Estrogenic and Pharmaceutical Septic System Discharge to Lakes

RICHARD L KIESLING [1], HEIKO L. SCHOENFUSS [2], PAUL SWEDNEBORG[3] and CARIN HUSSET[3]

1 U.S. Geological Survey, Mounds View, Minnesota 55112
2 Dept. of Biological Sciences, Saint Cloud State University, Saint Cloud, MN 56301.
3 Minnesota Department of Health, Saint Paul, MN 55164

SUMMARY:
Pharmaceuticals and endocrine active compounds, including estrogenic compounds, were found in water and sediment of 12 Minnesota lakes as part of a recent study by USGS and St. Cloud State University in cooperation with the Minnesota Pollution Control Agency. Lakes with a high density of septic systems had the most frequent detections of these chemicals. Low-levels of estrogenic compounds in lakes have caused the extinction of species of forage fish and are known to cause abnormal sexual development in bass and walleye in Minnesota Rivers. Given these results, there is a need to know how estrogenic and pharmaceutical compounds in Minnesota lakes affect fish populations. To answer this question, the USGS Minnesota Water Science Center, in partnership with St. Cloud State University and the MN Department of Health, will survey 20 additional Minnesota lakes with high densities of septic systems for water and sediment contamination from pharmaceuticals and estrogenic compounds. Each lake in the study will also be assessed for watershed and groundwater hydrology characteristics that may contribute to the observed patterns of water and sediment concentrations of estrogenic and pharmaceutical compounds. A subset of lakes with high contaminant concentrations will be chosen for detailed analysis of exposed fish populations, and native fish populations will be assessed during the spawning season for endocrine disruption and mating success. The project will provide details on what pharmaceutical and estrogenic compounds are present in lakes and whether these compounds affect the native fish populations in these same lakes.

PROBLEM:

Pharmaceuticals (Phac) and endocrine active compounds (EACs), including estrogenic compounds, were found in water and sediment of 12 Minnesota lakes as part of a recent study by USGS, MPCA, and St. Cloud State University (Writer and others, in review). Lakes with a high density of septic systems (i.e., onsite SSTS) had the most frequent detections of these chemicals (Ferrey and others, under review) and recent work has identified on-site septic systems as potential sources of emerging contaminants to lakes (Carrara and others, 2008; Godfrey and others, 2007) at concentrations capable of causing ecosystem stress such as reproductive failure of short-lived species of fish (Kidd and others 2007). Detailed studies of septic system performance have shown how Phac and EAC compounds survive treatment and discharge into shallow groundwater. Given these results, there is a need to know how vulnerable Minnesota lakes are to septic discharge of Phacs and EACs. We propose to survey Minnesota lakes with a high-density of septic systems for water and sediment contamination from Phacs and EACs. For a subset of these lakes, we will assess groundwater conditions and watershed characteristics that could influence contaminant distributions and assess adverse biological impacts associated with the presence of these compounds.

BACKGROUND:

Recent results from whole-lake experimental exposures to endocrine disruptors have documented complete reproductive failure of short-lived species of fish (Kidd and others 2007), raising questions about the effects of these compounds on longer-lived species of fish common to Minnesota lakes. Data from a statewide survey of endocrine disruption in Minnesota lakes have found biomarkers of endocrine disruption in fathead minnows and bluegills fish from urban storm-water lakes and rural septic-system lakes receiving EACs and Phacs (Schoenfuss and others, unpublished data) raising questions about the source of the stressors and the nature of the fish response. Given the large number of lakes in Minnesota that are potentially receiving emerging contaminants from septic tank discharge or nonpoint sources, there is a need for relevant data on how fish populations in lakes will respond to EAC exposure. An ongoing mesocosm study by Kiesling, Schoenfuss and Gaikowski is investigating the endocrine and reproductive responses of

representative fish species (i.e., fathead minnow, bluegill sunfish, and walleye pike) under controlled exposure to a known endocrine disruptor (17-beta estradiol). The work outlined below will extend the results from the mesocosm study to wild populations of one of these fish species (i.e., bluegill sunfish) by measuring contaminant occurrence, endocrine response, and reproductive success of the target fish species. The work will also build on the results from the preliminary survey of 12 Minnesota.

OBJECTIVES AND SCOPE:

Objective 1: Quantify the occurrence of estrogenic or pharmaceutical compounds in 30 Minnesota lakes that receive groundwater inputs from septic systems.

Pharmaceuticals and EACs will be measured in water and sediment from 20 additional Minnesota lakes, and the results will be combined with the results from the 12 lakes sampled as part of the Statewide Survey. Lakes chosen will have significant numbers of septic systems (>35% shoreline development) and will be distributed among the hydrologic and ecological regions of Minnesota. Sampling will follow the general protocol from the recently completed Statewide Survey with MPCA, with site selection and sample distribution designed to follow gradients in groundwater and surface water hydrology within and between lakes. Results from the proposed survey will be combined with the results from the recently completed Statewide Survey to develop a geo-spatial EAC contaminant database for Minnesota lakes. The database will be used to analyze patterns in contaminant occurrence relative to watershed characteristics.

Objective 2: Assess the hydrology and ecology of the watershed contributing to water or sediment concentrations of estrogenic/pharmaceutical compounds in the surveyed lakes.

Each Minnesota lake sampled under Objective 1 will be classified by geologic, hydrologic, and ecological characteristics. Groundwater hydrology, including depth to groundwater, and groundwater contribution to lake water balance, determined using $\delta^{18}O$ and $\delta^{2}H$ isotopes, will be coupled with surface water hydrology (contributing area, morphometry) and watershed characteristics (land use, land cover; fragmentation) to

develop categorical classifications for each lake. Groundwater level data will be collected for each lake in the vicinity of sampling sites, and seasonal (three times per year) groundwater isotope sampling in each lake will provide an estimate of groundwater contribution to water balance.

Objective 3: Assess biological exposure and response to known estrogenic and pharmaceutical compound contamination in Minnesota lakes.

