2005 Project Abstract
For the Period Ending June 30, 2008

PROJECT TITLE:	Unwanted Hormone Therapy: Protecting Water and Public Health
PROJECT MANAGER:	Paige J. Novak, Ph.D., P.E.
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FUNDING SOURCE:	Minnesota Environment and Natural Resources Trust Fund
LEGAL CITATION:	ML 2005 First Special Session, Chapter 1, Article 2, Sec. 11, Subd. 7(e)

APPROPRIATION AMOUNT: $300,000

Overall Project Outcome and Results

Endocrine disruptors have been linked to numerous problems in ecosystems and humans, particularly with respect to reproductive function and development. The effluent from the Western Lake Superior Sanitary District (WLSSD) Wastewater Treatment Plant in Duluth, Minnesota and the Metropolitan (Metro) Treatment Plant in St. Paul, Minnesota have been observed to be estrogenic. The goal of this project was to conduct mass balances across the two treatment plants to determine where estrogenic compounds come from and how they are distributed. For the Metro plant, the estrogenicity entering the plant was relatively consistent and was removed effectively, as measured by a receptor binding assay (the YES assay) (96%±2%). The estrogenicity leaving the plant consisted mainly of estrone, nonylphenol, and bisphenol A. Hormones (estriol and ethynylestradiol) were detected on two occasions (410 and 18 ng/L, respectively). At the WLSSD plant, the estrogenicity throughout the plant varied extensively over time. This was expected as the plant receives about 2/3 of its flow from industrial sources. The estrogenicity in the effluent also varied, as measured by the YES assay (3-34 ng/L or 0.4-4.3 g/day estradiol equivalent), but did appear to be treated within the plant. The estrogenic compounds most often detected in the effluent were estrone, nonylphenol, and bisphenol A. Unlike the Metro plant, bisphenol A did not appear to degrade appreciably in two out of three samples. This could be a result of competition, as the levels of other organic compounds would be high. Therefore, more research is required to determine how the presence of competing organic compounds, such as phytoestrogens, affects the microbial transformation of problematic compounds such as bisphenol A. Other removal methods (*e.g.*, sorption for nonylphenol) will also be complicated by the presence of competing compounds; additional research will also be required to better facilitate such processes.

Project Results Use and Dissemination

Results have been disseminated at several conferences. In addition, two manuscripts are being written and will be submitted for publication in September, 2008. This project also resulted in the generation of three Master's theses.

Date of Report: June 30, 2008

LCCMR 2005 Work Program Final Report

I. PROJECT TITLE: Unwanted Hormone Therapy: Protecting Water and Public Health

Project Manager: Paige J. Novak, Ph.D., P.E.
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Location: Minneapolis, Minnesota 55455; Additional work (sampling) will take place in St. Paul, Minnesota 55101 and Duluth, Minnesota 55806. See attached map.

Total Biennial LCCMR Project Budget:		
	LCCMR Appropriation:	$ 300,000
	Minus Amount Spent:	$ 293,963
	Equal Balance:	$ 6,037

Legal Citation: ML 2005 First Special Session, Chapter 1, Article 2, Sec. 11, Subd. 7(e)

Appropriation Language:
7 (e) Unwanted Hormone Therapy: Protecting Water and Public Health - 300,000
$150,000 the first year and $150,000 the second year are from the trust fund to the University of Minnesota to determine where behavior-altering estrogenic compounds come from and how they are distributed in wastewater treatment plants. This appropriation is available until June 30, 2008, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. and III. FINAL PROJECT SUMMARY:

Endocrine disruptors have been linked to numerous problems in ecosystems and humans, particularly with respect to reproductive function and development. The effluent from the Western Lake Superior Sanitary District (WLSSD) Wastewater Treatment Plant in Duluth, Minnesota and the Metropolitan (Metro) Treatment Plant in St. Paul, Minnesota have been observed to be estrogenic. The goal of this project was to conduct mass balances across the two treatment plants to determine where estrogenic compounds come from and how they are distributed. For the Metro plant, the estrogenicity entering the plant was relatively consistent and was removed effectively, as measured by a receptor binding assay (the YES assay) (96%±2%). The estrogenicity leaving the plant consisted mainly of estrone, nonylphenol, and bisphenol A. Hormones (estriol and ethynylestradiol) were detected on two occasions (410 and 18 ng/L, respectively). At the WLSSD plant, the estrogenicity throughout the plant

varied extensively over time. This was expected as the plant receives about 2/3 of its flow from industrial sources. The estrogenicity in the effluent also varied, as measured by the YES assay (3-34 ng/L or 0.4-4.3 g/day estradiol equivalent), but did appear to be treated within the plant. The estrogenic compounds most often detected in the effluent were estrone, nonylphenol, and bisphenol A. Unlike the Metro plant, bisphenol A did not appear to degrade appreciably in two out of three samples. This could be a result of competition, as the levels of other organic compounds would be high. Therefore, more research is required to determine how the presence of competing organic compounds, such as phytoestrogens, affects the microbial transformation of problematic compounds such as bisphenol A. Other removal methods (*e.g.*, sorption for nonylphenol) will also be complicated by the presence of competing compounds; additional research will also be required to better facilitate such processes.

