiment, *Potamopyrgus* growth decreased from low to high density treatments, indicating intraspecific competition. Additionally, we did not observe an effect of *Baetis* on *Potamopyrgus* growth.

Potamopyrgus negatively affected the survivorship but not the growth of *Diphetor*. For *Baetis*, we did not observe an effect of conspecific density on survivorship, which indicates a lack of intraspecific competition. However, *Baetis* survivorship was lower in combination treatments with *Potamopyrgus*, indicating the

presence of interspecific competition. Additionally, high and low densities with *Potamopyrgus* did not differentially affect *Baetis* survivorship, indicating that the intensity of competition did not increase as density increased. In contrast to survivorship, we did not detect any effects on *Baetis* growth, either intra- or interspecific.

One reason we did not detect any effects of *Potamopyrgus* on the growth of *Diphetor* or *Baetis* was the low survivorship of both mayflies in the experiments. Low survivorship resulted in fewer individuals from which to estimate growth in each replicate; i.e., a small sample size. Additionally, if we assume that only the most healthy individuals survived, these many be less affected by competition than by unhealthy individuals and result in a biased sample.

Objective 4.—In the fish growth experiment, *S. trutta* gained weight and *C. bairdi* lost weight. It seems probable that *C. bairdi* lost weight in this experiment due to density-dependent effects because three *C. bairdi* were held in each cage. Additionally, *C. bairdi* lost less weight in low densities of *P. antipodarum* compared to high densities of *P. antipodarum.* On the other hand, there was no difference in mean growth for *S. trutta* between low and high densities of *P. antipodarum.* High variability of trout growth in the low-density *P. antipodarum* reaches reduced our ability to detect any effect *of P. antipodarum* on *S. trutta*.

We are currently working on diet analysis and selectivity indices for the *S. trutta* and *C. bairdi,* but we can report some preliminary results. Diet analysis of 29 *S. trutta* and 17 mottled *C. bairdi* removed from a reach containing high snail densities (>50,000 m^{-2}) yielded only 1 *Potamopyrgus antipodarum* in the stomach of a *S. trutta* (greater than 23 cm length). This *P. antipodarum* individual appeared to be a newly hatched juvenile less than 1mm in length. Additionally, diet composition of trout and *C. bairdi* seemed to change between low- and high density reaches with *Potamopyrgus.* That is, the diet of *S. trutta* tended to eat more amphidpods in low-snail densities than in high snail densities. In contrast*, S. trutta* ate more chironomids in low-snail than in high-snail density reaches. Additionally, *C. bairdi* tended to eat a more varied diet in high-snail than in low-snail reaches, where only two taxa were eaten (Isopoda and Chironimidae).

The experiments in Objective 4 will be repeated in summer 2004 for *S. trutta* and *C. bairdi*, as well as for *Oncorhynchus mykiss* (rainbow trout) with funds from the US Fisheries and Wildlife Service.

Significance of findings:

Although this study does not demonstrate a clear effect of *Potamopyrgus* on baetid mayflies, it does suggest that the effect of *Potamopyrgus* on baetids in Darlinton may change on a temporal scale, having a greater effect during times of lower productivity or during different developmental ages of baetid larvae. This conclusion is supported by the results of competition experiments that demonstrate a negative effect of *Potamopyrgus* on baetid mayfly survivorship. Decreased survivorship will affect population dynamics of baetid species and may ultimately have negative implications for the persistence of certain mayfly (*Baetis* and *Diphetor*) populations in the presence of *Potamopyrgus*. Additionally, this study shows that *Potamopyrgus* can depress periphyton food resources, but whether to a level that limits other species will depend upon biological attributes and competitive abilities of each species. Because *Potamopyrgus* appears to be a strong competitor, it is likely to affect other

macroinvertebrates that rely on periphyton as a food source. Finally, this study does not demonstrate a strong effect of *Potamopyrgus* on the growth of either *S. trutta* or *C. bairdi*, but it does suggest that insectivorous fishes may adjust their diet according to changes in macroinvertebrate abundances caused by *Potamopyrgus*. As with many invasive species, *Potamopyrgus* is likely to reduce the distribution and abundance of many resident species.

Publications/Citations:
The research offered in this report has been presented publicly at two conferences, as well as an informal gathering between biology/ecology departments at Montana State University and University of Montana.

Cada, C., J. Smith, and B. L. Kerans. 2003. "What about the fish?" 3rd Annual *Potamopyrgus antipodarum* Conference. Montana State University—Bozeman.

Cada, C. A. and B. L. Kerans. 2004. Competitive interactions between the invasive gastropod *Potamopyrgus antipodarum* and baetid mayflies. Annual Meeting of the North American Benthological Society. Vancouver, B.C.

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Pharmaceuticals in septic system effluent

Basic Information

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Screening Level Study of Pharmaceuticals in Septic Tanks, Ground Water, and Surface Water in Missoula, Montana

By

Emily Godfrey

B.S Union College, 2002

presented in partial fulfillment of the requirements

for the degree of

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Chairperson

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Dean, Graduate School

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Screening Level Study of Pharmaceuticals in Septic Tanks, Ground Water and Surface Water in Missoula, Montana

Committee Chair: Dr. William W. Woessner

Individual septic systems and wastewater treatment plants (WWTP) are used to collect and treat sewage. Concern has been raised as to the fate of pharmaceuticals and personal care products found in sewage, yet their fate in household or community septic systems is poorly known. The use of septic tanks is widespread as approximately 25-35% of homes rely on them for waste disposal. This study attempts to characterize the occurrence and estimate concentrations of pharmaceuticals in septic system effluent, and examine the potential for the contamination of shallow aquifers. Sewage entering a wastewater treatment plant was also sampled. The occurrence of 19 drug residues and three drug metabolites of both prescription and non-prescription drugs in wastewater, ground water and surface water were analyzed by Time-of-Flight High Performance Liquid Chromatography/ Mass Spectrometry (ToF-HPLC-MS). Target compounds were acetaminophen, antipyrine, caffeine, carbamazepine, cimetidine, codeine, cotinine, diltiazem, erythromycin-18, fenofibrate, fluoxetine, hydrocodone, ketoprofen, metformin, nicotine, nifedipine, paraxanthine, ranitidine, salbutamol, sulfamethoxazole, trimethoprim and warfarin. Of all raw sewage samples, only 18 of the 22 pharmaceutical compounds were present in septic tanks, 12 were detected in WWTP influent, and nine were detected in WWTP effluent. The most frequently detected (>50%) non-prescription drugs in the raw sewage samples were acetaminophen, caffeine, nicotine and a caffeine metabolite (paraxanthine), and a nicotine metabolite (cotinine) These compounds occurred at concentrations that were estimated to be higher than 1570-ug/L, 500-ug/L, 100-ug/L, 1000-ug/L, and 100-ug/L, respectfully. Prescription drugs examined in the raw sewage were detected in about 30% of the samples with the exception of warfarin which was detected in approximately 77% of the samples. Other frequently detected prescription drugs were codeine, trimethoprim and carbamazepine. Ground water receiving septic effluent from a high school drain field contained measurable quantities of caffeine, carbamazepine and sulfamethoxazole (<210-ng/mL). Samples of shallow ground water within the unconfined aquifer underlying the city of Missoula and the adjacent county exhibited detectable concentrations of caffeine, carbamazepine, cotinine and trimethoprim.

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I would like to thank Mark Benotti. Without his patience and guidance this paper would have not been possible. I dedicate my results section to you. Reggie Spaulding, thank-you for helping me start the analysis and the procedure. Your time did not go unrecognized.

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To all my friends, new and old, thank-you for helping me relax and enjoy my time in Montana. Your support was vital for metal sanity: Rachele, Michelle, Emily, Andrew, Doug, Tara, and Margaret. Last, but not least, a special thanks to John for your unconditional support, listening to complaints and coming up with solutions. You make my life happier and I am grateful to have you.

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INTRODUCTION

During the last three decades, an increased focus on water pollution from organic chemicals such as toxic/carcinogenic pesticides and industrial byproducts has emerged (Christensen 1998). In recent years, pharmaceuticals and personal care products (PPCP's) and their metabolites are appearing in surface water, ground water and drinking water as a result of wastewater contamination (Raloff 1998; Buser et al. 1999; Hartig et al.1999; Seiler et al. 1999; Heberer 2002a and 2002b; Holm et al. 1995; Kolpin et al. 2002; Scheytt et al. 1998; Eckel et al. 1998; McQuillan et al. 2000, Buerge et al. 2003; Clara et al. 2004; Petrovic et al. 2003). Human, industrial and agricultural wastewaters contain low levels of antibiotics, prescription and non-prescription drugs, hormones, synthetic steroids, stimulants, detergent metabolites, fire retardants and personal care products. This includes compounds such as sulfamethoxazole, carbamazepine, acetaminophen, 17β-estradiol, coprostanol, caffeine, 4-nonylphenol monoethoxylate, tri(2-chlorethyl) phosphate and acetophenone, respectively (Ternes et al. 1998; Seiler et al. 1999; Daughton and Ternes 1999; Hirsch et al. 1998; Jones-Lepp et al. 2001; Huang and Sedlack 2001; Ternes et al. 2001; Kolpin et al. 2002).

To evaluate the pharmaceuticals released into the aquatic environment, studies have estimated the amount of prescription and non-prescription drugs consumed each year (Buerge et al. 2003, Ternes 1998; Hirsh 1998; Fisher and Boland, 2003). Hirsh et al. (1998) estimated German annual production of antibiotics to be in the range of 2000 tons per year, while Fisher and Borland (2003) estimated 56 tons per year of prescription drugs were sold in Sydney, Australia. In the United States 22,680 tons of antibiotics are prescribed annually (Levy 1998). However, prescription drug estimates are only a small

portion of the pharmaceuticals used on a daily basis. Large quantities of non-prescription drugs are sold without regulation through out the world (Christensen 1998). It is estimated that 1000 tons per year of ibuprofen are consumed in countries such as the United Kingdom and Germany. Globally caffeine average consumption (estimated from consumption of coffee, tea and soft drinks) is about 70-mg per person per day (Buerge et al. 2003). Both prescription and non-prescription drugs are beginning to be detected in water systems all over the world.

Recent Studies of Pharmaceuticals in the Environment

Over 20 years ago the first report of pharmaceutically active compounds found in sewage influent and effluent were clofibric acid, nicotine and caffeine. These compounds were reported to re-enter and persist in the aquatic environment from wastewater contamination (Daughton and Ternes 1999) (Appendix 1- Pathways of Pharmaceuticals in the Environment and Expanded Data for Analytical Difficulties). In recent studies on German sewage effluent, eighty percent of the human drugs studied, were detected in the part per billion range (Ternes 1998). In southwestern United States, Drewes et al. (2003) reported carbamazepine, primidone, ibuprofen, and naproxen to be commonly found in secondary and tertiary treated wastewater effluents and in surface water. Drewes et al. (2003) reported that antiepileptic drugs (such as carbamazepine and primidone) persisted in ground water under both anoxic and aerobic conditions. A literature review of 22 targeted pharmaceuticals detected in WWTP influent and effluent, surface water and ground water are reported (Table 1).

Table 1. Literature search of pharmaceuticals in surface and ground water, sewage influent and effluent

Compound	Surface Water and Ground Water (maximum reported concentration)	Raw Sewage WWTP (Max Reported Concentrations)	Outflow WWTP (max. reported concentrations)
Acetaminophen	Kolpin et al. 2002 (10 ug/L); Verstraeten et al. DRAFT (GW*-0.015 ug/L)	NF**	Ternes 1998 (6.0ug/L)
Antipyrine	Ternes 1998 (0.95 ug/L)	NF**	NF**
Caffeine	Seiler et al. 1999 (0.23 ug/L); Mcquillan et al. 2001(1.5 ug/L); Kolpin et al. 2002 (6.0 ug/L); Buerge et al. 2003 (250 ng/L); Clara et al. 2004 (0.10 ug/L); Sacher et al. (900 ng/L); Ternes et al. 2001 (0.88 ug/L); Buerge et al. 2003 (several different studies 100, 1440, 115, 47, 6000, 370, 880 1270, 171, 160, 2400 ng/L GW*- 80, 230ng/L); Verstraeten et al. DRAFT (GW*-0.12 ug/L); McQuillan et al. 2003 (1500 ng/L)	Ternes et al. 2001 (1.9 ug/L); Heberer, 2002 (640ug/L); Buerge et al. 2003 (several studies 73, 300, 20 and 147ug/L); Benotti et al. 2003 (109ng/L)	Heberer, 2002 (3ug/L) Buerge et al. 2003 (several studies 9480, 0.08, 6.7, 0.19 and 2ug/L), Drewes et al. 2003 (15700 ng/L); McQuillan et al. 2003 (1000 ng/L)
Carbamazepine	Ternes 1998 (1.1 ug/L), Heberer 2002 (7.3ug/L); Drewes et al. 2003 (235 ng/L); Clara et al. 2004 (GW* 900ng/L)	Heberer, 2002 (3.8ug/L); Benotti et al. 2003 (119ng/L); Clara et al. 2004 (2000ng/L)	Drewes et al. 2003 (610 ng/L); Heberer, 2002 (5ug/L) Ternes 1998 (6.3 ug/L); Clara et al. 2004 (1510ng/L)
Cimetidine	Kolpin et al. 2002 (0.58 ug/l)	Benotti et al. 2003 (240 ng/L)	NF**
Codeine	Verstraeten et al. DRAFT (GW*-0.080 ug/L)	NF**	NF**
Cotinine	Verstraeten et al. DRAFT (GW*-0.060 ug/L)	Benotti et al. 2003 (22 ng/L)	NF**
Diltiazem	Kolpin et al. 2002 (0.049 ug/L); Clara et al. 2004 (0.033 ug/L); Ternes et al. 2001 (0.033 ug/L)	Ternes et al. 2001 (0.053 ug/L); Benotti et al. 2003 (52.4 ng/L)	NF**
Erythromycin-18	Kolpin et al. 2002 (1.7ug/L); Castiglioni et al. 2004 (15.9 ng/L); Sacher et al. 2001 (ng/mL); Verstraeten et al. DRAFT (GW*-0.75 ug/L)	NF**	NF**
Fenofibrate	NF**	Zwiener et al. 2000 (1.19ug/L)	Zwiener et al. 2000 (0.17 ug/L); Ternes 1998 (0.03 ug/L); Drewes et al. 2003 (35 ng/L)
Fluoxetine	Kolpin et al. 2002 (0.012ug/L)	NF**	NF**
Hydrocodone	NF**	NF**	NF**
Ketoprofen	Ternes, 1998 (0.12 ug/L)	NF**	Drewes et al. 2003 (45 ng/L); Ternes 1998 (0.12 ug/L);
Metformin	Kolpin et al. 2002 (0.15 ug/L)	NF**	NF**
Nicotine	NF**	NF**	NF**
Nifedipine	NF**	Ternes et al. 2001 (0.089 ug/L)	NF**
Paraxanthine (1,7-dimethylanthine)	Kolpin et al. 2002 (3.1 ug/L) Called 1,7-dimethylxanthine	Benotti et al. 2003 (154 ng/L)	NF**
Ranitidine	Kolpin et al. 2002 (0.01 ug/L); Castiglioni et al. 2004(0.002 ug/L),	Benotti et al. 2003 (91ng/L)	NF**
Salbutamol	Ternes, 1998 (0.035 ug/L) Castiglioni et al. 2004 (4.6ng/L)	Benotti et al. 2003 (35.6 ng/L)	NF**
Sulfamethoxazole	Kolpin et al. 2002 (1.9 and 0.52 ug/L) Sacher et al. 2001 (410 ng/L), Castiglioni et al. 2004 (0.9ng/L); Verstraeten et al. DRAFT (GW*-0.15 ug/L); Hartig et al., 2000(231 ng/L)	Benotti et al. 2003 (458 ng/L)	Hartig et al. 2000 (799ng/L)
Trimethoprim	Kolpin et al. 2002 (0.3 ug/L); Verstraeten et al. DRAFT (GW*-0.58 ug/L)	Benotti et al. 2003 (105ng/L)	NF
Warfarin	Verstraeten et al. DRAFT (GW*-0.009 ug/L)	NF**	NF**
GW*=ground water	NF**= not found in literature search or not analyzed		

Pharmaceuticals in surface water may impact aquatic biota, raising concern over their presence. A reproductive hormone 17β-estradiol, detected in the outfall of sewage treatment plants, negatively impact fish reproductive systems at trace levels (Huang and Sedlak 2001; Sedlak et al. 2000; Plesner et al. 2002). Daughton and Ternes (1999) suggested that humans exposed to trace concentrations of biologically active drugs, for example synthetic antibiotic medicines such as sulfonamides, could also suffer adverse impacts. Long-term exposure of non-target organisms to trace concentrations of antibiotics may contribute to the maintenance and spread of antibiotic resistance (Levy 1998).

To date, efforts have focused on the detection and fate of pharmaceuticals in surface water. The U.S.G.S recently sampled 139 streams in 30 states for compounds including plasticizers, pharmaceuticals and hormones (Kolpin et al. 2002). Of the 95 wastewater contaminants examined, one or more compounds were present in 80% of the streams or rivers tested. Only a few studies (e.g. Holm et al. 1995, Umari et al. 1995, Eckel et al. 1998, Seiler et al. 1999, Drewes et al. 2003, Verstraeten et al. Draft, Benotti et al. 2003) have examined the concentration of pharmaceuticals in raw sewage. To date, no published research has examined PPCP concentrations from individual septic systems (Verstraeten et al. 2004). According to Knowles (1998), approximately 10% of septic tanks in the United States are malfunctioning: over 7000 faulty tanks per day. This raises concerns that trace pharmaceuticals may be entering the ground water underlying these systems.

GOALS AND OBJECTIVES

This study characterizes the occurrence and estimates the concentration of pharmaceuticals in septic system effluent, and examines the potential for contamination of shallow aquifers. It examines pharmaceutical concentrations in: (1) single family and community septic tanks; (2) influent and effluent of a wastewater treatment plant; (3) eight ground water samples from monitoring wells in a highly productive sand and gravel aquifer; and (4) in a septic system and monitoring well network serving a rural high school. The specific study objectives were to: (1) identify target compounds; (2) develop sampling and analyses procedures; (3) characterize individual and community septic tank effluent; (4) sample ground water in a sole source aquifer that is overlain by areas containing sewer lines and septic systems. The data from this effort provide an inventory of pharmaceuticals found in septic waste and, with limited data, examine the transport and fate of pharmaceuticals in the associated ground water systems.

METHODS

Identify target compounds of concern

Pharmaceuticals selected for this study were based on the following criteria: 1) they are commonly used drugs; 2) the compound has been reported to occur in the environment; 3) the compound ionizes well under positive electron spray mode (analytical consideration). Certain compounds, like ibuprofen, that fit criteria 1) and 2), were not included as they cannot be easily detected using the chosen analytical technique. Target compounds including 19 pharmaceuticals, both prescription and non-prescription drugs, and three metabolites were selected for evaluation (Table 2, Appendix 2- Structures and Molecular Weights of Pharmaceuticals).

Table 2. Pharmaceuticals analyzed. The last two columns report the maximum recommended dose for an adult and maximum urinary excretion percentage

Compound	Type	Use	Recommended Dose for adult (mg/day)	Maximum Urinary Excretion (%) (Goodman and Gilman, 1990)
Acetaminophen	Non-prescription drug	Antipyretic	600	3 +/-1
Antipyrine (Phenazone)	Prescription	Analgesic	54	ND*
Caffeine	Non-prescription drug	Stimulant	210-440	1.1 +/- 0.5
Carbamazepine	Prescription drug	Anticonvulsant, antineuralgic, antimanic, antidepressant, antipsychotic	100-400	<1, 3 (PDR**)
Cimetidine	Prescription drug	Antiasthmatic	300-800, 2-4 times daily	62 +/- 20
Codeine	Prescription drug	Analgesic (anti-cough)	12-60, 1-4 times daily	Negligible
Cotinine	Metabolite	Nicotine metabolite	Metabolite	ND*
Diltiazem	Prescription drug	Antihypertensive	30-120	<4
Erythromycin-18	Metabolite of Prescription drug	Antibiotic	250	12 +/- 7
Fenofibrate	Prescription	Lipid Metabolism regulator	54-200 daily	ND
Fluoxetine	Prescription drug	Antidepressant, antiobsessional, and antibulimic	10-40 daily or weekly	<2.5
Hydrocodone	Prescription drug	Analgesic (anti-cough) and antitussive	5-7.5	ND*
Ketoprofen	Non-Prescription	Anti-inflammatory	25-200	<1
Metformin	Prescription drug	Antihyperglycemic	500-1000, twice a day	ND*
Nicotine	non prescription drug		4	16.7 +/- 8.6
Nifedipine	Prescription drug	Antianginal (blood pressure control)	10-90, daily	~0
Paraxanthine (1,7-dimethylanthine)	Metabolite	Caffeine metabolite	Metabolite	ND*
Ranitidine	Non- Prescription drug	Histamine	25-300, three a day	69 +/- 6
Salbutamol	Prescription drug	Relax restricted airways	2-5	ND*
Sulfamethoxazole	Prescription drug	Antibiotic	200-800	14 +/-2
Trimethoprim	Prescription Drug	Antibiotic	40-160	69 +/- 17
Warfarin	Prescription drug	Anticoagulant	1-10	<2

ND*= no data PDR**= Physicians desk reference, 2001

Field Sampling and Site Description

Five types of sites were sampled for pharmaceuticals: 1) individual and community septic systems 2) the city wastewater treatment plant; 3) the Frenchtown High School research site; 4) shallow monitoring wells in the Missoula Aquifer, and 5) the Clark Fork River. A more detailed description of each site is given below.