A subset of the 20 lakes sampled under Objective 1 will be chosen for detailed biological analysis based of the severity of contamination. Adult bluegill sunfish will be sampled during the spring reproductive period from active nesting areas in each lake. Nesting areas will be associated with developed and undeveloped shorelines. Adult male and female fish will be evaluated using a variety of biomarkers, including condition factors, blood-chemistry (e.g., plasma vitellogenin and reproductive hormones) and histo-pathological indices of abnormal gonad development in fish (e.g., intersex). Nesting sites will be evaluated for selected Phac and EAC residues in food-web components at the time of active reproduction. Samples from major trophic levels including adult bluegill will also be collected for stable isotope analysis to determine bluegill food web structure during the nesting period.

Individual fish will be marked or tagged during the spawning period using Passive Integrated Transponder (PIT) tags. These tags will allow us to track individual fish movements through the spawning beds, and to positively identify recaptured individuals for re-sampling throughout the spawning season. As described by the manufacturer, PIT tag microchips remain inactive until read with a scanner. The scanner sends a low frequency signal to the microchip within the tag providing the power needed to send its unique code back to the scanner and positively identify the animal. Detection grids are set-up using flat plate antennas up to 20 feet in length. Fish can be detected up to 18 inches above the antennas making this design ideal for fish oriented to bottom substrates like spawning bluegill sunfish. Passive tags are designed to last the life of the animal providing a reliable, long term identification method.

Detailed data on the tagged fish will be collected during the spawning period. Effects of EACs on reproductive success (i.e, mating behavior and spawning success) will be measured in a two-way, balanced design using replicate, tagged fish from septic-influenced and control spawning beds within the same lake. The sampling design will be replicated between lakes. Water and sediment concentrations will be monitored multiple times during the spawn using ELISA chemical assay kits. For a sub-set of tagged fish, gene transcription microarrays will be used to assess gene activation associated with chemical exposure. Individual fish will be evaluated for cyclical hormone activity and blood-chemistry biomarkers (e.g., plasma vitellogenin) during the spawn (e.g., Knapp and Neff 2007). All fish sampled will be evaluated for histo-pathological indices of abnormal reproductive physiology before and after spawning where possible and appropriate.

Objective 3: Enhance EAC analytical capabilities at the Minnesota Department of Health (MDH).

MDH currently has the capability to quantify a number of Phacs and organic compounds including the EACs bisphenol A, nonyl phenol (NP), and octyl phenol (OP). As part of this project, MDH will enhance its existing Phacs and EAC methods while implementing advanced laboratory techniques to quantify NP precursors. All MDH Phac and EAC analytical capabilities will be used to analyze water samples from the 20 Minnesota lakes in this study. The following compounds will be analyzed by MDH in water samples collected from the spawning beds:

Analyte	Use
fluoxetine	antidepressant
norfluoxetine	antidepressant
sertraline	antidepressant
norsertraline	antidepressant
paroxetine	antidepressant
citalopram	antidepressant
fluvoxamine	antidepressant
duloxetine	antidepressant
buproprion	antidepressant
venlafaxine	antidepressant

carbamazepine	epilepsy drug
sulfamethoxazole	anti-infective
triclosan	anti-microbial
bisphenol A	used in plastics
methyl paraben	Preservative
ethyl paraben	Preservative
propyl paraben	Preservative
butyl paraben	Preservative
benzyl paraben	Preservative
benzophenone	sunscreen agent
benzotriazole	corrosion inhibitor
5-methyl benzotriazole	corrosion inhibitor
octyl phenol	surfactant breakdown
nonyl phenol	surfactant breakdown

RELEVANCE AND BENEFITS:

In Minnesota, endocrine disruption has been observed in short- and long-lived fish species including vitellogenin induction in male fathead minnows, male carp, and walleye (Folmar and others, 1996, 2001; Lee and others, 2000). Vitellogenin in male carp was also observed at numerous sites downstream of WWTP discharges throughout central Minnesota (Lee and others, 2000). Two ongoing studies in Minnesota have recently identified additional fish species affected by EACs in tributaries of the Mississippi and the St. Croix Rivers (Jahns and others in prep; Lee and others in review) as well as urban lakes (Schoenfuss and others – unpublished data). Taken as a whole, these results indicate that Minnesota fish communities are vulnerable to reproductive impacts from EACs. This study helps answer how vulnerable a common lake species is to EAC exposure during spawning in lakes across Minnesota. The study takes advantage of the specific life-history characteristics of bluegill sunfish to investigate the impact of EAC exposure on the spawning activity and reproductive output. As a whole the study will provide the following benefits:

1. Most EACs are found at very low concentrations in water but reach higher concentrations in sediment. Despite these low concentrations, research has identified developmental and reproductive effects on fish species at environmentally relevant concentrations. The proposed work will determine if lakes under the influence of septic

systems are at risk for significant contamination from Phacs and EACs while providing details on what pharmaceutical and estrogenic compounds are present in lakes with high numbers of SSTS systems.

2. In Minnesota, pharmaceuticals and EACs have been observed in a range of lake types from a diverse set of background conditions. This study will provide a comprehensive analysis of the frequency and magnitude of contamination relative to important factors including hydrology and geology, groundwater hydrology and watershed characteristics.

3. Three recent studies in Minnesota indicate river and lake fish communities are vulnerable to reproductive impacts from EACs. The proposed project uses a representative fish (bluegill) to determine how vulnerable adult fish are to pharmaceutical and estrogenic compounds exposure during spawning. The study assesses the role of food-web structure and function when measuring the ecological characteristics that might mitigate EAC exposure while providing an estimate of how pharmaceuticals and estrogenic compounds affect biological communities, and whether effects are limited in space and time.

APPROACH:

Sample Collection Methods.

All samples will be collected using protocols and procedures to obtain a representative sample and avoid sample contamination. Specific protocols and methods are documented for the collection and processing of water-quality and sediment samples (U.S. Geological Survey, 2006). All samples will be collected with inert materials such as Teflon, glass, or stainless steel. A multi-parameter probe will be used to measure field parameters (specific conductance, pH, water temperature, and dissolved oxygen) at each site.