IV. OUTLINE OF PROJECT RESULTS:

Result 1: Plant Mass Balance and Characterization

Description:

Field sampling will be conducted at the WLSSD and Metro Wastewater Treatment Plants and will be designed to provide a mass balance, for both specific compounds and non-specific compounds with estrogenic activity, across the plants. Samples of the major liquid streams around the unit operations and samples of the solids streams around the solids processing unit operations will be taken. Flow information will also be obtained at each sampling point. Samples will be taken roughly once each "season" (summer (high water use/flow, potential chlorination, high temperatures), fall (lower water flow, high temperatures), winter (low water flow, low temperatures), and spring (higher water flow, low temperatures)) during the first two years of the project; additional samples may be taken in the last year of the project. Samples, both whole and fractionated, will be analyzed for estrogenicity and organic content. Inspection of mass/time values across the plant will show in general how the estrogenic compounds partition and where they are formed. Laboratory experiments will be performed to establish how these compounds are formed. Laboratory experiments may consist of experiments designed to test the impact of a particular industrial waste component, particular unit operations, and basic operating parameters (such as hydraulic loading, organic loading, nitrification/denitrification, or mean cell residence time) on the estrogenicity of the plant effluent.

Summary Budget Information for Result 1:	**LCMR Budget**	**$ 272,211**
	Balance	**$ -32**

Completion Date: April 30, 2008

Final Report Summary:

This project required us to develop new methods to determine the estrogenicity across wastewater treatment plants, including in the raw influent sewage and solids streams, both of which are extremely difficult to work with. This necessitated the investigation and comparison of several methods so that we could be confident in our results. This comparison is presented below and served as the basis of two Master's theses (one included and one to be

submitted to LCCMR upon its completion early this fall) and one manuscript that will be submitted to either *Environmental Science and Technology* or *Environmental Chemistry and Toxicology* this September. This portion of the work was unexpected, but provides a deeper understanding of the mass balance results and is a contribution to the field in general. In addition, laboratory experiments had been proposed to verify removal mechanisms that were suggested by field data. These experiments were not performed because the complexity of the analysis required for the field samples was much greater than expected and required much more time than anticipated. The results from the mass balance study at the field scale are presented below as well. This will serve as the basis of a third Master's thesis (to be submitted to LCCMR upon its completion this fall) and a second manuscript submitted to either *Environmental Science and Technology* or *Environmental Chemistry and Toxicology* this September.

Comparison of Analysis Methods

Three commonly-used methods for measuring estrogens in wastewater were compared. The assays under scrutiny were two estrogen receptor binding/response assays (the YES (yeast estrogen screen) assay and a rainbow trout estrogen receptor binding assay (rtERB)) and the standard chemical analytical method of liquid chromatography coupled to mass spectrometry (LC-MS). The YES assay uses a recombinant yeast cell that contains the human estrogen receptor. When estrogen is present, it binds to the receptor and triggers a color reaction in the assay reagents, which is then measured. The rtERB assay is similar to the YES assay in that it measures the amount of estrogens in the sample that bind to receptors; the receptors, however, are from trout liver cells, thereby providing a measurement of potential ecological (fish) impact as opposed to potential human impact.

The YES results for the Metro plant sampled on February 28, 2007 showed a decrease in estrogenicity across the plant from 10.7 ng/L estradiol equivalents (EEQ) to 7.3 ng/L EEQ in the influent and effluent, respectively. The rtERB data for that same sampling date gave very different results. The influent was 749.4 ng/L EEQ, while the effluent was 22.6 ng/L EEQ. EEQ values were also calculated from the LC-MS data using potency values from the literature for the selected compounds measured (estradiol, estrone, estriol, ethynylestradiol, nonylphenol, octylphenol, bisphenol A, and genistein, *1-4*). The influent contained 12.5 ng/L EEQ, while the effluent contained 5.46 ng/L.

The WLSSD plant was sampled on August 3, 2007. The YES results showed an increase in estrogenicity from 3 ng/L in the influent to 38.5 ng/L in the effluent. The rtERB data, however, showed the influent to be 206.9 ng/L and the effluent to be 60.1 ng/L. The LC-MS data for the influent was 13.5 ng/L, and for the effluent it was 31.3 ng/L. Therefore, there was a large increase in estrogenicity as measured by the YES assay, a decrease as measured by the rtERB assay, and a moderate increase when analyzed using the YES assay.