Thirty-two single-family and ten community septic tanks were sampled in the City of Missoula (Figure 1). The single-family 3,785-L septic tanks are classified as STEP systems (Septic Tank Effluent Pump) and are used to collect household wastewater (Figure 2). When the liquid effluent reaches a volume of 2,600-L, it is pumped from the septic tank to the city sewer line. Solids that settle to the bottom of the tank are pumped out as needed. The community septic tanks, which hold 11,300 to 30,300 liters, are designed to catch wastewater from approximately 10-75 apartments and/or homes. These STEP systems discharge to the city sewer line.

Septic tank effluent samples were collected using a parastolic pump, equipped with new 30-cm length of silicon tubing and a section of 1.5 to 7.6-m clean polyethylene tubing. Samples pumped from the tanks were collected in a 2.5-L glass bottle. All bottles were pre-washed with methanol and Milli-Q water and dried overnight. All tubing used for pumping samples was new and discarded after sample collection.

The municipal wastewater treatment plant (WWTP) in Missoula, Montana is connected to about 57,000-population equivalents. The WWTP consists of commonly used treatment steps, preliminary sedimentation followed by activated sludge treatment and final clarification by chlorination.

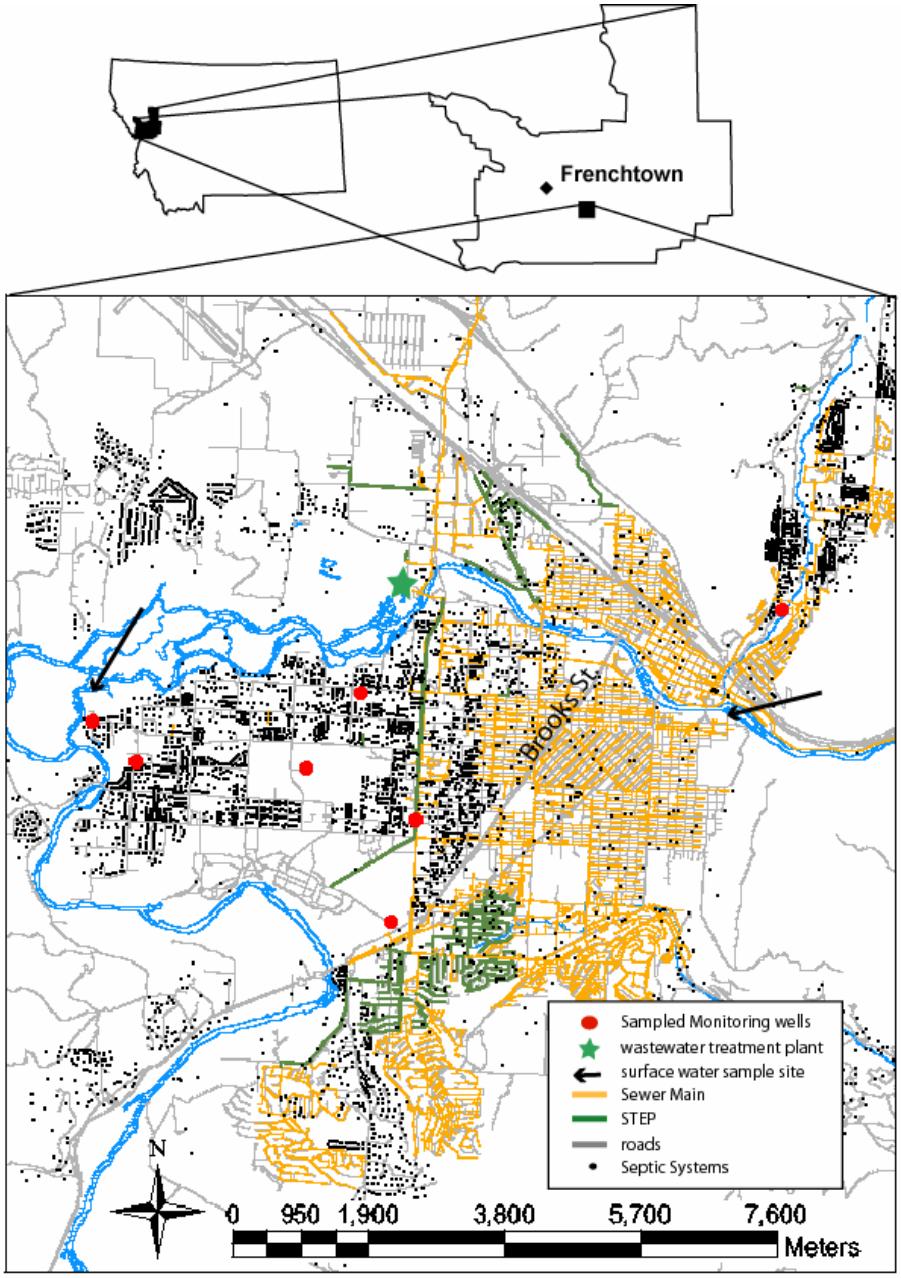

Figure 1. Location map of the CIty of Missoula. Shown are sewer systems (gravity flow and STEP), unsewered areas, monitoring wells, the wastewater treatment plant and location of the surface water samples in the city of Missoula, Montana (Map Source: Department of Water Quality Missoula, Montana)

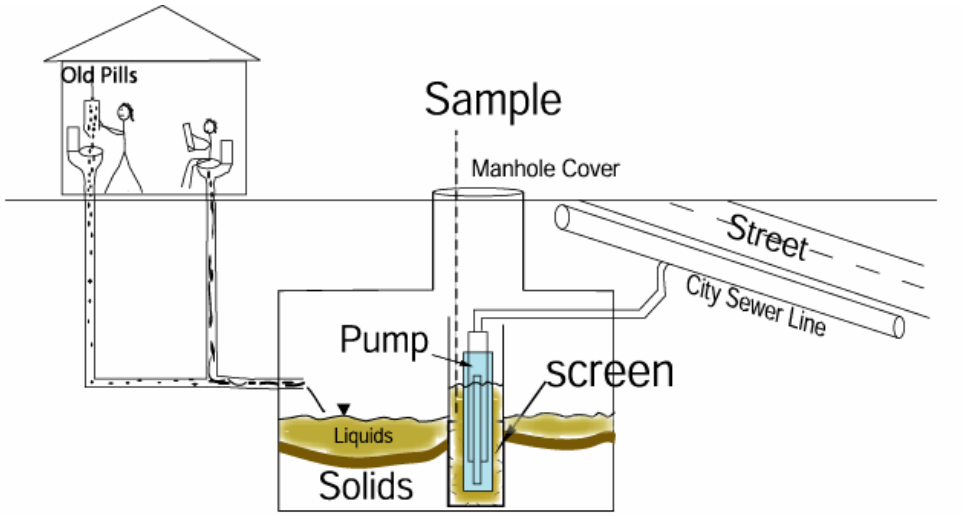

Figure 2. Schematic diagram of STEP (Septic Tank Effluent Pumping) system for a single-family residence.

Two influent samples were obtained at the WWTP, after primary sedimentation, by submersing a 2.5-L glass bottle into the liquid flowing into the secondary treatment basin. As an advanced wastewater treatment, Missoula WWTP uses ultraviolet treatment during the summer months to help destroy photoreactive compounds. Two effluent samples were taken before and after ultraviolet treatment. Effluent from the WWTP is then discharged into the Clark Fork River.

Samples of effluent from the 22,712-L septic tank of Frenchtown High School (350 students and staff) were collected using the same process as described for sampling individual STEP systems. At the high school, four shallow monitoring wells were sampled from a well-documented wastewater-impacted aquifer located beneath the drain field, and along the ground water flow path (Deborde et al. 1998; Lauerman 1999) (Figure 3). The drain field is constructed of PVC pipe with 26 laterals buried in trenches 0.6-m below land surface and surrounded by washed 5-cm diameter cobbles. The subsurface contains medium sand to a depth of 2.4 to 3.4-m, and 7.6-m of sand and gravel that is saturated at 1.5 to 3-m below land surface (DeBorde et al. 1998). Prior to sampling ground water, all 2.5-L glass bottles were silanized and clean tubing was used for each sample (Cras et al. 1999). Samples were obtained from wells using a parastolic pump and a length of new silicon and polyethylene tubing.

Eight shallow ground water wells used to monitor the water quality of the sole-source Missoula Aquifer were sampled to characterize the ground water near the water table. The Missoula aquifer is a coarse-grained gravel, unconfined aquifer that supplies potable water to the city and county. The aquifer varies from 15.2 to 36.6-m in thickness and the water table occurs between 21.3 and 30.5-m below land surface.

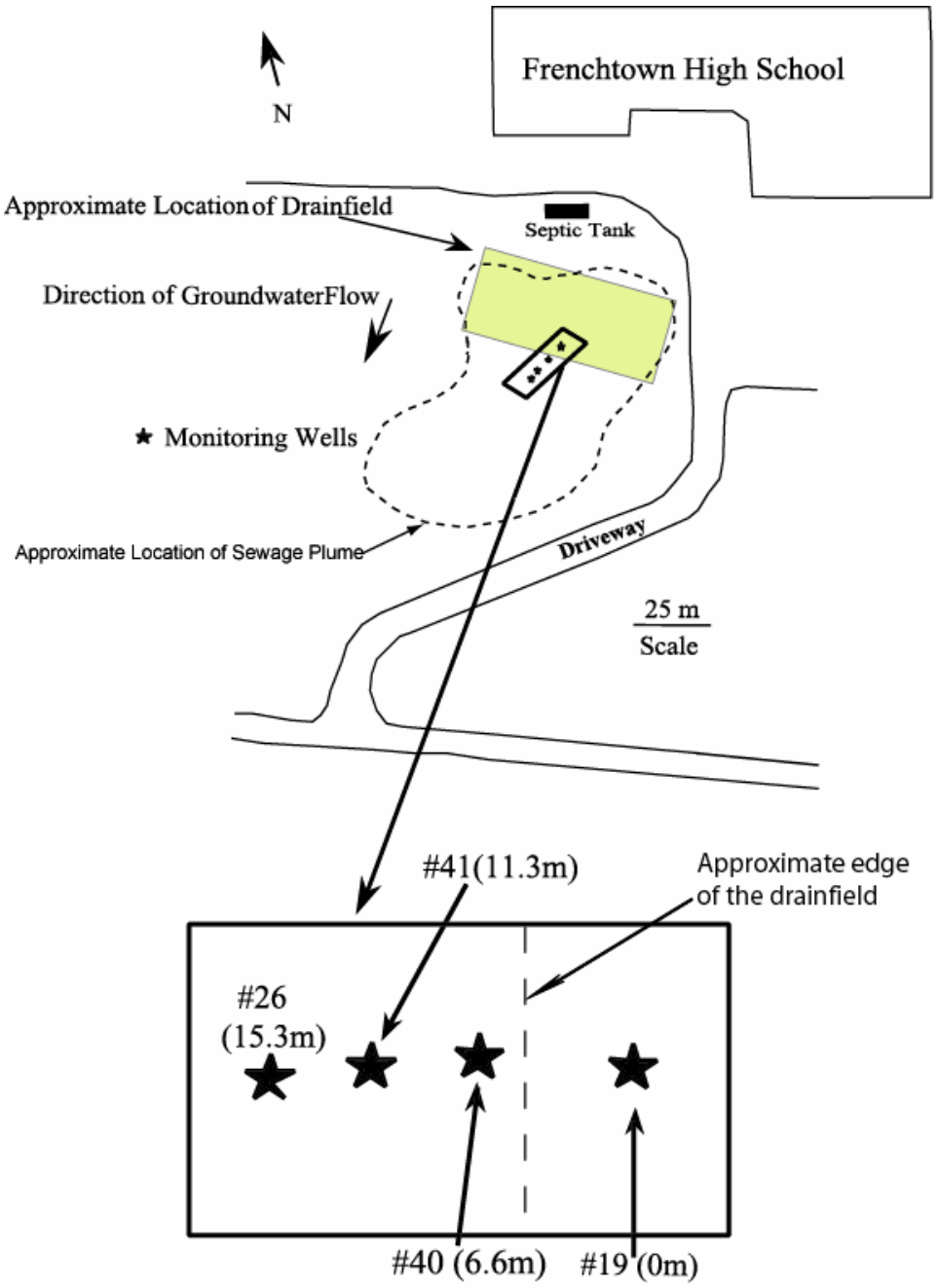

Figure 3. Frenchtown high school septic system. Approximate location of ground water sewage plume located below the drainfield. Insert of ground water wells sampled for pharmaceutical analysis, with approximate distance from each other in parenthesis. Well 19 is located directly beneath the drain field (Adapted from Deborde et al. 1998).

The wells were purged for more than five minutes at a rate of approximately 10-L/min, to ensure that well casings were flushed. Samples were collected using disposable polyethylene bailers and placed in 2.5-L silanized glass bottles. One additional ground water sample representing potable ground water supplied by Mountain Water Company was obtained from the faucet of a local home in the City of Missoula.

In an attempt to examine the concentrations of target compounds in the Clark Fork River two samples were collected, one at the headwaters of the Missoula Valley and another downstream from the sewage treatment plant (~5.6-km) (Figure 1). Samples were obtained by submersing 2.5-L silanized glass bottles into the river.

All samples were transported to the lab and stored in coolers (4˚C), after sample preparation samples were stored in lab freezers until analyzed.

Sample Preparation

At this time no standardized procedure has been adapted for sample preparation and analysis. I prepared samples within 1-3 days of collection, using adjusted methods described by Kolpin et al. (2002) (pharmaceutical extraction method 3). This method was designed to target human prescription and non-prescription drugs and their metabolites (Appendix 3). In brief, first a pre-filtration step was initiated by passing the sample through a 0.45-um glass fiber filter. Then 1-L of sample was processed through a solid phase extraction (SPE) cartridge that contained 6-cc, 500-mg of sorbant Hydrophilic-Lipophilic-Balance (Oasis, HLB) at a flow rate of 15 to 25-mL/min (Appendix 4). Next, compounds were extracted from the SPE cartridge using two 3-mL aliquots of CH_3OH and two 3-mL aliquots of CH_3OH acidified with trifluoroacetic acid (0.1% trifluoroacetic acid, $C_2HF_3O_2$). Compounds were slowly reduced to near dryness

under N_2 and then brought to a 1-mL solution volume with the starting mobile phase for the high performance liquid chromatography (HPLC) analysis, 10-mM ammonium formate/formic acid, (pH=3.7). All effluent samples were filtered with a 0.2-um PTFE syringe filter then diluted to a 10% solution, prior to analysis. Compounds were separated and measured by Time-of-Flight, High Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-TOF-MS, Waters HPLC system) in the laboratory of SUNY at Stony Brook, using a polar (neutral silanol) reverse-phase octylsilane (C8) HPLC column (Metasil Basic 3um, 150*2.0mm; Metachem Technologies). This preparation procedure was used for all samples (Benotti et al., 2003).

Ground water sample recoveries are between 3% and 110%, depending on the compound (reported by personal communication with Mark Benotti of SUNY-Stony Brook University) (Appendix 4). Standard curves used for quantification and calibration reports of all compounds are found in Appendix 6. For quality control one internal standard, $^{13}C_3$- labeled caffeine, was used (Cambridge Isotope Laboratories in Cambridge, Ma). Pharmaceutical standards were obtained from Aldrich and prepared by the personnel of the SUNY at Stony Brook lab. Analyses were conducted in ESP+ mode with a selected mass range of 100 to 800 Da. A lock mass, leucine enkephalin (Sigma #P9003), added post-column at a flow rate of 1-uL/min with a concentration of 5-ng/mL, was used to compensate for drift of the external calibration during analysis due to possible temperature fluctuations and instabilities of the power supply by a single point correction of the base calibration file after analysis (Benotti et al. 2003, Ferrer and

Thurman 2003). Quantification of compounds was estimated from the internal standard ($^{13}C_3$- labeled caffeine) injected into the sample prior to analysis.

Resultant concentrations from the above procedure must be qualified before discussion. This study attempted to characterize PPCP concentrations in an environmental compartment for which little data exist (septic tanks). Generally speaking, PPCPs in the septic tanks exhibited a wide range of concentrations (from ng/L to high µg/L). While this offers interesting discussion, it must be noted that both the extraction procedure and HPLC-TOF-MS analysis was designed to study trace levels of contaminants. Thus, reported concentrations, especially high values, represent a low-end concentration. The actual value cannot be quantitatively determined because phenomenon such as over-loading of SPE cartridges, ionization suppression/enhancement, and detector saturation are likely clouding high environmental concentrations (Benotti et al. 2003). Although studies to qualify detector saturation and ionization suppression were outside the scope of this project, observation of such phenomenon indicate that concentrations to 500-ng/L are within the error of the analysis. Systematic error increases linearly for concentrations above 500-ng/L, but considering the worst case, probably underestimate the highest concentrations. Therefore, values reported in this study should be compared to other environmental concentrations with this in mind.

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Analytical results

As part of the method development and to maximize the resolution and sensitivity of the HPLC-TOF-MS, three samples were prepared at sample concentrations of 10%, 50% and 100% solution. The 10% solution was chosen for it produced chromatograms

with the least amount of matrix interference and a discernable internal standard peak. Thus for all effluent samples, prior to HPLC analysis, a 10% standard solution was used. Because standards examined during sample analysis did not produce reliable results, they were run again on a later date for better correlation (Appendix 6).

Analytical difficulty occurred during sample preparation and SPE concentration. Using the stated preparation methodology, target compounds were captured from a 1-L filtered effluent sample using a 6-cc, 500-mg HLB sorbant. The ability for the HLB cartridges to capture all target compounds was evaluated by passing one sample through two HLB cartridges in series. Compounds such as acetaminophen, caffeine, cotinine and paraxathine were detected in the second processing of 1L samples, while ketoprofen, nicotine and warfarin were not detected (Table 3).

Table 3. Double runs through cartridges. Samples A and B are samples from two septic tanks. A1 and B1 are the results of effluent processed on a HLB cartridge and A 2 and B 2 are processed on a second HLB cartridge. **All concentrations represent minimum concentrations.**

Samples	Acetaminophen	Caffeine	Cotinine	Ketoprofen	Nicotine	Paraxathine	Warfarin
	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
A 1	1.09	8.26	Nd	Nd	Nd	67.67	1.81
A 2	0.64	1.39	0.12	Nd	Nd	40.88	Nd
B 1	140.01	60.84	5.36	147.64	0.87	71.84	5.84
B 2	427.73	13.621	2.44	Nd	nd	196.43	Nd

Table 4. Sample splits. These are reported by compound, total mean % comparisons, number of positive identified compounds parenthesis. **All values compared represented minimum concentrations**

Compound (n=)	Acetaminophen (9)	Caffeine (9)	Carbamazepine (3)	Cimetidine (2)	Cotinine (8)	Diltiazem (2)
Total mean (%)	88.4	83.2	78.1	91.7	83.9	83.2
Compound (n=)	**Erythromycin-18 (3)**	**Codeine (4)**	**Hydrocodone (1)**	**Ketoprofen (1)**	**Metaformin (3)**	**Nicotine (8)**
Total mean (%)	87.5	90.9	90.4	70.6	87.5	81.7
Compound (n=)	**Paraxathine (9)**	**Ranitidine (1)**	**Sulfamethoxazole (2)**	**Trimethoprim (3)**	**Warfarin (6)**	
Total mean (%)	83.8	69.4	46.0	80.6	70.7	

Two samples were available for preparation from each site. Of all effluent samples, nine splits were prepared and analyzed in duplicate to determine method reproducibility (Table 4). All compounds exhibited reproducibility above 50% with the exception of sulfamethoxazole, which was only detected in two samples.

During the evaporation of the sample, solids were observed to form in the test tube. Visually, these samples were a dark brown color and collected on the bottom and sides of the glass vial. Adding the mobile phase (10-mM ammonium formate/formic acid, pH=3.7) to the near dry sample re-dissolved a portion of the solid phase, but in some samples the solid phase remained in the vial. It is likely that the residue remaining in the sample vial contained target compounds. These conditions may have created analytical results that are lower than their actual values (Appendix 1).

Instrument detection limits and recovery data for ground water samples are reported for method and analysis (Personal communication with Benotti 2004) in Appendix 4. Recovery data for septic effluent were not completed in this study.

RESULTS

Results from the analysis of pharmaceuticals in septic system effluent are presented for both single-family and community tanks, WWTP influent and effluent, Frenchtown and Missoula Valley ground water.

Single Family and Community Septic Tanks

This study analyzed for 22 pharmaceuticals in each sample. Of those; only 18 were found above their detection limit (Figure 4).

