

Understanding Sources of Aquatic Contaminants of Emerging Concern

A. Introduction/Background

Detection of Emerging Contaminants in the Environment

Protecting the integrity and supply of our water resources is one of the most important environmental and human health issues we will face this century. Continued exponential growth in our national and world population has put increasing demand on land and water use for the production of food, fiber and fuel, and to meet industrial and community needs. With the development of more sensitive analytical methodologies [1], new contaminants of environmental concern (CECs) have been recognized, raising questions of their source, persistence, transport and potential adverse human and ecological health effects. Many of these emerging contaminants result from the use and disposal of natural and synthetic compounds that offer improvements in agriculture, industry, medical treatment, personal care and residential conveniences [2]. They may include, but are not limited to, pharmaceuticals and antibiotics that are used for treating humans and domesticated animals, natural or synthetic hormones excreted into the environment, phytoestrogens from certain plants, additives to consumer and personal care products, and recently identified industrial contaminants [1, 3-5].

State, national and international monitoring studies have reported detection of CECs in surface and ground waters [1, 3-10]. In a study of the Mississippi River from its headwaters at Lake Itasca through the metropolitan area of the Twin Cities to downstream Brownsville, Minnesota, water and bed-sediment samples contained a total of 24 and 40 compounds, respectively, with individual samples containing up to 8 and 31 contaminants. The most frequently detected water contaminants included a common plant and animal sterol (cholesterol), several ingredients found in personal care products (N,N-diethyl-meta-toluamide, hexahydrohexamethylcyclopent-abenzopyran) and two agricultural herbicides (atrazine, metolachlor). Bed-sediments contained components of household or industrial products (acetophenone, indole, 4-nonylphenol, phenol), plant sterols or fecal indicators (beta-sitosterol, beta-stigmastanol, 3-beta coprostanol, cholesterol), a wood preservative (p-cresol), and compounds found in asphalt and kerosene (fluoranthene, pyrene, and 2,6-dimethylnaphthalene) [5]. In a nationwide survey of 139 rivers in 30 states, Kolpin et al. [1] reported the occurrence of pharmaceuticals, hormones, or other organic wastewater contaminants in 80% of the streams sampled. These contaminants represent a wide range of biologically active compounds originating from residential, industrial, and agricultural sources. Analyses targeted 95 organic wastewater contaminants, of which 82 were detected. The most frequently detected compounds were a stimulant (caffeine), fecal steroid (coprostanol), a plant and animal sterol (cholesterol), an antimicrobial disinfectant (triclosan), insect repellent (N,N-diethyltoluamide), fire retardant (tri(2-chloroethyl)phosphate), and a nonionic detergent metabolite (4-nonylphenol). Studies evaluating septic system discharge (i.e., groundwater down-gradient of the septic systems) measured estrogens, pharmaceuticals, and personal care products at concentrations ranging from ng/L to mg/L levels [11-13]. Of particular concern is the study of Swartz et al. [12] that showed levels of the potent human steroidal hormones 17 β -estradiol (E2) and estrone (E1) as high as 30 and 100 ng/L, respectively.

Ecological and Human Health Concerns

The frequency of detection of emerging contaminants reported in surface waters has invoked concern of their potential ecological and human health effects.

Bioactive (Medicinal) Contaminants: Pharmaceuticals, including over-the-counter and prescription formulations, are bioactive medicinal compounds designed to treat humans and animals. By the year 2010 it is estimated that pharmaceutical sales in the United States will reach \$330 billion annually [14]. Pharmaceuticals are absorbed after intake then undergo metabolic conversions. Nevertheless, significant portions may be excreted unmetabolized or as bioactive derivatives [14-19]. Antibiotics are also utilized in the restoration and promotion of health in treating bacterial infections and diseases. An estimated 23,000 tons of antibiotics are produced in the United States each year, of which approximately 40% have prophylactic use in agriculture as growth enhancers in livestock feed and to prevent bacterial infections in fish rearing [14, 20-21]. Similar to pharmaceuticals, antibiotics administered to humans and animals may undergo incomplete metabolism; resulting in excretion of the parent product along with its metabolites [16]. Research has shown up to 90% of antibiotics added to animal feed can be excreted [22, 23]. Genetic selection of resistant bacteria is believed to be induced by exposure to low levels of antibiotics, similar to what has been found in surface waters [19, 24, 25]. In fact, bacteria resistant to sulfonamide and trimethoprim, antibiotics used for humans and animals, have been reported in rivers in the United States [26]. Six classes of antibiotics (aminoglycoside, fluoroquinolone, β -lactam, macrolide, sulfonamide, and tetracycline) are used in both animal husbandry and human medicine [23]; this raises concern about the development and proliferation of antibiotic-resistant pathogens from the animal-related use of these compounds [16, 19, 27, 28]. Disposal of unused pharmaceuticals and antibiotics as well as excretion of residues and degradation products can result in contamination of surface waters. This occurs through insufficient removal at sewage treatment plants, with runoff following land application of either animal-waste or biosolids, or with leakage from storage structures associated with animal feeding operations [14-19, 29].

Endocrine Disrupting Contaminants: A number of CECs have been shown to be hormonally-active, blocking or mimicking the function of natural hormones and interfering with the regulation of the endocrine system [30, 31]. Factors contributing to endocrine disruption are often complex and may involve biochemical, structural, and behavioral mechanisms [5, 32]. Biomarkers associated with endocrine disruption include the expression of measurable vitellogenin concentrations in males, the occurrence of altered sex steroids, or observed intersex characteristics (oocytes present in testes tissue) [33-37]. Examples of known endocrine-disrupting compounds include natural or synthetic sterols and hormones and an assortment of pesticides and components of personal care, household and industrial products (fragrances, nonionic detergent metabolites, and plasticizers) [33, 34, 37-40].

