Assessment of the endocrine disruption potential of an advanced tertiary treated sewage effluent using multiple lines of evidence

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Abstract

In Australia, due to increased uncertainties over security of water supply because of unpredictable drought and flood cycles, alternative water sources are being investigated for commercial, agricultural, industrial and domestic supply, including the option of reusing treated sewage effluents. However, sewage effluent is a known source of estrogenic endocrine disrupting chemicals (EDCs) in the environment. Exposure to sewage effluents containing steroid estrogens and xenoestrogens can cause developmental and behavioural reproductive abnormalities in fish and other aquatic animals. As such, risk of endocrine disruption is one of the water quality issues that needs to be evaluated when assessing the appropriate level of treatment required for reuse applications. The Gerringong-Gerroa sewage treatment plant (GGSTP), currently employs advanced tertiary treatment technology to treat domestic sewage from two small coastal towns (Gerringong and Gerroa), which receive large seasonal influxes of holiday makers. In this study, the efficacy of the treatment at the GGSTP in removing estrogenically active chemicals was assessed using a multi-tiered assessment approach, incorporating chemical analysis, *in vitro* bioassays and *in vivo* fish exposure studies.

The raw sewage influent was found to contain steroidal estrogens; 17β-estradiol (E_2), estrone (E_1) and estriol (E_3) as well as synthetic phenolic xenoestrogens; 4-tert-octylphenol, Bisphenol A and technical nonylphenol at concentrations commonly found in sewage influents. The influent also displayed high levels of activity in the two-hybrid yeast *in vitro* bioassay. However, the final effluent had no detectable concentrations of steroidal estrogens, no estrogenic activity in the two-hybrid yeast assay and only infrequent occurrence of low concentrations of synthetic phenols. Biodegradation by activated sludge treatment provided significant, but incomplete removal of measured EDCs and estrogenic activity, with the in-line combination of ozone oxidation and biologically activated carbon filtration reducing the remaining estrogenic activity to undetectable levels. EDCs in both the dissolved and particulate phases of the effluent were removed by the treatment process and the efficacy of treatment was not compromised by increases in influent flow during the peak holiday seasons. Treatment of the effluent at the GGSTP was also successful at reducing retinoic acid receptor

(RAR) activity and genotoxicity to below detection limits and greatly reducing arylhydrocarbon receptor (AhR) activity.

On-site real-time exposure tests using the mosquitofish (*Gambusia holbrooki*) and rainbowfish (*Melanotaenia fluviatilis*) demonstrated that the final effluent did not elicit up-regulation of vitellogenin, a well known biomarker of exposure to estrogenic EDCs. Despite the presence of residual concentrations of E_1 and the *in vitro* activity in effluent after being processed through activated sludge treatment, clarification and sandfiltration, vitellogenin up-regulation was not detected in fish exposed to this partially treated effluent. Overall, the results provide evidence that the application of advanced tertiary treatment technology to domestic sewage can produce a final effluent that is unlikely to pose an endocrine disruption risk to the aquatic biota.

Abbreviations

4nNP	4-*n*-nonylphenol
4nOP	4-*n*-octylphenol
4NQO	4-nitroquinoline-N-oxide
4tAP	4-*tert*-amylphenol
4tOP	4-*tert*-octylphenol
AhR	Arylhydrocarbon receptor
AP	Alkylphenol
APEO/APE	Alkylphenol polyethoxylates
AS	Activated sludge
atRA	*all-trans* retinoic acid
BAC	Biological activated carbon
BaP	Benzo[a]pyrene
BDL	Below detection limits
BPA	Bisphenol A
COA	Chemical advanced oxidants
DAFF	Dissolved air floatation filtration
DCM	Dichloromethane
DE	Diethylether
DECCW	Department of Environment Climate Change and Water
DMSO	Dimethylsulfoxide
DN	Denitrification
DNA	Dioxyribose nucleic acid
E_1	Estrone
E_2	17β-estradiol
E_3	Estriol
ED	Endocrine disruption
EDC	endocrine disrupting chemical/compound
EE_2	17α-ethynylestradiol
EEq	Estradiol equivalent (concentration)
EPA	Environmental Protection Agency
ER	Estrogen receptor
ERBA	Estrogen receptor binding assay
ERE	Estrogen response element
EROD	Ethoxyresorufin-O-deethylase
ESI	Electrospray ionisation
GAC	Granular activated carbon
GC-MS	Gas chromatography mass spectrometry
GC-MS-MS	Gas chromatography tandem mass spectrometry
GGSTP	Gerringong-Gerroa Sewage Treatment Plant
GPC	Gel permeation chromatography

H_2SO_4	Sulphuric acid
HAH	Halogenated aromatic hydrocarbon
hER	Human estrogen receptor
HRT	Hydraulic retention time
ISTD	Internal standard
Kow	Octanol-water partitioning coefficient
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LOD	Limits of detection
LOQ	Limits of quantification
MCF-7	Michigan Cancer Foundation -7
medER	Medaka (*Oryzias latipes*) estrogen receptor
MeOH	Methanol
MLSS	Mixed liquor suspended solids
mRNA	messanger ribonucleic acid
MSTFA	n-methyl-N-(trimethyl-silyl) trifluroacetamide
NP	Nonylphenol
NPE	Nonylphenol polyethoxylates
NSW	New South Wales
O3	Ozone
OP	Octylphenol
OPE	Octylphenol polyethoxylates
PAC	Particulate activated carbon
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PNEC	Proposed no effect concentration
PPCPs	Pharmaceuticals and personal care products
QLD	Queensland
qPCR	Quantitative polymerase chain reaction
RAR	Retinoic acid receptor
RNA	Ribonucleic acid
RO	Reverse osmosis
RT-PCR	Reverse-transcriptase polymerase chain reaction
SPE	Solid phase extraction
SRT	Solids retention time
STP	Sewage treatment plant
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TFAA	Trifluroacetic anhydride
TIE	Toxicity Identification Evaluation
Tm	Melting temperature
TNP	Technical nonylphenol
UV	Ultraviolet light
Vtg	Vitellogenin

YES	Yeast estrogen screen
αE_2	17α-estrdaiol
β-NP	β-naphtoflavone