Okanagan Estrogens Project Update November 2009

Background information brief research review

Endocrine Disrupting Compounds enter waterways through sewage and industrial treatment plant effluent, overland runoff from both urban and agricultural land and input from cattle feedlot facilities (1-4). Since the origination of the concept, the definition of Endocrine Disrupting Compound (EDC) has not been clear (5). For the purpose of this discussion, EDCs are both natural and synthetic exogenous compounds that elicit an unnatural response by an organism's endocrine system (2, 6).

The problem with EDCs in the environment is that regardless of a short or long contaminant half life, they are persistent due to the continual disposal into the environment. Even though it was found that 17α-ethinylestradiol (a synthetic estrogen) bioaccumulates in Mediterranean Mussel, the main problems arise from chronic low dose exposure to EDCs (6). It has long been noted that these chronic levels cause feminization of male fish and amphibians and even the collapse of fish populations (7-9). With respect to humans, the recent increases in certain cancers, a declining boy to girl sex ratio and a decrease in sperm quality and quantity have been linked to EDCs (6). It is unresolved as to whether or not these affects can be as a result of exposure through drinking water, indicating a need for further exploration (6).

The four EDCs of interest for this project are the natural steroidal estrogens, estrone (E1), estradiol (E2), and estriol (E3) and the synthetic ethinylestradiol (EE2). The three steroid hormones are mainly produced by females and to a lesser extent, males (8). Estradiol is a sex hormone produced by the ovaries, placenta and the testes. Estrone is a metabolite of E2 and is commonly found in urine, the ovaries, and the placenta (10). Estriol is produced mainly during pregnancy by the placenta and to a smaller extent by the ovaries (2). EE2 is derived from E2 and is an orally bio-active estrogen used in almost all modern formulations oral contraceptive pills (11).

All four of the studied compounds are not equally potent, but in the field of EDCs natural estrogens are the most potent (12)(3, 7, 13, 14). Estriol is the least potent, then Estrone, Estradiol and Ethinylestradiol (15). Estradiol is only about 1.5 times more estrogenic that Estrone (16). This is important to consider when we look at the levels exiting treatment facilities.

Once EDCs are in the environment they are extremely difficult to remove. Conventional treatment with coagulation and filtration has been found to only remove up to 25% of EDCs (6). Additional methods such as Granular Activated Carbon and Reverse Osmosis may prove more effective but are costly and have their own associated problems (6). Biological wastewater treatment does degrade EDCs to a point but steroidal EDCs in particular are not fully broken down (17). In general, adsorption of EDCs to sediments removes more EDCs than does biodegradation (17).

Project description update

The Okanagan Endocrine Disruptor research project began in September 2008 with the hiring of a Masters Student. Since this time, much progress has been made with respect to method development, sampling procedures and general research reviews.

The objectives of the study are: (1) to determine the estrogen concentrations leaving three Okanagan wastewater treatment facilities, (2) to determine if concentrations in the receiving waters are a concern, and (3) depending on the results point to a best practices approach with respect to endocrine disruption.

Since September 2008 the following has been carried out:

(1) Sampling continuously at the three sewage treatment plants on a fairly consistent time schedule (monthly),
(2) Sampling of receiving waters from spring 2009 to early fall 2009 on a monthly basis,
(3) Development and tweaking of analytical methods using the HPLC only and then using the HPLC-MS-MS, and
(4) Initial determination of estrogen concentrations in treated sewage outfall

The plans for the remainder of 2009 and forward are as follows:

(1) Continue sampling wastewater effluents through the winter months,
(2) Sample the MacKay reservoir in Vernon throughout winter,
(3) Sample Okanagan Lake until lake stratification breaks down completely, and
(4) Increase sample volumes of all of the receiving waters to push the detection limit

Obstacles

To date, most of the analytical procedures for analyzing for steroidal estrogens employ the use of a Solid Phase Extraction (SPE) step and then Liquid Chromatography-tandem Mass Spectroscopy (LC-MS-MS)(18). GC-MS and LC-MS are noted as the most sensitive and reliable instruments for analysis of compounds such as EDCs (18). Since there is no standard method developed, there has been a process of strategic elimination to establish a method that will work for all wastewater and receiving water samples. However, there have been obstacles along the way.