A survey of 139 streams in the United States resulted in the detection of 18 hormones and steroids [1]. Seventeen β -ethinylestradiol (EE2), an ovulation inhibitor in oral contraceptives, was measured at levels near those reported to disrupt reproductive function in male fish [10, 14]. Low dose life-long exposure of fathead minnows (*Pimephales promelas*) to EE2 reduced offspring's hatching success and demasculinized male fish [41, 42]. Indeed, researchers have documented the near extinction of the natural population of fathead minnows from a lake to which extremely low and environmentally-relevant levels (5-6 ng/L) of EE2 were added over a

three-year period [43]. Sampling two years after the addition of EE2 had stopped showed no recovery of the minnows in this lake [43]. Male Japanese Medaka (*Oryzias latipes*) exposed to EE2 or 17 β -estradiol (E2) for 21 days demonstrated histological changes and vitellogenin induction, respectively [44, 45]. Seventeen α -trenbolone and 17 β -trenbolone, synthetic androgens associated with growth-promoting implants in beef, are potent endocrine disrupters to fathead minnows (*P. promelas*) and resulted in vitellogenin reduction in female medaka (*O. latipes*), fathead minnows (*P. promelas*), and zebrafish (*D. rerio*) [44, 45].

Phytoestrogens, plant-derived estrogen mimics, have also been shown to interfere with the function of the endocrine system [31]; resulting in immunosuppression [46], vitellogenin gene expression [47], decreased testosterone production [48] and changes in behavior [49]. Japanese Medaka exposed to the phytoestrogens genistein and equol showed delayed oocyte maturation and exhibited gonadal intersex and alterations in external physical sexual characteristics [50].

Components of personal care, household, and industrial products as well as plant and animal protection products (pesticides) have been detected in surface waters and shown to disrupt hormone activity. In a reconnaissance study evaluating 91 contaminants in Minnesota, thirteen endocrine disrupting compound were detected including: a fragrance (acetyl-hexamethyl-tetrahydro-naphthalene), plant sterol (beta-sitosterol), pesticide (diazinon), preservative (3-tert-butyl-4-hydroxyanisole), plasticizer (bisphenol-A), polyaromatic hydrocarbon (benzo[a]pyrene) and 7 nonionic detergent metabolites (4-cumylphenol, 4-normal-octylphenol, 4-tert-octylphenol, nonylphenol diethoxylate, octylphenol diethoxylate, octylphenol monoethoxylate, para-nonylphenol) [3]. Evaluation of fish from the Mississippi River found no occurrence of intersex; however, measurable concentrations of vitellogenin were reported in 33 to 63 percent of male fish [5]. On several occasions, Kolpin et al. [1] observed concentrations of a plasticizer (bis(2-ethylhexyl)phthalate) at quantities above established maximum contaminant levels for the protect of aquatic-life.

Pesticides are utilized in agricultural and residential settings to control a number of pests including fungi, weeds, insects, and rodents. Approximately 25% of pesticides used in the United States are for non-agricultural purposes; including urban pesticide applications to roadsides, structures, lawns and gardens, golf courses, for mosquito control and in pet shampoos [51]. Application rates on lawns and golf courses are considerably higher than the rates reported for agricultural purposes; however, agricultural uses represent a much greater area of application [52, 53]. Hoffman et al. [54] compared measured pesticides levels in surface waters from eight urban streams with six paired agricultural streams. Carbaryl, chlorpyrifos, diazinon, and malathion were the most frequently detected insecticides in urban surface waters; along with the herbicides atrazine, metolachlor, simazine, and tebuthiuron. At times carbaryl and diazinon exceeded criteria for the protection of aquatic life [54, 55]. In addition, each of these insecticides and the herbicide atrazine are known or suspected endocrine disrupting compounds [56, 57].

Sources of Contaminants of Emerging Concern (CECs)

Environmental contaminants originate from a variety of sources and may enter surface waters by numerous transport pathways. Examples include direct discharge into surface waters via wastewater treatment effluents, industrial effluents, or leakage of storage lagoons [58, 59]. Contaminants can also be transported to surface waters by indirect routes such as runoff from

agricultural, residential or urban landscapes [54, 60, 61]; contributions from groundwater polluted with landfill, septic or feedlot leachates [3]; or atmospheric deposition and drift [62]. Lee et al. [3] evaluated the occurrence and distribution of contaminants in Minnesota surface waters and leachates. Summation of organic contaminants in all the surface water samples resulted in 9 pharmaceuticals, 7 antibiotics, 4 sterols and 36 household-, industrial- or agricultural-use compounds. The agricultural herbicide metolachlor was the most frequently detected followed by caffeine. Water underlying feedlots contained a pharmaceutical, an antibiotic, and 9 household-, industrial- or agricultural-use compounds. Three landfill leachates contained a total of 4 pharmaceuticals, 3 antibiotics, 4 sterols and 35 household-, industrial- or agricultural-use compounds. Overall, more contaminants were detected in surface water than ground water with wastewater treatment effluent as a major pathway of contaminants to surface waters.

Despite documentation of the ecological impacts of phytoestrogens, to our knowledge, no research has been completed on the presence of these compounds in surface water at a watershed scale. In fact, very little work has been performed evaluating the point or non-point sources of phytoestrogens in the environment. Although there are numerous plant-based industries, only one, the pulp and paper industry, has been confirmed as a phytoestrogen point source. Effluents from pulp and paper mills have been shown to contain high levels of phytosterols and isoflavones, with elevated genistein concentrations (13.1 µg/L) in particular [50]. In addition, fish exposed to mill effluents have displayed developmental and reproductive impairments [63].