The YES assay showed a mild decrease in estrogenicity (in terms of concentration) at Metro, but a marked increase at WLSSD. The nature of the samples being analyzed greatly affects the YES assay, and the assay requires the yeast to be alive to obtain results. Influents could be highly toxic to the yeast (we observed this with many of our samples) because they have not yet undergone treatment. Yeast cell death as a result of toxicity leads to an underestimate of the estrogenicity in the sample. This would account for the lower-than-expected removal of estrogenicity across the Metro plant for the February 2007 samples. The WLSSD plant

receives a large proportion of its influent (about 66%) from industrial sources; our observations showed that this waste tends to contain compounds that are toxic to the yeast cells. The large increase in estrogenicity across the WLSSD plant for August 2007 samples can be explained by the toxicity of the influent—this waste contained compounds that were clearly toxic to the yeast and the influent therefore appeared to be artificially low in estrogenicity. The effluent EEQ values for both Metro and WLSSD were consistent with the range of values measured at these and other plants *(5-9)*.

The rtERB assay gave percent removal values closer to those found in the literature for both plants. Despite the similar nature of the YES and the rtERB assays, toxicity is not a factor in the rtERB assay because the liver cells used are not alive. This prevents underestimates in estrogenicity as a result of the presence of toxic compounds, as was the case with the YES assay. The rtERB assay, by using trout liver cells, also more closely approximates the actual interaction of estrogens with receptor sites in an aquatic species. Because the YES assay uses yeast cells to which the human estrogen receptor has been added, the interaction of estrogens with the two different receptors used in these assays might vary considerably.

The LC-MS data showed that estrogenicity decreased moderately at Metro but increased at WLSSD. Samples (36 L equivalent) of the raw influent and effluent were taken and concentrated to 600 μL (60,000 times). Although the samples were cleaned, this degree of concentration resulted in a large amount of non-target material in the sample, leading to noise in the chromatogram. With such noise, the compounds of interest are harder to detect and quantify accurately. Also, in the case of an analytical method such as LC-MS, one only quantifies the compounds that they target; if unknown estrogenic compounds exist, they will not be quantified by LC-MS, but will be detected by a non-specific binding assay such as the rtERB. These factors could lead to an underestimate of estrogenicity in the influent (in the case of a great deal of noise in the chromatogram) and the effluent (in the case of unknown estrogenic compounds). The compounds measured and their concentrations in the influent and effluent on two sampling dates are shown below in Tables 1 and 2. These results are discussed below with the other results obtained from four additional Metro samples and two additional WLSSD samples.

Table 1. Concentration of target estrogenic compounds in the influent and effluent of the Metro Plant in the February, 2007 samples

Compound	Concentration (ng/L) in the Metro Plant Samples	
	Influent	Effluent
Estradiol	BDL	BDL
Estriol	57	BDL[a]
Estrone	30	19
Ethynylestradiol	BDL	BDL
Genistein	BDL	5.5
Bisphenol A	6900	18
Nonylphenol	8700	3200
Octylphenol	BDL	BDL
Triclosan	1400	170

[a] Below detection limit

Table 2. Concentration of target estrogenic compounds in the influent and effluent of the WLSSD Plant in the August, 2007 samples

Compound	Concentration (ng/L) in the WLSSD Plant Samples	
	Influent	Effluent
Estradiol	BDL	BDL
Estriol	BDL	BDL
Estrone	66	190
Ethynylestradiol	BDL	BDL
Genistein	BDL	BDL
Bisphenol A	7400	940
Nonylphenol	3000	3100
Octylphenol	BDL	BDL
Triclosan	530	250

Mass Balance Study, Results from the Yeast Estrogen Screen Assay

The YES assay was used to create seasonal mass balances of total estrogenicity across the various treatment processes at the Metro and WLSSD plants. Using the EEQ concentrations found using the YES assay, along with the flow rates provided by the facility, mass balances for the Metro plant were calculated for April, July, and November of 2006, and February, May, and July of 2007. Samples analyzed included primary influent, secondary influent (post-settling tank), centrate (centrifuge filtrate), pre-chlorination effluent, and post-chlorination effluent. Mass balances for the WLSSD plant were established for September 2006, and January, May, and August 2007. The WLSSD samples included municipal influent (regional residential and business sources), total influent (municipal with industrial sources), influent with recycle stream (total influent post-bar screens and after introduction of internal recycle streams), supernatant (digester supernatant), secondary effluent (pre-mixed media filtration), pre-chlorination effluent, and post-chlorination effluent.