All collection and processing equipment will be cleaned between samples with a succession of native water, soapy tap water, tap water, de-ionized water, methanol, and organic-free water rinses. To avoid contamination of samples, use of personal care items

(such as insect repellent, sunscreen, cologne, aftershave, and perfume) will be avoided for personnel collecting and processing samples. Caffeinated products and tobacco products will not be consumed during (or immediately prior to) collection or processing of samples. Powder-less, disposable gloves will be worn during bottom-sediment sample collection to avoid contamination of samples. Standard labeling and packing techniques (double foam sleeves placed around bottles) will be used to ensure sample integrity. When not in use, sample processing equipment will be stored in double-bagged clean polyethylene bags.

Water samples will be split into two chemical and one bioassay fractions. The chemical aliquots of the water sample will be sent to USGS laboratories for analyses of nutrients, major ions, EACs, hormones, and pharmaceuticals and to the Minnesota Department of Health lab for analyses of serotonin inhibitors and selected endocrine disruptors. (Table 1). Major ions and nutrients will be analyzed at the USGS–NWQL using standard analytical techniques described in Fishman and Friedman (1989), Patton and Truitt (1992), Fishman (1993), and Fishman and others (1994). Samples analyzed for dissolved major-ion and nutrient concentrations will be filtered using 0.45-µm-pore-size encapsulated filters. Nutrient samples will be preserved and maintained at 4°C until analyzed at the USGS–NWQL. Samples analyzed to determine total nutrient concentrations will not be filtered. Dissolved phosphorus will be analyzed using U.S. Environmental Protection Agency method 365.1, low-level persulfate digestion. Alkylphenol, nonylphenol ethoxy carboxylates, wastewater indicators, and hormones will be analyzed according to Barber and others (2000); Barber, Furlong, and others (2003); and Barber, Keefe, and others (2003). Other wastewater indicators will be analyzed at the USGS NWQL using methods outlined in Zaugg and others (2002, 2006).

Bottom sediment will be collected once at each site using techniques to obtain the most recent bottom sediment deposition (top 5 cm of bottom sediment). At least 5 bottom sediment samples will be composited for each sample site. Bottom-sediment samples will be collected according to established protocols (U.S. Geological Survey, 2006). Samples will be collected with a stainless steel Eckman grab sampler or other stainless steel coring

equipment. The bottom-sediment sample will be discarded if it contains a large amount of vegetation or appeared to be disturbed. Bottom-sediment samples will be transferred to a glass container and homogenized for 5 minutes, and 500ml grams of unsieved wet material will be placed in wide-mouth, glass containers, chilled, and sent to the appropriate USGS Laboratory.

Briefly, the method used to analyze bottom sediment is an accelerated solvent extraction (ASE), using either Soxhlet extraction or pressurized solvent extraction (PSE), followed by solid-phase extraction (SPE) for sample preparation, with analysis by LC/mass spectrometry or capillary-column gas chromatography/mass spectrometry. Both methods identify and quantify individual compounds using retention times and spectral matches, along with standard calibration curves. Compounds included are a group of antibiotics and other pharmaceuticals and a broad group of emerging contaminants including alkylphenol ethoxylate nonionic surfactants and their degradates, food additives, fragrances, antioxidants, flame retardants, plasticizers, industrial solvents, and disinfectants. Interim method detection limits for the 61 individual compounds range from about 12 to 850 ug/kg. Many of the individual compounds have an interim method reporting level of 50 ug/kg.

Quality Assurance (QA) Plan

All data collection and sampling will follow established scientific methods including the use of experimental replication and published analytical methods as outlined in the Workplan. Chemical analyses will include the use of blanks, replicates, spikes, and standards as outlined in the Minnesota WSC QA/QC document and in the individual SOPs for each analytical method.

PERSONNEL:

This project is a continuing partnership between the United States Geological Survey (USGS) and Dr. Heiko L. Schoenfuss, Professor and Director of the Aquatic Toxicology Laboratory, Department of Biological Sciences, St. Cloud State University, with the addition of team members from the MN department of Health and Dr. Dalma Martinovic

from St. Thomas University. USGS personnel include Dr. Richard Kiesling (project Leader) with Kathy Lee and Dr. Mindy Erikson. Team members from the USGS will manage lake sampling and characterization, chemical analysis, and n-lake spawning experiments. Team members from St. Cloud State University (Schoenfuss plus a post-doctoral research fellow and graduate student) will manage histopathology analysis. All team members will participate in writing the final report and communicating results to state user groups.

PRODUCTS

Details of results will be available as a final project report to LCCMR in the form of a USGS data series report and a scientific journal article manuscript. Results will be communicated to local groups, state agencies and national peer groups through presentations at regional and national meetings including state resource management meetings.

REFERENCES:

Barber, L.B., Brown, G.K., and Zaugg, S.D., 2000, Potential endocrine disrupting organic chemicals in treated municipal wastewater and river water, chap. 7 of Keith, L.H., Jones-Lepp, T.L., and Needham, L.L., eds., Analysis of environmental endocrine disruptors: Washington, D.C., American Chemical Society, American Chemical Society Symposium Series 747, p. 97–123.

Barber, L.B., Furlong, E.T., Keefe, S.H., Brown, G.K., and Cahill, J.D., 2003, Natural and contaminant organic compounds in the Boulder Creek watershed, Colorado, during high-flow and low-flow conditions, 2000, chap. 5 of U.S. Geological Survey Water-Resources Investigations Report 2003–4045, p. 103–144.

Barber, L.B., Keefe, S.H., Brown, G.K., Taylor, H.E., Antweiler, R.C., Peart, D.B., Plowman, T.I., Roth, D.A., and Wass, R.D., 2003, Organic and trace element contaminants in water biota, sediment, and semipermeable membrane devices at the Tres Rios Treatment Wetlands, Phoenix, Arizona: U.S. Geological Survey Water-Resources Investigations Report 03–4129, 89 p.