In our ongoing research, we investigated the phytoestrogen (genistein, daidzein, coumestrol, formononetin, biochanin-A, and zearalenone) content of the waste streams of 18 plant-processing industries and three wastewater treatment plants (influent and effluent) from locations in the Midwestern United States. Coumestrol, formononetin, biochanin-A, and zearalenone were minor constituents of the phytoestrogen balance for both industrial and wastewater treatment samples. Genistein and daidzein, however, were present in some samples in very high concentrations, ranging from non-detectable to 4.0×10^5 ng/L (combined). In particular, soy-based industries (biodiesel, soy oil, and soy milk effluents) contained high levels of genistein and daidzein, ranging from 1.5×10^4 ng/L to 4.0×10^5 ng/L. This is not surprising as soy contains high phytoestrogen levels. Of interest is the fact that dairy cattle is often fed soy-based silage and soy meal as a protein supplement, potentially leading to high phytoestrogen levels in the waste of dairy cattle and the subsequent run-off from dairy-rearing facilities.

The result is that surface waters contain complex mixtures of dozens, if not hundreds, of CECs coming from a variety of agricultural, industrial, and residential sources. It is imperative to understand the sources of these different compounds so that appropriate reduction and remediation strategies can be implemented.

B. Project Objectives and Hypothesis

We hypothesize that various agricultural and residential land uses will result in diverse and predictable surface water contaminants where the presence of unique marker compounds and relative concentration and temporal occurrence of contaminants will differentiate agricultural sources (crop and livestock production) from non-agricultural sources (residential sewer and septic, metropolitan wastewater treatment plant effluent, hospital discharges).

To test our hypothesis, we will:

- (1) Identify and quantify CECs including pharmaceuticals, antibiotics, hormones, pesticides, and other wastewater contaminants, indicative of agricultural and residential uses, in surface water sampled from 7 sub-watershed streams in the Zumbro River watershed. (RESULT 1)
- (2) Correlate CEC profiles from each river with land use in the surrounding area, developing chemical markers for source identification. (RESULT 2)
- (3) Apportion CECs to various land uses and determine the comparative magnitude of impact from agricultural and non-agricultural land uses on the watershed. (RESULT 2)

We will also develop recommendations to state policy makers for strategies to reduce sources, mitigate sources, and remediate sources of CECs. We will engage policymakers, resource managers, and the regulatory community in discussions using a workshop format where our findings will be the scientific basis of the recommendations. (RESULT 3)

C. Project Impact and Long-Term Goals

Confirmation of contaminant signatures associated with land use will provide a tool that can be easily implemented in other watersheds to identify and track pollution sources. Once contaminants and sources are revealed, management and mitigation strategies can be identified to reduce their occurrence in soil and water; protecting our natural resources and reducing their impact on aquatic habitats and potentially, human health. In addition, contaminant signatures associated with land uses will provide valuable information to regulators, who may need to categorize these pollutants and establish water quality standards.

D. Rational and Significance

The established occurrence of pharmaceuticals, antibiotics, hormones, and other CECs in surface waters, nationally and internationally raises questions of their source, fate, and potential ecological and human health effects. A number of CECs have been shown to disrupt the normal function of the endocrine system or promote antimicrobial resistance at environmentally relevant levels.

Dominant sources of CECs are thought to be wastewater treatment plants (WWTPs point source) and agriculture (non-point source) but with different chemical contributions in their discharges. The Zumbro River watershed in southeastern Minnesota provides a unique opportunity to study CECs from both agricultural and non-agricultural sources due to its sub-watershed configuration and differing land uses. Five first-order streams and two second-order streams have been identified with surrounding land uses differing in percent and type of agriculture, percent of urban sewer- or suburban septic-systems, and upstream or downstream from a wastewater treatment plant. We have also identified unique marker compounds that will differentiate agricultural sources (crop and livestock production) from non-agricultural sources (residential sewer and septic, metropolitan waste water treatment plant effluent, hospital discharges). Several monitoring studies have reported a number of these emerging contaminants in surface waters; however, we will be the first to target identification of contaminant signatures associated with agricultural and non-agricultural land use as well as evaluate phytoestrogens at a watershed scale. We first have to answer the question “Is there a problem?” followed by “What is the source?” Upon confirmation of a problem and source, management and mitigation strategies

can be implemented to reduce contaminants, protect the environment, and improve the sustainability of agricultural practices.

E. Approach

Summary of Proposed Activities

Surface water samples will be collected from seven sub-watersheds of the Zumbro River watershed and analyzed for selected CECs including pharmaceuticals, antibiotics, hormones, pesticides, phytoestrogens and wastewater contaminants. Compounds of interest and land use profiles will be compared to develop chemical markers for determining source identification and to assess the magnitude of impact of agricultural and non-agricultural land use on surface waters.

Study Location (Zumbro River Watershed)

The headwaters of the South Fork of the Zumbro River (SFZR) Watershed encompasses 56,742 hectares (ha) in southern Olmsted and eastern Dodge Counties of southeastern Minnesota [64] (Figure 1). Seven sub-watersheds dominated by different agricultural land uses come to a confluence in the City of Rochester, MN. Six of the seven sub-watersheds in the study area are listed as impaired waters by the Minnesota Pollution Control Agency (MPCA).