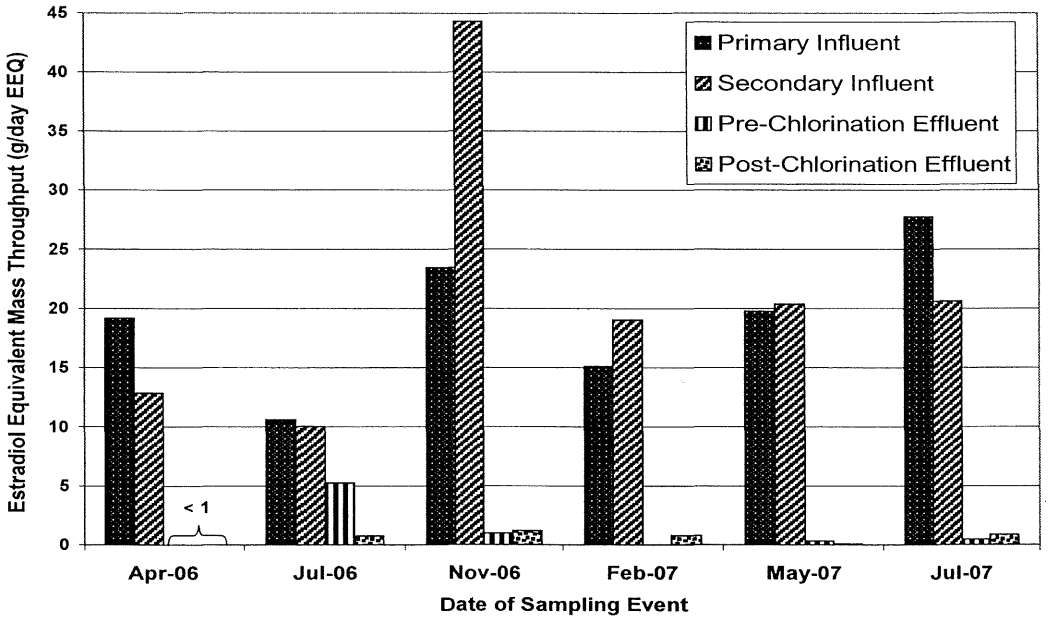
In general, the YES assay provided useful results regarding total estrogenicity throughout the plants and overall treatment efficiency. At the Metro Plant, the EEQ throughputs of the primary and secondary influents were similar, averaging 19 ± 6 and 21 ± 12 ng/day EEQ, respectively. The EEQ mass throughputs of the pre- and post-chlorination effluents were also very similar, though considerably less than the influents, with averages of 1.8 ± 2.3 and 0.7 ± 0.4 ng/day EEQ, respectively. The centrate samples, some of which had relatively high EEQ values (up to 26 ng/L EEQ), contributed little to the relative mass transfer across the plant (0.08 ± 0.06 ng/day EEQ) due to their relatively minor volumes. The EEQ mass throughput for each of the Metro samples is shown in Table 3 and Figure 1.

Table 3. Calculated estradiol equivalent (EEQ) mass throughput of Metro plant samples based on results of the YES assay

	Metro Plant Estradiol Equivalent Mass Throughput (g/day EEQ)					
	Apr-06	Jul-06	Nov-06	Feb-07	May-07	Jul-07
Primary Influent	19	11	23	15	20	28
Secondary Influent	13	10	44	19	20	21
Centrate	0.03	0.1	0.1	0.01	0.04	0.1
Pre-Cl Effluent	< 0.9	5	1.0	*	0.3	0.5
Post-Cl Effluent	< 0.8	1	1.2	0.8	0.1	0.9

* No pre-chlorinated effluent sample was taken during the February 2007 sampling event (the plant was not disinfecting)

Figure 1. Comparison of Estradiol equivalent mass throughput of Metro plant samples based on results of the YES assay



The Metro EEQ mass throughput data indicate that the plant was consistent at reducing the total estrogenicity of the influent throughout the year, with an average efficiency of 96% ± 2%. In addition, given the significant decrease in EEQ mass throughput in the effluents compared to the influents, and the relatively minor estrogenicity associated with centrifugation solids, it appears that most of this reduction occurs in the activated sludge tanks during biological treatment. This observation agrees with previous studies at other treatment plants which concluded that biological treatment is the primary removal mechanism for many estrogenic compounds.

For the WLSSD plant, application of the YES assay presented additional challenges. Relative to the Metro plant, WLSSD samples exhibited greater toxicity and inhibition when analyzed by the YES assay. Because the YES assay relies on yeast cell viability and reproduction, samples containing high concentrations of inhibiting/toxic compounds will