Carrara, C.; Ptacel, C.J.; Robertson, W. D.; Blowes, D. W.; Moncur, M. C.; Sverko, E.; and Backus,S. 2008. Fate of Pharmaceutical and Trace Organic Compounds in Three Septic System Plumes, Ontario, Canada. Environ. Sci. Technol. 2008, 42, 2805–2811

Fishman, M.J., and Friedman, L.C., 1989, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A1, 545 p.

Fishman, M.J., and Friedman, L.C., 1989, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A1, 545 p.

Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory--Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p.

Fishman, M.J., and Friedman, L.C., 1989, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A1, 545 p.

Folmar, L. C.; Denslow, N. D.; Kroll, K.; Orlando, E. F.; Enblom, J.; Marcino, J.; Metcalfe, C.; Guillette, L. J. Jr. Altered serum sex steroids and vitellogenin induction in walleye (Stizostedion vitreum) collected near a metropolitan sewage treatment plant. Arch. Environ. Contam. Toxicol. 2001, 40, 392-398.

Folmar, L. C.; Denslow, N. D.; Rao, V.; Chow, M.; Crain, D. A.; Enblom, J.; Marcino, J.; Guillette, L. J. Jr. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (Cyprinus carpio) captured near a major metropolitan sewage treatment plant. Environ. Health Perspect. 1996, 104, 1096-1101.

Godfrey, E.; Woessner, W. W.; and Benotti, M. J. 2008. Pharmaceuticals in On-Site Sewage Effluent and Ground Water, Western Montana. Ground Water 45: 263–271.

Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., and Flick, R. W. 2007. Collapse of a fish population after exposure to a synthetic estrogen PNAS 104: 8897-8901.

Kim, S.C., Carlson, K.H., 2007. Quantification of human and veterinary antibiotics in water and sediments using SPE/LC/ MS/MS method. Anal. Bioanal. Chem. 387: 1301-1315.

Knapp, R and B. D. Neff. 2007. Steroid hormones in bluegill, a species with male alternative reproductive tactics including female mimicry. Biol. Lett. 3: 628-631.

Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 2002, 36, 1202-1211.

Lee, K. E.; Barber, L. B.; Furlong, E. T.; Cahill, J. D.; Kolpin, D. W.; Meyer, M. T.; Zaugg, S. D. Presence and distribution of organic wastewater compounds in wastewater, surface, ground, and drinking waters, Minnesota, 2000-02: U.S. Geological Survey Scientific Investigation Report 2004-5138, 2004; pp 47.

Lee, K. E.; Blazer, V. S.; Denslow, N. D.; Goldstein, R. M.; Talmage, P. J. Use of biological characteristics of common carp (Cyprinus carpio) to indicate exposure to hormonally active agents in selected Minnesota streams, 1999. U.S. Geological Survey Water-Resources Investigations Report 00-4202, 2000; pp 47.

Mayer, T.; Bennie, D.; Rosa, F.; Rekas, G. ; Palabrica, V.; and Schachtschneider, J. Occurrence of alkylphenolic substances in a Great Lakes coastal marsh, Cootes Paradise, ON, Canada. Environmental Pollution 147: 683-690

Patton, C.J., and Truitt, E.P., 1992, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory--Determination of total phosphorus by a Kjeldahl digestion method and an automated colorimetric finish that includes dialysis: U.S. Geological Survey Open-File Report 92-146, 39 p.

Pojana, G., Gomiero, A.; Jonkers, N.; and Marcomini, A. 2007. Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environment International 33 (2007) 929–936

U.S. Geological Survey. 2006. National field manual for the collection of water-quality data: Techniques of Water-Resources Investigations, book 9, available at http://water.usgs.gov/owq/FieldManual/, variously paged.

Zaugg, S. D., S. G. Smith, M. P. Schroeder, L. B. Barber, and M. R. Burkhardt. 2002. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry. U.S. Geological Survey, WRIR 01-4186, Denver, Colorado.

Zaugg, S.D., Smith, S.G., and Schroeder, M.P., 2006, Determination of wastewater compounds in whole water by continuous liquid–liquid extraction and capillary-column gas chromatography/mass spectrometry: U.S. Geological Survey Techniques and Methods, book 5, chap. B4, 30 p.

Table 1. Physical properties and chemicals analyzed for in water or bottom sediment samples.

[MRL, minimum reporting level; EDP, endocrine-disrupting potential; K_{ow}, octanol-water partition coefficient; CASRN, Chemical Abstracts Services Registry Number; µS/cm, microsiemens per centimeter at 25 degrees Celsius (°C); FIELD, field sample; mg/L, milligrams per liter; --, not available; NWQL, National Water Quality Laboratory; USGS–NRP, U.S. Geological Survey National Research Program (laboratory); EDTA, ethylenediaminetetraacetic acid; NTA; nitrilotriacetic acid; NPEC, nonlyphenol ethoxycarboxylate; µg/L, micrograms per liter; ng/L, nanograms per liter;; S, suspected; K, known; E or e, remark code estimated concentration reported; F, fungicide; H, herbicide; I, insecticide; GUP, general-use pesticide; FR, flame retardant; WW, wastewater; manuf., manufacturing; %, percent; >, greater than; CP, combustion product; PAH, polycyclic aromatic hydrocarbon; UV, ultraviolet; NA, not applicable; Compounds measured in both water and sediment samples unless otherwise marked].