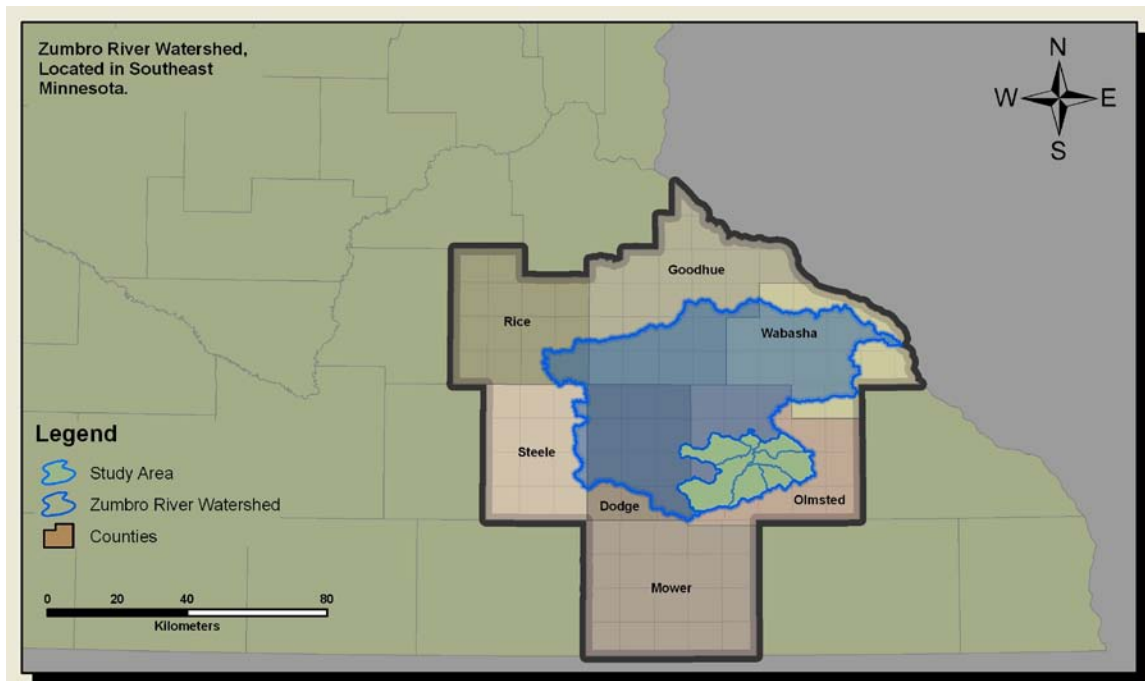


Figure 1. Study area in the Zumbro River Watershed, Minnesota, USA.

Agricultural Production

The up-gradient SFZR agricultural watersheds have approximately 5,200 households with 14,400 residents living in suburban developments with two to five acre lots and on farmsteads that average 87 acres in size [65]. The agricultural land use is dominated by cropland (76.9%), pasture (9.5%), woodland (6.9%) and other uses (6.7%). Free flowing streams and rivers with

narrow floodplains constitute less than 0.3% of the land area. There are no natural lakes and over 80% of the pre-settlement wetlands have been drained.

Olmsted County encompasses 169,903 hectares. According to the 2007 Agriculture Census, Olmsted County cropland is dominated by corn (46,363 ha), soybeans (23,249 ha) and forage (8,624 ha) with 3,570 ha of vegetables and 1,783 ha of corn for silage. Animal production estimates were 46,449 hogs and pigs, 42,434 beef cows and calves, 9,300 dairy cows, 4,150 geese and an undisclosed number of turkeys and other poultry. The SFZR study area encompasses approximately 15 percent of the County.

The number of animal units and mixture of animals in confined animal feedlot operations (CAFOs) varies by watershed within the SFZR study area (Table 1). Some watersheds are urbanized and have no CAFOs, while others are dominated by hogs, dairy or beef cattle. The MPCA regulates feedlots exceeding 300 animal units. Olmsted County tracks and regulates the smaller facilities with 30 to 299 animal units. Federal and State rules require a minimum of 9 months of manure storage for CAFOs. Best Management Practices currently allow winter applications of manure on frozen ground with less than 6% slopes, a time when a large proportion of manure is applied to the surface. Pre-planting and post harvest manure is injected or surface applied in the spring and fall with some manure spread in the summer following the harvest of vegetable crops.

Table 1: Stream characteristics and streamflow; land use and land cover percentages; dominant CAFO and drainage areas by sub-watershed. [CAFO, Confined Animal Feeding Operation; WWTP, wastewater treatment plant; (1)Mayowood Lake to source; (2) Data includes West Branch only; (3) Data is estimated.]

Site Id	Site Name	Length of Stream (km)	Gradient (m/km)	Flow at Mouth (m ³ /s)	Percent Urban Sewered	Percent Suburban ISTS	Percent Forest/Shrub	Dominant CAFO by animal type	Percent Agriculture	Percent Wetlands	Basin Area (ha)
1	Cascade Creek	36.1	2.6	0.12	18	5	5	beef	69	<3	9,977
2	South Fork Zumbro River @ HWY 14	99.1	1.6	0.89	19	4	8	hogs	66	<3	18,255
3	South Fork Zumbro River downstream WWTP	99.1	1.6	0.89	19	4	8	hogs	66	<3	18,255
4	Willow Creek	20.9	4	0.08	45	4	8	dairy hogs beef turkeys	40	<3	5,757
5	Badger Run	14.4	4.8	0.34	1	16	4	beef turkey	76	<3	4,206
6	Bear Creek	26.9	1.9	1.44	12	8	8	dairy hogs	69	<3	9,240
7	Silver Creek	14.8	4.7	0.03	11	4	15	dairy	67	<3	5,068

Residential and Urban Demographics

The SFZR headwaters study area encompasses all or part of nine Townships up-gradient of the City of Rochester [66], an Urban Service Area measuring 10,259 hectares with a total of 46,636 households and 115,231 residents [67]. The City of Rochester, population 100,845 (41,403 households), lies in the valley below the confluence of the six sub watersheds that are the subject of the SFZR study. Rochester is the home of the Mayo Clinic, a world renowned medical center boasting over 1,500 physicians and scientists, 500,000 annual visitors and 300,000 annual patient visits to the Clinic and three hospitals that have 2,136 hospital beds [68]. Due to the large number of patients, transient lodging is a major industry in the Rochester area.

The City of Rochester is served by a centralized sewer system that reflects the local watersheds and a recently expanded waste water treatment plant and water reclamation plant (WWTP) that discharges 49.2 million liters/day of treated effluent from a single outfall into the SFZR down gradient of the City. The adjoining suburban and agricultural areas rely on in-ground individual septic treatment systems (ISTS). The entire region relies on groundwater for the potable water supply that is susceptible to contamination.