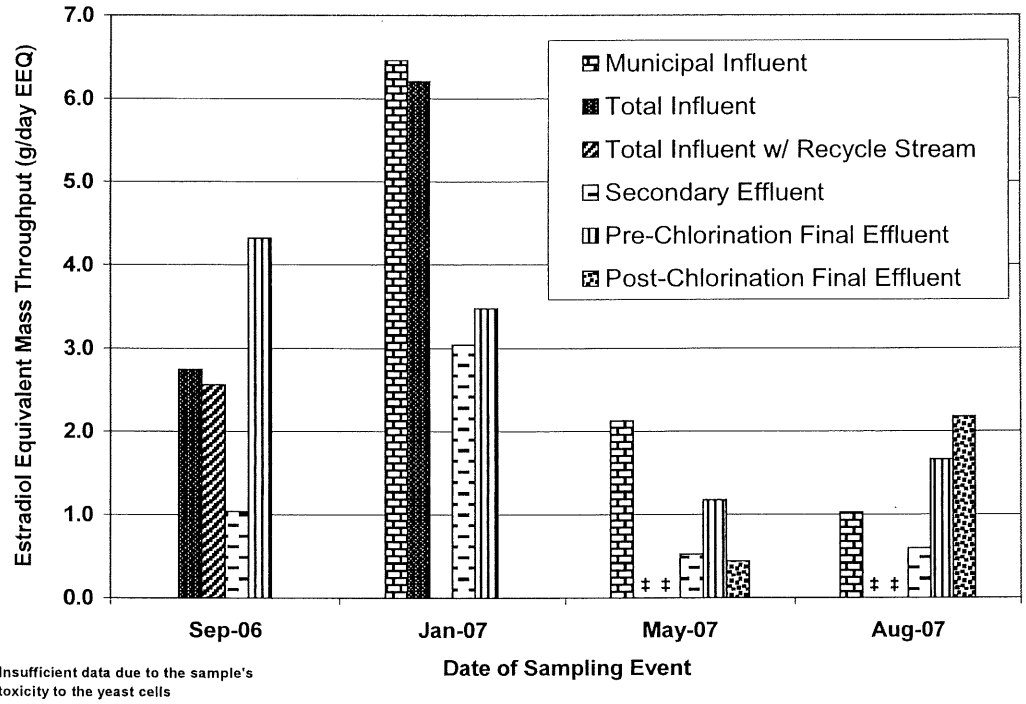
decrease the assay's response, resulting in artificially low estimates of total estrogenicity, or possibly preventing any estimate at all. Two of the total influent samples and two of the influent with recycle stream samples collected at WLSSD exhibited toxicity such that there was insufficient response to estimate estrogenicity. Inhibition may have been a factor with the remaining influent samples, leading to reduced estimates of total estrogenicity. Nevertheless, despite the possibility of slight inhibition, the YES assay data from WLSSD do show important elements in the plant's treatment of environmental estrogens. The EEQ mass throughput of the WLSSD samples is shown in Table 4 and Figure 2.

Table 4. Calculated estradiol equivalent (EEQ) mass throughput of WLSSD plant samples based on results of the YES assay

	WLSSD Plant Estradiol Equivalent Mass Throughput (g/day EEQ)			
	Sep-06	**Jan-07**	**May-07**	**Aug-07**
Municipal Influent	*	6.5	2.1	1.0
Total Influent	2.7	6.2	(toxic)	(toxic)
Total Influent w/ Recycle Stream	2.6	*	(toxic)	(toxic)
Centrate	0.02	0.1	0.02	(toxic)
Secondary Effluent	1.0	3.0	0.5	0.6
Pre-Cl Final Effluent	4.3	3.5	1.2	1.7
Post-Cl Final Effluent	*	*	0.4	2.2

* No sample was taken

Figure 2. Comparison of Estradiol equivalent mass throughput of WLSSD plant samples based on results of the YES assay



For WLSSD, the pattern of estrogenicity (as measured by the YES assay) was quite different. The total influent EEQ mass throughput averaged 4.5 ± 2.5 ng/day EEQ, which was similar to the influent with recycle stream sample (2.6 ng/day EEQ). Relative to the Metro plant, the WLSSD plant had much less (approximately one-fifth) and more variable total estrogenic input. This was expected given that this plant treats approximately one-fifth of the Metro plant's volume, and is less consistent in terms of daily volume. In addition, the increased levels of toxicity and inhibition observed in the WLSSD samples add to the variability of the YES assay results.

The WLSSD plant's municipal influent, which is comprised mostly of non-industrial sources, had an average mass of 3.9 ± 2.9 ng/day EEQ, which is similar to the average for the total influent samples (4.5 ± 2.5 ng/day EEQ). This may be an indication that most of the estrogenic response observed in the YES assay is derived from compounds in the municipal, rather than industrial, inputs. Indeed, for the only sampling event in which there are both municipal and total influent data, the municipal influent appears to contribute all of the EEQ mass throughput (6.5 ng/day EEQ from the municipal, 6.2 ng/day EEQ for the total influent).

The WLSSD plant's secondary effluent, pre-chlorination effluent, and post-chlorination effluent had average mass throughputs of 1.3 ± 1.2, 2.7 ± 1.5, and 1.3 ± 1.2 g/day EEQ, respectively. Comparing the municipal and total influent samples to the pre- and post-chlorination effluents yields significant differences in treatment, ranging from an approximate 80% reduction (May 2007) to an increase of over 100% (August 2007). As mentioned above, it is highly likely that the inhibition seen with the WLSSD samples in the YES assay skewed the results of the influent to appear lower than they actually were, which would have the effect of decreasing the apparent removal efficiency. The YES assay results consistently showed that the WLSSD plant effluent had the same or slightly greater estrogenic equivalent mass throughput as the Metro plant, despite its much smaller flow.