Property/chemical name	MRL[1]	Log K_{ow}[3]	CASRN[4]	Possible compound uses or sources[5]
Nutrients Analyzed in Water at the USGS National Water Quality Laboratory (mg/L)				
Ammonia plus organic nitrogen, filtered	0.1	--	17778-88-0	Nutrient
Ammonia plus organic nitrogen, unfiltered	0.1	--	17778-88-0	Nutrient
Ammonia as nitrogen	0.02	--	7664–41–7	Nutrient
Nitrite plus nitrate	0.04	--	--	Nutrient
Nitrogen, nitrite	0.002	--	14797–65–0	Nutrient
Orthophosphate	0.008	--	14265–44–2	Nutrient
Total phosphorus, filtered	0.006	--	7723–14–0	Nutrient
Total phosphorus, unfiltered	0.008	--	7723-14-0	Nutrient
Chemicals Analyzed in Water at USGS National Research Program Laboratory(ng/L)				
4-Nonylphenol[1]	100	5.92	25154–52–3	Surfactant metabolite.
4-Nonylphenolmonoethoxylate[1]	50	5.58	27986–36–3	Surfactant metabolite.
4-Nonylphenoldiethoxylate[1]	50	--	9016–45–9	Surfactant metabolite.
4-*tert*-Octylphenol[1]	5	5.28	140–66–9	Surfactant metabolite.
4-*tert*-Octylphenolmonoethoxylate[1]	5	--	9036–19–5	Surfactant metabolite.
4-*tert*-Octylphenoldiethoxylate[1]	5	--	2315–64–0?	Surfactant metabolite.
Bisphenol A[1]	5	3.64	80–05–7	FR, manuf. polycarbonate resins, antioxidant.
Fluoxetine[1]	50	--	58–08–2	Stimulant.
Fluvoxamine[1]	5	--	57–88–5	Animal steroid
Diethylpthalate[1]	5	--	--	Pharmaceutical
Triclocarban[1]	50	--	134–62–3	I, urban uses, mosquito repellent.
Triclosan[1]	5	--	3380–34–5	Antimicrobial.
17β-estradiol[1]	5	--	--	Reproductive Hormone
17α-ethynylestradiol[1]	5	--	--	Ovulation inhibitor
Estrone[1]	5	--	--	Reproductive hormone

Estriol[1]	5	--	--	Reproductive hormone
1,4-Dichlorobenzene[1]	5	3.28	106–46–7	Moth repellent, fumigant, deodorant.
2,6-Di-*tert*-butyl-1,4-benzoquinone[1]	5	4.07	719–22–2	Antioxidant by-product.
5-methyl-1H-benzotriazole[1]	5	1.71	136–85–6	Antioxidant in antifreeze and deicers
N,N,diethyl-*meta*-toluamide[1]	5	2.26	134–62–3	I, urban uses, mosquito repellent.
4-Androstene-3,17-dione [1]	5	--	--	Anabolic agent and natural steroid
Testosterone [1]	5	--	--	Reproductive hormone
D6-Bisphenol A (percent)	--	--	86588–58–1	Surrogate standard.
D21-2,6-Di-*tert*-butyl-4-methylphenol (percent)	--	--	64502–99–4	Surrogate standard.
4-*n*-Nonylphenol (percent)	--	--	104–40–5	Surrogate standard.
4-*n*-Nonylphenolmonoethoxylate (percent)	--	--	--	Surrogate standard.
4-*n*-Nonylphenoldiethoxylate (percent)	--	--	--	Surrogate standard.
D4-17-*β*-Estradiol (percent)	--	--	66789–03–5	Surrogate standard.
D7-Cholesterol (percent)	--	--	--	Surrogate standard.
Pharmaceuticals Analyzed in Water at the USGS National Water Quality Laboratory(ug/L)				
1,7-Dimethylxanthine[1]	0.12	-0.39	611-59-6	Precursor is a stimulant
Acetaminophen[1]	0.08	0.27	103-90-2	Analgesic
Albuterol	0.06	0.64	18559-94-9	Bronchodilator
Caffeine[1]	0.20	.16	58–08–2	Beverages, diuretic, very mobile/biodegradable
Carbamazepine[1]	0.04	2.25	298-46-4	Antiepileptic
Codeine[1]	0.04	1.28	76-57-3	Opiate agonist
Cotinine[1]	0.026	.34	486–56–6	Primary nicotine metabolite
Dehydronifedipine[1]	0.08	--	67035-22-7	Precursor is an antiangial
Diltiazem[1]	0.08	2.79	42399-41-7	Antihypertensive
Diphendydramine[1]	0.04	3.11	58-73-1	Antipruritic
Fluoxetine[1]	--		54739-18-3	SSRI Antidepressant
Sulfamethoxazole[1]	0.16	0.48	723-46-6	Antibiotic
Thiabendazole[1]	0.06	2.00	148-79-8	Anthelmintic, fungacide
Trimethoprim[1]	0.02	0.73	738-70-5	Antibiotic
Warfarin[1]	0.1	2.23	81-81-2	Anticoagulant, rodenticide
Carbamazepine-d10 (percent)	--	--		Surrogate standard
Ethyl Nicotinate-d4 (percent)	--	--		Surrogate Standard
Chemicals analyzed in Bottom Sediment at the USGS National Water Quality Laboratory (ng/g)				
1,4-Dichlorobenzene[1]	50	3.28	106–46–7	Moth repellent, fumigant, deodorant
1-Methylnaphthalene[1]	50	3.72	90–12–0	2–5% of gasoline, diesel fuel, or crude oil