A 2004 Scientific Investigation Report published by the United States Geological Survey (USGS), “Presence and Distribution of Organic Wastewater Compounds (OWC’s) in Waste Water, Surface, Ground and Drinking Waters, Minnesota, 2000-2002” employed five analytical techniques to identify 91 OWC’s across Minnesota. The USGS and cooperators sampled the effluent of the Rochester WWTP where they identified 19-25 OWC compounds that displayed temporal changes in the compounds and concentrations. No wastewater influent sampling was conducted in Rochester. The USGS sampled four surface water sites on the SFZR in the City of Rochester. Samples were taken upstream and downstream of the water reclamation plant and in one up gradient tributary, Bear Creek [3]. The USGS analysis identified the presence of OWC’s in all surface water samples, including 19 OWC compounds from surface water up gradient of the WWTP, 12 to 24 OWC’s down-gradient of the WWTP and three OWC’s in Bear Creek.

Landscape and Hydrogeology

The Rochester area is characterized as a mature landscape dissected by a dendritic drainage network flowing to the Zumbro River. Deeply dissected stream valleys cut through glacial sediments and Paleozoic age bedrock that consist of interbedded carbonates, sandstone and shale. The ground elevation in the study area falls from 415 meters above Mean Sea Level at the headlands to the south and west and falls to 297 meters down gradient of Rochester near the WWTP outfall.

The SFZR study encompasses the Rochester Plateau Ecological Classification System that defines the eastern edge of the glaciated plain, the northern extent of loess deposits, and the eastern edge of the bedrock controlled karst bluff lands. The uplands and headwaters in the east half of the study area are bedrock controlled karst hills and bluff lands with thin loess and colluvium soils. Precipitation infiltrates rapidly into the bedrock recharging the groundwater. Base flow of the surface waters is sustained by groundwater discharge from the Paleozoic bedrock aquifers but seasonal flood flows are controlled by melt water and storm water runoff. The headwaters and uplands to the west are a more gently rolling till plan with loess covered, pre-Wisconsin age glacial till that forms a thick soil mantle over the buried bedrock. On the west

the base flow of the surface waters are sustained by shallow confined alluvial aquifers and the discharge from agricultural drain tile.

The surface and groundwater hydrology are influenced by the bedrock aquifers and the presence of a 12-meter thick shale-confining unit, the Decorah shale that separates the Upper Carbonate Aquifer from the deeper St. Peter-Prairie du Chein and Jordan aquifers. The Upper Carbonate aquifer has a plentiful water supply that is susceptible to contamination and was largely abandoned as a potable water source in the late 1950's due to high levels of nitrate and bacterial contamination. Studies by the USGS [69] suggest that the Upper Carbonate Aquifer recharges the lower aquifers at the terminal edge of the Decorah shale within the SFZR study area. The USGS estimated that about half of Rochester's drinking water recharge occurs on the Decorah edge.

Within the SFZR study area, small springs, seeps, wetlands and calcareous fens, often on side hills and slopes at the top of the impervious Decorah Shale, are the surface manifestation of groundwater and surface water interaction and cause cold-water discharge that forms the headwaters of the SFZR. On the western glaciated plain, wetlands do occur in floodplains and once occurred as prairie potholes. The western base flow is from water stored and discharged from the glacial-alluvial units in the till.

Climate

The study area is in the interior climate region of North America characterized by cold winters and mild summers with greatly variable daily and weekly temperature extremes. The area receives an average annual rainfall of 30 inches, mean summer temperature of 20.1 °C and mean winter temperature of -8.8 °C.

The United States Geological Survey estimates that 25% of the annual precipitation infiltrates and recharges the groundwater aquifers [4].

Collection of Water Samples

The study area consists of the following sub-watersheds within the Zumbro River Watershed: (1) Cascade Creek, (2) two sites of the South Fork Zumbro River, (3) Willow Creek, (4) Badger Run, (5) Bear Creek, and (6) Silver Creek (Figure 2). The confluence of all of the watersheds is centrally located within the Rochester City Limits, where each sub-watershed's dominant land uses provide the opportunity to investigate CECs in surface waters from a variety of agricultural landscapes. Water samples (5-40L) will be collected three times a year (winter, spring and late summer) over the two year project period from the sub-watersheds up-gradient of the Rochester WWTP, and one from the SFZR down-gradient of the WWTP.

Surface water samples will be collected in accordance with the protocols and procedures as outlined in the USGS National Field Manual for the Collection of Water-Quality Data [70]. Sample collection techniques, quality control/quality assurance, contaminant prevention, sample preservation and handling, chain-of-custody procedures will be conducted in accordance with USGS and University of Minnesota protocol. All samples will be collected in inert materials such as Teflon, fluorocarbon polymers, glass, aluminum or stainless steel. This team has extensive experience in large and small volume field water collections.