In February 2009 the worldwide shortage of the solvent Acetonitrile (ACN) started to affect the project as orders could not be filled. Up to this point, ACN had been used as the main solvent for instrument operation. Established methods most commonly employed ACN as an analytical solvent. Consequently, a new method employing methanol was developed and tested. While the new method continues to produce good results, the ACN method needs to be tested to ensure that the results are comparable. Supply/demand for ACN increased in October 2009 making this possible.

While it was expected that the three different treatment plant effluents would not behave the same, we observed dramatic matrix effects among wastewaters. Thus, it has been difficult to standardize the methods for the analysis. While the cause of the difference has not been specifically determined, it is likely to do with the incoming sewage and not the variation in the treatment process. Initial evaluations of the three treatment plants show that they are very similar in their principle operation. The incoming water quality parameters which are sampled for monthly are similar; however nutrients are the main analyte. It is likely that the observed matrix effects are caused by differences in the loading of organics.

Achievements and Results to Date

As part of the method development and evaluation, theoretical concentrations of the four compounds of interest were calculated. Based on the model created by Johnson and Williams (2004), theoretical concentrations of Estrone, Estradiol and Ethinylestradiol were calculated (table 1) (19). The model was based on a review of natural and synthetic estrogen excretion by humans using European and American data (19). Although the paper was written to explain and estimate United Kingdom values, the model is useful to us as population demographics and values with respect to birth control are expected to be similar.

Table 1 Theoretical concentrations of estrone, estradiol and ethinylestradiol in the treated wastewater effluent

Analyte	Concentrations at Vernon Wastewater Treatment Facility (ng/L)	Concentrations at Kelowna Wastewater Treatment Facility (ng/L)	Concentrations at Penticton Wastewater Treatment Facility (ng/L)
Estrone	13.6 ± 5.3	15.8 ±6.2	12.2 ± 4.7
Estradiol	1.7 ± 0.6	1.9 ±0.6	1.5 ± 0.5
Ethinylestradiol	0.36 ± 0.05	0.39 ±0.06	0.32 ± 0.05

The results to date (table 2) compared with the theoretical results show that for Estrone, Vernon and Penticton wastewater are much lower than expected, while Kelowna wastewater is higher.

Table 2 Measured concentrations of estrogens in treated wastewater effluent

Analyte	Measured Concentrations at Vernon Wastewater Treatment Facility (ng/L)		Measured Concentrations at Kelowna Wastewater Treatment Facility (ng/L)		Measured Concentrations at Penticton Wastewater Treatment Facility (ng/L)
	Pre UV treatment	Post UV treatment	Pre UV Treatment	Post UV Treatment	
Estrone	1.05 ± 2	0.46 ± 2	NA[i]	39 ±5	3.9 ± 2
Estradiol	1.4 ± 6	0.8 ± 6	NA	4 ± 8	ND
Estriol	ND[i]	ND	NA	ND	ND
Ethinylestradiol	ND	ND	NA	ND	ND

Note i ND = Non Detect, NA = no value to date

Okanagan River

Initial results from the Okanagan River Channel in Penticton (data still in progress) show very low levels of Estrone, which are near the detection limit. We are currently working on different methods to improve the detection limit so that we can more confidently determine the concentration. The present detection limit is estimated at 0.2 ng/L for Estrone and 0.5 ng/L for Estradiol.

The Okanagan River Channel system is analogous to a plug flow reactor. In these systems reactions and degradation rely on a long “reaction tube”. The preliminary results suggest that the downstream levels (up to 3300m) are a result of dilution as opposed to additional degradation in the “reaction tube” or river.

Should the levels in the Penticton wastewater effluent reach the levels seen in the Kelowna wastewater facility there would be a problem in the river channel. For example, in average flow conditions (8 m^3/s) the river channel the concentration would be 0.7 ng/L Estrone; during low flow (4 m^3/s), the concentration would be 1.4 ng/L Estrone (20) . These levels are higher than the 0.5 ng/L that causes problems in fish populations.