2,6-Dimethylnaphthalene[1]	50	4.26	581–42–0	Percent in diesel/kerosene (trace in gasoline)
2-Methylnaphthalene[1]	50	3.72	91–57–6	2–5% of gasoline, diesel fuel, or crude oil
3-*beta*-Coprostanol[1]	500	8.82	360–68–9	Carnivore fecal indicator.
3-Methyl-1H-indole (skatol) [1]	50	2.60	83–34–1	Fragrance, stench in feces, and coal tar
3-*tert*-Butyl-4-hydroxyanisole[1]	150	3.50	25013–16–5	Antioxidant
4-Cumylphenol[1]	50	4.12	599–64–4	Surfactant metabolite
4-*n*-Octylphenol[1]	50	5.50	1806–26–4	Surfactant metabolite
4-Nonylphenol[1]	750	5.92	84852–15–3	Surfactant metabolite
4-Nonylphenoldiethoxylate[1]	1000	--	--	Surfactant metabolite
4-Nonylphenolmonoethoxylate[1]	500	5.58	--	Surfactant metabolite
4-*tert*-Octylphenol[1]	50	5.28	140–66–9	Surfactant metabolite
4-tert-Octylphenoldiethoxylate[1]	50	--	--	Surfactant metabolite
4-tert-Octylphenolmonoethoxylate[1]	250	--	--	Surfactant metabolite
Acetophenone [1]	150	1.67	98–86–2	Fragrance and flavor
Acetyl-hexamethyl-tetrahydronaphthalene (tonalide) [1]	50	6.35	21145–77–7	Musk fragrance
Anthracene [1]	50	4.35	120–12–7	CP, component of tar, diesel, or crude oil
Anthraquinone[1]	50	3.34	84–65–1	Manuf. dye/textiles, seed treatment, bird repellent
Atrazine[1]	100	2.82	1912–24–9	Selective triazine herbicide
Benzo[a]pyrene [1]	50	6.11	50–32–8	CP, regulated PAH
Benzophenone[1]	50	3.15	119–61–9	Fixative for perfumes and soaps
beta-Sitosterol[1]	500	9.65	83–46–5	Plant sterol
beta-Stigmastanol[1]	500	9.73	19466–47–8	Herbivore fecal indicator (digestion of sitosterol)
Bisphenol A[10]	50	3.64	80–05–7	FR, manuf. polycarbonate resins, antioxidant
Bromacil[1]	500	1.68	314–40–9	H (GUP), >80% noncrop usage on grass/brush
Camphor[1]	50	3.04	76–22–2	Flavor, odorant, ointments.
Carbazole[1]	50	3.23	86–74–8	I, manuf. dyes, explosives, and lubricants
Chlorpyrifos [1]	50	4.66	2921–88–2	I, historically for domestic pest and termite control
Cholesterol[1]	250	8.74	57–88–5	Often a fecal indicator, also a plant sterol
Diazinon [1]	50	3.86	333–41–5	I, > 40% nonagricultural usage, ants, flies
d-Limonene[1]	50	4.83	5989–27–5	F, antimicrobial, antiviral, fragrance in aerosols
Fluoranthene[1]	50	4.93	206–44–0	CP, in coal tar, asphalt (traces in gasoline or diesel fuel)
Hexahydrohexamethyl-cyclopenta-benzopyran (galaxolide) [1]	50	6.26	1222–05–5	Musk fragrance
Indole[1]	100	2.05	120–72–9	Pesticide inert ingredient, fragrance in coffee
Isoborneol[1]	50	2.85	124–76–5	Fragrance in perfumery, in disinfectants

Isophorone[1]	50	2.62	78–59–1	Solvent for lacquer, plastic, oil, silicon, resin
Isopropylbenzene (cumene) [1]	100	3.45	98–82–8	Manuf. phenol/acetone, fuels and paint thinner
Isoquinoline[1]	100	2.14	119–65–3	Flavors and fragrances
Menthol[1]	50	3.38	89–78–1	Cigarettes, cough drops, liniment, mouthwash
Metolachlor[1]	50	3.24	51218–45–2	H (GUP), indicator of agricultural drainage
N,N-diethyl-*meta*-toluamide[1]	100	2.26	134–62–3	I, urban uses, mosquito repellent
Naphthalene [1]	50	3.17	91–20–3	Fumigant, moth repellent, component (10%) of gasoline
p-Cresol (4-Methylphenol) [1]	250	2.06	106–44–5	Disinfectant
Phenanthrene [1]	50	4.35	85–01–8	CP, manuf. explosives, in tar, diesel fuel, or crude oil
Phenol [1]	50	1.51	108–95–2	Disinfectant, manuf several products, leachate
Prometon[1]	50	3.57	1610–18–0	H (non-crop only), applied prior to blacktop
Pyrene [1]	50	4.93	129–00–0	CP , In coal tar, asphalt (traces in gasoline or diesel fuel)
Tributyl phosphate[1]	50	3.82	126–73–8	Antifoaming agent, flame retardant
Triclosan[1]	50	4.66	3380–34–5	Disinfectant, antimicrobial
Triphenyl phosphate[1]	50	4.70	115–86–6	FR, plasticizer, resin, wax, finish, roofing paper
Tris(2-butoxyethyl) phosphate[1]	150	3.00	78–51–3	Flame retardant
Tris(2-chloroethyl) phosphate [1]	100	1.63	115–96–8	Plasticizer, flame retardant
Tris(dichloroisopropyl) phosphate[1]	100	3.65	13674–87–8	Flame retardant
Bisphenol A-d3, (percent)	--	--	--	Surrogate standard
Caffeine-13C, (percent)	--	--	--	Surrogate standard
Decafluorobiphenyl, (percent)	--	--	--	Surrogate standard
Fluoranthene-d10, (percent) [1]	--	--	--	Surrogate standard
Hormones Analyzed in Water and Bottom Sediment at the USGS National Water Quality Laboratory (ng/g)				
11-Ketotestosterone [1]	0.26	--	--	Reproductive hormone
17-alpha-Estradiol [1]	0.1	--	--	Reproductive hormone
17-beta-Estradiol (E2) [1]	0.1	--	--	Reproductive hormone
17-alpha-Ethynylestradiol (EE2) [1]	0.1	--	--	Ovulation inhibitor
Norethindrone [1]	0.1	--	--	Ovulation inhibitor
4-Androstene-3,17-dione [1]	0.1	--	--	Anabolic agent and natural steroid
cis-Androsterone [1]	0.1	--	--	Urinary steroid
Cholesterol [1]	250	--	--	Plant/animal steroid
3-beta-Coprostanol [1]	250	--	--	Fecal steroid
Dihydrotestosterone [1]	0.1	--	--	Metabolite of testosterone

