THE IMPACT ENDOCRINE DISRUPTING CHEMICALS (EDC'S) DISCHARGED FROM WASTE WATER TREATMENT PLANTS HAVE ON RIVER SYSTEMS AND AQUATIC LIFE

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ABSTRACT

The variability and extremes of the Australian climate often results in high summer water temperatures, very seasonal flows and sometimes stressed rivers. Dealing with this variability poses many challenges for our river managers. In some river systems in Australia, treated effluent can be a major component of the total flow and discharges from Waste Water Treatment Plants (WWTPs) are increasingly being regarded as a potential source of environmental flows.

Overseas studies have indicated that Endocrine Disrupting Chemicals (EDCs) and other micro pollutants (pharmaceuticals) may not be fully removed by existing waste water treatment technologies. In the Australian context, there was very little understanding of what impact the range of micro pollutants present in WWTPs effluent could have on native aquatic species.

To obtain information in Australian conditions, a collaborative study was conducted by CSIRO and the U.S. Geological Survey at the West Wodonga WWTP. This plant receives waste water from the Wodonga Township as well as several other trade waste customers. As the plant discharges treated effluent to the Murray River, it was an ideal site at which to undertake such a study and the results are included in this paper.

KEY WORDS

Endocrine Disrupting Chemicals (EDCs), Mobile Fish Unit (MFU), North East Water (NEW), Waste Water Treatment Plant (WWTP), EGL Management Services (EGLms)

1.0 INTRODUCTION

Intersex fish (such as male fish with eggs), and other evidence of reproductive disruption including behavioural changes, have been observed in fishes collected downstream from wastewater treatment plants (WWTPs) outfalls in the United Kingdom, Europe, Asia, and North America. It was shown that some of these disruptive changes were associated with effluent from supposedly well-functioning sewage treatment works. The effects appeared to be mostly estrogenic and associated with an up-regulation of the vitellogenin gene in male fish. Vitellogenin is an egg-yolk protein and is only present in female fish. Under the influence of chemicals with estrogenic properties in the WWTP effluent, the male fish were found to be expressing female characteristics. Studies indicated the most potent components were estradiol (natural hormone secreted by females) and ethinylestradiol (active ingredient in birth-control pill) as excretory products of high density human and domestic animal populations.

Endocrine disruption in fishes native to Australia has not been investigated so far.

CSIRO staff from Adelaide (Dr Anu Kumar, Dr Mike Williams and Mr Hai Doan) in collaboration with a scientist from U.S. Geological Survey (Dr Larry Barber) established a trial project at the West Wodonga WWTP which discharges treated effluent to the Murray River.

The trial was to monitor if the survival and reproduction in fish is affected by EDCs present in the treated effluent. This would assist in assessing if the treated effluent has any effect on the receiving waters which is the Murray River in this case-study. The trial focused on the estrogenic compounds and their affect on the reproductive system in a native fish species, the Murray Rainbowfish and a standard fish test model used in the overseas studies, the Zebra fish.

The Wodonga site was chosen for the trial as its raw waste water comes from both domestic and trade waste sources. The trade waste component comes from an abattoir and pet food factory, both increasing the potential for hormones to be present in the effluent.

1.1 What is an Endocrine Disruptor?

Endocrine Disrupting Chemicals (EDCs) can be both natural and man-made and can interfere with the hormonal system and disrupt the normal functioning of the endocrine system. An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, corresponding to a change in endocrine function (*excluding, however, non-specific toxic effects*).

1.2 Endocrine Disrupting Chemical Studies and Effects

Studies at WWTPs overseas indicate that even highly treated effluents are likely to have enough natural and/or synthetic hormones present to cause impacts in fish unless diluted significantly at discharge.

Researchers in the United States and Europe have demonstrated a number of effects from EDCs in wildlife populations, often involving the endocrine (hormone) system and damaging the reproductive processes. This is because EDCs mimic, block or disrupt the actions of hormones and disrupt the normal functioning of the endocrine system.

Endocrine disrupting effects include altered hormone and specific protein levels, intersex (both male and female reproductive organs), imposex (females with male sex organs), reduced reproductive success and abnormal growth or reproductive development. For most of the reported effects in wildlife, the evidence for a causal link with endocrine disruption is still weak or non-existent, although in some instances a more definitive link has been made.