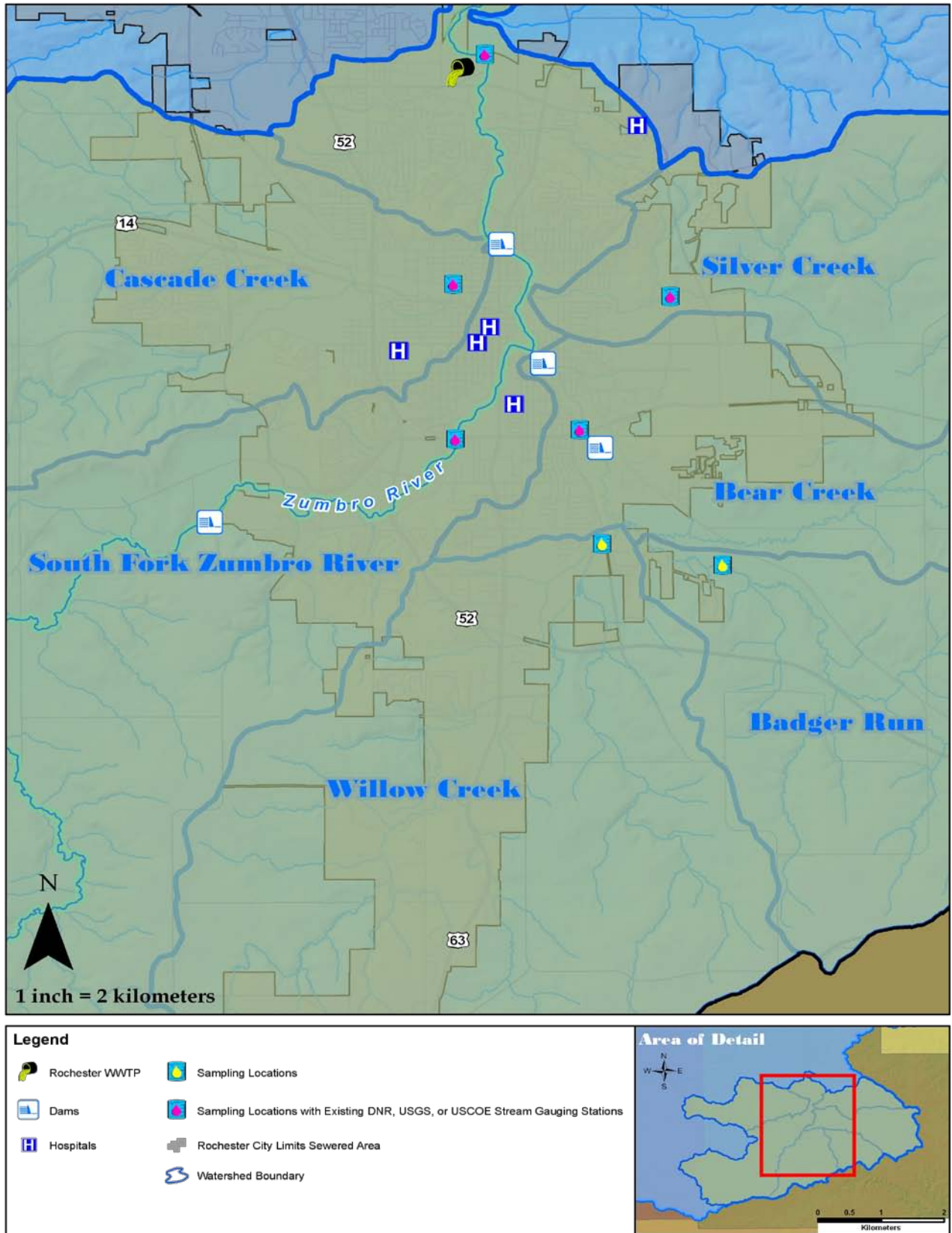


Figure 2. Location of sampling sites

In order to determine the natural condition of the surface water samples at the time of sampling and the profile of variability across each stream section the following water quality parameters will be assessed *in situ*: 1) temperature, 2) dissolved oxygen, 3) pH, 4) turbidity, 5) nitrate, 6) chloride, and 7) ammonia using a portable multi-parameter instrument (Hydrolab Sonde). The multi-parameter instrument will be calibrated and tested before each field use according to the manufacturer's specifications and thoroughly cleaned between each sampling location. Equipment, field and source solution blanks will be routinely collected for Quality Assurance/Quality Control purposes.

Stream Flow

For selected sites (Cascade Creek, South Fork Zumbro River (HWY 14, 37th St Bridge and downstream WWTP), Bear Creek and Silver Creek), stream flow data will be obtained from existing USGS, DNR, or MPCA stream gauging stations. On the two streams without existing gauging stations, stream flow measurements will be recorded concurrently with water sampling using current meters calibrated to the stream cross sections.

Sample Collection and Processing

We will initially collect small volume samples (1 – 2 L) to test our detection limits for this study. Water will be pumped from the subsurface into cleaned containers, and refrigerated for return to the laboratory. Samples will be refrigerated during transfer from Rochester to the University of Minnesota. Prior to water analysis, total suspended solids will be removed from each water sample by filtering through a glass-fiber filter (0.7 μm) to separate the operationally defined dissolved- and particulate-phase [60].

In the case that larger volumes are needed for detection purposes, we will use an Infiltrax large volume high pressure automated sampler (Axys, Vancouver, British Columbia, Canada). Water is pumped through a glass fiber filter cartridge and then through absorbent resin (XAD) to capture dissolved phase contaminants. The resin will be refrigerated and returned to the laboratory for analysis. Up to 100 L can be processed using this system.

Analyte Selection

Although hundreds of compounds are associated with both residential and agricultural use (i.e. pharmaceuticals and antibiotics with human and veterinary use, pesticides with urban and agricultural application, hormones common to humans and animals) we have selected compounds that are exclusive to agricultural or non-agricultural sources as well as other expected compounds. A list of our initial targeted compounds is provided in Table 2.

Analyte Extraction and Analysis

HPLC-grade solvents (i.e. acetonitrile, methanol, and water) will be used. Analyte standards will be obtained from commercial sources such as Chem Service. Analytical methods utilized to measure the various analytes will be those previously developed in our laboratory [71-73] or adapted from recent literature [1, 10, 11, 13, 54]. Briefly, target compounds and added surrogate compounds will be extracted from the filtered water using two basic extraction methods with minor modifications depending on the analyte: solid-phase extraction (SPE) or liquid-liquid extraction (LLE), followed by appropriate solvent reduction. Samples will be extracted as a set of

11 environmental samples, one duplicate sample, two fortified ultrapure water spikes (check standards), and two ultrapure water blanks.