Mass Balance Study, Analytical Results

In general, the LC-MS results show how variable estrogenic compounds can be throughout each plant and throughout time. Based on our results, the most common estrogenic compounds in the Metro plant effluent were estrone, nonylphenol, bisphenol A, and triclosan. Overall removal percentages were high for nonylphenol, bisphenol A, and triclosan; nevertheless, the quantity of these compounds entering the plant was very high, resulting in residual material exiting the plant. Estrone was either produced within the plant (observed in 3 out of 4 samples) or was poorly removed (63% removal observed on May, 2007). Estrone could be formed from the oxidation of estradiol or estradiol conjugates, often found in wastewater influent but not included in our analysis. This oxidation would be expected to occur in an aerobic activated sludge plant such as Metro. Estradiol was never detected in Metro effluent and genistein was only detected once at very low concentrations (5 ng/L, February, 2007). Ethynylestradiol was detected in the effluent on one date (18 ng/L, May, 2007) and estriol (a natural human estrogen of relatively high potency) was detected on another (410 ng/L, July, 2006). In general, the concentrations of specific compounds were higher in the secondary influent (after primary sedimentation), possibly because of the presence of recycle streams. Concentrations dropped significantly across the activated sludge tank (35-100% overall removal), as was observed for general estrogenicity with the YES assay.

At WLSSD fewer compounds were detected with certainty, which was a result of the extremely noisy chromatograms, and those that were detected degraded to a lesser extent (4-100%). The most common estrogenic compounds in the WLSSD plant effluent were estrone and bisphenol A. One sample also had high levels of triclosan and nonylphenol in the effluent (Table 2). Estrone increased in concentration across the plant in one sample (to 190 ng/L, August, 2007) and remained constant in another. Nonylphenol (August, 2007) and bisphenol A remained roughly constant from the influent to the effluent in two other samples (August, 2007 and May, 2007). Because of the higher loading of organic material to the WLSSD Plant in general (primarily from the industrial effluent entering the plant), it is not surprising that some of these trace compounds degraded to a lesser extent than that observed at Metro—there is simply a larger quantity of "food" present for the microorganisms and it is therefore less likely that these trace organic compounds would degrade to the same extent. Based on the potency of the compounds that we analyzed, estrone would result in the majority of the EEQ of the effluent, but given the YES and rtERB results, it is likely that other compounds were present that were not detected by LC-MS. Indeed, the clean-up required to analyze the samples by LC-MS could have removed some of these compounds, namely phytoestrogens. We are continuing to look at this issue and as it is resolved these results will be included in the manuscripts submitted for publication in September (to be mailed to the LCCMR office upon completion). Estriol, estradiol, genistein, ethynylestradiol were never detected in the WLSSD effluent.

Conclusions and Recommendations

Because of the YES assay's inability to accurately measure estrogenicity in toxic samples, it is not recommended for general use on wastewater samples, other than effluent samples. Nevertheless, the assay is well suited to measuring fairly clean samples that can contain complex mixtures of chemicals (surface waters, etc.). The broad measurement of estrogenicity provided by the assay allows quantification of all chemicals capable of binding to the human estrogen receptor, whether a given researcher knows to look for them or not. This is an advantage of the assay and makes it particularly useful as a preliminary screening tool for relatively clean samples (effluents, surface waters, etc.). The LC-MS requires that the researcher know exactly which chemicals to look for during analysis. There is no way to capture overall estrogenicity using LC-MS analysis. Nevertheless, with its low detection limits and high accuracy, it is advantageous over the *in vitro* assays in its ability to measure low levels of specific compounds and observe specific phenomena, such as the formation of estrone in the plants. Because dirty and/or toxic samples hinder the YES assay through toxicity and the LC-MS through noise, rtERB is preferred for such samples (influents, solids, etc.). This shows that each assay can provide different information and the most powerful results come when these methods are combined.

Overall the plants appeared to perform well, removing overall estrogenicity (as measured by the YES assay) as well as specific compounds. Removal occurred for the most part in the activated sludge tanks. The LC-MS seemed to detect many of the estrogenic compounds in the Metro effluent, but did not capture those compounds in WLSSD effluent that were causing the estrogenic response in the YES or rtERB assays. This is probably because of the complexity of the WLSSD influent (about 66% industrial), which most likely contains phytoestrogens and other estrogenic compounds of industrial origin. In fact, very few specific compounds were detected in the effluent from WLSSD (bisphenol A, estrone, and on one occasion, nonylphenol and triclosan).

We can conclude several things from these results. First, the use of a combination of methods (analytical and more general binding assays) is critical for understanding the estrogenicity discharged in wastewater. Second, well-functioning activated sludge plants, as WLSSD and Metro are, do a very good job removing estrogenicity, although estrone does tend to be produced, likely from the oxidation of estradiol conjugates. Finally, the majority of the estrogenicity leaving these plants exists as estrone, nonylphenol, bisphenol A, and triclosan (which is only weakly estrogenic). Little research has been performed on the degradation of these compounds in a complex matrix (i.e. wastewater) at low concentrations. Therefore, more research is needed before concrete recommendations for further improving performance can be made.