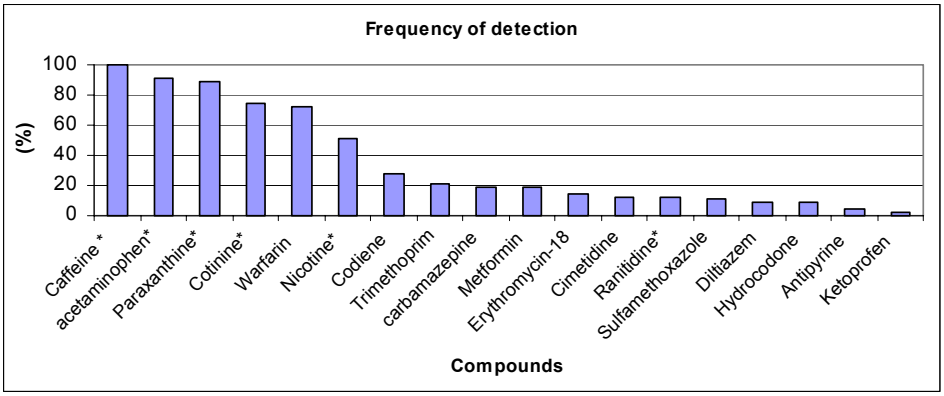
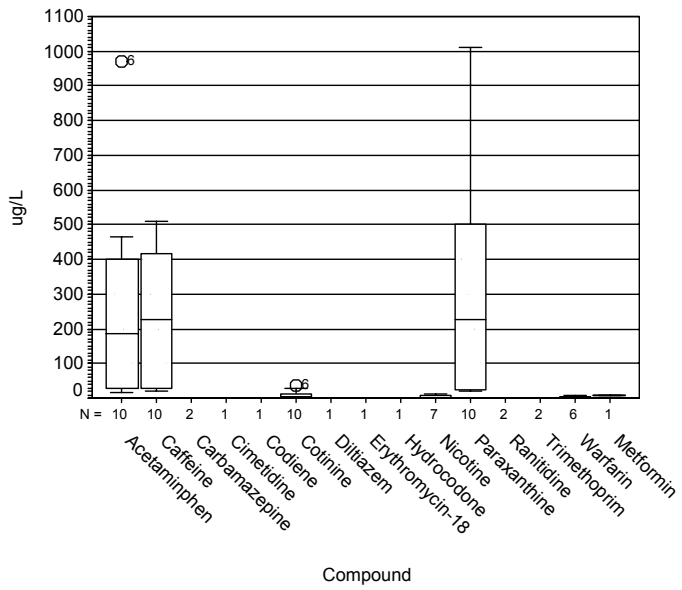


Figure 4. Most frequently detected compounds in raw sewage samples (community, single family, school septic effluent and WWTP influent). Marked (*) compounds are nonprescription drugs and/or there metabolites.

Concentration ranges and numbers of occurrence are provided for all compounds detected in community and single-family septic tank effluent (Figure 5 and 6)

Compounds not detected were fenofibrate, fluoxetine, nifedipine and salbutamol. In all community tank effluent the most detected compounds (>60%) were acetaminophen, caffeine, cotinine, paraxanthine and warfarin (Figure 5). In single-family tanks the most detected compounds (>60%) were caffeine, acetaminophen, cotinine, paraxanthine and warfarin (Figure 6).

a.



b.

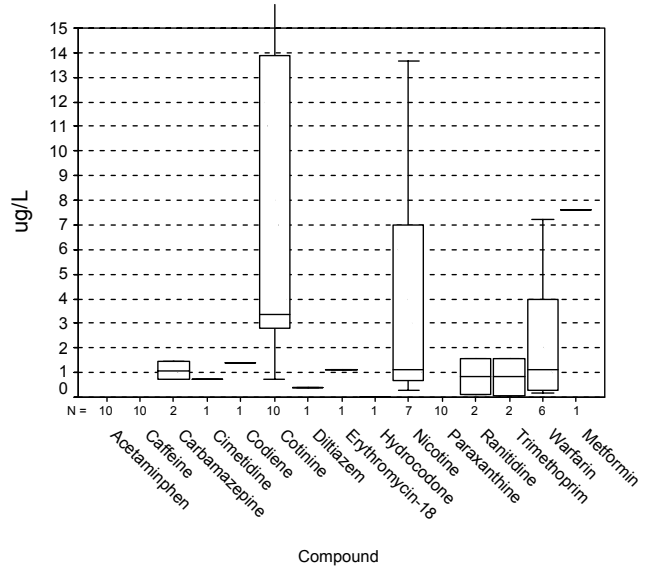
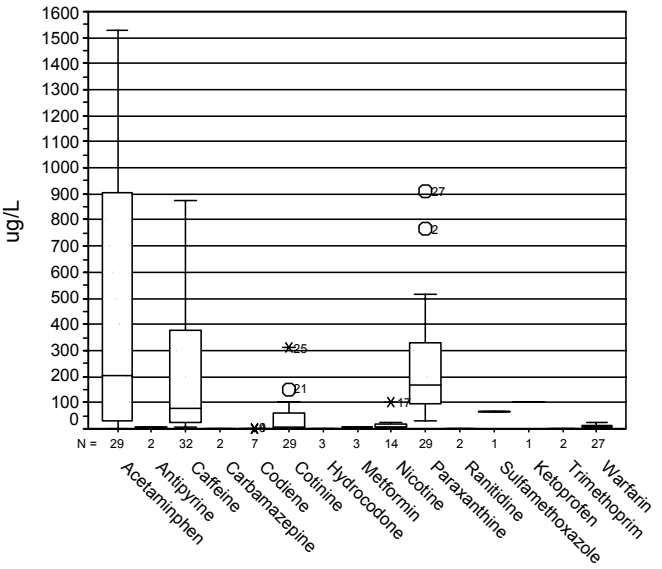


Figure 5. Pharmaceuticals detected in community septic tanks. Box plots report median, 75, 25 quantities and maximum and minimum values and O_{xx} represent outliers. The numbers of detections in samples are reported above the compound name. Two box plots are used to show all concentration ranges of samples (a) higher concentrations and (b) lower concentrations. **All concentrations represent minimum concentrations.**
a.



b.

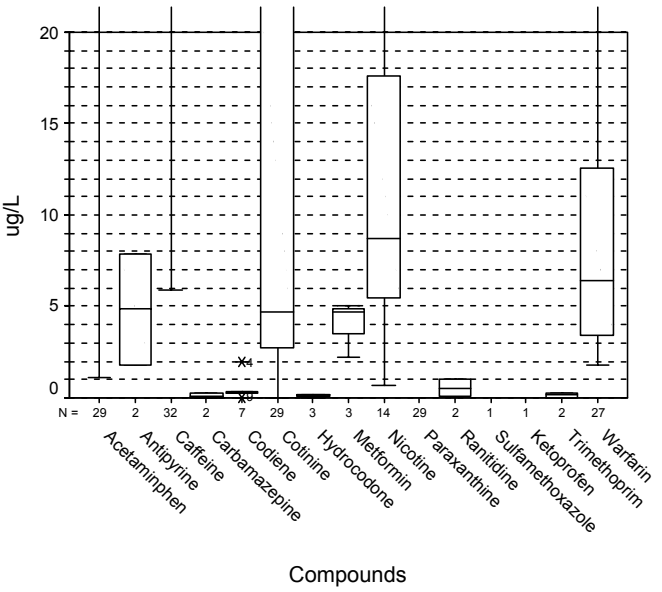


Figure 6. Pharmaceuticals detected in single-family septic tank. Box plots report median, 75, 25 quantities and maximum and minimum values. O_{xx} and represent outliers and $*_{xx}$ represent extreme values. The numbers of detections in samples are reported above the compound name. Two box plots are used to show all concentration ranges of samples (a) higher concentrations and (b) lower concentrations. **All concentrations represent minimum concentrations.**

Wastewater Treatment Plant

Comparisons of pharmaceutical concentrations from influent and effluent sewage of the city's WWTP are reported, including concentrations of before and after ultraviolet treatment (Figure 7). Acetaminophen, diltiazem, nicotine, paraxathine and warfarin were not detected in the outflow of the WWTP.

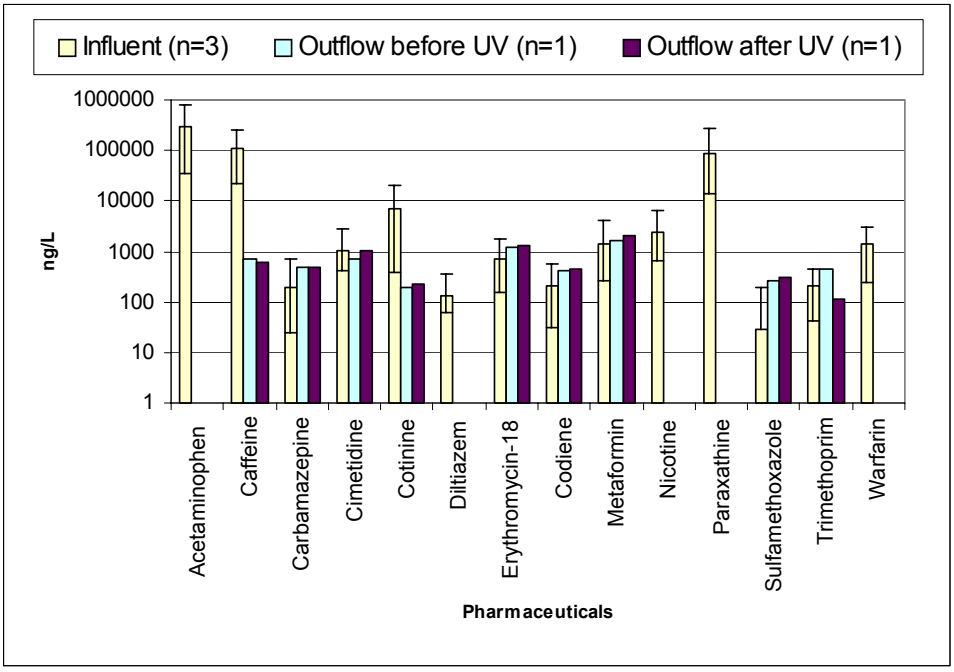


Figure 7. Concentrations of pharmaceuticals at the WWTP. Bars and boxes in the influent column represent a range of three sampling period's. Two concentrations are plotted of outflow samples before and after ultraviolet treatment. **All concentrations represent minimum concentrations.**

Frenchtown High School Site

Results from the Frenchtown High School site represent two consecutive sampling events conducted on 10/30/03 and 11/05/03, respectively. Only twelve of the twenty-two pharmaceuticals were detected in the septic tank. Pharmaceutical levels found in the school's septic effluent had comparable concentrations between sampling

periods. The exceptions to this were erythromycin-18 and sulfamethoxazole, which appeared to be higher during the second sampling period (Figure 8).

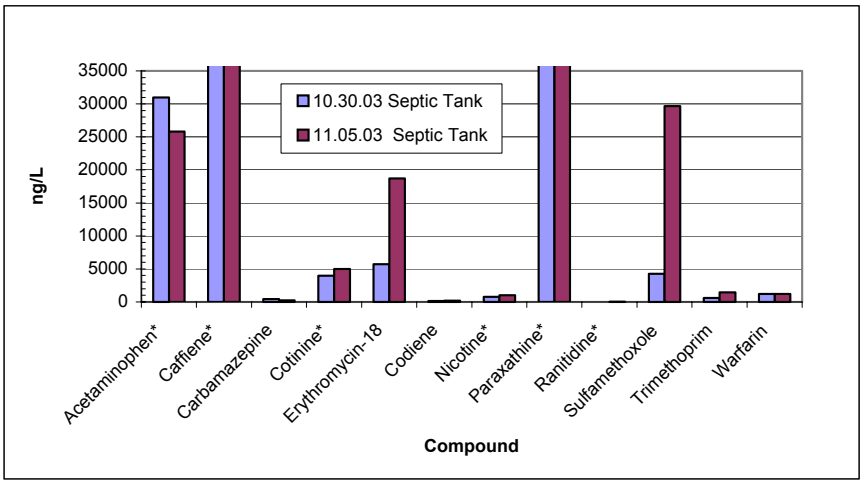
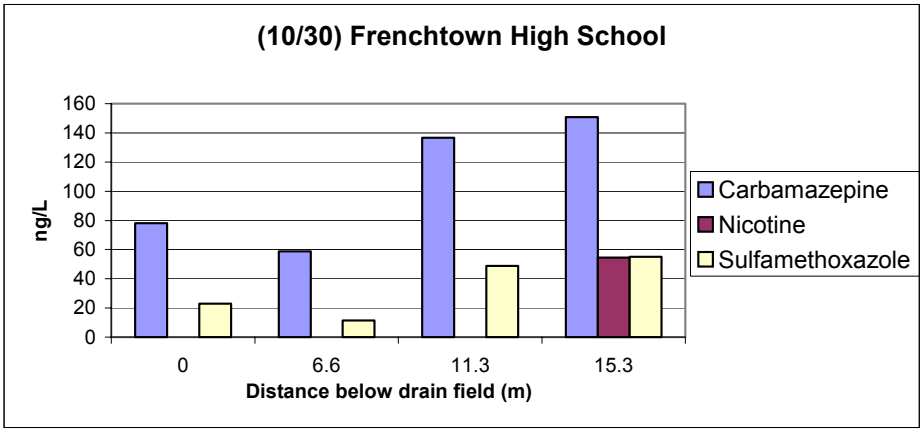


Figure 8. Concentrations of pharmaceuticals from septic effluent at Frenchtown high school taken on 10/30/03 and 11/05/05. Marked (*) compounds are nonprescription drugs and there metabolites. **All concentrations represent minimum concentrations.**

Ground water from four ground water wells finished below the drain field and within the plume of impacted ground water, tested positive for four of the twenty-two compounds analyzed (Figure 9). Carbamazepine and sulfamethoxazole were the most frequently detected compounds in ground water at Frenchtown (Figure 9). Nicotine was positively detected in ground water samples but at levels below the limit of quantification, with the exception of one ground water sample (Figure 9).

a.



b.

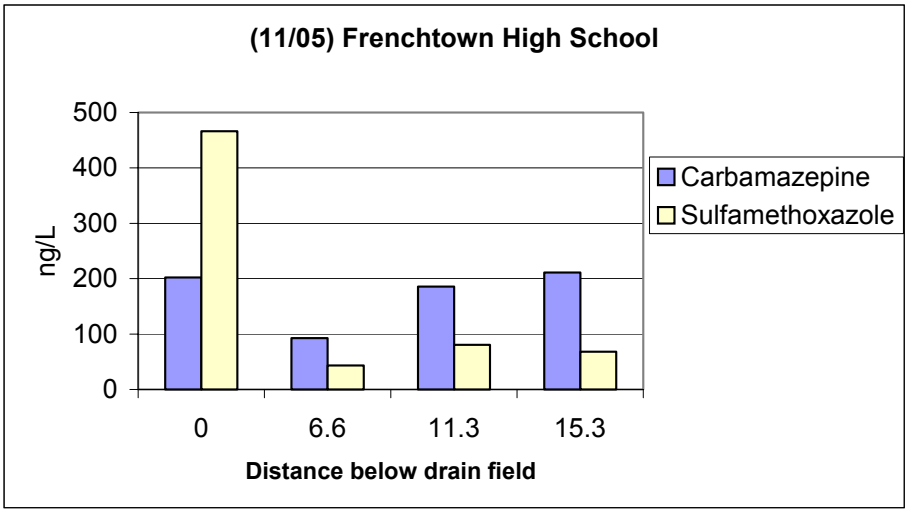


Figure 9. Concentrations of pharmaceuticals from Frenchtown high school ground water, taken 10/30/03(a) and 11/05/03(b) of pharmaceuticals detected below drain field (0 meters), just outside of drain field (6.6m), further down the flow line (11.3m) and furthest away from drain field (15.3m). **All concentrations represent minimum concentrations.**

Missoula Valley Ground Water

Ground water samples taken in the Missoula Valley exhibited concentrations of pharmaceuticals in the low ng/L range (Figure 10).

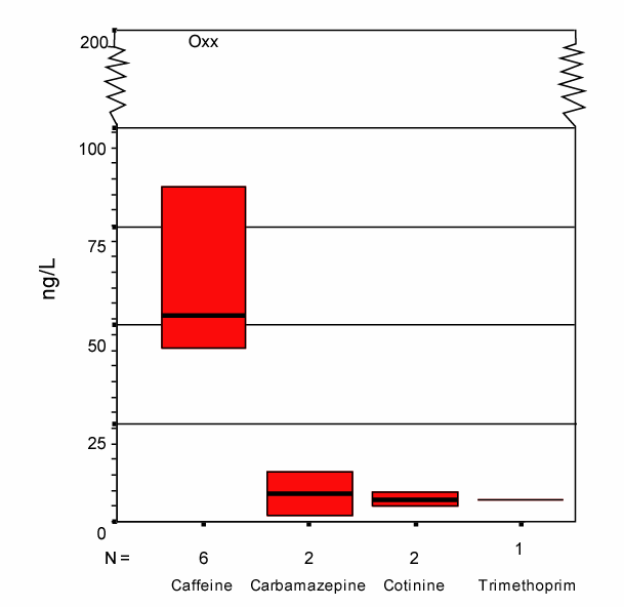


Figure 10. Ground water box plot of pharmaceuticals in the Missoula Valley. Box plots report median, 75, 25 quantities and maximum and minimum values. Oxx represent extreme values. **All concentrations represent minimum concentrations.**

Single samples of the Clark Fork River were collected at headwaters of the Missoula Valley and 3.5 miles below the outfall of the WWTP. Only two pharmaceuticals were above detection limit below the WWTP, caffeine at 1.37-ug/L and carbamazepine at 0.003-ug/L. Results from a sample of potable ground water supplied by Mountain Water Company, did not show the presence of any target compounds.

DISCUSSION

This screening level study evaluated the occurrence of 22 pharmaceuticals in septic tanks, the city WWTP, along with a limited evaluation of their persistence in ground water and the Clark Fork River in Missoula, Montana. These data provide unique information about the range of pharmaceutical concentrations found in community and single-family septic tanks and effluent. Results from ground water sampling suggest that specific pharmaceuticals enter and persist in the subsurface.

Effluent samples

(Community, single family, school septic tanks and WWTP samples)

Non-prescription drugs

Non-prescription drugs examined in this study include acetaminophen, caffeine, nicotine, ranitidine, paraxanthine (caffeine metabolite), and cotinine (nicotine metabolite). Five of these compounds are among the most frequently detected compounds in sewage for this study (Figure 4). Acetaminophen, caffeine and paraxanthine in community and single family tanks were detected most frequently in the samples, with concentrations estimated at greater then 1530-ug/L, 877-ug/L, and 910-ug/L, respectively (Figure 5a, 6a). High concentrations detected in WWTP were estimated to be lower than septic effluent, acetaminophen at 525-ug/L, caffeine at 137-ug/L, and paraxanthine at 183-ug/L.

Concentrations in septic systems appear to be more variable (have a larger range) than samples from the WWTP. Variations in concentrations are likely the result of the septic tank effluent's susceptibility to fluctuation and/or perturbations from the people it

serves. It is likely that WWTP's have more stable concentrations and fluctuations are more subtle as it serves a diverse population and wastes are diluted.

The greater frequency of detection and higher presumed concentrations for non-prescription drugs compared to prescription drugs in both septic waste and WWTP influent, is related to their suspected greater annual use (Kolpin et al. 2002). Kolpin et al. (2002) observed similar findings when testing streams and rivers across the US. Their work reports that non-prescription drugs were detected more frequently than other organic contaminants such as antibiotics, prescription drugs and reproductive hormones. They also frequently detected concentrations of drug metabolites and noted the importance of expanding analysis to include the possible degradates of parent compounds (Kolpin et al. 2002). For example, there are more than 20 metabolites of caffeine produced in the human liver (Buerge et al. 2003).

Prescription Drugs

Prescription drugs in effluent were detected less than 30% of the time, with the exception of warfarin which was detected in 77% of the samples (Figure 7). The highest concentrations of prescription drugs found in both single-family and community tank effluent were estimated to be greater than; 6.4-ug/L for carbamazepine, 1.9-ug/L for codeine, 0.1-ug/L for hydrocodone, 104-ug/L for ketprofen, 64-ug/L for sulfamethoxazole, 1.5-ug/L for trimethoprim and 23-ug/L for warfarin (Figure 5 and 6). The apparent lower concentrations and frequency of detection for prescription drugs could be the result of their limited use and accessibility. Heberer (2001a) states that a reliable predictor of environmental concentrations of pharmaceuticals is the overall

consumption and the fate of individual compounds in the human body. The observations made in this study seem to agree with this hypothesis.

In an attempt to predict the concentrations of a pharmaceutical in single-family septic tank effluent it was assumed that 1) no degradation occurred; 2) one adult in the household is consuming maximum dosage of each drug; 3) no drugs are being excreted in feces; 4) all drugs are being released at maximum urinary excretion levels as listed in Table 2, and 5) no drugs are reacting or degrading in the septic tank. A comparison of predicted septic tank concentrations to the median pharmaceutical concentrations found in the single-family septic effluent proved to be variable (Table 5).

Table 5. Predicted and examined pharmaceutical concentrations. These represent single-family septic tanks and assume one healthy adult is consuming each pharmaceutical. **Median concentrations represent minimum concentrations.**

Compound	Median concentration of compound in single family septic tank* (ng/L)	Estimated concentration of compound in septic tank* (ng/L)
Acetaminophen	206081	69230
Caffeine	79870	1292
Carbamazepine	80	1538
Cimetidine	8667	18923
Ketoprofen	104211	769
Nicotine	8710	389
Ranitidine	517	259615
Sulfamethoxazole	64767	49231
Trimethoprim	132	52923
Warfarin	6419	77
*=Assumptions	* = no degradation of compound, one adult is consuming drug,	* = no drugs are being excreted in solid phase, 2600L septic tank dilution

Predicted concentrations varied considerably from the recorded concentrations in single-family septic effluent. The assumption that one adult is consuming the target compound seems to be insufficient because concentrations found in the effluent are

considerably higher than predicted. For example, acetaminophen, caffeine, ketoprofen, sulfamethoxazole and warfarin are recorded to greater than 206,080; 79,870; 64,767 and 6,419-ng/L in the septic tank, and predicted values are 6,923, 1,292, 769, 49,231 and 77-ng/L (Table 5). This could be the result of either more than one person consuming the drug, retention times of aqueous septic effluent being longer than 24 hours, or direct disposal of drugs into a septic tank.

Predicted concentrations of trimethoprim, ranitidine and carbamazepine are considerably higher than median septic effluent concentrations. This could be due to either the adult dosage being lower than the maximum concentration used in the calculation, or that some removal process (be it degradation or sorption) occurs in the septic tank (Table 5).