For SPE, an amount of surrogate chemical will be added to 500- to 1000-mL of filtered water samples and the sample pH will be adjusted to 3 using concentrated H₂SO₄. The sample will be passed through either a preconditioned Oasis Hydrophilic-Lipophilic-Balance (HLB) cartridge (60-500 mg, depending on chemical of interest), or a HLP cartridge that will be placed on top of a mixed mode, HLB-cation exchange (MCX) cartridge (60 mg) (Waters Inc., Milford, MA) (operating in tandem). The HLB and MCX cartridges will be conditioned with CH₃OH, CH₃OH with 5% NH₄OH, and distilled H₂O. After the sample is passed through the SPE cartridges, the HLB cartridges will be eluted with CH₃OH, followed by acidic CH₃OH for selected chemicals, and the MCX cartridges will be eluted separately using CH₃OH with 5% NH₄OH. This SPE procedure has been automated using a Zymark Autotrace system. The methanol extracts will be evaporated just to dryness under nitrogen at 45 °C using a Zymark Turbovap.

For LLE, an amount of surrogate chemical will be added to 500- to 1000-mL of filtered water samples and the sample pH will be adjusted to 3 using concentrated H₂SO₄. The sample will then be extracted multiple times using dichloromethane (DCM) (4:1 sample :DCM v:v). The DCM extracts will be evaporated just to dryness under nitrogen at 45 °C using a Zymark Turbovap, then redissolved in internal standard spiked solvent appropriate for analysis by analyzed by capillary-column GC/MS. A portion of the LLE extracts will be derivatized by the formation of trimethylsilyl (TMS) ethers of the hydroxyl groups and oximes of the keto groups, which enhances the analysis of steroid and hormone compounds by GC/MS. The samples will be reanalyzed after derivatization.

The XAD from any high volume samples will be extracted by Soxhlet extractor for 24 hours using dichloromethane, following a 4 hour extraction in acetone. The combined extract is then handled as described above for the LLE samples.

Depending on the analyte, analysis of the extracted chemicals will be by a Waters Alliance high performance liquid chromatography/mass spectrometry with electrospray interface operating in positive-ion (LC/MS-ESI(+)), or by an Agilent 6890 gas chromatograph with capillary column coupled to a 5973 mass selective detector (GC/MS) operating in selected ion mode. LC and GC operating conditions (i.e. column specifications, carrier gas and mobile phase details, etc.) and MS operating conditions and masses monitored are detailed in the cited references. Each sample will have a surrogate and internal standard which will also be analyzed. Positive identification of a compound will be determined by peaks with a signal-to-noise ratio of 3:1 in the sample matrix eluting within the expected retention time (RT ± 5%) and having the presence of the confirmation ion(s) within ± 20% of the ratio of the qualifying ion(s) as found in the reference standard. Concentrations of the target compounds will be calculated from 5 point calibration curves utilizing internal standard quantification.

Table 2. Emerging contaminants and their source.

	Source			
	Agriculture		Residential	
Pharmaceuticals and Antibiotics	Animal	Plant	Human	Plant
Acetaminophen			X	
Caffeine			X	
Cotinine			X	
Erythromycin-H ₂ O (metabolite)	X		X	
Monensin	X			
Sulfamethoxazole	X		X	
Trimethoprim	X		X	
Tylosin	X			
Virginiamycin	X			
Hormones and Steroids				
17 α -ethynyl estradiol (EE2)	X		X	
17 α -estradiol	X		X	
17 β -estradiol (E2)	X		X	
17 α -trenbolone	X			
17 β -trenbolone	X			
Cholesterol	X		X	
Coprostanol	X		X	
Estriol	X		X	
Phytoestrogens				
Daidzein	X	X	X	X
Equol	X	X	X	X
Formonetin	X	X	X	X
Genistein	X	X	X	X
Personal Care, Household and Industrial Products				
4-Nonylphenol			X	
N,N-diethyl-meta-toluamide			X	
Triclosan			X	
Pesticides				
Acetochlor		X		
Atrazine		X		
Carbaryl		X		X
MCPP, dimethylamine salt				X
Iprodione				X

The water composition of stream samples will be determined a Perkin Elmer Optima 300DV dual view inductively coupled plasma spectrograph (ICP), which has the capability to measure 74 different elements. A Dohrmann Carbon Analyzer will be used for the analysis of total organic carbon in a water sample. An Alpkem RFA 300- Rapid Flow Analyzer will be used for low level anion analysis, including nitrate-N, nitrite-N, ammonia-N, total Kjeldahl N, total phosphorus, ortho-P, and chloride. Standard methods used by the Research Analytical Laboratory in the Department of Soil, Water & Climate, University of Minnesota will be followed.

Potential Pitfalls

We have proposed a list of chemical markers based on local land use and contaminant profiles reported in state and national surface water assessments. It is possible we will not detect the anticipated contaminants in our river samples. The analytical methodology outlined in the proposed research allows for the detection of a large suite of compounds. Therefore if the proposed chemical markers are not found we will have identified other contaminants that can be used in place of the original marker chemical.

Limitations to Proposed Procedures

The cost of sample collection and analysis limits the proposed research to examination of contaminants in the dissolved phase. Extraction and analysis of filtered particulates and the addition of bed sediment samples would allow for the evaluation of contaminants associated with the particulate phase. Particulate samples will be archived for potential future study.

F. Interpretation of Results, Expected Outcomes

Results will be interpreted using the diverse expertise and experience of our research team. We anticipate the detailed characterization of contaminant concentrations and temporal occurrences for each river will reflect the surrounding land use and provide contaminant signatures associated with specific land uses. We will develop a suite of chemical markers that can be utilized by scientists, producers, extension specialists, and natural resource managers to identify problems and develop and implement management and mitigation strategies. In addition, the information will be valuable to regulators who might need to categorize these pollutants and establish water quality standards.