References

(1) Matsui, S.; Takigami, H.; Matsuda, T.; Taniguchi, N.; Adachi, J.; Kawami, H.; Shimizu, Y., Estrogen and estrogen mimics contamination in water and the role of sewage treatment. *Water Science & Technology* **2000**, *42*, 173-179.

(2) Jungbauer, A.; Beck, V., Yeast reporter system for rapid determination of estrogenic activity. *Journal of Chromatography B* **2002**, *777*, 167-178.

(3) Coldham, N. G.; Dave, M.; Sivapathasundaram, S.; McDonnell, D. P.; Connor, C.; Sauer, M. J., Evaluation of a recombinant yeast cell estrogen screening assay. *Environmental Health Perspectives* **1997**, *105*, 734.

(4) Gaido, K. W.; Leonard, L. S.; Lovell, S.; Gould, J. C.; Babai, D.; Portier, C. J.; McDonnell, D. P., Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicology & Applied Pharmacology* **1997**, *143*, 205-212.

(5) Svenson, A.; Allard, A. S.; Ek, M., Removal of estrogenicity in Swedish municipal sewage treatment plants. *Water Research* **2003**, *37*, 4433-4443.

(6) Martinovic, D.; Denny, J. S.; Schmieder, P. K.; Ankley, G. T.; Sorensen, P. W., Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance. *Environmental Science & Technology* **2008**, *42*, 3421-3427.

(7) Tanaka, H.; Yakou, Y.; Takahashi, A.; Higashitani, T.; Komori, K., Comparison between estrogenicities estimated from DNA recombinant yeast assay and from chemical analyses of endocrine disruptors during sewage treatment. *Water Science & Technology* **2001**, *43*, 125-132.

(8) Pawlowski, S.; Ternes, T.; Bonerz, M.; Kluczka, T.; van der Burg, B.; Nau, H.; Erdinger, L.; Braunbeck, T., Combined in situ and in vitro assessment of the estrogenic activity of sewage and surface water samples. *Toxicological Sciences* **2003**, *75*, 57-65.

(9) Murk, A. J.; Legler, J.; van Lipzig, M. M. H.; Meerman, J. H. N.; Belfroid, A. C.; Spenkelink, A.; van der Burg, B.; Rijs, G. B. J.; Vethaak, D., Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. *Environmental Toxicology & Chemistry* **2002**, *21*, 16-23.

Result 2: Analysis and Development of Treatment Alternatives

Description:
Based on the results from Result 1, we will develop a comprehensive listing and description of treatment alternatives designed to avoid or minimize the formation of estrogenic compounds or to treat them based on how they partition. It is expected that there will be a variety of treatment alternatives that, given the information generated from the field and laboratory studies, should result in minimizing this problem.

Summary Budget Information for Result 2:	LCCMR Budget	$ 27,789
	Balance	$ 6,069

Completion Date: June 30, 2008

Final Report Summary:
Results have been disseminated at several conferences. In addition, two manuscripts are being written and will be submitted for publication next month (September, 2008), likely to the journal *Environmental Science and Technology* or *Environmental Chemistry and Toxicology*, both excellent journals with a high impact factors. These papers will be forwarded to the LCCMR office once they have been accepted for publication. This project also resulted in the generation of three Master's theses (**1 included** and two to be sent to the LCCMR office when they are completed, approximately October 15, 2008).

Recommendations for improved treatment are difficult to make as there is little in the literature on the biodegradation of the primary estrogens leaving the plant (estrone, nonylphenol, and bisphenol A). Nonylphenol is quite hydrophobic, and will partition to either the sludge or another hydrophobic surface such as activated carbon. It is possible that this could be used to further remove nonylphenol from the effluent. Nevertheless, if a high concentration of organic matter is present in the effluent (as is certainly the case at WLSSD and likely at Metro as well), the active sorption sites on the carbon would quickly become filled by other compounds and nonylphenol may not be effectively removed.

Little research has been conducted on the transformation of bisphenol A under realistic conditions. It did appear to degrade across the activated sludge tank at the Metro Plant, but did not degrade appreciably in two out of three samples from WLSSD. It is unclear whether the lack of transformation at WLSSD was a result of increased competition from other organic compounds (such as phytoestrogens present from the pulp and paper effluent) or because the retention time was simply too low. Increasing the hydraulic and/or solids retention time could perhaps increase bisphenol A removal, although this should be studied further before changes are made on a full-scale. Such changes may also increase estrone degradation, allowing enough time for estrone to first form and then degrade within the plant.

Remaining Balance:
Deb Swackhamer did not take all of her salary for Result 2, leaving a balance. This was a result of her appointment as Interim Director of the Institute on the Environment, which paid 100% of her salary, negating the need for her to take summer salary for her work on the project. In addition, no out-of-state travel funds were used on the project.