To compare the amount of prescription drugs entering the Missoula Valley to the compounds reported in this study, a pharmacy in the Missoula Valley that serves approximately 7% of the population estimated dosages prescribed during a 4-week period (Figure 11).

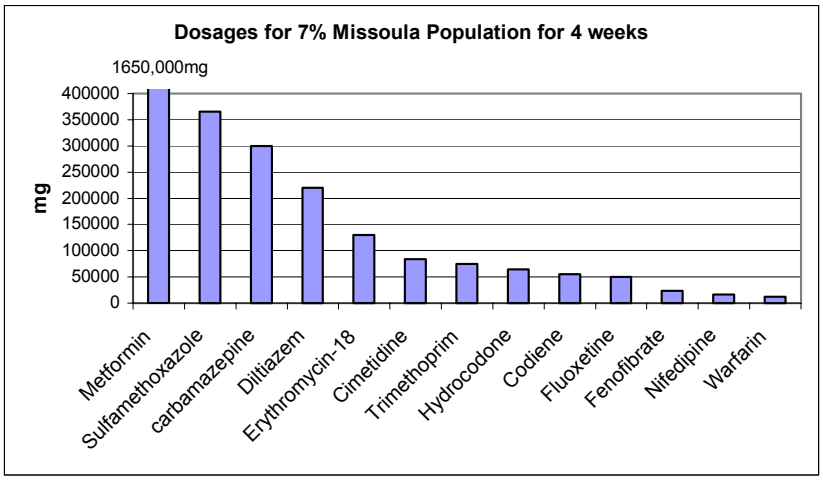


Figure 11. Prescription drug dosages for Missoula Valley

Of the compounds analyzed for in this study, metformin, sulfamethoxazole and carbamazepine are the most prescribed drugs in the Missoula Valley. It was unanticipated that carbamazepine would be one of the top 3 prescribed drugs based on its reported use in medicine (Table 11).

Wastewater treatment plant

The effluent samples at the WWTP were taken synoptically. However, pharmaceutical concentrations entering the plant were generally higher than levels leaving the plant (Figure 5). Ultraviolet treatment did not seem to significantly alter the apparent pharmaceutical concentrations (Figure 5). Acetaminophen, diltiazem, nicotine, paraxanthine and warfarin were below detection limits in WWTP outflow samples. This could be the result of degradation processes by microorganisms, elimination by the wastewater treatment process or the stated recovery issues. Ternes (1998) noted the lack of acetaminophen in surface water due to high removal efficiencies by WWTP's. Buerge et al. (2003) and Heberer et al. (2002) reported ~99.3% and 99.9% removal rates of WWTP for caffeine, respectively.

Missoula's WWTP discharges water into the Clark Fork River. A single river water sample was taken 3.5-miles below the WWTP, contained only two pharmaceuticals: caffeine greater than 1.36-ug/L, and carbamazepine greater than 2.7-ng/L.

Hypothetical pharmaceutical concentrations down stream from the WWTP were determined using: 1) concentrations of compounds detected in the WWTP effluent; 2) discharge of the Clark Fork River in September (2003) at 1,374-million liter/day; and 3) discharge of the WWTP into the Clark Fork River, 28-million liter/day (Table 6). This

prediction assumes that neither degradation nor retardation occurs and that no upstream sources of target compounds are influencing concentrations in the river. Only two of the nine pharmaceuticals detected in the WWTP effluent were detected downstream of the WWTP. This could be the result of degradation and/or retardation, depending on the compound. An apparent high concentration of caffeine detected in river water, compared to WWTP effluent, could be from upstream sources of human activities, septic effluent influence from unsewered homes near the river or the result of analytical uncertainty.

Table 6. Predicted and actual downstream from wastewater treatment plant concentrations. **Actual concentrations represent minimum concentrations.**

Compound	Outflow (ng/L)	Predicted Concentrations (ng/L)	Found 3.5-miles downstream (ng/L)
Caffeine	615	7.0	1370
Carbamazepine	498	5.7	2.7
Cimetidine	1027	11.7	Nd
Cotinine	228	2.6	Nd
Erythromycin-18	1269	14.4	Nd
Codeine	458	5.2	Nd
Metaformin	2049	23.3	Nd
Sulfamethoxazole	297	3.4	Nd
Trimethoprim	115	1.3	Nd
nd=not detected			

Ground Water

Sand and Gravel Waste Impacted Shallow Aquifer

To examine how pharmaceuticals behave in the subsurface several ground water wells were sampled below and near the Frenchtown High school drain field. Sampling a septic tank provides "snapshots" of concentrations moving through a septic system at a specific time. For example, if someone is prescribed antibiotics for five days, while the

drug is being consumed it will be present in the septic effluent at high concentrations. In an ideal wastewater system once consumption of the antibiotics and elimination from the body ceased, septic effluent pharmaceutical concentrations should be undetectable. Applying this reasoning to ground water contaminated by septic effluent, pulses of antibiotics may travel through an aquifer.

The shallow aquifer below the Frenchtown High School drain field is impacted from septic waste as evidenced by the elevated concentrations of nitrate, chloride and ammonium (Lauerman 1999; Fink 2000). Only 12 of the 22 compounds were detected in the school's septic tank effluent, while only 4 of those 12 were detected in the ground water (Figure 9). Both carbamazepine and sulfamethoxazole have the highest recorded concentrations of the pharmaceuticals detected in the ground water at the high school. Underneath the drain field the 2-3-m thick vadose zone is eliminating approximately 65-75% of the pharmaceuticals detected in the school septic tank effluent. Concentrations of prescription drugs, carbamazepine and sulfamethoxazole may show some reduction after traveling through the vadose zone. This may be the result of dilution, degradation, or the beginning or end of a "pulse" of drugs moving through the septic system. Removal or retardation of sulfamethoxazole in the vadose zone appears to be greater than carbamazepine. Concentrations of sulfamethoxazole fell from greater than 29600-ng/L in the effluent to greater than 460-ng/L in the underlying ground water, a reduction of 98%. Carbamazepine appears to be relatively persistent in this specific subsurface environment of anoxic ground water (DO < 0.1-3.0-mg/L), as it is found in ground water 15.3-m down gradient of the drain field (DeBorde 1998).

Carbamazepine and sulfur containing drugs are reported by previous studies to be more persistent in the environment. Other studies have reported the persistence of carbamazepine through WWTP (Ternes 1998). Clara et al. (2004) examined both lab-scale and the full-scale effect of sewage treatment plants on carbamazepine, and reported no significant degradation or adsorption of carbamazepine during the wastewater treatment processes. Heberer (2002b) reported 8% removal rate of carbamazepine from the Berlin wastewater treatment plants. Verstraeten et al. (2004) suggests anaerobic conditions could either aid in the persistence of or slow down degradation of antibiotics in ground water. Drewes et al. (2003) reported that carbamazepine persisted through anoxic and aerobic conditions during travel times of up to eight years. Scheytt (2004), along with other literature, states that sulfur-containing drugs, such as sulfamethoxazole and salphaxalazine, are relatively persistent in the environment, (Halling-Sorensen et al. 1998; Huang et al. 2000; Hartig et al. 1999; Hartig and Jekel 2000; Lindsey et al. 2001). The presence of carbamazepine and sulfamethoxazole in the Frenchtown High School ground water may be partially attributed to their resistance to degradation in the ground water system (Figure 9). This data also correlates with the high prescription rates for both carbamazepine and sulfamethoxazole, in the Missoula area.

The low level of occurrence of non-prescription drugs acetaminophen, caffeine and other similarly structured compounds in ground water could be partially due to their adsorption onto the aquifer media or their degradation in the subsurface, especially if aerobic conditions are present (Verstraeten et al. Draft; Drewes et al. 2003). Concentrations for caffeine were reduced from a detectable range (18-ng/L) to concentrations that were below detection (BDL). Nicotine showed a similar trend. These

shallow aquifer wells illustrate the direct impact that drain field effluent has on a shallow unconfined aquifer (Figure 9).

Missoula Valley Shallow Observation Wells

Shallow ground water samples of the Missoula aquifer were taken from wells finished near the water table (Figure 1). Ground water samples from near the water table (~6-15.2-m below land surface) of the Missoula Aquifer contained five of the 22 pharmaceuticals being investigated. Of the eight ground water wells sampled, six contained low levels (ng/L) of pharmaceuticals. These included caffeine, carbamazepine, cotinine, nicotine and trimethoprim (Figure 12). This could be the consequence of impacts from septic system effluent in unsewered areas or the leakage of effluent from damaged sewer lines also found in some areas (Figure 12).

Large Production Drinking Water Well

High yield production wells extracting water from the eastern portion of the Missoula Aquifer provide potable water for a portion of the city. These wells typically extract water from the base of the aquifer. A single tap water sample obtained in downtown Missoula found no target compounds above the analytical detection limit.

Ground Water Summary

Ground water was analyzed in 3 settings for PPCP's: a shallow waste impacted aquifer, monitoring wells for the Missoula Aquifer and the community water supply system. Pharmaceuticals were only observed in the monitoring wells. The most persistent compounds in ground water were carbamazepine and sulfamethoxazole.

ANALYTICAL DIFFICULTIES

There are thousands of tons of pharmaceuticals produced and used in human and veterinary medicinal practices (Daughton and Jones-Lepp 2001). This can lead to potentially thousands of different molecules belonging to different chemical classes, structures and behaviors that could re-enter the environment. It would be unrealistic and costly to produce analytical methods for measuring all pharmaceuticals in the environment. To date no single analytical procedure has been set as an accepted method to measure quantities of pharmaceuticals in the environment (Castiglioni et al. 2004).

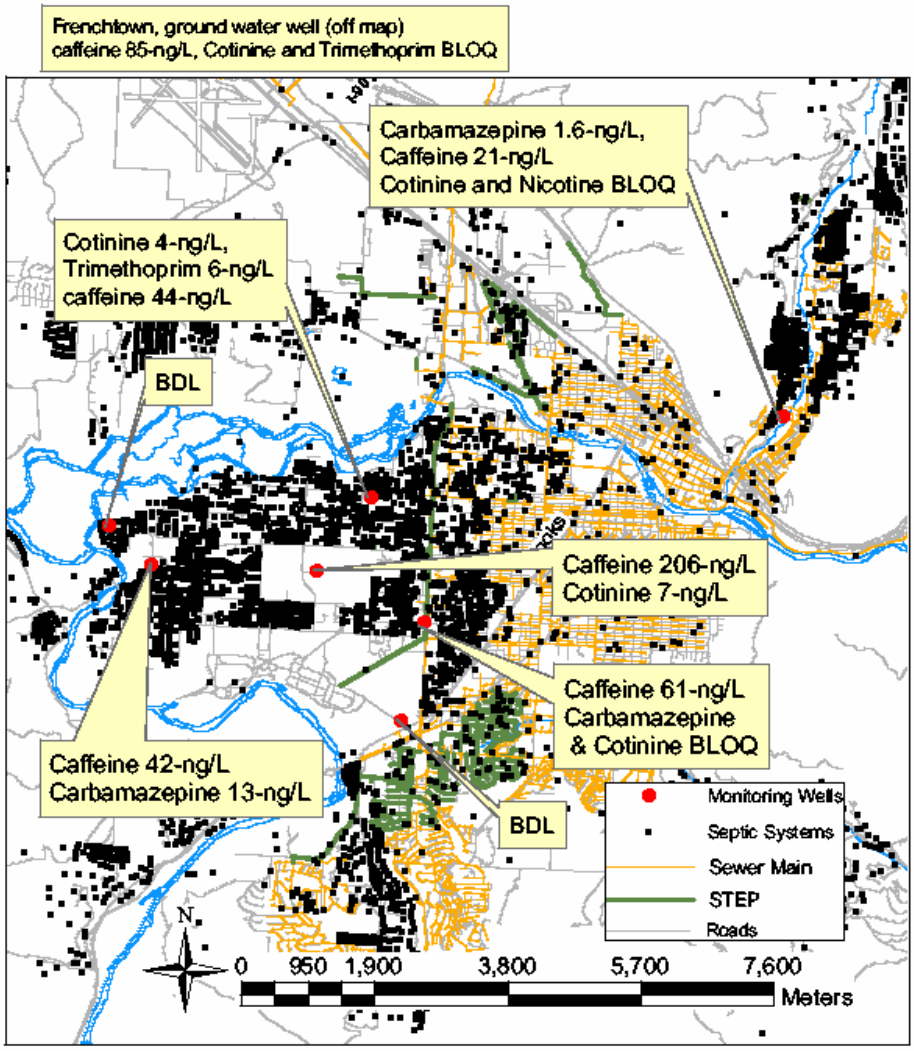


Figure 12. Mapped pharmaceutical concentrations in the Missoula Valley. BDL = below detection limit and BLOQ= below limit of quantification (Map source: Department of Water quality Missoula, Montana)

Due to the analytical difficulties mentioned earlier, this study reports a range of concentrations for all raw sewage samples. Reasons for error include: 1) over saturation of the 500-mg, 6-cc HLB sorbant by sewage effluent samples; 2) loss of target compounds during filtration 3) loss of target compounds to the glass vial; and 4) concentrations of target compound over saturating the detector, causing suppression of ions during analysis.

Recovery data for ground water samples are reported in Appendix 5. Recovery data for raw sewage effluent matrix are not reported yet a limited number of recoveries are reported by Ternes (2001). Ternes (2001) reported 70% recovery of caffeine in sewage treatment plant effluent with other pharmaceuticals ranging from 30-142% recovery. Clearly, additional effort is needed to standardize analytical techniques.

FURTHER RESEARCH

The presence of PPCP's in our waterways and ground water is a growing concern. With increased sensitivity of analytical equipment, we are able to report concentrations in the low ng/L range (Benotti et al. 2003). This low level of detection also leads to questions about cleaning glassware and sample preparation. Methods that address sample preparation for raw sewage are needed. Methodology that addresses preparation and analysis of samples with raw sewage matrix are in need. In addition, other compounds that may be important to evaluate in ground water and wastewater include: primidone, naproxen, gemfibrozol, and metoprolol (Scheytt 1998; Ternes 1998; Drewes et al. 2003; Heberer 2001; Castiglioni et al. 2004). Certainly a follow up study of Missoula's ground water that more clearly quantifies the occurrence and concentration of pharmaceuticals

and personal care products should be conducted. This screening level study should be used to design such an effort.

CONCLUSION

Based on the analysis of all sewage effluent samples, 18 of the 22 compounds studied, were detected above the detection limit. These 18 compounds include both prescription and non-prescription drugs, with prescription drugs being most frequently detected. This is most likely the result of greater annual use by the general population. Compounds most frequently detected in ground water within the waste impacted FHS aquifer were compounds such as carbamazepine and sulfamethoxazole. These compounds corresponded to pharmaceuticals prescribed in large quantities in the Missoula area as well as compounds known to be more persistent in the environment. Ground water obtained from shallow monitoring wells throughout the Missoula Valley contained low levels of pharmaceuticals. Most likely these compounds are from sewage effluent originating from residences not connected to the city sewer and/or from leaks in sewer lines. The possible short- and long-term effects of pharmaceuticals being recycled through the water environment are unknown.

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APPENDIX 1.

Pathways of Pharmaceutical into the Environment and Expanded Data for Analytical Difficulties

PPCP's can re-enter the environment through sewage disposal via excretion, incorrect disposal of old drugs and rinsing of topical drugs. Depending on the compound in this study, pharmaceuticals can be excreted in the urine with efficiencies as high as 62% to negligible amounts (Table 7). Table 7 reports the percent of pharmaceuticals that is excreted in urine from a healthy young adult.

Table 7. Urinary excretion of unchanged pharmaceuticals from the body

Compound	Urinary Excretion (%) (Goodman and Gilman 1990)	Compound	Urinary Excretion (%) (Goodman and Gilman 1990)
Acetaminophen	3 +/-1	Hydrocodone	Na
Antipyrine	Na	Ketoprofen	<1
Caffeine	1.1 +/- 0.5	Metformin	Na
Carbamazepine	<1	Nicotine	16.7 +/- 8.6
Cimetidine	62 +/- 20	Nifedipine	~0
Codeine	negligible	Paraxanthine	Na
Cotinine	Na	Ranitidine	69 +/- 6
Diltiazem	<4	Salbutamol	Na
Diphenhydramine	1.9 +/- 0.8	Sulfamethoxazole	14 +/-2
Erythromycin	12 +/- 7	Trimethoprim	69 +/- 17
Fenofibrate	Na	Warfarin	<2
Fluoxetine	<2.5		
Na = No data			

Once these pharmaceuticals leave the house they enter the municipal or septic tank system. These molecules can then be cleaved during sewage treatment causing the original pharmaceutical to be released into the environment (Heberer 2002b). Pharmaceuticals take several pathways to reach groundwater and surface water sources (Figure 1).

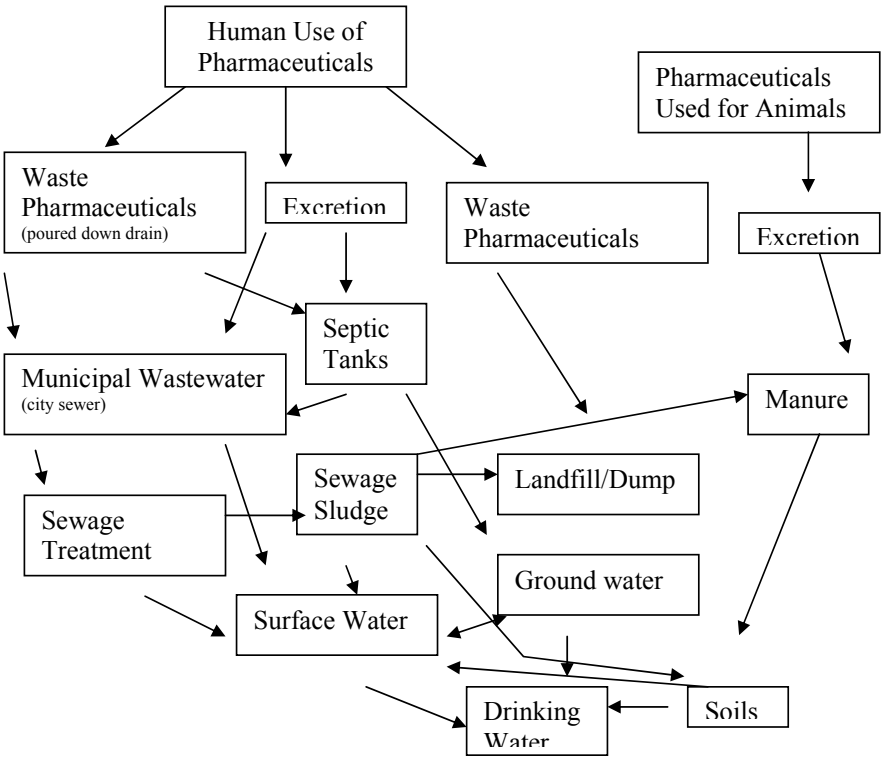


Figure 13. Pathways of pharmaceuticals into the environment (adapted from Heberer 2002b)

Another possible pathway for pharmaceuticals to enter the environment is through medicinal products for animal use, which are excreted and used as fertilizer for soil and can leach into groundwater or rivers and streams via run-off.

Importance of Water Resources

In the United States, ground water alone is used in ¾ th of all American cities and 90% of all rural households as the sole source of drinking water. (Nizeyimana et al. 1996) According to Verstraeten et al. (2004), 25-30% of households use septic systems for wastewater disposal. In Montana, 38% of households depend on septic and cesspool

systems for wastewater disposal (U.S. Census 2000). Within a given year it is estimated that ~10% of septic tanks in the United States are malfunctioning, which equals to more than 7000 faulty tanks per day (Knowles 1998). Leaky or malfunctioning septic tanks have been known to cause disease outbreaks from groundwater contamination (Scandura and Sobsey 1997). Past studies have focused mainly on bacteria, nitrogen and phosphorous as the major pollutants from leaky septic tanks or sewage disposal, but another suite of bioactive chemicals such as pharmaceuticals and personal care products (PPCP) are receiving attention, from both human and veterinary practices.

Sample Preparation

It is also important to note that in certain samples, during preparation, after elution from the HLB cartridges and during evaporation under N_2 gas, a few samples solidified and turned a dark brown color. This dark brown solid would stick to the sides of the glass vial or float in solution. Adding a mobile phase to the near dry sample, re-dissolved a portion of solid phase, but in some samples the solid phase remained on the glass vial. To re-dissolve all of the solid phase from the vial, 1mL of mobile phase was used to re-dissolve the solidified sample (Table 8).

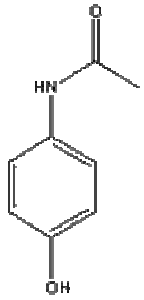
Table 8. Results of re-dissolving solidified samples. Sample C is first sample prepared using reported sample preparation; C 2 is the re-dissolved solid phase sample. **All concentrations represent minimum concentrations.**

	Acetaminophen	**Caffeine**	**Codeine**	**Hydrocodone**	Ketoprofen	**Paraxathine**
Sample	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
C	1197	1008	0.33	0.13	13	765
C 2	85	345	0.03	0.04	4	34

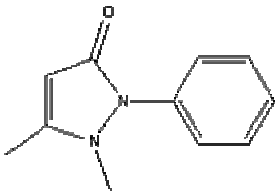
The recoveries from the solid phase of caffeine, ketoprofen and hydrocodone were 34, 35 and 28%, respectively. The other pharmaceuticals, acetaminophen, codeine and paraxathine, were below 10% recovery.

APPENDIX 2.