G. Dissemination and Use

We will publish these results in the peer-reviewed literature, and distribute the results to policy makers, regulators, and water managers throughout the state. We will offer to hold workshops for interested parties in the potential use and applicability of the resulting tool (e.g. at MPCA, MDH, MDA). We will brief the LCCMR on the outcomes and utility of the project, and also present the findings at the annual Water Resources Conference attended by more than 650 water professionals from around the state. Also, results of this project will be available on the Water Resources Center website, prepared in a lay-technical style for widest dissemination and use by the broader technical community.

Finally, as part of this project we will hold a workshop to engage policy makers, resource managers, and the regulatory community in discussions of identifying and evaluating strategies for reducing sources, mitigating sources, and remediating sources of CECs to surface waters. The

scientific basis of this discussion will be founded in the source determination research from this project. The outcome of the workshop will be a series of recommendations for policy makers.

H. Proposed Timeline

Collection, extraction, and analytical methods will all be verified prior to sample collection. Particular attention will be paid to the volume of water needed to provide satisfactory results. Collection of samples will begin in fall of 2010 and proceed through fall of 2012. Sample processing and analysis will begin immediately following the collection of the initial water samples and proceed continuously until completed. Data analysis and interpretation will begin upon receipt of analytical results and continue until the completion of the project in June 2013. The workshop for policy makers will occur in winter 2013. Progress and final reports will be prepared and delivered to LCCMR as required. We anticipate preparing a peer-reviewed manuscript on the occurrence, and temporal and spatial distributions of the CECs from the study, and a peer-reviewed manuscript on the development of the signature of chemical markers that can be used for source determination.

I. Credentials of Investigators and Role in Project

Deborah Swackhamer, PhD (Professor and Charles M. Denny Chair, Humphrey Institute of Public Affairs; Professor, Environmental Health Sciences; Co-director, Water Resources Center, University of Minnesota). Dr. Swackhamer is an expert in the analysis, and transport and fate, of organic environmental contaminants. As Principle Investigator (PI), she will oversee project progress and administration; assist with data interpretation and synthesis, and lead the team in the development of chemical markers for source determination. She will be responsible for the preparation and delivery of all required progress and final reports, and will participate in the development and publication of peer-reviewed manuscripts.

Pamela Rice, PhD (Adjunct Assistant Professor, Department of Soil, Water, and Climate, University of Minnesota and Scientist with Agricultural Research Service of the USDA). Dr. Rice is an environmental analytical chemist and toxicologist. She will co-coordinate sample extraction; i.e. she will be responsible for the extraction of organic analytes from water. She will help coordinate sample analysis, data analysis and interpretation, provide expertise on plant and animal protection products, provide interpretation of toxicological significance of measured residues, and participate in the development and publication of peer-reviewed manuscripts.

William Koskinen, PhD (Adjunct Professor, Department of Soil, Water, and Climate, University of Minnesota and Senior Scientist with Agricultural Research Service of the USDA). Dr. Koskinen is an analytical chemist, specializing in agricultural chemical analysis. He will provide expertise in analytical sample analysis. He will be responsible for organic and inorganic instrumental analyses. He will help co-coordinate sample extraction, and provide expertise on plant and animal protection chemicals. He will assist in student advising, data interpretation and analysis and participate in the development and publication of peer-reviewed manuscripts.

Paige Novak, PhD (Associate Professor, Department of Civil Engineering, University of Minnesota). Dr. Novak is an environmental microbiologist and environmental engineer. She will provide expertise on wastewater contaminants, particularly endocrine disrupting chemicals and phytoestrogens. She will assist in student advising, data interpretation and analysis, and participate in the development and publication of peer-reviewed manuscripts.

William Arnold, PhD (Associate Professor, Department of Civil Engineering, University of Minnesota). Dr. Arnold is an environmental engineer with expertise on wastewater contaminants, particularly antibiotics and personal care products. He will assist in student advising, data interpretation and analysis, and participate in the development and publication of peer-reviewed manuscripts.

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Attachment A: Budget Detail for 2010 Projects											
Project Title: Understanding Sources of Aquatic Contaminants of Emerging Concern											
Project Manager Name: Deborah Swackhamer											
Trust Fund Appropriation: \$ 640,000											
1) See list of non-eligible expenses, do not include any of these items in your budget sheet											
2) Remove any budget item lines not applicable											
2010 Trust Fund Budget	<u>Result 1 Budget:</u>	Amount Spent <i>(date)</i>	Balance <i>(date)</i>	<u>Result 2 Budget:</u>	Amount Spent <i>(date)</i>	Balance <i>(date)</i>	<u>Result 3 Budget:</u>	Amount Spent <i>(date)</i>	Balance <i>(date)</i>	TOTAL BUDGET	TOTAL BALANCE
	Characterization of CECs from different land uses			Development of "source signature" for CECs in water			Development of recommendations to state policy-makers				
BUDGET ITEM											
PERSONNEL: wages and benefits											
Deborah Swackhamer, PI (4%)	\$ 9,210			14,230			5,102				
Paige Novak (4%)	7,750			11,974			4,293				
William Arnold (4%)	6,848			10,580			3,793				
Brian Barber (50%)	98,174			0			0				
3 graduate students (50% each)	97,718			150,975			54,536				
Contracts											
Professional/technical (for hydrology and GIS; TBD by RFP)	50,000										
Other direct operating costs (instrumental costs, workshop costs)	20000			20,000			3,000				
Printing	400						297				
Supplies (sampling, laboratory)	68,000										
Travel expenses in Minnesota	3,120										
COLUMN TOTAL	\$361,221	\$0	\$361,221	\$207,758	\$0	\$207,758	\$71,021	\$0	\$71,021	\$0	\$0