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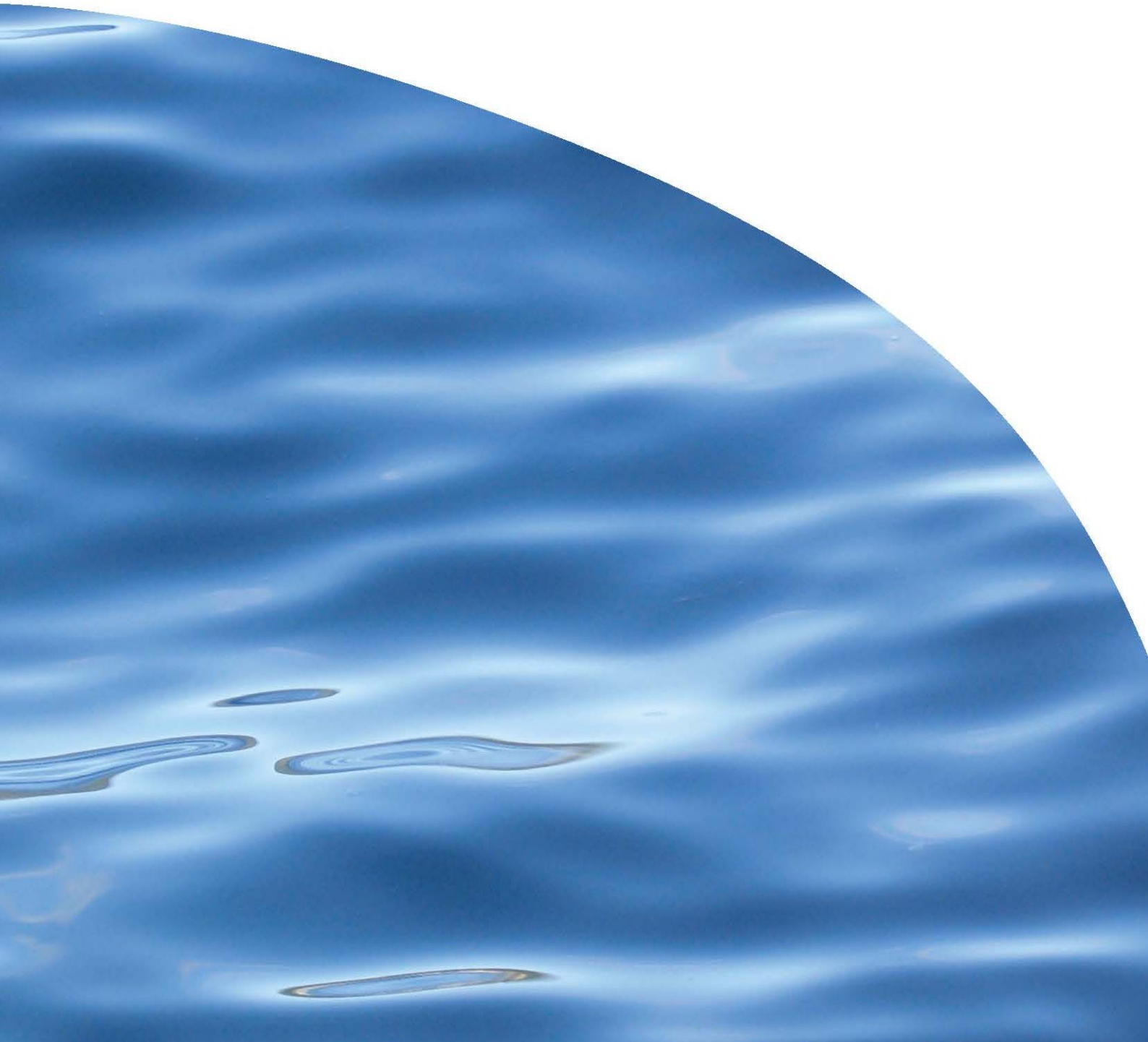
ANALYSIS OF HORMONAL ACTIVITY AND SELECTED ENDOCRINE DISRUPTING CHEMICALS IN ROTORUA DISTRICT COUNCIL SEWAGE TREATMENT PLANT WASTEWATER AND STREAM WATER SAMPLES



ANALYSIS OF HORMONAL ACTIVITY AND SELECTED ENDOCRINE DISRUPTING CHEMICALS IN ROTORUA DISTRICT COUNCIL SEWAGE TREATMENT PLANT WASTEWATER AND STREAM WATER SAMPLES