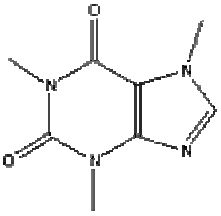
Structures and molecular weights of pharmaceuticals



Acetaminophen $C_8H_9NO_2$ 151.0633 Positive Calculation using Monoisotopic weight 152.0711 –uses atomic weight of most abundant isotope of each element

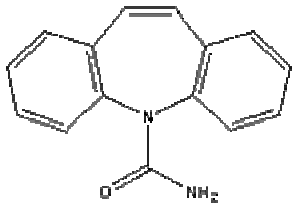


Antipyrine $C_{11}H_{12}N_2O$ 188.0950
Pos.189.1028

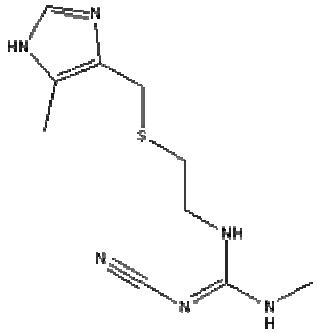


Caffeine $C_8H_{10}N_4O_2$ 194.0804

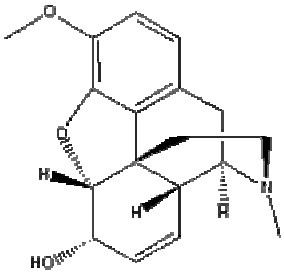
Pos 195.0882



Carbamazapine $C_{15}H_{12}N_2O$ 236.0950
Pos 237.1028

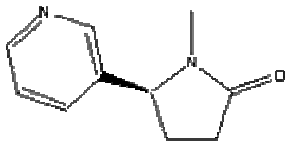


Cimetidine $C_{10}H_{16}N_6S$ 252.1157
Pos 253.1235

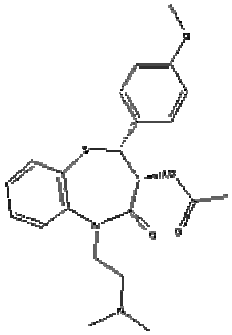


Codiene $C_{18}H_{21}NO_3$ 299.3688

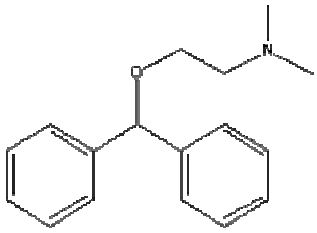
Pos 300.1599



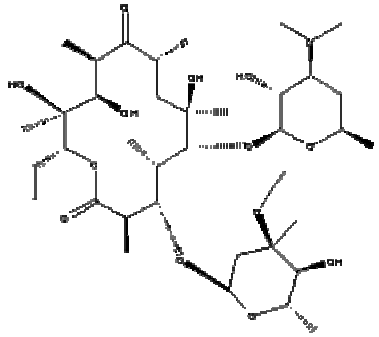
Cotinine $C_{10}H_{12}N_2O$ 176.0950
Pos 177.1028



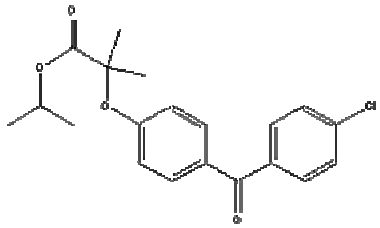
Diltiazem $C_{22}H_{26}N_2O_4S$ 414.1613
Pos 415.1691



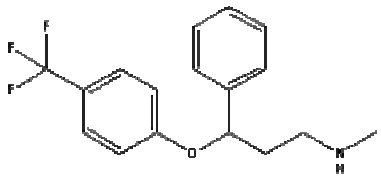
Diphenhydramine $C_{17}H_{21}NO$ 255.1623
Pos 256.1701



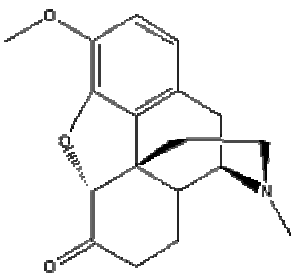
Erythromycin $C_{37}H_{67}NO_{13}$ 733.4612
Pos. 734.4690



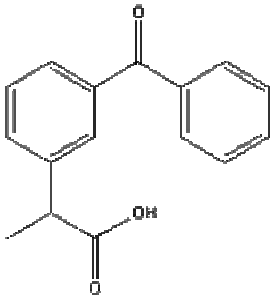
Fenofibrate $C_{20}H_{21}O_4Cl$ 360.1128
Pos. 361.1206



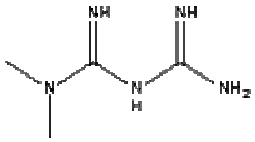
Fluoxetine $C_{17}H_{18}NOF_3$ 309.1340
Pos. 310.1418



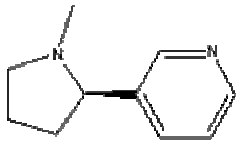
Hydrocodone C18H21NO3 299.3688
Pos 300.1599



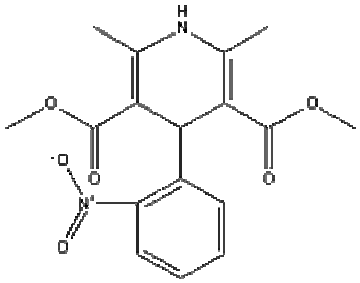
Ketoprofen $C_{16}H_{14}O_3$ 254.0943
Pos. 255.1021



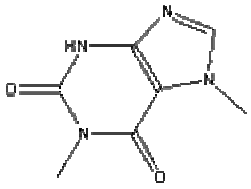
Metformin $C_4H_{11}N_5$ 129.1014
Pos. 130.1092



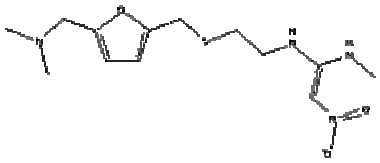
Nicotine C10H14N2 162.1157
Pos. 163.1235



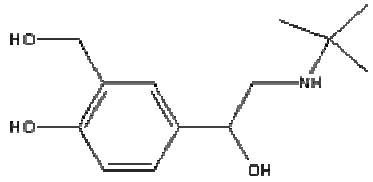
Nifedipine $C_{17}H_{18}N_2O_6$ 346.1165
Pos 347.1243



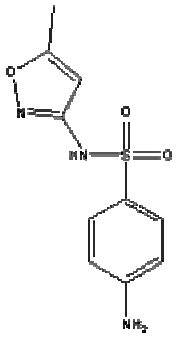
Paraxanthine $C_7H_8N_4O_2$ 180.0647
Pos. 181.0725



Ranitidine $C_{13}H_{22}N_4O_3S$ 314.1413
Pos. 315.1491

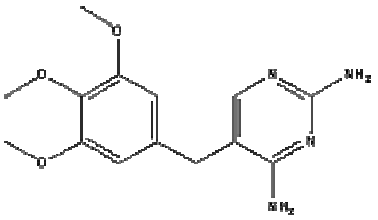


Salbutamol $C_{13}H_{21}NO_3$ 239.1521
Pos 240.1599

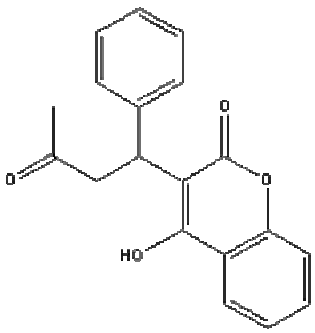


Sulfamethoxazole $C_{10}H_{11}N_3O_3S$ 253.0521

Pos 254.0599



Trimethoprim $C_{14}H_{18}N_4O_3$ 290.1379
Pos 291.1457



Warfarin $C_{19}H_{16}O_4$ 308.1049
Pos 309.1127

APPENDIX 3.

Pharmaceutical Analysis

Filter Process

1. Filter 1 L of sample through 0.45-um PTFE glass fiber filter.
    a. this may require up to 15 different filters depending on the suspended solid concentration of sample

2. Using SPE cartridge filter 1L through HLB cartridge (I ordered a 6cc/500mg cartridge)
    a. Suction filtration apparatus through HLB cartridge (15-20mL/min)
    b. Catch container for discard of liquid

3. Elute twice
    a. 6 mL methanol
        i. 3 mL pipette pushing's
        ii. Let methanol gravity fall through HLB
    b. 6 mL acidified methanol 0.1% TFA in MeOH (TFA=trifluoroacetic acid)
        i. 3mL pipette pushing's
        ii. Let methanol gravity fall through HLB
4. Reduce compounds to near dryness under N_2 gas (immerse the samples in a warm water bath ~30 degrees) For Reference use 100uL of MeOH in a separate test tube and stop the samples when they look like the 100uL test tube
    a. This can take up to 6 hours depending on N2 stream, want to take hours to avoid volatilization of compounds

5. Fill test tube to 1mL of a final volume with mobile phase (50%actonitrile and 50% formic acid adjusted to pH of 3.7)
    a. 890uL of mobile phase

6. Filter with syringe filter: for HPLC: 0.2um PTFE syringe filter

7. Dilutions of raw sewage samples were run at 10% of concentrated sample.
    a. Raw Sewage preparation: 20uL of concentrated sample + 180uL of mobile phase + 4uL of internal standard at 5ug/mL (13-C Caffeine)
    b. Ground water and surface water samples: 200uL of sample + 4uL of internal standard at 5ug/mL

HPLC- ToF MS

Sample Prep and Calibration

Three concentrations of samples
100% = 200ul sample + 4ul 5ug/mL solution 13C
50% = 100ul sample + 100ul mobil + 4 ul 13C
10% = 20ul sample + 180ul mobile + 4 ul 13C

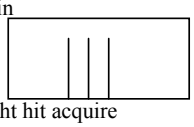
To determine the best resolution

Calibration (calibrate everyday)

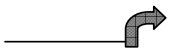
To start Machine

(screw in line to detector)
Cap. (V) = 2600
Sample cone = 30
Extract cone = 5
Desolve T = 350
Source T = 150
HIT GAS BUTTON!!!!!
Put in Concentration of each compound (all that you know)

Before- polyalah pos match the weights 10ul/min
Small amount of polyalah diluted with 50*50 solution
Don't go over 200-300 counts
Play with desolvation gas and sample cone to get stable sample
Manually~ 200L/hr
Play with Cap (V) on screen until TIC is below ~300counts
Zoom in the middle of spectrum
→Options
Acquisition setup
Lteff Trial = error to get peak near know weight
→Then Aquire
file name
mass 100-800
Calibration (no select)
Run for 1 min



Once all at same height hit acquire
Go to chromatogram then right click, and drag over chromatogram
Go to spectrum (to calculate resolution) zoom in on 556peak (Poly al)
Choose around Lock mass ~ peak m/z / 50% of peak
In Chromatogram ->Centroid data
Process → center
Resolution # calculate (ie. 6900)



Compare to liturature values
Tools → make calibration → find ref of polyal (which should be created)
Select tools -> Make calibration file

When injecting Leu before running standards
Make sure ~30hits +- 10
1ml syringe 3ul/min 5ug/mL leu

Once samples are run- APPLY CALIBRATION FILE!

<u>To Finish Run for day</u>
Faucet button-turn off
Syringe off
API gass off
Temp to 100
Move files
- AFAMM files- All file accurate mass measure

Quantify off Afamm files (all file accurate mass measure)

Accurate mass on every mass spectral scan under the entire chromatogram

USING QUANTIFICATION PROGRAM

Edit -> quantify
->Method editor (For 13-C Caff)

1. Quantify trace [---] sec (click on --- then chromatogram where peak is 198.)
2. General Parameters
    a. External relative
    b. Polytype-→ avg. RF (click on)
    c. Point of origin (not force)
    d. Uncheck propagate general parameters

Method editor (for all compounds standards)

1. internal ref (13 C caffeine)
2. General parameters
    a. Response type (internal relative)
    b. Poly type (linear)
    c. Point of origin (include)
3. Append (not modify)
4. Conc of standards
    a. Conc A = C13
    b. Conc B = Ace
    c. Conc C = Caff

Save File under MethDB

Make sure you have a column which lists sample type (eg. Analyte and standard)

GO to Quantify in chromatogram page
Select
1. Integrate
2. Calibrate
3. Quantify

Rename curve file (where u place curve file)

Calculate Accurate Mass

Combine Spectrum under saturation for peak
Mass Measure
TOF
Np multiplier- 0.8 or 0.85

Subtract
=actual compound weight – found compound mass
=0.0022 Da
(=195.0882-195.0904)
(= -0.0022Da or 2.2 mDa (ie 4.5e-)
Tools
-elemental compound → click on peak

APPENDIX 4.

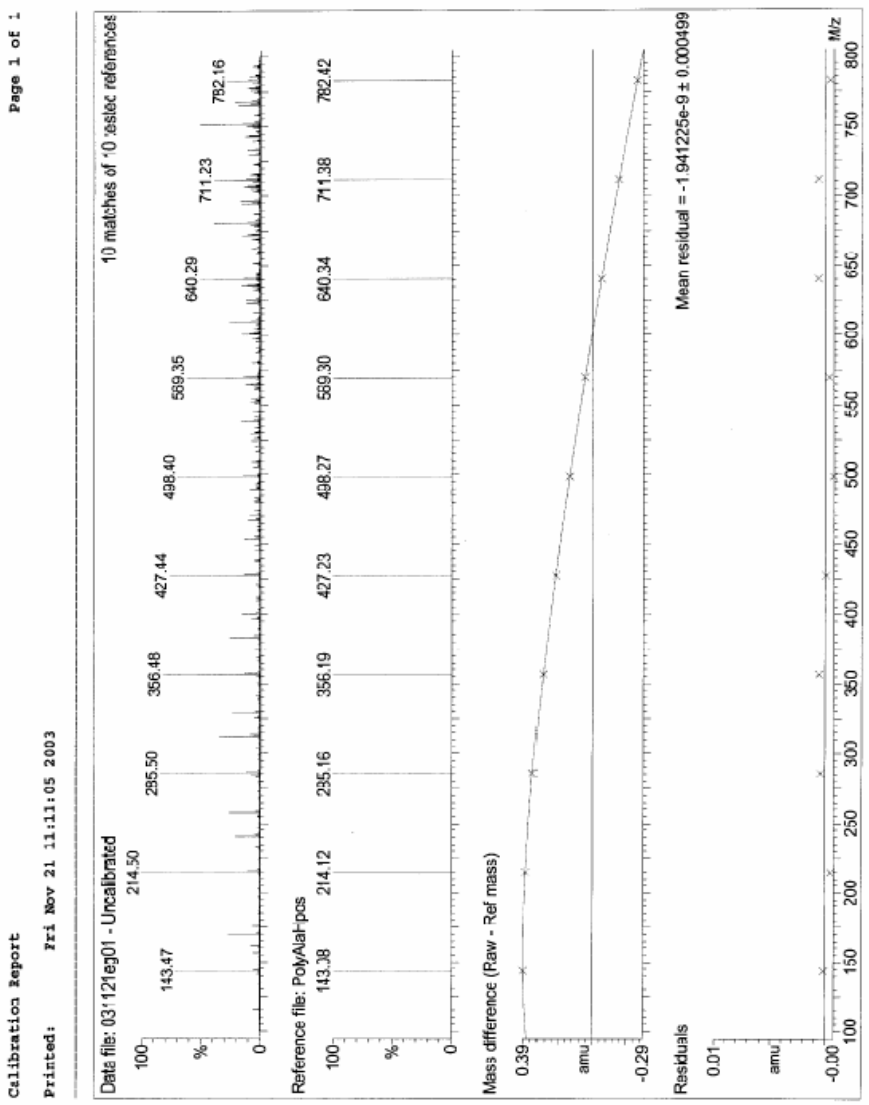
	Instrument detection limits in ng/L		* = <60% recovery		% recovery (n=8)	standard deviation (n=8)	**relative standard deviation**
acetaminophen	11.34			acetaminophen	110.08	6.44	**5.85**
antipyrine	0.27		*	antipyrine	5.51	1.55	**28.16**
caffeine	4.26			caffeine	100.68	6.04	**6.00**
carbamezipine	0.47			carbamezipine	71.49	9.56	**13.38**
cimetidine	1.91			cimetidine	93.22	15.74	**16.88**
cotinine	2.71			codeine	103.31	11.83	**11.45**
diltiazem	0.78			cotinine	105.82	8.77	**8.28**
erythromycin - 18	2.18			diltiazem	65.63	9.32	**14.20**
fenofibrate	1.59		*	diphenhydramine	54.53	11.76	**21.56**
fluoxetine	3.87			erythromycin	3.56	2.30	**64.54**
ketoprofen	19.06			erythromycin - 18	64.18	19.39	**30.21**
metformin	4.38		*	fenofibrate	3.26	1.76	**53.96**
nifedipine	5.05		*	fluoxetine	56.39	13.50	**23.94**
paraxanthine	21.16		*	hydrocodone	8.52	3.11	**36.47**
ranitidine	1.11			ketoprofen	83.91	15.80	**18.82**
salbutamol	9.60		*	metformin	59.60	9.84	**16.52**
sulfamethoxazole	2.53		*	nifedipine	39.89	12.75	**31.96**
trimethoprim	0.13			paraxanthine	102.97	6.31	**6.13**
warfarin	0.77			ranitidine	66.81	10.41	**15.59**
nicotine	4.49			nicotine	120.03	15.54	**12.94**
				salbutamol	108.86	10.24	**9.41**
			*	sulfamethoxazole	37.73	3.55	**9.42**
			*	trimethoprim	12.36	3.67	**29.69**
			*	warfarin	48.71	16.43	**33.73**

Compound	Type	Use	Maximum Recommended Dose for adult (mg/day)	Maximum Urinary Excretion (%) (Goodman et al. 1990)
Acetaminophen	non prescription drug	Antipyretic	600	3 +/-1
Antipyrine (Phenazone)	Prescription	Analgesic	54	Na
Caffeine	Non-prescription drug	Stimulant	210-440	1.1 +/- 0.5
carbamazepine	Prescription drug	Anticonvulsant, antineuralgic, antimanic, antidiuretic, antipsychotic	100-400	<1
Cimetidine	Prescription drug	Antiasthmatic	300-800, 2-4 times daily	62 +/- 20
Codiene	Prescription drug	Analgesic (anti-cough)	12-60, 1-4 times daily	Negligible
Cotinine	metabolite	Nicotine metabolite		Na
Diltiazem	Prescription drug	Antihypertensive	30-120	<4
Erythromycin-18	Metabolite of Prescription drug	Antibiotic	250	12 +/- 7
Fenofibrate	Prescription	Lipid Matabolism regulator	54-200 daily	Na
Fluoxetine	Prescription drug	antidepressant, antiobsessional, and antibulimic	10-40 daily or weekly	<2.5
Hydrocodone	Prescription drug	analgesic (anti-cough) and antitussive	5-7.5	Na
Ketoprofen	Prescription	Anti-inflammatory	25-200	<1
Metformin	Prescription drug	antihyperglycemic	500-1000, twice a day	Na
Nicotine	non prescription drug		4	16.7 +/- 8.6
Nifedipine	Prescription drug	antianginal (blood pressure control)	10-90, daily	~0

Paraxanthine (1,7-dimethylanthine)	Metabolite	Caffeine metabolite		Na
Ranitidine	Non-Prescription drug	Histimine	25-300, three a day	69 +/- 6
Salbutamol	Prescription drug	relax restricted airways	5-Feb	Na
Sulfamethoxazole	Prescription drug	Antibiotic	200-800	14 +/-2
Trimethoprim	Prescription Drug	Antibiotic	40-160	69 +/- 17
Warfarin	Prescription drug	anticoagulant	10-Jan	<2

APPENDIX 5

Calibration of ToF-HPLC-MS during sample analysis



APPENDIX 6.