V. TOTAL LCCMR PROJECT BUDGET:

All Results: Personnel:	**$**	**227,926**
All Results: Equipment:	**$**	**61,751**
All Results: Development:	**$**	**0**
All Results: Acquisition:	**$**	**0**
All Results: Other:	**$**	**10,323**
TOTAL LCCMR PROJECT BUDGET:	**$**	**300,000**

Explanation of Capital Expenditures Greater Than $3,500: Initial plans were to purchase an Estrogen-receptor Binding Sensor manufactured by Threefold Sensors. Problems came to light with the sensor's ability to function in environments containing high quantities of organic carbon (e.g., wastewater). We therefore decided not to purchase the sensor. We did purchase a piece of equipment that was capable of filtering and extracting compounds from 40 liters of water in a short time period, which was critical and used throughout the project.

In addition, we sought permission to use funds from LCCMR to purchase part of a liquid chromatograph coupled to a mass spectrometer (LC-MS). We used the LC-MS to quantify specific estrogenic compounds in our samples (such as nonylphenols, estradiol, and genistein); it was therefore critical instrumentation for our LCCMR project. Such equipment is prohibitively expensive if purchased new, but we found a vendor that had a used LC-MS that met our needs at a reasonable price (i.e. $45,000-$50,000), and colleagues in our building who were interested in sharing the cost of a used LC-MS that could be used for analyzing wastewater and biosolids samples. We will continue to use the LC-MS for similar analyses for its useful lifetime. If not, we commit to pay back the Environment and Natural Resources Trust Fund an amount equal to either the cash value received or the residual value approved by the LCCMR director if it is sold.

VI. OTHER FUNDS & PARTNERS:

A. **Project Partners:** Project partners include Tim Tuominen, Environmental Services, Western Lake Superior Sanitary District (WLSSD), and Michael Rieth, Principal Engineer, Metropolitan Council Environmental Services.
B. **Other Funds being Spent during the Project Period:** No other funds will be spent during the project period.
C. **Required Match (if applicable):** Not applicable
D. **Past Spending:** Funds have not been spent on the project described above.
E. **Time:** The proposed project will be completed in the allotted three-year period.

VII. DISSEMINATION:

The target audience for results from this research will be professionals in the area of wastewater treatment and estrogenic compounds. Specific targets will be environmental engineers and scientists in academia, state agencies such as the MDA and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in

peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional and national conferences such as the *Minnesota Water* conference and *The International Water Association World Water Congress*.

VIII. REPORTING REQUIREMENTS:

Periodic work program progress reports will be submitted not later than January 2006, July 2006, January 2007, July 2007, January 2008. A final work program report and associated products will be submitted by June 30, 2008.

IX. RESEARCH PROJECTS:

See Attachment B.

Attachment A: Budget Detail for 2005 Projects

Proposal Title: Unwanted Hormone Therapy: Protecting Water and Public Health (W-05)

Project Manager Name: Paige J. Novak

LCMR Requested Dollars: $300,000

2005 LCMR Proposal Budget	Amended Result 1 Budget (approved):	Amount Spent *(06/30/08)*	Balance *(06/30/08)*	Result 2 Budget:	Amount Spent *(06/30/08)*	Balance *(06/30/08)*	
	Plant Mass Balance and Characterization			Analysis and Development of Treatment Alternatives			
BUDGET ITEM							**TOTAL FOR BUDGET ITEM**
PERSONNEL: Staff Expenses, wages, salaries							
Paige Novak							
Michael Semmens							
Deborah Swackhamer							
Graduate Assistant							
Graduate Assistant							
Administrative Assistance (assistance that is essentially needed and directly related to the project such as accounting for the project, ordering supplies and invoicing for the project, assisting with analytical method development on the project, etc.)							
PERSONNEL: Staff benefits							
Paige Novak							
Michael Semmens							
Deborah Swackhamer							
Graduate Assistant, health benefits and summer FICA							
Graduate Assistant tuition							
Graduate Assistant, health benefits and summer FICA							
Graduate Assistant tuition							
	185,137	188,075	-2,938	27,789	21,720	6,069	3,131
Other direct operating costs							
Laboratory supplies (including, but not limited to, gas cylinders, glassware, syringes, chemical standards, membrane filters, fractionation columns, and supplies for the estrogenicity detector)	50,751	50,930	-179				-179
Laboratory equipment maintenance (for equipment used on this project only)	2,000	1,766	234				234
Equipment / Tools							
Estrogenicity detector	29,000	28,978	23				23
Printing (publication costs and copying costs directly related to this project only)	323	750	-427				-427
Travel expenses in Minnesota (to sampling sites)	2,000	1,745	255				255
Travel outside Minnesota (for dissemination at national and regional conferences)	3,000	0	3,000				3,000
COLUMN TOTAL	**272,211**	**272,243**	**-32**	**27,789**	**21,720**	**6,069**	**6,037**