Bisphenol A [1]	12	--	--	Plastic component
Epitestosterone [1]	0.5	--	--	Form of testosterone
Equilenin [1]	0.26	--	--	Hormone replacement
Equilin [1]	0.5	--	--	Hormone replacement
Estriol [1]	0.26	--	--	Reproductive hormone
Estrone [1]	0.1	--	--	Reproductive hormone
Mestranol [1]	0.1	--	--	Ovulation inhibitor
Progesterone [1]	0.5	--	--	Reproductive hormone
trans-Diethylstilbestrol [1]	0.1	--	--	Synthetic metabolic intermediate of diethylstilbestrol
Testosterone [1]	0.1	--	--	Reproductive hormone
Testosterone-2,2,4,6,6-d5	--	--	--	Surrogate Standard
17-alpha-Ethynylestradiol-2,4,16,16-d4	--	--	--	Surrogate Standard
Mestranol-2,4,16,16-d4	--	--	--	Surrogate Standard
Dihydrotestosterone-1,2,4,5a-d4	--	--	--	Surrogate Standard
Bisphenol-A-d16	--	--	--	Surrogate Standard
4-Androstene-3,17-dione-2,2,4,6,6,16,16-d7	--	--	--	Surrogate Standard
Norethindrone-2,2,4,6,6,10-d6	--	--	--	Surrogate Standard
Cholesterol-d7	--	--	--	Surrogate Standard
Progesterone-2,2,4,6,6,17a,21,21,21-d9	--	--	--	Surrogate Standard
Estriol-2,4,17-d3	--	--	--	Surrogate Standard
Estrone-2,4,16,16-d4	--	--	--	Surrogate Standard
17-beta-Estradiol-d4	--	--	--	Surrogate Standard
trans-Diethyl-1,1,1',1'-d4-stilbesterol-3,3',5,5'-d4	--	--	--	Surrogate Standard

[1]Chemicals with and "E" following the number indicate a compound with low recovery, unstable instrument response, or reference standard prepared from a technical mixture for water analyses (Zaugg and others, 2006). Chemicals with an "e" following the number are estimated if the spike recovery or expected continuing calibration verification concentrations for each set of samples are not within control limits (Zaugg and others, 2006).

[2]Endocrine disrupting potential (EDP) from the following sources: Kime, 1998; Tremblay and Van der Kraak, 1998; EC-BKH, 2000; Nishihara and others, 2000; Global Water Research Coalition, 2003; Versonnen and others, 2003; Institute of Environmental Health, 2005; Korner and others, 2005; and Terasaki and others, 2005; Scheurs and others, 2004.

[3]Log K_{ow} is the octanol-water partition coefficient and is a measure of the equilibrium concentration of a compound between octanol and water. A high value indicates a compound that will preferentially partition into soil organic matter rather than water. It was calculated using the U.S. Environmental Protection Agency's exposure assessment tools and models (EPI-suite software, WSKOWWINTM version 1.40; U.S. Environmental Protection Agency, 2005b).

[4]This report contains Chemical Abstracts Services Registry Numbers (CASRN)®, which is a Registered Trademark of the American Chemical Society. A CASRN is a numeric identifier that can contain up to nine digits, divided by dashes into three parts. For example, 58–08–2 is the CASRN for caffeine. The online database provides a source for the latest registry number information: http://www.cas.org/index.html. Chemical Abstracts Services recommends the verification of the CASRNs through CAS Client Services℠.

[5]Sources are Kime, 1998; Tremblay and Van der Kraak, 1998; EC-BKH, 2000; Nishihara and others, 2000; Zaugg and others, 2007 Versonnen and others, 2003; Barber, Furlong, and others, 2003; Global Water Research Coalition, 2003; Institute of Environmental Health, 2005; Furlong and others, 2008; Korner and others, 2005; Terasaki and others, 2005.

APPENDIX A: SAFETY PLAN

The following preliminary job Hazard Assessment has been performed for this project.

Proposal Job Hazard Analysis – Central Region

Project Title: Estrogenic and Pharmaceutical Septic System Discharge to Lakes
Project Chief or Proposal Author: Richard Kiesling

√	*Potential Project Safety Elements*
1.X	Wading, bridge, or cableway measurements or sampling (WRD 99.32 & 01.05)
2.	Working on ice covered rivers or lakes (see WRD 00.03)
3.	Measuring or sampling during floods
4.	Well drilling; coring, augers, hydro-punch, borehole logging
5.	Electrical hazards in the work area – above and below ground
6.	Construction – including cableways, trenching and demolition
7.X	Working in remote areas, communication, office call in procedures (OP94.02)
8.X	Ergonomics, Office issues, carpal tunnel syndrome
9.	Field Vehicles appropriate for task?- Safety screens, equipment restraints.
10.	All terrain vehicles, snowmobiles, fork lifts,
11.	Helicopter or fixed wing aircraft usage (see OAS at: http://www.oas.gov/)
12.	Site access: Federal, State, County and private lands
13.	Hypothermia or Hyperthermia (heat stress)
14.	Hantavirus, Lyme Disease, Histoplasmosis, Pfiesteria, Others?
15.X	Contaminated water or soil with sanitary, biological, or chemical concerns
16.	Immunizations - voluntary programs
17.X	Laboratory or mobile laboratory. Chemical hygiene plan, HazComm & MSDS's
18.X	Hazardous waste disposal – Lab and Field
19.	Hazardous waste site operations (RCRA, CERCLA) HASP, HAZWOPPER
20.	Confined space – Stilling Wells, Well Pits, Sample sites
21.	Radioactivity – Borehole logging – Soil Moisture -
22.	Respiratory protection – Dusts, Vapors, Fumes, Biologic (medical monitoring)
23.	Water levels – wells, well pits, pumps and electrical issues
24.	Electrofishing (see http://1stop.usgs.gov/safety/Topic/jha/electrofishing.htm)
25.	High pressure compressed gas cylinders – handling and transport
26.X	Boating – operator training, equipment, requirements, inspections
27.	Special Training
28.X	Location and Phone # of emergency medical and rescue facilities.