Standard curves for standards run on 02/02/02 after samples which were run on 11/20/03

Compound 1: 13C-caffeine Sample List: 20040202a Method File: ppcp_1_caf
Response Factor: 1.04740
RRF SD: 0.125148, % Relative SD: 11.9485
Response type: External Std, Area
Curve type: RF

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	100	9.666	94.088	94.088	89.83
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	100	9.703	99.132	99.132	94.65
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	100	9.684	95.396	95.396	91.08
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	100	9.666	104.956	104.956	100.21
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	100	9.703	106.746	106.746	101.91
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	100	9.666	128.124	128.124	122.33

Compound 6: acetaminophen Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.999417
Calibration curve: 0.398109 * x + 1.04441
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	7.428	1.275	1.355	0.78
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	7.538	4	4.035	7.51
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	7.391	6.153	6.45	13.58
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	7.428	20.163	19.211	45.63
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	7.52	68.602	64.267	158.81
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	7.391	255.26	199.229	497.81

Compound 7: antipyrine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.997968
Calibration curve: 33.2392 * x + 141.160
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5				
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	11.922	404.761	408.305	8.04
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	11.96	673.169	705.657	16.98
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	11.923	1867.694	1779.502	49.29
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	11.922	5470.678	5124.949	149.94
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	11.941	9741.431	7603.127	224.49

Compound 8: caffeine Sample List: 20040202a Method File: ppcp_1_caf

Coefficient of Determination: 0.996364
Calibration curve: 1.39359 * x + 5.26508
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	9.666	8.118	8.628	2.41
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	9.703	18.307	18.467	9.47
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	9.684	27.459	28.784	16.88
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	9.666	71.771	68.382	45.29
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	9.703	230.581	216.009	151.22
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	9.666	932.311	727.663	518.37

Compound 9: carbamazapine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.994068
Calibration curve: 28.6802 * x + 209.797
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	17.077	183.424	194.949	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	17.022	467.785	471.881	9.14
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	17.078	745.749	781.74	19.94
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	17.077	1764.309	1680.999	51.3
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	17.022	4784.229	4481.881	148.96
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	17.059	7897.733	6164.132	207.61

Compound 10: cimetidine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.995573
Calibration curve: 3.27345 * x + 20.7305
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	7.63	18.322	19.473	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	7.722	48.988	49.417	8.76
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	7.612	77.685	81.434	18.54
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	7.63	200.604	191.132	52.06
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	7.703	542.265	507.996	148.85
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	7.63	1435.299	1120.242	335.89

Compound 11: cotinine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.998948
Calibration curve: 8.88401 * x + 27.6540
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	5.851	32.418	34.455	0.77
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	5.832	90.286	91.077	7.14
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	5.832	151.856	159.185	14.81
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	5.832	429.579	409.294	42.96
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	5.851	1563.841	1465.011	161.79
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	5.851	5694.307	4444.372	497.15

Compound 12: diltiazem Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.993240
Calibration curve: 38.8243 * x + 296.030
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	16.655	256.773	272.907	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	16.619	612.121	617.481	8.28
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	16.674	965.565	1012.165	18.45
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	16.692	2557.658	2436.886	55.14
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	16.637	6443.525	6036.315	147.85
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	16.674	10146.1	7918.972	196.34

Compound 13: erythromycin-18 Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.988260
Calibration curve: 2.00115 * x + 34.5099
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	17.132	14.851	15.784	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	17.206	35.222	35.53	0.51
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	17.132	56.133	58.842	12.16
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	17.169	155.663	148.313	56.87
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	17.224	446.029	417.841	191.56
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	17.242	1292.884	1009.088	487.01

Compound 14: fenofibrate Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.991857
Calibration curve: 7.44159 * x + 112.478
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	29.313	35.188	37.399	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	29.35	107.759	108.703	0

5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	29.277	171.117	179.375	8.99
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	29.331	751.419	715.937	81.09
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	29.368	1424.723	1334.685	164.24
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	29.35	4843.545	3780.357	492.89

Compound 15: fluoxetine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.988688
Calibration curve: 2.30158 * x + 22.7278
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	18.747	18.138	19.278	0
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	18.728	44.151	44.538	9.48
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	18.783	65.432	68.59	19.93
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	18.802	159.433	151.905	56.13
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	18.747	386.269	361.858	147.35
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	18.802	838.408	654.372	274.44

Compound 16: ketoprofen Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.995968
Calibration curve: 0.409053 * x + 0.471100
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	19.939	1.112	1.182	1.74
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	19.847	4.069	4.105	8.88
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	19.921	7.023	7.362	16.85
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	19.939	19.064	18.164	43.25
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	19.847	66.844	62.62	151.93
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	19.921	292.119	227.997	556.23

Compound 17: metformin Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.998085
Calibration curve: 1.24376 * x + 1.60357
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	1.925	4.455	4.735	2.52
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	1.943	10.539	10.631	7.26
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	1.925	20.892	21.9	16.32
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	1.943	60.906	58.03	45.37
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	1.943	202.623	189.818	151.33
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	1.943	360.462	281.338	224.91

Compound 18: nicotine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.974948
Calibration curve: 1.35489 * x + -6.53392
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	3.337	0.63	0.67	5.32
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	3.337	3.506	3.537	7.43
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	3.282	15.487	16.234	16.8
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	3.301	37.417	35.65	31.13
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	3.337	218.63	204.813	155.99
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	3.264	1100.906	859.25	639.01

Compound 19: nifedipine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.989167
Calibration curve: 1.20130 * x + 11.0205
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	20.544	8.394	8.921	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	20.471	20.146	20.322	7.74
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	20.544	34.835	36.516	21.22
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	20.563	82.431	78.539	56.2
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	20.452	200.59	187.913	147.25
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	20.545	436.994	341.071	274.74

Compound 20: paraxanthine Sample List: 20040202a Method File: ppcp_1_caf
Coefficient of Determination: 0.992360
Calibration curve: 0.122040 * x + 1.32657
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	8.089	0.349	0.371	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	8.162	1.607	1.621	2.41
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	8.089	2.932	3.074	14.31
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	8.089	7.638	7.277	48.76
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	8.144	25.519	23.906	185.02
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	8.07	78.269	61.088	489.69

Compound 6: ranitidine Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.997248
Calibration curve: 12.0738 * x + 54.1978

Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	8.107	53.803	57.396	0.26
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	8.18	167.097	168.949	9.5
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	8.107	249.623	260.842	17.12
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	8.125	680.473	648.002	49.18
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	8.162	1985.344	1864.348	149.92
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	8.125	4944.368	3854.356	314.74

Compound 7: salbutamol Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.997604
Calibration curve: 0.734530 * x + 1.87635
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	7.08	2.944	3.141	1.72
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	7.19	8.627	8.723	9.32
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	7.08	12.861	13.439	15.74
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	7.098	37.635	35.839	46.24
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	7.171	120.137	112.815	151.03
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	7.098	326.807	254.761	344.28

Compound 8: sulfamethoxazole Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.986111
Calibration curve: 2.34738 * x + 55.8413
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	14.564	27.511	29.348	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	14.527	62.063	62.751	2.94
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	14.693	91.566	95.681	16.97
6	040130mb07afamm	50 ng/mL std. aol'n	Standard	50	14.546	199.76	190.228	57.25
7	040130mb08afamm	150 ng/mL std. sol'n	Standard	150	14.509	542.038	509.004	193.05
8	040130mb09afamm	500 ng/mL std. sol'n	Standard	500	14.509	1536.17	1197.513	486.36

Compound 9: trimethoprim Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.989710
Calibration curve: 23.1741 * x + 227.928
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

#	Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3	040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	10.803	195.744	208.816	0
4	040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	10.822	439.798	444.672	9.35
5	040130mb06afamm	15 ng/mL std. sol'n	Standard	15	10.859	663.629	693.454	20.09

6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	10.822	1578.395	1503.076	55.02
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	10.822	3887.545	3650.62	147.69
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	10.822	6245.866	4868.932	200.27

Compound 10: warfarin Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.979874
Calibration curve: 10.3899 * x + 121.351
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	21.48	85.54	91.252	0
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	21.388	203.37	205.624	8.11
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	21.462	325.635	340.27	21.07
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	21.48	796.72	758.701	61.34
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	21.37	1739.654	1633.631	145.55
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	21.48	4353.2	3393.514	314.94

Compound 11: codeine Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.994776
Calibration curve: 15.7700 * x + 86.1003
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	9.226	108.286	115.517	1.87
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	9.263	238.535	241.178	9.83
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	9.245	366.744	383.227	18.84
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	9.245	843.118	802.885	45.45
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	9.262	2626.948	2466.849	150.97
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	9.245	5520.744	4303.667	267.44

Compound 12: hydrocodone Sample List: 20040202b Method File: ppcp_2_caf
Coefficient of Determination: 0.997200
Calibration curve: 22.2776 * x + 87.0299
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

# Name	Sample Text	Type	Std Conc	RT	Area	Response	ng/mL
3 040130mb04afamm	1.5 ng/mL std. sol'n	Standard	1.5	10.583	106.315	113.415	1.18
4 040130mb05afamm	5.0 ng/mL std. sol'n	Standard	5	10.602	287.355	290.539	9.14
5 040130mb06afamm	15 ng/mL std. sol'n	Standard	15	10.639	455.72	476.201	17.47
6 040130mb07afamm	50 ng/mL std. aol'n	Standard	50	10.602	1190.884	1134.056	47
7 040130mb08afamm	150 ng/mL std. sol'n	Standard	150	10.602	3665.86	3442.445	150.62
8 040130mb09afamm	500 ng/mL std. sol'n	Standard	500	10.602	7312.643	5700.532	251.98

APPENDIX 7.

		WATER QUALITY DISTRICT						
		Monitoring Well Network						
		(revised 10/99)						
WQD ID	**LEGAL ID**	**PHYSICAL LOCATION**	**TOTAL DEPTH**	**SCREEN INTERVAL**	**Latitude**	**Longitude**	**x-coordinates**	**y-coordinates**
			(Feet)	(Feet)				
WQD-1	W152129A	Touchette Ln., Frenchtown	22.7	5-25	47 01 50.705	-114 16 17.906	237668.1666	320027.6417
WQD-5	W131919C	Hawthorne School	35.45	10-35	46 51 58.428	-114 03 01.094	253391.5562	300779.6998
WQD-6	W131931D	Larchmont (shallow)	50.02	32-52	46 50 16.687	-114 02 32.972	253803.8004	297610.5784
WQD-7	W132026D	Humble / Mount	25.96	5-25	46 51 21.469	-114 05 26.724	250247.4055	299820.8464
WQD-8	W131930D	C.S. Porter School	53.76	35-55	46 51 02.617	-114 02 20.539	254148.7777	299010.4061
WQD-11	W131914C	Alvina Park	24	4-24	46 52 46.774	-113 58 27.737	259253.3796	301936.1411
WQD-33	U132025D	Tower Street (DSL) (MV-40)	50.38	38-48	46 51 23.391	-114 03 34.546	252621.7552	299741.3966
WQD-36	W132026B	Spurgin/Kelly Island	28.5	8.5-28.5	46 51 38.6	-114 05 57.1	249644.6561	300386.351

WQD ID	LEGAL ID	PHYSICAL LOCATION	Results
WQD-1	W152129A	Touchette Ln., Frenchtown	Caffeine 85-ng/L, cotinine and Carbamazepine BLOQ
WQD-5	W131919C	Hawthorne School	Caffeine 44-ng/L, Cotinine 4-ng/L and trimethoprim 6-ng/L
WQD-6	W131931D	Larchmont (shallow)	BDL
WQD-7	W132026D	Humble / Mount	Caffeine 42-ng/L, carbamazepine 13-ng/L
WQD-8	W131930D	C.S. Porter School	caffeine 61-ng/L, carbamazepine and cotinine BLOQ
WQD-11	W131914C	Alvina Park	caffeine 21-ng/L, carbamazepine 1.6-ng/L, cotinine and nicotine BLOQ
WQD-33	U132025D	Tower Street (DSL) (MV-40)	caffeine 206-ng/L, cotinine 7-ng/L
WQD-36	W132026B	Spurgin/Kelly Island	BDL

APPENDIX 8.

Masslynx Name	Type of Sample	Date and sample ID	Acetaminophen	Antipyrine	Caffiene	Carbamazepine
			ng/L	ng/L	ng/L	ng/L
031117eg04afamm	Community Tank	06 17 03 1a	42261		30875	
031117eg05afamm	Community Tank	06 17 03 1b	42063		14559	
031117eg06afamm	Community Tank	06 17 03 2a	28562		19862	
031117eg07afamm	Community Tank	06 17 03 2b	23440		17382	
031117eg08afamm	Community Tank	06 17 03 3a	19478		30234	
031117eg09afamm	Community Tank	06 17 03 3b	14908		40015	
031117eg10afamm	Community Tank	06 17 03 4a	16772		27659	1448
031117eg11afamm	Community Tank	06 17 03 4b	12351		25602	808
031117eg12afamm	Community Tank	06 17 03 6a	463344		456626	
031117eg13afamm	Community Tank	06 17 03 6b	385010		344824	
031117eg14afamm	Community Tank	06 17 03 8a	970639		388941	
031117eg15afamm	Community Tank	06 17 03 8b (Spiked 5000ug/mL)	1243706		579460	28628
031117eg16afamm	Community Tank	06 17 03 9a	365530		508898	
031117eg17afamm	Community Tank	06 17 03 9b (spiked 1mL 5000ug/mL)	793214		946714	48488
031118eg10afamm	Community Tank	06 17 10a	400741		414130	
031118eg11afamm	Single Home Tank	07 01 1a	4596147		50291	
031118eg12afamm	Single Home Tank	07 01 2a	1322401		877587	
031118eg13afamm	Single Home Tank	07 01 2b (lots stuck on vial)	1196897		1007822	
031118eg14afamm	Single Home Tank	07 01 2b2 (890uL of mobile phase to redissolve)	84960		345019	
031118eg15afamm	Single Home Tank	07 01 3a	4871		18210	

031118eg16afamm	Single Home Tank	07 01 4a	1310272		508944	
031118eg17afamm	Single Home Tank	07 01 5a	55283		16305	6
031118eg18afamm	Single Home Tank	07 01 6a	2959		239614	
031118eg19afamm	Single Home Tank	07 01 7a			71302	
031118eg20afamm	Single Home Tank	07 01 8a	161708		349382	
031118eg21afamm	Single Home Tank	07 01 9a	1269618	1834	172985	
031118eg22afamm	Single Home Tank	07 01 10a	5486		17849	
031118eg23afamm	Single Home Tank	07 16 1a	21864		8559	
031118eg24afamm	Single Home Tank	07 16 2a	902979		463198	
031118eg25afamm	Single Home Tank	07 16 3a	41751		52955	
031118eg26afamm	Single Home Tank	07 16 4a	46479	7854	80970	
031119eg04afammA	Single Home Tank	07 16 5a	516654		138970	
031119eg05afamm	Single Home Tank	07 16 6a	30696		70514	
031119eg06afamm	Single Home Tank	07 16 7a	408090		78770	
031119eg08afamm	Single Home Tank	07 16 9a	15399		17750	
031119eg09afamm	Single Home Tank	07 16 10a	206081		157670	
031119eg10afamm	Single Home Tank	07 16 11a	471217		407085	
031119eg11afamm	Single Home Tank	07 16 12a	424720		410212	
031119eg12afamm	Single Home Tank	07 16 13a	1530156		87613	
031119eg13afamm	Single Home Tank	07 16 14a	41567		5855	231
031119eg14afamm	Single Home Tank	07 16 15a	738053		668556	
031119eg15afamm	Single Home Tank	09 02 1a	2692358		338233	
031119eg16afamm	Single Home Tank	09 02 2a	233221		30809	
031119eg18afamm	Single Home Tank	09 02 4a	1409801		436467	
031119eg19afamm	Single Home Tank	09 02 5a			75796	
031119eg20afamm	Inflow	09 30 1	525079		137607	486
031119eg21afamm	Outflow before ultraviolet treatment	09 30 2			719	470

031119eg22afamm	Outflow after ultraviolet treatment	09 30 3			616	498
031120eg04afamm	Single Home Tank	09 02 3a			422328	
031121eg04afamm	Single Home Tank	09 02 8b	1086		8255	
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)	639		1395	
031121eg07afamm	non-silinized Glassware wwtp	10 27 1a	261582		109136	175
031121eg08afamm	non- silinized Glassware wwtp	10 27 1b	257830		103151	205
031121eg09afamm	Silinized Glassware wwtp	10 27 2a	296188		87584	200
031121eg10afamm	silinized glassware wwtp	10 27 2b	291914		87071	215
031121eg15afamm	Frenchtown High school	10 30 1	30998		53684	454
031121eg20afamm	Frenchtown High school	11 05 1	25814		62192	262
031121eg27afamm	Single Home Tank	9 02 2b	148151		22667	
031121eg28afamm	Single Home Tank	09 02 10a	130392		54581	
031121eg29afamm	Single Home Tank	09 02 10b	140017		60844	
031121eg30afamm	Single Home Tank	09 02 10b2 (two runs through HLB)	427739		13621	
030723mb08afamm	Single Home Tank	07 01 03 3b 10%	10717		21170	
030723mb09afamm	Single Home Tank	07 01 03 3b 50%	24370		35253	
030723mb10afamm	Single Home Tank	07 01 03 3b 100%	13678		19124	
030724mb03afamm	Single Home Tank	6.17.03-5a	144993		63263	
030724mb04afamm	Single Home Tank	6.17.03-5b (spiked)	533558		278121	23511
030724mb08afamm	Single Home Tank	6.17.03-7a	230808		418338	726

030724mb09afamm	Single Home Tank	6.17.03-7b spiked	708103		621381	33668
031121eg04afamm	Single Home Tank	09 02 8b	1086		8255	
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)	639		1395	
		Blanks = BDL				

Masslynx Name	Type of Sample	Date and sample ID	Cimetidine	Cotinine	Diltiazem	Erythromycin-18	Codiene
			ng/L	ng/L	ng/L	ng/L	ng/L
031117eg04afamm	Community Tank	06 17 03 1a		2238			
031117eg05afamm	Community Tank	06 17 03 1b		2793			
031117eg06afamm	Community Tank	06 17 03 2a		2866		1107	
031117eg07afamm	Community Tank	06 17 03 2b		2366		1284	
031117eg08afamm	Community Tank	06 17 03 3a	713	2838	398		
031117eg09afamm	Community Tank	06 17 03 3b		3825			5
031117eg10afamm	Community Tank	06 17 03 4a		3377			
031117eg11afamm	Community Tank	06 17 03 4b		2781			
031117eg12afamm	Community Tank	06 17 03 6a		28763			1384
031117eg13afamm	Community Tank	06 17 03 6b		18973			1127
031117eg14afamm	Community Tank	06 17 03 8a		38276			
031117eg15afamm	Community Tank	06 17 03 8b (Spiked 5000ug/mL)		32204	5159		
031117eg16afamm	Community Tank	06 17 03 9a		662			
031117eg17afamm	Community Tank	06 17 03 9b (spiked 1mL 5000ug/mL)		1139			
031118eg10afamm	Community Tank	06 17 10a		3260			
031118eg11afamm	Single Home Tank	07 01 1a					
031118eg12afamm	Single Home Tank	07 01 2a					277
031118eg13afamm	Single Home Tank	07 01 2b (lots stuck on vial)					329
031118eg14afamm	Single Home Tank	07 01 2b2 (890uL of mobile phase to redissolve)					28

031118eg15afamm	Single Home Tank	07 01 3a					318
031118eg16afamm	Single Home Tank	07 01 4a		67470			1958
031118eg17afamm	Single Home Tank	07 01 5a					
031118eg18afamm	Single Home Tank	07 01 6a		256			
031118eg19afamm	Single Home Tank	07 01 7a					
031118eg20afamm	Single Home Tank	07 01 8a		4181			246
031118eg21afamm	Single Home Tank	07 01 9a		370			5
031118eg22afamm	Single Home Tank	07 01 10a		103			
031118eg23afamm	Single Home Tank	07 16 1a		2952			
031118eg24afamm	Single Home Tank	07 16 2a		80642			
031118eg25afamm	Single Home Tank	07 16 3a		151079			262
031118eg26afamm	Single Home Tank	07 16 4a		101029			291
031119eg04afamm A	Single Home Tank	07 16 5a		59170			
031119eg05afamm	Single Home Tank	07 16 6a		8915			
031119eg06afamm	Single Home Tank	07 16 7a		309821			
031119eg08afamm	Single Home Tank	07 16 9a					
031119eg09afamm	Single Home Tank	07 16 10a		6507			
031119eg10afamm	Single Home Tank	07 16 11a		4733			
031119eg11afamm	Single Home Tank	07 16 12a		2735			
031119eg12afamm	Single Home Tank	07 16 13a		62782			
031119eg13afamm	Single Home Tank	07 16 14a		1052			
031119eg14afamm	Single Home Tank	07 16 15a		78810			
031119eg15afamm	Single Home Tank	09 02 1a					
031119eg16afamm	Single Home Tank	09 02 2a					
031119eg18afamm	Single Home Tank	09 02 4a		45948			
031119eg19afamm	Single Home Tank	09 02 5a					
031119eg20afamm	Inflow	09 30 1	1733	13118	233	1073	343

031119eg21afamm	Outflow before ultraviolet treatment	09 30 2	696	198		1235	428
031119eg22afamm	Outflow after ultraviolet treatment	09 30 3	1027	228		1269	458
031120eg04afamm	Single Home Tank	09 02 3a					
031121eg04afamm	Single Home Tank	09 02 8b					
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)		116			
031121eg07afamm	non-silinized Glassware wwtp	10 27 1a	1014	7248	134	704	215
031121eg08afamm	non- silinized Glassware wwtp	10 27 1b	1069	7046	178	823	219
031121eg09afamm	Silinized Glassware wwtp	10 27 2a	605	6872	73	554	183
031121eg10afamm	silinized glassware wwtp	10 27 2b	683	7097	80	502	182
031121eg15afamm	Frenchtown High school(septic tank)	10 30 1		3999		5713	151
031121eg20afamm	Frenchtown High school(septic tank)	11 05 1		4994		18712	219
031121eg27afamm	Single Home Tank	9 02 2b					
031121eg28afamm	Single Home Tank	09 02 10a		4911			
031121eg29afamm	Single Home Tank	09 02 10b		5359			
031121eg30afamm	Single Home Tank	09 02 10b2 (double runs through HLB)		2439			
030723mb08afamm	Single Home Tank	07 01 03 3b 10%					
030723mb09afamm	Single Home Tank	07 01 03 3b 50%					

030723mb10afamm	Single Home Tank	07 01 03 3b 100%					
030724mb03afamm	Single Home Tank	6.17.03-5a		13873			
030724mb04afamm	Single Home Tank	6.17.03-5b (spiked)	8589	23543	19851		
030724mb08afamm	Single Home Tank	6.17.03-7a		7911			
030724mb09afamm	Single Home Tank	6.17.03-7b spiked		9940	77314		
031121eg04afamm	Single Home Tank	09 02 8b					
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)		116			
		Blanks= BDL					