1.3 The Main Objectives

The project had the following main objectives:

- To determine whether effluents from WWTPs can cause reproductive disruption in two fish species (one native) within 28 days in an on-site mobile laboratory.
- To quantify the estrogenic and androgenic load and accumulation in the creeks and rivers receiving treated effluent as environmental flows.
- To ensure that the reproductive health and sustainability of indigenous fish populations would not be adversely affected in environments receiving treated effluents as environmental flows.
- To inform operators of the impact their treated effluent has on the receiving waters and aquatic life.

2.0 THE WEST WODONGA WWTP PROCESS

This plant is located on Old Barnawartha Road, Wodonga West in Victoria. The plant has advanced secondary treatment by Biological Nutrient Removal using a 5 stage Bardenpho, process followed by UV disinfection. The plant receives 11 ML/d on average that is made up of domestic and industrial components and has a capacity of around 130,000 EP on a BOD basis.

Treatment consists of 3mm step screens with flow through to two biological nutrient removal reactors. The solids stream is then sent through dissolved air floatation for thickening, aerobic digestion, centrifuge dewatering with air drying of the sludge which is then taken off site for reuse. The process has another stream that flows through sand filters then chlorination to offsite reuse customers. The liquid stream is sent to secondary clarification with the final effluent being disinfected by ultraviolet light prior to discharging to the Murray River downstream of the Lake Hume.

3.0 THE FISH TRIAL

One hundred and thirty of each, adult male Murray Rainbowfish and Zebrafish fish were utilised in on-site experiments within a flow-through mobile exposure laboratory. These fish were used as they can be easily maintained under laboratory conditions, are found in South Australia, Victoria and New South Wales with the male and females easily identified. Ten fish of each species were collected as initial controls. The remaining fish were exposed to reference water or dilutions of de-chlorinated WWTP effluent 100% effluent - 100% river water - 50%: 50% (effluent: river water) 25%: 75% (effluent: river water) - 10%: 90% (effluent: river water) for up to 28 days (7, 14 and 28 days).

Murray Rainbowfish

Zebrafish

The laboratory was equipped to conduct on-site experiments under conditions of controlled temperature, lighting, feed, aeration, and flow. All surfaces in contact with test solutions were glass, stainless steel, or Teflon to minimise any contamination. The water was collected from upstream of the WWTP outflow (Reference) or from the effluent channel (100%-Effluent) into separate 200 L stainless steel holding tanks. Thermally equilibrated water then flows to stainless steel splitter tanks, and then to 15-L glass aquarium. The flow rates ensure that retention time in the holding tanks did not exceed two hours. Throughout this experiment, fish were maintained at 24 ºC (+/- 1) under flow-through conditions in the glass aquariums. Flow to the aquariums was maintained at 200 mL/min, providing 99% replacement of test volume every hour. Aeration of test water in each aquarium at 100 bubbles per minute guarantees a minimum dissolved oxygen concentration of > 85% throughout the experiment.

Mobile Fish Unit Set Up External View ***Sample collection***

3.1 The Sampling Schedule

Effluent samples were collected everyday to characterise the chemistry during the on-site fish exposure experiment in April-May, 2009. Basic water quality parameters (chemical oxygen demand, total suspended solids, ammonium, and major ions) were analysed using standard techniques. The effluent and river water samples were analysed for a range of EDCs, pharmaceuticals, and consumer product chemicals.
The fish were sacrificed at day 1, 7, 14 and 28. At each sampling time, fish were anaesthetised before being decapitated and put in to dry ice. They were then transported to a laboratory where the fish samples were processed to assess the estrogenic impacts of being exposed to treated effluent over the various days. Samples of the liver, gonads, blood /plasma were frozen to -80°C for protein and nucleotide analysis. They were then checked for gross abnormalities, intersex status, Vitellogenin detection (mRNA and protein) using standard molecular techniques.

3.2 Results

The maximum concentration column represents the maximum levels detected in the effluent at the Wodonga Plant and these are shown in the table below.