Okanagan Lake

Samples were taken on Okanagan Lake in July, August and September. In preparation for sampling the effluent plume, a limnological modeling study was used (HayCo study) (21) to site the most appropriate sample locations.

In order to track the plume fluorescence, conductance and temperature measurements were used. Fluorescence measurements can be used to trace wastewater as a result of the optical properties of detergents (22, 23). Conductance and temperature are also commonly used to trace wastewater due to the high conductance and temperature of effluent water. Following the suggested plume outlined in the HayCo study, intensive sampling was performed both vertically and horizontally on different days to ensure that potential hot pockets were not missed. The results from our tracking showed little evidence of a coherent plume. The authors suggest that the plume could vary considerably in both the vertical and horizontal direction (21). We intend to sample more intensely in proximity the outfall, with special attention to the perforations in the transmission pipe.

Using the HayCo-derived scenario, we calculated that there would be an approximate concentration of 0.0195 ng/L Estrone about in a plume at about 25 meters depth, and within 1000m horizontal distance from the diffuser. This level below our current detection limit.

Ultraviolet Treatment

Preliminary results indicate that the addition of UV treatment to the liquid stream further degrades the estrogens. There has been a lot of other research to suggest this as well but so far it has been determined that it is not a reliable treatment option as the dosage would have to be dramatically increased to produce consistent results (17).

Putting it all together

The reduction of EDCs in Okanagan River Channel is likely through dilution and not through degradation. While the preliminary results for Okanagan Lake still need to come in, the vectors for degradation are dilution and potentially minor ultraviolet irradiation and biodegradation. This means there is potential for a biologically relevant estrogenic plume in Okanagan Lake. It appears that the depth distribution of a potential plume overlaps with the distribution of Kokanee in Okanagan Lake (24). In terms of Vernon's wastewater, there is dilution and increased biodegradation in MacKay reservoir and increased scavenging via the land application of effluent. This suggests that it will be very difficult to detect estrogens in Vernon effluent "receiving waters". From the perspective of receiving waters, it appears that waste water treatment and effluent management in Vernon are likely superior to those in Kelowna and Penticton.

Upcoming tasks to be completed

Over the next year there are still many tasks to be completed. As mentioned some of the first tasks will be to lower the detection limit of the methods. This will allow for more confidence in very low values and detection of estrogens in previous non-detect waters. We will expand and intensify our sampling of MacKay reservoir , continue sampling the Okanagan River (all seasons), and conclude a second sample season for the stratified Okanagan Lake values, Vernon "receiving waters (summer) in 2010. Sampling in Okanagan Lake will include high resolution mapping of the effluent plume.

References

(1) LINTELMANN, J.; KATAYAMA, A.; KURIHARA, N.; SHORE, L.; WENZEL, A. Endocrine Disruptors in the Environment. *Pure Appl. Chem.* **2003,** *75* (5), 681.

(2) US Environmental Protection Agency. Endocrine Disruptors Research | Science Topics | NCER | ORD | US EPA. *2009* (10/30/2009).

(3) Servos, M. R.; Bennie, D. T.; Burnison, B. K.; Jurkovic, A.; McInnis, R.; Neheli, T.; Schnell, A.; Seto, P.; Smyth, S. A.; Ternes, T. A. Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. *Sci. Total Environ.* **2005,** *336* (1-3), 155-170.

(4) Zhang, Y.; Zhou, J. L. Occurrence and removal of endocrine disrupting chemicals in wastewater. *Chemosphere* **2008,** *73* (5), 848-853.

(5) Foster, W. G.; Agzarian, J. Toward Less Confusing Terminology in Endocrine Disruptor Research. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews* **2008,** *11* (3), 152.

(6) Rahman, M. F.; Yanful, E. K.; Jasim, S. Y. Endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in the aquatic environment: implications for the drinking water industry and global environmental health. *Journal of Water & Health* **2009,** *7* (2), 224-243.