Masslynx Name	Type of Sample	Date and sample ID	Cimetidine	Cotinine	Diltiazem	Erythromycin -18	Codiene
			ng/L	ng/L	ng/L	ng/L	ng/L
031117eg04afamm	Community Tank	06 17 03 1a		2238			
031117eg05afamm	Community Tank	06 17 03 1b		2793			
031117eg06afamm	Community Tank	06 17 03 2a		2866		1107	
031117eg07afamm	Community Tank	06 17 03 2b		2366		1284	
031117eg08afamm	Community Tank	06 17 03 3a	713	2838	398		
031117eg09afamm	Community Tank	06 17 03 3b		3825			5
031117eg10afamm	Community Tank	06 17 03 4a		3377			
031117eg11afamm	Community Tank	06 17 03 4b		2781			
031117eg12afamm	Community Tank	06 17 03 6a		28763			1384
031117eg13afamm	Community Tank	06 17 03 6b		18973			1127
031117eg14afamm	Community Tank	06 17 03 8a		38276			
031117eg15afamm	Community Tank	06 17 03 8b (Spiked 5000ug/mL)		32204	5159		
031117eg16afamm	Community Tank	06 17 03 9a		662			
031117eg17afamm	Community Tank	06 17 03 9b (spiked 1mL 5000ug/mL)		1139			
031118eg10afamm	Community Tank	06 17 10a		3260			
031118eg11afamm	Single Home Tank	07 01 1a					
031118eg12afamm	Single Home Tank	07 01 2a					277
031118eg13afamm	Single Home Tank	07 01 2b (lots stuck on vial)					329
031118eg14afamm	Single Home Tank	07 01 2b2 (890uL of mobile phase to redissolve)					28

031118eg15afamm	Single Home Tank	07 01 3a					318
031118eg16afamm	Single Home Tank	07 01 4a		67470			1958
031118eg17afamm	Single Home Tank	07 01 5a					
031118eg18afamm	Single Home Tank	07 01 6a		256			
031118eg19afamm	Single Home Tank	07 01 7a					
031118eg20afamm	Single Home Tank	07 01 8a		4181			246
031118eg21afamm	Single Home Tank	07 01 9a		370			5
031118eg22afamm	Single Home Tank	07 01 10a		103			
031118eg23afamm	Single Home Tank	07 16 1a		2952			
031118eg24afamm	Single Home Tank	07 16 2a		80642			
031118eg25afamm	Single Home Tank	07 16 3a		151079			262
031118eg26afamm	Single Home Tank	07 16 4a		101029			291
031119eg04afammA	Single Home Tank	07 16 5a		59170			
031119eg05afamm	Single Home Tank	07 16 6a		8915			
031119eg06afamm	Single Home Tank	07 16 7a		309821			
031119eg08afamm	Single Home Tank	07 16 9a					
031119eg09afamm	Single Home Tank	07 16 10a		6507			
031119eg10afamm	Single Home Tank	07 16 11a		4733			
031119eg11afamm	Single Home Tank	07 16 12a		2735			
031119eg12afamm	Single Home Tank	07 16 13a		62782			
031119eg13afamm	Single Home Tank	07 16 14a		1052			
031119eg14afamm	Single Home Tank	07 16 15a		78810			
031119eg15afamm	Single Home Tank	09 02 1a					
031119eg16afamm	Single Home Tank	09 02 2a					
031119eg18afamm	Single Home Tank	09 02 4a		45948			
031119eg19afamm	Single Home Tank	09 02 5a					
031119eg20afamm	Inflow	09 30 1	1733	13118	233	1073	343
031119eg21afamm	Outflow before	09 30 2	696	198		1235	428

	ultraviolet treatment						
031119eg22afamm	Outflow after ultraviolet treatment	09 30 3	1027	228		1269	458
031120eg04afamm	Single Home Tank	09 02 3a					
031121eg04afamm	Single Home Tank	09 02 8b					
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)		116			
031121eg07afamm	non-silinized Glassware wwtp	10 27 1a	1014	7248	134	704	215
031121eg08afamm	non- silinized Glassware wwtp	10 27 1b	1069	7046	178	823	219
031121eg09afamm	Silinized Glassware wwtp	10 27 2a	605	6872	73	554	183
031121eg10afamm	silinized glassware wwtp	10 27 2b	683	7097	80	502	182
031121eg15afamm	Frenchtown High school(septic tank)	10 30 1		3999		5713	151
031121eg20afamm	Frenchtown High school(septic tank)	11 05 1		4994		18712	219
031121eg27afamm	Single Home Tank	9 02 2b					
031121eg28afamm	Single Home Tank	09 02 10a		4911			
031121eg29afamm	Single Home Tank	09 02 10b		5359			
031121eg30afamm	Single Home Tank	09 02 10b2 (double runs through HLB)		2439			
030723mb08afamm	Single Home Tank	07 01 03 3b 10%					
030723mb09afamm	Single Home Tank	07 01 03 3b 50%					
030723mb10afamm	Single Home Tank	07 01 03 3b 100%					
030724mb03afamm	Single Home Tank	6.17.03-5a		13873			
030724mb04afamm	Single Home Tank	6.17.03-5b (spiked)	8589	23543	19851		

030724mb08afamm	Single Home Tank	6.17.03-7a		7911			
030724mb09afamm	Single Home Tank	6.17.03-7b spiked		9940	77314		
031121eg04afamm	Single Home Tank	09 02 8b					
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)		116			
		Blanks= BDL					

Masslynx Name	Type of Sample	Date and sample ID	Ranitidine	Sulfamethoxazole	Trimethoprim	Warfarin
			ng/L	ng/L	ng/L	ng/L
031117eg04afamm	Community Tank	06 17 03 1a				364
031117eg05afamm	Community Tank	06 17 03 1b				456
031117eg06afamm	Community Tank	06 17 03 2a				286
031117eg07afamm	Community Tank	06 17 03 2b				237
031117eg08afamm	Community Tank	06 17 03 3a				145
031117eg09afamm	Community Tank	06 17 03 3b				
031117eg10afamm	Community Tank	06 17 03 4a				1901
031117eg11afamm	Community Tank	06 17 03 4b				682
031117eg12afamm	Community Tank	06 17 03 6a			50	
031117eg13afamm	Community Tank	06 17 03 6b	103		44	
031117eg14afamm	Community Tank	06 17 03 8a				3987
031117eg15afamm	Community Tank	06 17 03 8b (Spiked 5000ug/mL)			45362	4724
031117eg16afamm	Community Tank	06 17 03 9a				
031117eg17afamm	Community Tank	06 17 03 9b (spiked 1mL 5000ug/mL)			193439	
031118eg10afamm	Community Tank	06 17 10a				7241
031118eg11afamm	Single Home Tank	07 01 1a				
031118eg12afamm	Single Home Tank	07 01 2a				3603
031118eg13afamm	Single Home Tank	07 01 2b (lots stuck on vial)				
031118eg14afamm	Single Home Tank	07 01 2b2 (890uL of mobile phase to redissolve)				

031118eg15afamm	Single Home Tank	07 01 3a				3910
031118eg16afamm	Single Home Tank	07 01 4a				3293
031118eg17afamm	Single Home Tank	07 01 5a				
031118eg18afamm	Single Home Tank	07 01 6a				12618
031118eg19afamm	Single Home Tank	07 01 7a				13253
031118eg20afamm	Single Home Tank	07 01 8a				4837
031118eg21afamm	Single Home Tank	07 01 9a			5	4314
031118eg22afamm	Single Home Tank	07 01 10a				2354
031118eg23afamm	Single Home Tank	07 16 1a				7026
031118eg24afamm	Single Home Tank	07 16 2a				3450
031118eg25afamm	Single Home Tank	07 16 3a				18529
031118eg26afamm	Single Home Tank	07 16 4a				11666
031119eg04afammA	Single Home Tank	07 16 5a				
031119eg05afamm	Single Home Tank	07 16 6a				2205
031119eg06afamm	Single Home Tank	07 16 7a				18263
031119eg08afamm	Single Home Tank	07 16 9a				
031119eg09afamm	Single Home Tank	07 16 10a	50			4732
031119eg10afamm	Single Home Tank	07 16 11a				12048
031119eg11afamm	Single Home Tank	07 16 12a				
031119eg12afamm	Single Home Tank	07 16 13a				8380
031119eg13afamm	Single Home Tank	07 16 14a	985	64767		6419
031119eg14afamm	Single Home Tank	07 16 15a				14322
031119eg15afamm	Single Home Tank	09 02 1a				16437
031119eg16afamm	Single Home Tank	09 02 2a			259	2462
031119eg18afamm	Single Home Tank	09 02 4a				7014
031119eg19afamm	Single Home Tank	09 02 5a				23297
031119eg20afamm	Inflow	09 30 1			213	1686
031119eg21afamm	Outflow before	09 30 2		268	464	

	ultraviolet treatment					
031119eg22afamm	Outflow after ultraviolet treatment	09 30 3		297	115	
031120eg04afamm	Single Home Tank	09 02 3a				12592
031121eg04afamm	Single Home Tank	09 02 8b				1811
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)				
031121eg07afamm	non-silinized Glassware wwtp	10 27 1a		30	251	1444
031121eg08afamm	non- silinized Glassware wwtp	10 27 1b	119	234	208	1799
031121eg09afamm	Silinized Glassware wwtp	10 27 2a	84	165	171	1193
031121eg10afamm	silinized glassware wwtp	10 27 2b	121	208	250	1354
031121eg15afamm	Frenchtown High school(septic tank)	10 30 1	5	4266	628	1217
031121eg20afamm	Frenchtown High school(septic tank)	11 05 1	21	29690	1472	1203
031121eg27afamm	Single Home Tank	9 02 2b		-52	193	
031121eg28afamm	Single Home Tank	09 02 10a				3340
031121eg29afamm	Single Home Tank	09 02 10b				5840
031121eg30afamm	Single Home Tank	09 02 10b2 (double runs through HLB)				
030723mb08afamm	Single Home Tank	07 01 03 3b 10%				
030723mb09afamm	Single Home Tank	07 01 03 3b 50%				
030723mb10afamm	Single Home Tank	07 01 03 3b 100%				
030724mb03afamm	Single Home Tank	6.17.03-5a	129			
030724mb04afamm	Single Home Tank	6.17.03-5b (spiked)	101		25619	

030724mb08afamm	Single Home Tank	6.17.03-7a	1590		1581	
030724mb09afamm	Single Home Tank	6.17.03-7b spiked	1527		65041	
031121eg04afamm	Single Home Tank	09 02 8b				1811
031121eg05afamm	Single Home Tank	09 02 8b2 (two runs through HLB)				
		Blanks= BDL				

APPENDIX 9.

Masslynx Name	Type	Date and sample identification	Acetaminophen	Antipyrine	Caffiene	Carbamazepine	Cimetidine	Cotinine
			ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
031119eg24afamm	DWQ samples (Legal ID= W131919C)	10 15 1a- Hawthorne	BDL	BDL	44.0	BDL	BDL	4
031119eg25afamm	DWQ samples (Legal ID= U132025D)	10 15 2a- Tower and Spurgin	BDL	BDL	206.9	BDL	BDL	7
031119eg26afamm	DWQ samples (Legal ID= W132026B)	10 15 3a- Kelly isl. And Spurgin	BDL	BDL	BDL	BDL	BDL	BDL
031119eg27afamm	DWQ samples (Legal ID= W132026D)	10 15 4a- Humble and Mount	BDL	BDL	42.0	13	BDL	BDL
031120eg05afamm	DWQ samples (Legal ID= W131930D)	10 15 5a- (Spiked 1mL 50 ng/mL)	8440	BDL	12175	581	95	8
031120eg06afamm	DWQ samples (Legal ID= W131930D)	10 15 5b- Central and Reserve	BDL	BDL	61.0	BDL	BDL	1
031120eg07afamm	DWQ samples (Legal ID= W152129A)	10 15 6a- Touchette Ln, Frenchtown	BDL	BDL	85.0	BDL	BDL	2
031120eg08afamm	DWQ samples (Legal ID= W131931D)	10 15 7a- Larchmont	BDL	BDL	BDL	BDL	BDL	BDL
031121eg06afamm	DWQ samples (Legal ID= W131914C)	10 15 8a- Alvina Park	BDL	BDL	21	2	BDL	BLOQ
031121eg15afamm	Frenchtown High school (septic tank)	10/30/2003 1	30998		53684	454	BDL	3999

031121eg16afam m	Frenchtown High school (well #19)	10/30/2003 2	BDL	BDL	BDL	78	BDL	BDL
031121eg17afam m	Frenchtown High school (well #40)	10/30/2003 3	BDL	BDL	BDL	59	BDL	BDL
031121eg18afam m	Frenchtown High school (well #41)	10/30/2003 4	BDL	BDL	BDL	137	BDL	BDL
031121eg19afam m	Frenchtown High school (Well #26)	10/30/2003 5	BDL	BDL	BDL	151	BDL	BDL
031121eg20afam m	Frenchtown High school (septic tank)	11/05/2003 1	25814	BDL	62192	262	BDL	4994
031121eg21afam m	Frenchtown High school (well #19)	11/05/2003 2	BDL	BDL	18	202	BDL	BDL
031121eg22afam m	Frenchtown High school (well #40)	11/05/2003 3	BDL	BDL	BDL	93	BDL	BDL
031121eg23afam m	Frenchtown High school (well #41)	11/05/2003 4	BDL	BDL	BDL	186	BDL	BDL
031121eg24afam m	Frenchtown High school (Well #26)	11/05/2003 5	BDL	BDL	BDL	211	BDL	BDL
031119eg20afam m	Inflow	09 30 1	525079		137607	486	1733	13118
031119eg21afam m	Outflow before ultraviolet treatment	09 30 2	BDL	BDL	719	470	696	198
031119eg22afam m	Outflow after ultraviolet treatment	09 30 3	BDL	BDL	616	498	1027	228
031119eg23afam m	Kelly Island sample (Clark Fork down stream from WWTP)	09 30 4	BDL	BDL	1367	3	BDL	0
031121eg25afam	CF River	11 08 1	BDL	BDL	BDL	BDL	BDL	BDL

m								
031121eg26afam m	Tap water	11 08 2a	BDL	BDL	BDL	BDL	BDL	BDL
Masslynx Name	Type	Date and sample identification	Diltiazem	Erythromycin-18	Codiene	Hydrocodone	Ketoprofen	Metaformin
			ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
031119eg24afam m	DWQ samples (Legal ID= W131919C)	10 15 1a- Hawthorne	BDL	BDL	BDL	BDL	BDL	BDL
031119eg25afam m	DWQ samples (Legal ID= U132025D)	10 15 2a- Tower and Spurgin	BDL	BDL	BDL	BDL	BDL	BDL
031119eg26afam m	DWQ samples (Legal ID= W132026B)	10 15 3a- Kelly isl. And Spurgin	BDL	BDL	BDL	BDL	BDL	BDL
031119eg27afam m	DWQ samples (Legal ID= W132026D)	10 15 4a- Humble and Mount	BDL	BDL	BDL	BDL	BDL	BDL
031120eg05afam m	DWQ samples (Legal ID= W131930D)	10 15 5a- (Spiked 1mL 50 ng/mL)	4303	BDL	BDL	BDL	BDL	BDL
031120eg06afam m	DWQ samples (Legal ID= W131930D)	10 15 5b- Central and Reserve	BDL	BDL	BDL	BDL	BDL	BDL
031120eg07afam m	DWQ samples (Legal ID= W152129A)	10 15 6a- Touchette Ln, Frenchtown	BDL	BDL	BDL	BDL	BDL	BDL
031120eg08afam m	DWQ samples (Legal ID= W131931D)	10 15 7a- Larchmont	BDL	BDL	BDL	BDL	BDL	BDL
031121eg06afam m	DWQ samples (Legal ID= W131914C)	10 15 8a- Alvina Park	BDL	BDL	BDL	BDL	BDL	BDL
031121eg15afam m	Frenchtown High school (septic tank)	10/30/2003 1	BDL	5713	151	BDL	BDL	BDL

031121eg16afam m	Frenchtown High school (well #19)	10/30/2003 2	BDL	BDL	BDL	BDL	BDL	BDL
031121eg17afam m	Frenchtown High school (well #40)	10/30/2003 3	BDL	BDL	BDL	BDL	BDL	BDL
031121eg18afam m	Frenchtown High school (well #41)	10/30/2003 4	BDL	BDL	BDL	BDL	BDL	BDL
031121eg19afam m	Frenchtown High school (Well #26)	10/30/2003 5	BDL	BDL	BDL	BDL	BDL	BDL
031121eg20afam m	Frenchtown High school (septic tank)	11/05/2003 1	BDL	18712	219	BDL	BDL	BDL
031121eg21afam m	Frenchtown High school (well #19)	11/05/2003 2	BDL	BDL	BDL	BDL	BDL	BDL
031121eg22afam m	Frenchtown High school (well #40)	11/05/2003 3	BDL	BDL	BDL	BDL	BDL	BDL
031121eg23afam m	Frenchtown High school (well #41)	11/05/2003 4	BDL	BDL	BDL	BDL	BDL	BDL
031121eg24afam m	Frenchtown High school (Well #26)	11/05/2003 5	BDL	BDL	BDL	BDL	BDL	BDL
031119eg20afam m	Inflow	09 30 1	233.2734911	1073	343	BDL	BDL	2687
031119eg21afam m	Outflow before ultraviolet treatment	09 30 2	BDL	1235	428	BDL	BDL	1676
031119eg22afam m	Outflow after ultraviolet treatment	09 30 3	BDL	1269	458	BDL	BDL	2049
031119eg23afam m	Kelly Island sample (Clark Fork down stream from WWTP)	09 30 4	BDL	BDL	BDL	BDL	BDL	
031121eg25afam	CF River	11 08 1	BDL	BDL	BDL	BDL	BDL	BDL

m								
031121eg26afam m	Tap water	11 08 2a	BDL	BDL	BDL	BDL	BDL	BDL
		BDL= Below detection limit						
		BLOQ=Detected compound with a signal to noise ratio > 3 (>S/N=3) but below limit of quantitation						
		Spiked= Caffiene, Actaminophen, carbamazepine, cimetidine, diltiazem, trimethoprim						

Masslynx Name	Type	Date and sample identification	Nicotine	Paraxathine	Ranitidine	Sulfamethoxazole	Trimethoprim	Warfarin
			ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
031119eg24afam m	DWQ samples (Legal ID= W131919C)	10 15 1a- Hawthorne	BDL	BDL	BDL	BDL	6	BDL
031119eg25afam m	DWQ samples (Legal ID= U132025D)	10 15 2a- Tower and Spurgin	BDL	BDL	BDL	BDL	BDL	BDL
031119eg26afam	DWQ samples (Legal	10 15 3a- Kelly isl.	BDL	BDL	BDL	BDL	BDL	BDL

m	ID= W132026B)	And Spurgin						
031119eg27afam m	DWQ samples (Legal ID= W132026D)	10 15 4a- Humble and Mount	BDL	BDL	BDL	BDL	BDL	BDL
031120eg05afam m	DWQ samples (Legal ID= W131930D)	10 15 5a- (Spiked 1mL 50 ng/mL)	BDL	BDL	BDL	BDL	2589	BDL
031120eg06afam m	DWQ samples (Legal ID= W131930D)	10 15 5b- Central and Reserve	BDL	BDL	BDL	BDL	BDL	BDL
031120eg07afam m	DWQ samples (Legal ID= W152129A)	10 15 6a- Touchette Ln, Frenchtown	BDL	BDL	BDL	BDL	BDL	BDL
031120eg08afam m	DWQ samples (Legal ID= W131931D)	10 15 7a- Larchmont	BDL	BDL	BDL	BDL	BDL	BDL
031121eg06afam m	DWQ samples (Legal ID= W131914C)	10 15 8a- Alvina Park	BDL	BDL	BDL	BDL	BDL	BDL
031121eg15afam m	Frenchtown High school (septic tank)	10/30/2003 1	783	87773.6341	5	4266	628	1217
031121eg16afam m	Frenchtown High school (well #19)	10/30/2003 2	BDL	BDL	BDL	23	BDL	BDL
031121eg17afam m	Frenchtown High school (well #40)	10/30/2003 3	BDL	BDL	BDL	11	BDL	BDL
031121eg18afam m	Frenchtown High school (well #41)	10/30/2003 4	BDL	BDL	BDL	49	BDL	BDL
031121eg19afam m	Frenchtown High school (Well #26)	10/30/2003 5	55	BDL	BDL	55	BDL	BDL
031121eg20afam m	Frenchtown High school (septic tank)	11/05/2003 1	1002	84129.8287	21	29690	1472	1203
031121eg21afam m	Frenchtown High school (well #19)	11/05/2003 2	BDL	BDL	BDL	466	BDL	BDL

031121eg22afam m	Frenchtown High school (well #40)	11/05/2003 3	BDL	BDL	BDL	44	BDL	BDL
031121eg23afam m	Frenchtown High school (well #41)	11/05/2003 4	BDL	BDL	BDL	81	BDL	BDL
031121eg24afam m	Frenchtown High school (Well #26)	11/05/2003 5	BDL	BDL	BDL	68	BDL	BDL
			BDL					
031119eg20afam m	Inflow	09 30 1	4132	183393.021	BDL		213	1686
031119eg21afam m	Outflow before ultraviolet treatment	09 30 2	BDL	BDL	BDL	268	464	BDL
031119eg22afam m	Outflow after ultraviolet treatment	09 30 3	BDL	BDL	BDL	297	115	BDL
031119eg23afam m	Kelly Island sample (Clark Fork down stream from WWTP)	09 30 4	BDL	BDL	BDL	BDL	BDL	BDL
031121eg25afam m	CF River	11 08 1	BDL	BDL	BDL	BDL	BDL	BDL
031121eg26afam m	Tap water	11 08 2a	BDL	BDL	BDL	BDL	BDL	BDL
	BDL= Below detection limit							
	BLOQ=Detected compound with a signal to noise ratio > 3 (>S/N=3) but below							

	limit of quantitation							
	Spiked= Caffiene, Actaminophen, carbamazepine, cimetidine, diltiazem, trimethoprim							

Recharge assessment of the Anaconda Mine near Belt, Montana

Basic Information

Title:	Recharge assessment of the Anaconda Mine near Belt, Montana
Project Number:	2002MT4B
Start Date:	3/1/2002
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	At-Large
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Geochemical Processes, Toxic Substances
Descriptors:	Acid Mine Drainage, Ground-Water Recharge, Tritium, CFC, Age Dating
Principal Investigators:	Jon C. Reiten, Shawn Reddish

Publication

1. Reddish, Shawn, and Jon Reiten, 2003, A continued study of the hydrogeologic characterization of AMD production along Belt Creek near Belt, MT, "in" Montana Section of the American Water Resources Association Poster Session, Butte, Montana October 2

Title: Recharge Assessment of the Anaconda Mine near Belt, Montana

Start Date: March 1, 2002

End Date: February 28, 2004

Congressional District: Montana

Primary PI:Jon Reiten

Other Co-Investigator: Shawn Reddish

Project Class: Research

Research Synopsis
Recharge Assessment of the Anaconda Mine near Belt, Montana
A 2002 Grant Award

By

Jon Reiten, Assistant Research Hydrologist and

Shawn Reddish, Research Specialist

Montana Bureau of Mines and Geology

Billings, Montana

ABSTRACT

Decades of underground coal mining have resulted in acid mine drainage (AMD), which has contaminated ground-water and surface-water resources in Belt. The acid mine drainage is lowering the pH of Belt Creek and increasing trace metals concentration in the stream. The goal of this project is to define the Hydrogeologic regime in the vicinity of Belt so that recharge to old mine workings, the source of acid mine drainage, can be delineated with a reasonable level of certainty.