Box no.	**Planned Responses to Potential Project Safety Elements without National or State Response Plans.**
8.X	Ergonomics, Office issues, carpal tunnel syndrome
Concern	Repetitive motion injuries associated with computer use
Response	Awareness training and ergonomic furniture and aids as needed
15.X	Contaminated water or soil with sanitary, biological, or chemical concerns
Concern	Repetitive motion injuries associated with computer use
Response	Awareness training and ergonomic furniture and aids as needed
17.X	Laboratory chemical use.
Concern	Exposure to chemicals used in the laboratory and onsite
Response	Lab safety training, Introductory Hazmat Training, Chemical Hygiene plan, MSDS sheet availability, Mandatory fume hood and protective clothing use
18.X	Hazardous waste disposal – Lab and Field
Concern	Treatment of low-level chemical waste
Response	Approved onsite waste-water treatment and disposal at USGS Office
22.X	Respiratory protection – Dusts, Vapors, Fumes, Biologic (medical monitoring)
Concern	Exposure to chemicals during handling of field samples
Response	Prepare all samples using approved safety gear
28.X	Location and Phone # of emergency medical and rescue facilities.
Concern	Staff unfamiliar with location of facilities in sampling area
Response	Orientation and introductory training to include information on emergency contacts and medical emergency procedures

A project safety review will be conducted annually as part of the Minnesota Water Science Center annual review process. .

Summary of Reviewers Comments:

The following review comments were used to revise the current proposal. The comments are summarized below with author's responses.

Peer Review Comments with responses in italics:

Lake monitoring -

1. The proposal should specify the types of equipment that will be used, the sampling frequency, and the lab analyses that are planned. *Response – sampling equipment with references to standard sampling protocols have been described in the proposal. Samples will be collected once from each lake site. Table 1 provided a list of compounds and the text provides a list of method references for each class of compounds.*
2. Lake monitoring stations should be described in some detail because they are not standard installations. *Response – this comment refers to USGS protocols for site description. All sites will be fully described with specific location information and photographic records.*
3. Proposal includes using watershed and groundwater characteristics to predict effects of septic discharge on target species. This will require mechanistic models; statistical models will not be adequate for this type of forecasting. Do you have any particular models in mind, or will they be developed as part of the project. *Response – the models will be developed as part of the project.*

Biological assessment:

1. The proposal should indicate the type of sampling planned for fish studies. It isn't clear how the bluegills will be tracked during spawning periods. *Response – PIT tags will be used to mark fish and boat-mounted and in-lake antennas will be used to track fish movements. Details are provided in the proposal.*
2. Will the monitoring of breeding bluegills be maintained the entire time? *Response – fish movements will be recorded while the antennas are deployed. Samples*

will be collected from tagged fish at least twice during the spawning period – once during pre-spawn nest defense period and once during embryo development.

3. Specify the biological monitoring design in more detail. I assume the design involves separate monitoring for individual fish on the spawning beds. Are there contingencies built into the design in case of failure of one or more of the approaches? *Response – the fall-back position is to seine and electro-shock fish on the spawning beds for sampling with release back on to the beds if in-lake tracking does not work.*

Two areas of groundwater discharge to the lake near residences were chosen as locations where residential septic systems may be impacting the lake habitat (Figure 2). Groundwater was sampled as it passed upward through the lake sediment (Figure 3) and was analyzed for linear alkylbenzene sulfonates (LAS) and alkylphenol ethoxylates (APEs). Surface water was sampled at the four lake locations shown in Figure 2 â€“ areas influenced by road runoff, agricultural activities, and two locations influenced by residential septic drainfields â€“ and analyzed for a broad suite of contaminants.Â Fish from both septic influenced sites, however, showed more evidence of exposure to estrogenic chemicals, and changes in reproductive organs were observed in fish from these habitats. Septic systems are underground wastewater treatment structures, commonly used in rural areas without centralized sewer systems. They use a combination of nature and proven technology to treat wastewater from household plumbing produced by bathrooms, kitchen drains, and laundry. A typical septic system consists of a septic tank and a drainfield, or soil absorption field. The septic tank digests organic matter and separates floatable matter (e.g., oils and grease) and solids from the wastewater. Soil-based systems discharge the liquid (known as effluent) from the septic tank into a series of per Paleolimnologists collect lake sediments using special coring devices to study a lake's physical, chemical and biological history. Lake sediments are often dated using the radioisotopes lead-210 and carbon-14. The age of a given sediment sample is based on the radioactive decay of the isotope. Other dating methods are based on identifying sharp increases of pollen in the core from ragweed and other plants indicative of agricultural soil disturbances or deforestation. Stratigraphic analyses of sediments have been used increasingly to assess the history of lakes, especially with regard to h The estrogen equivalents (EEQs) ranged from nd (not detected) to 2.96 ng/L, and the estrogenic activities decreased along the processes. Among the 32 samples, DES prevailed in all samples, with concentrations ranging 1.46â€“12.0 ng/L, BPA, OP and NP were partially detected, with concentrations ranging from nd to 17.73 ng/L, nd to 0.49 ng/L and nd to 3.27 ng/L, respectively.Â The recombined yeast estrogen screen (YES) and liquid chromatography tandem mass spectrometry (LC-MS/MS) were applied to assess the estrogenicity and detect the estrogens in the samples. The estrogen equivalents (EEQs) ranged from nd (not detected) to 2.96 ng/L, and the estrogenic activities decreased along the processes. Aerobic Septic Systems Letâ€™s Talk About Them: How does an aerobic septic system work? How do you maintain an aerobic septic system?Â There are conventional septic systems and then there are the more complex aerobic septic systems. The difference being, in a word: Oxygen. While a conventional septic system uses only the septic tank to separate solids, fats and grease, an aerobic treatment unit (ATU) uses Oxygen infusion for digestion rather than the anaerobic process.Â Letâ€™s say you want to purchase an amazing property for a cottage, right by the lake of course. Now letâ€™s say that the lot space is rather small, but it will suit your needs just fine for what you have in mind. Wellâ€¦a conventional septic system may not work for this type of lot, letâ€™s see why