<u>Table 1:</u> ***Treated effluent analysis results***

Compound	Maximum Concentration in Treated Effluent	Relative Potency
17β-Estradiol	< 1 ng/L	1
Estrone	27 ng/L	0.22
17α-Ethynylestradiol	< 1 ng/L	1.03
4-Nonylphenol	1000 ng/L	0.0001
4-*t*-Octylphenol	<1 ng/L	0.0004
Bisphenol A	<1 ng/L	0.0001
4-Nonylphenolethoxylate	<1 ng/L	0.000006
Total estrogenicity as 17β-Estradiol Equivalent (EEQ)	< 6 ng/L	

17β-Estradiol is the natural estrogens secreted by females.
17α-Ethynylestradiol is the birth control pill.

The WWTP effluent and Murray River reference water did not affect fish survival during the 28-day trial. The fish were under minor physiological stress but we did not find intersex condition (male fish with eggs) in the male Murray rainbowfish or zebrafish exposed to the 100% effluent or the river water and their dilutions. The Vitellogenin gene and protein in male fish exposed to the 100% effluent were also close to the background levels found in the river water exposed fish.

Based on the laboratory studies conducted with Murray rainbowfish by Dr Marianne Woods and Dr Anu Kumar, it has been established that Vitellogenin induction in male rainbowfish can be induced at concentrations of ~ 10 ng/L 17 β-Estradiol. In light of these results, the absence of Vitellogenin induction at both the mRNA and plasma protein level after 28 days of exposure to either the Wodonga WWTP effluent or the Murray River reference water suggests that these sources of water are not significantly estrogenic. The estrogenic activity of the effluent was also low (between 2-6 ng/L) during exposures further confirming the low estrogenicity of the treated effluent from the Wodonga plant. The overall results are summarised in the table below based on the multiple lines of approaches undertaken in this study. Majority of the approaches over 28-day exposure period are represented by green colour suggesting no overall effect of the river water during 28 day exposure period. However, the treated effluent exhibited minor effect in fish on day 1, day 7 and day 28 of the exposure period. The variability of the effluent quality over 28-day exposure period could be responsible for the variable responses in exposed fish.

Table 2: ***Summary of Results – Tiered Approach with Multiple Lines of Evidence***

Treatment	Analytical	Estrogenic response	Androgenic Response	Fish mortality ZF	Fish mortality RF	Fish biomarker mRNA	Fish biomarker VTG	Fish histology	Overall summary
Reference Water	-	-	-	+	-	-	-	-	-
Effluent 100% Day1	++	+	-	+	-	-	-	-	+
Effluent 100% Day 7	+	-	-	+	-	+	+	+	+
Effluent 100% Day 14	-	-	-	+	-	-	+	-	-
Effluent 100% Day 28	+	-	-	+	-	+	+	+	+

-	No Effect	+	Minor Effect	++	Effect	+++	Major Effect

The results confirm that the tertiary treated Wodonga effluent has a very low estrogenic potential. The dilution of the Wodonga effluent in the Murray River is anticipated to be more than 90% and at such dilutions there is no risk to the reproductive success of rainbowfish downstream of this outfall.

4.0 RISK OF UNDERTAKING THE STUDY

There was a substantial risk in the trial as it was the first MFU experiment to be undertaken in Australia. Not knowing what the project outcome would be before it started, there was a risk that if there was an impact to the fish, the whole operation of the WWTP may need to be amended in order to protect our downstream users. Whatever the trial outcome, North East Water and EGLms were fully supportive of the trial and wanted to be made aware of any environmental impact that needed to be researched and reported.

5.0 CONCLUSION

The results from this experiment have provided valuable evidence for operators around Australia about how important their WWTP process and operating skill has on our environment. The MFU experiment was successfully conducted over 28 days with all fish surviving the 28 days exposure. This confirmed that there were no acute effects to fish when exposed to 100% treated effluent. After the laboratory had analysed the samples it was confirmed that there were no detrimental effects on the reproductive system of the fish and that the effluent from the Wodonga WWTP did not induce any endocrine disrupting effects. This approach can be further tested at other WWTPs to evaluate the estrogenic potential of the treated effluent before releasing it into the receiving environment. Although results from this investigation at a tertiary WWTP suggest little impact, they cannot necessarily be extrapolated to other less advanced treatment technologies still found throughout Australia.

I would strongly encourage other organisations to participate in other or similar trials as it was a very rewarding and informative exercise.