By inventorying, sampling, and age dating water from wells wells, springs, adits and seeps we intend to determine if the recharge is local or regional. Currently we have investigated and inventoried all important parts of the study area. The inventory process includes identifying GPS coordinates, measuring electric conductivity, pH, oxidation-

reduction potential, dissolved oxygen; and determining the geologic source. This information will be used to screen for the most useful sampling sites. All information is entered into a database accessible by the public.

Water levels at 20 wells and springs are measured monthly to monitor the fluctuations of local aquifers. Several of these wells and springs have been sampled for tritium and then Chlorofluorocarbons (CFC) to determine the age of the water. All wells have varying concentrations of tritium. This suggests ground water in the alluvial, Kootenai, Swift, and Madison aquifers are less than 50 years old. Samples have also been taken to determine CFC concentrations, but the results are not available yet. By determining the age of water in the mine workings and comparing overlying and underlying aquifers, methods can be developed to reduce recharge to the acid producing mine workings.

Stream flows at 11 sites are also measured monthly in the study area. Differences in flows can determine the gain or losses of surface-water to local aquifers. Field parameters including measuring specific conductivity, pH, oxidation-reduction, and dissolved oxygen are also taken at each site. The AMD discharge is monitored monthly for flow and field parameters and a continual pressure transducer monitors the AMD discharge.

Based on very preliminary interpretations a significant source of water to the Anaconda mine appears to be from the overlying Kootenai Formation. Figure 1 is a surficial geologic map of the area above and adjacent to the Anaconda mine. The Kootenai Formation is up to 200 feet thick in the Belt area.

Two general trends are apparent from existing water-level fluctuation data (Figure 2). Wells completed in the uplands up gradient of the mine have very minor water-level fluctuations trending flat to a slight decline. Wells completed near streams or small tributaries generally indicate a declining water level in response to the recent drought.

A potentiometric surface map of the Kootenai Formation was constructed based on measurements collected during the well inventory. This map was contoured using measurements from 37 wells and springs near the mine. This map shows only general water-level conditions in the mapped area. Additional wells at critical locations will be needed to accurately depict ground-water flow. In addition, a more accurate contour map requires monitoring of water levels at approximately the same time. The mapping depicts a ground-water divide located about 3.5 miles south of the Anaconda mine (Figure 3). Only

precipitation falling north of this divide has the potential to move towards the mine. Ground-water flow is perpendicular to the water-level contours. The upland between Belt Creek and Box Elder Creek is highly dissected by tributaries to the two streams. These tributaries plus the main stems of the two streams are discharge areas for ground water moving out of the Kootenai Formation. The potential recharge area to the Anaconda mine starts at the ground-water divide 3.5 miles south of the mine and extends to the region directly overlying the abandoned mine working. This forms a relatively narrow band following the axis of the surface water divide between Belt Creek and Box Elder Creek. The potential recharge area covers about 2,100 acres overlying and up gradient of the mine. The highly dissected nature of the upland appears to cause much of the precipitation falling on the upland to bleed out and discharge to the surface water drainages and springs in the valley walls. Much of this water is consumed by vegetation in the drainages as shown by the areas of dense plant cover depicted on Figure 4. Several of the springs appear to be related to the contact of the Sunburst Sandstone Member (aquifer) and the underlying unnamed fine grained unit (aquitard).

The ground-water divide south of the mine appears to be both topographically and structurally controlled. The topographic high area forming the ground-water divide is located just north of a paired anticline-syncline structure that trends North 45 degrees East.

Publications/Citations- AMERICAN WATER RESOURCES ASSOCIATION Poster Presentation October 2, 2003

Student Support - Jay Hanson at the Montana Bureau of Mines and Geology

Figure 1: Geologic Map of the Anaconda Mine Area, Belt Montana

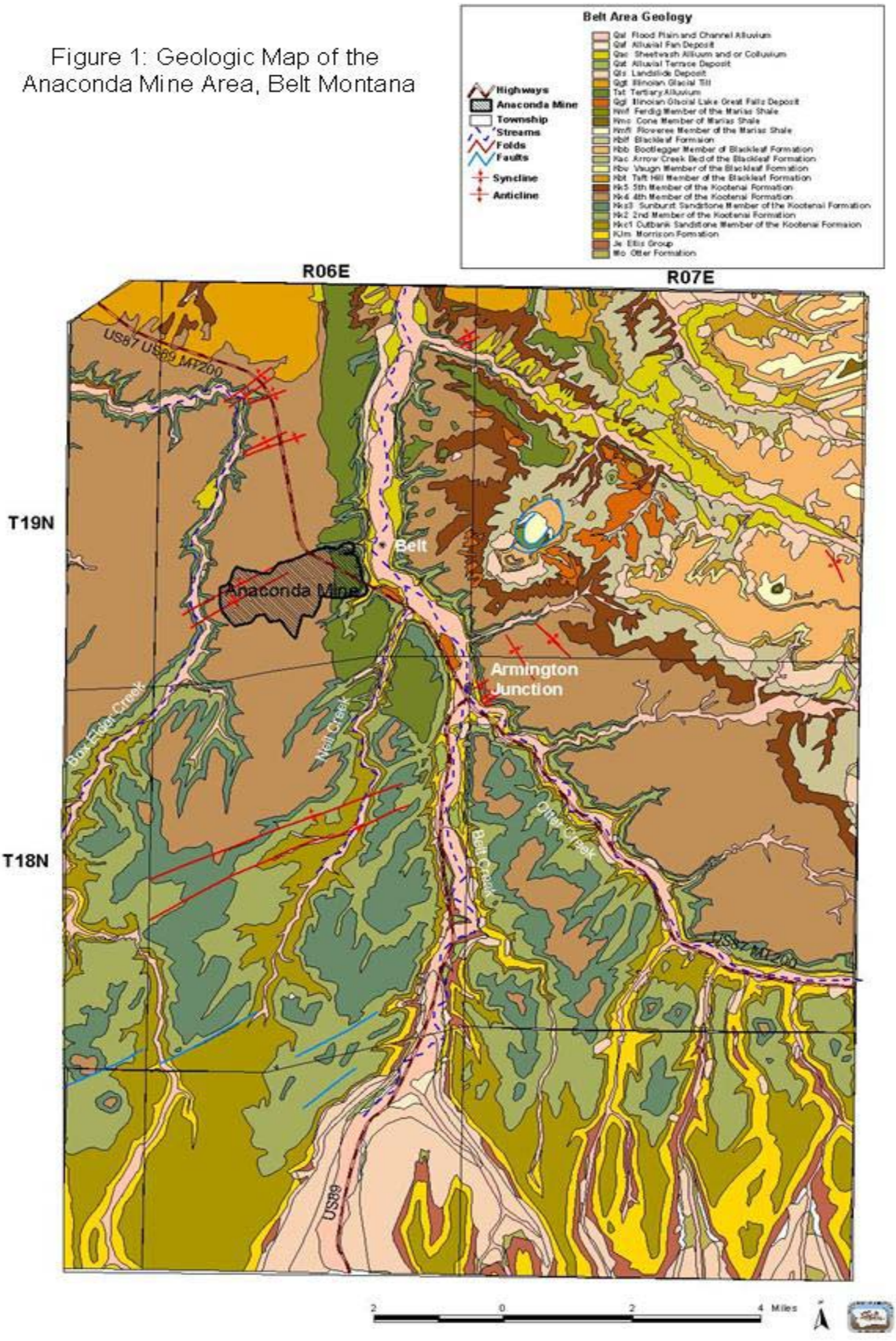


Figure 2. Hydrographs showing water level fluctuations in the Kootenai aquifer. Hydrographs are from two upland wells (M# 204516 and 199851) and two wells in tributaries (M# 180021 and 84937). These wells are identified on Figures 3 and 4.

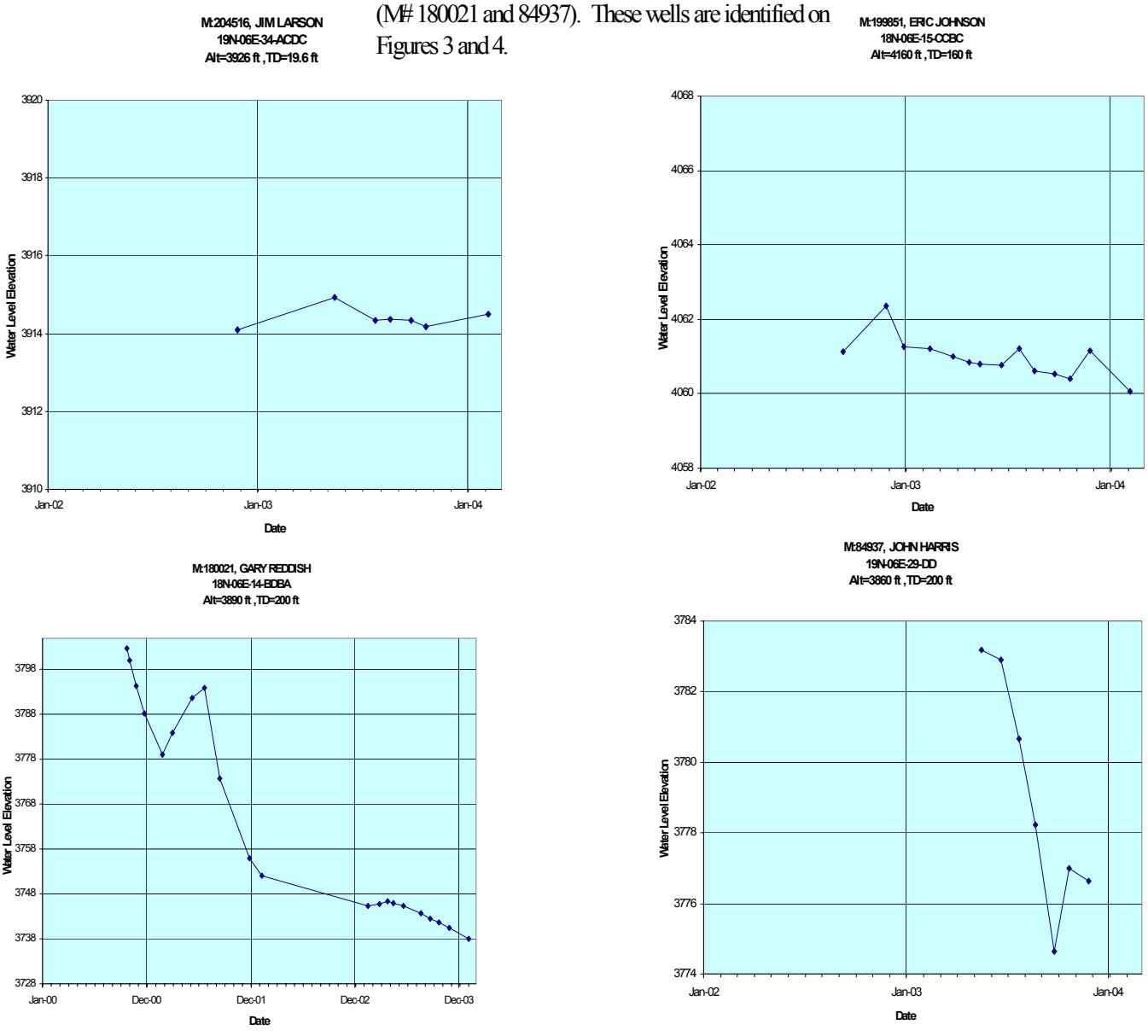
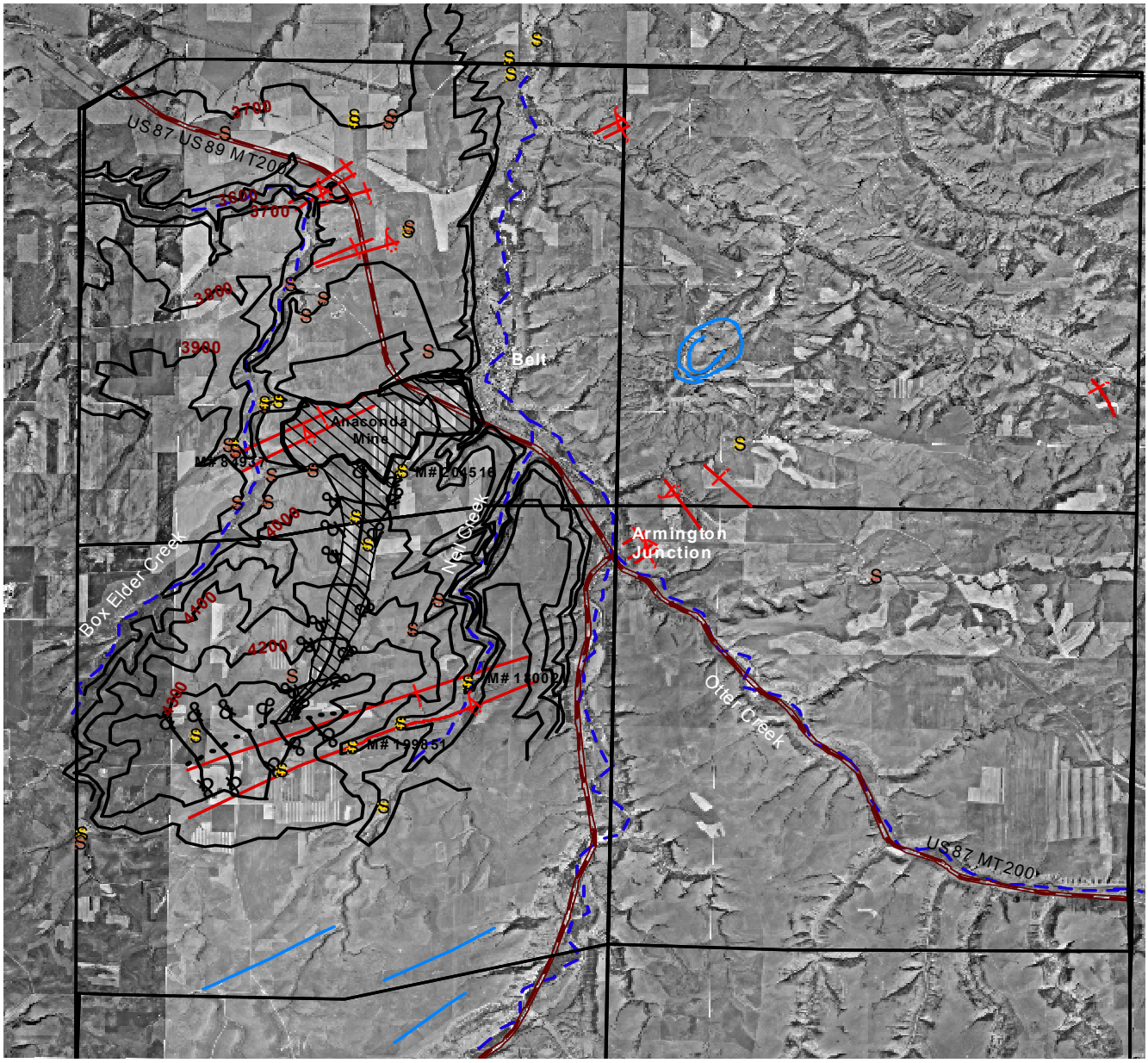


Figure 3. Potentiometric surface and ground water divide of the Kootenai Formation



Information Transfer Program

During FY 2003, the Montana Water Center developed or sponsored many tools to carry out its mission to mobilize the resources of Montanas public university researchers to resolve the states water problems. Because of 104b support, the Center was actively involved in these water information transfer activities:

1. coordinated all water research and information transfer activities in concert with the Centers Director at Montana State University (Gretchen Rupp), and Associate Directors at the University of Montana (Dr. Donald Potts) and Montana Tech (Dr. Marvin Miller) campuses;

2. administered the 104b research grants, and promoted interest in the 104g research grant program;

3. encouraged and enabled student involvement through nternships, research opportunities, trainings, and other efforts that provide practical experience for future water professionals;

4. initiated a monthly Montana Water e-newsletter series distributed to a database of nearly 1,000 people;

5. continued to maintain and expand MONTANA WATER, the Montana Water Centers web information network at http://water.montana.edu. This website includes an events page, news updates, an online library, water-resource forums, a Montana watersheds projects database, an expertise directory, water facts and more;

6. produced the Montana Water Centers 2002-2003 Annual Report, a booklet covering all of the programs accomplished through the Centers $1.9M budget;

7. produced a new brochure for the Center;

8. co-produced the Water Rights in Montana booklet for broad distribution in conjunction with the Montana Department of Natural Resources and Conservation and the Montana Legislative Environmental Quality Council;

9. coordinated two live teleconferences sponsored by the American Water Works Association. About 50 water-system professionals attended at downlink sites in Missoula, Havre, Great Falls, Billings, Helena, Butte and Bozeman. The November 2002 teleconference centered on emerging treatment technologies. In March 2003, participants learned the latest regarding water storage.

10. conducted the state-wide water research meeting in Butte, Montana in September 2003 for exchange of research information among water professionals. A record number of water professionals attended to hear more than 40 papers and 10 poster presentations. The web-based archive of this meeting is found at http://www.awra.org/state/montana;

11. served as a liaison among the university community and water professionals and decisionmakers in local, state, and tribal and federal governments, including attendance at all Montana Legislative Environmental Quality Council meetings and Montana University System research outreach coordination meetings;

12. invested time in partnering with other groups with similar goals of translating scientific information for effective problem-solving;

13. participated in the Annual Water School. This training for water and wastewater managers and operators has been offered for nearly seven decades by the Water School. Each year, operators from throughout Montana can receive four days of water and wastewater training for managing their local systems. The program features workshops and presenters from private consulting, industry, academia and government. At the close of the training, operators may sit for the water/wastewater certification exam administered by the Montana Department of Environmental Quality (DEQ). Along with DEQ, this program is conducted by the Montana Environmental Training Center, the Montana Water Center, and the MSU Department of Civil Engineering; and

14. developed the concept for a monthly Montana Water Center press research profile series.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	2	0	1	0	3
Masters	6	0	0	0	6
Ph.D.	1	0	0	0	1
Post-Doc.	0	0	0	0	0
Total	9	0	1	0	10

Notable Awards and Achievements

In addition to the productive research conducted under the Montana Water Centers USGS base grant, the Center gladly reports several notable achievements resulting from USGS and other funding sources:

The study by Dr. Paul Sturman was featured on the MSU Homepage in April 2004, with an accompanying press release. In addition, this project funding was instrumental in garnering an additional $50,000 support from the USEPA Mine Waste Technology Program (through a subcontract from MSE Technology Applications, Inc.) to further study the use of organic carbon as a remedial treatment at the Golden Sunlight Mine in Cardwell, Montana.

The post-fire research conducted by Dr. Scott Wood and Dr. Thomas DeLuca, University of Montana added to the limited literature available on the effectiveness of post-fire burned area erosion control techniques. in addition, graduate student Amy Groen, received the Best Student Presentation award for her presentation on this research at the Montana State AWRA Meeting in Butte, Montana in October 2003.

The proceedings of the 7th International Symposium on Fish Physiology, Toxicology, and Water Quality are now available follwing a successful conferecne convend by the Montana Water Center in Tallin Estonia on May 12-15, 2003. The proceedings of this international symposium can be found at the Montana Water Center webiste at: http://water.montana.edu/symposium/proceedings/default.htm

The Small Systems Technical Assistance -- Drinking Water Assistance Program administered by the Montana Water Center operates a flagship institution in the eight-center network of Small System Technology Assistance Centers. Project descriptions and resources from all eight centers can be accessed on the TACnet website maintained by the Montana Water Center at http://water.montana.edu/tacnet/deafult.htm

The Whirling Disease Research Initiative is overseen by the National Partnership for the Management of Wild and Native Coldwater Fisheries. In FY 2003 the Water Center managed the work of the Partnership through a competitive grant program which examines impacts of and solutions to the national whirling disease dilemma. Fourteen research project culminated in valuable solutions to the disease. The work of

this significant annual intitative can be viewed at
http://water.montana.edu/mwc/programs/fisheries/whirling/default.htm.

The Wild Fish Habitat Inititative website developed by the Montana Water Center was unveiled with major funding from the U.S. Fish and Wildlife Service. This site provides information on habitat restoration research as well as technical resources and successful restoration case studies from throughout the northwestern United States. Case studies include narrative descriptions, project goals, restoration methods, project costs, landowner contributions, and monitoring data, all with the intention of providing landowners and managers with information with which to make informed habitat restoration decisions. The site URL is: http://wildfish.montana.edu/

Publications from Prior Projects