(7) Fernandez, M. P.; Campbell, P. M.; Ikonomou, M. G.; Devlin, R. H. Assessment of environmental estrogens and the intersex/sex reversal capacity for chinook salmon (Oncorhynchus tshawytscha) in primary and final municipal wastewater effluents. *Environment International,* **2007,** *33* (3), 391-396.

(8) Kidd, K. A.; Blanchfield, P. J.; Mills, K. H.; Palace, V. P.; Evans, R. E.; Lazorchak, J. M.; Flick, R. W. Collapse of a fish population after exposure to a synthetic estrogen. *Proc. Natl. Acad. Sci. U. S. A.* **2007,** *104* (21), 8897-8901.

(9) Routledge, E. J.; Sheahan, D.; Desbrow, C.; Brighty, G. C.; Waldock, M.; Sumpter, J. P. Identification of Estrogenic Chemicals in STW Effluent. 2. In Vivo Responses in Trout and Roach. *Environ. Sci. Technol.* **1998,** *32* (11), 1559-1565.

(10) Fernandez, M. P.; Buchanan, I. D.; Ikonomou, M. G. Seasonal variability of the reduction in estrogenic activity at a municipal WWTP. *Water Research,* **2008,** *42* (12), 3075-3081.

(11) Lai, K. M.; Johnson, K. L.; Scrimshaw, M. D.; Lester, J. N. Binding of Waterborne Steroid Estrogens to Solid Phases in River and Estuarine Systems. *Environ. Sci. Technol.* **2000,** *34* (18), 3890-3894.

(12) Liu, Z.; Kanjo, Y.; Mizutani, S. Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: A review. *Sci. Total Environ.* **2009,** *407* (18), 4975-4985.

(13) Koh, . Treatment and removal strategies for estrogens from wastewater. *Environmental technology* **2008,** *29* (3), 245.

(14) Harries, . Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environmental Toxicology and Chemistry* **1997,** *16* (3), 534.

(15) Huo, C. X.; Hickey, P. EDC demonstration programme in the UK Anglian Water's approach. *Environmental technology* **2007,** *28* (7), 731.

(16) Metcalfe C.D.; Metcalfe T.L; Kiparissis Y.; Koenig B.G.; Khan C., ; Hughes R.J.; Croley T.R.; March R.E.; Potter T., .
Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with
Japanese medaka (Oryzias latipes). *Environmental Toxicology and Chemistry* **2001,** *20* (2), 297.

(17) Bolong, N.; Ismail, A. F.; Salim, M. R.; Matsuura, T. A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination* **2009,** *239* (1-3), 229-246.

(18) Briciu, R. D.; Kot-Wasik, A.; Namiesnik, J. Analytical Challenges and Recent Advances in the Determination of Estrogens in Water Environments. *J. Chromatogr. Sci.* **February 2009,** *47*, 127-139(13).

(19) Johnson, C.; Williams, R. J. A Model To Estimate Influent and Effluent Concentrations of Estradiol, Estrone, and Ethinylestradiol at Sewage Treatment Works -. *Environ. Sci. Technol.* **2004,** *38* (13), 3649-3658.

(20) Environnment Canada. Real-Time Hydrometric Data. **2006,** *2009* (11/10/2009).

(21) HAY & COMPANY CONSULTANTS INC. ***City of Kelowna: Influence of Limnology on Domestic Water Intakes*** 2001.

(22) Managaki, S.; Takada, H.; Kim, D.; Horiguchi, T.; Shiraishi, H. Three-dimensional distributions of sewage markers in Tokyo Bay water—fluorescent whitening agents (FWAs). *Mar. Pollut. Bull.* **2006,** *52* (3), 281-292.

(23) Ahmad, S. R.; Reynolds, D. M. Monitoring of water quality using fluorescence technique: prospect of on-line process control. *Water Res.* **1999,** *33* (9), 2069-2074.

(24) Sebastien, D.; Scholten, G.; Woodruff, P. Okanagan Lake Kokanee Abundance, Size and Age Structure Based on Acoustic and Trawl Surveys, 1988 to 2004. Okanagan Lake Action Plan Year 9 (2004) Report. BC Ministry of Environment, Fisheries Project Report No. RD 111 **2005**.