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1. INTRODUCTION

Rotorua District Council (RDC) contracted the Cawthron Institute (Cawthron) to estimate the efficacy of two wastewater treatment plant (WWTP) technologies to remove endocrine disrupting chemicals (EDCs). Municipal sewage wastewater is potentially a major source of contaminants depending on the nature of the pollutants entering the system and the treatment technologies used. Endocrine disrupting chemicals are one important 'family' of contaminants that are cause for concern because of their potential to disrupt endocrine functions in wildlife and human populations. EDCs have been defined as "exogenous substances that causes adverse health effects in an intact organism, or its progeny, secondary to endocrine function" (European Commission 1996). While sewage effluents can contain EDCs, a local study investigating the fate of estrogenic and androgenic activities in New Zealand and Australian sewage treatment plants found that secondary WWTPs (and particularly activated sludge treatment) removed up to 99% of the estrogenic and androgenic activity originally present within influents (Leusch *et al* 2006a).

The approach taken in this work combined the use of analytical chemistry and biological assays (bioassays) to measure EDCs activity in concentrated sample extracts. Bioassays using reporter gene technologies have been successfully used to estimate estrogenicity, anti-estrogenicity androgenicity and anti-androgenicity in environmental samples (Balaguer *et al*. 1999; Muller *et al*. 2008). Such bioassays are now commonly used because they are easy to perform, rapid and relatively cheap, which makes them a good choice for large-scale screening to assess hormonal activity (Leusch *et al.* 2010; Mnif *et al*. 2010; Tremblay *et al*. 2005; Tremblay *et al*. 2010).

While bioassays provide estimates of the total estrogenic or androgenic activity of a sample extract, they do not identify the specific compounds responsible. The biologically active chemicals can be identified by chemical analysis on a selection of common steroid hormones and other known EDCs. This approach has previously been used in New Zealand to assess the endocrine disruption potential of wastewater (Leusch *et al* 2006b) as well as dairy shed and dairy oxidation pond effluents (Sarmah *et al*. 2006; Gadd *et al*. 2010).

1.1. Objective of this study

This report will provide the results of the assessment of the levels of EDCs in effluents released from the Rotorua District Council WWTP and the receiving environment using chemical and biological methods.

2. METHODOLOGIES

2.1. Sample collection and extraction

The membrane bioreactor (MBR) system and Bardenpho effluent samples were collected by staff from the RDC WWTP laboratory on 13 November 2012. Grant Northcott, with assistance from an RDC staff member, collected two stream water samples from reference site-10 in the Waipa Forest and the Puarenga Stream. A sample volume of 16 L was obtained from each location by filling four replicate, 4 L amber glass Winchesters.

Blank samples to provide background levels consisted of:

- Field blanks: Solid phase extraction (SPE) cartridge blank (includes solvents, Milli-Q conditioning washes and rinses)
- Solvent blanks: Dimethyl sulfoxide (DMSO; sample of the DMSO solvent used to prepare the final extracts for bioassay analysis).

2.1.1. Sample preparation for bioassay

The samples were adjusted to pH 2.5 by the addition of concentrated sulphuric acid and vacuum filtered through GFC filters to remove particulates. Six litres of filtered sample was extracted using an Oasis HLB 1 g 20 mL SPE cartridge. The sample bottle was rinsed three times with each wash passing through the SPE cartridge. The cartridges were then dried under full vacuum. Organic chemicals, including EDCs, were eluted from the SPE cartridge with a solvent mixture of dichloromethane/methanol (95:5) and purified by passing through a sequential florosil cartridge (IST, 2 g, 12 mL) into a collection vial. The solvent extract was blown to dryness at a temperature of 30^{o}C under a gentle stream of nitrogen gas, redissolved in 0.5 mL of DMSO and transferred to 2 mL amber glass vials. With a sample volume of 6 L and final extract volume of 0.5 mL, the four effluent and stream samples were concentrated by a factor of 12,000 (relative enrichment factor).

2.1.2. Sample preparation for trace chemical analysis

Ten litres of acidified and filtered sample was spiked with a solution of carbon-13 labelled surrogate standards and extracted using an Oasis HLB 1 g 20 mL SPE cartridge. The SPE cartridges were dried under full vacuum and organic chemicals, including EDCs, were eluted from the SPE cartridge with a solvent mixture of dichloromethane/methanol (95:5) and purified by passing through a sequential florosil cartridge (IST, 2 g, 12 mL) into a collection vial. The solvent extract was concentrated and exchanged into dichloromethane and further purified using gel permeation chromatography. The purified extract was blown to dryness at a temperature of 30^{o}C under a gentle stream of nitrogen gas. A mixture of isotopically labeled internal

standards was added before the steroid hormones and other polar chemical residues were derivitised to their respective trimethylsilyl ethers.

2.2. Estrogenic and androgenic bioassays

Estrogenic and androgenic activity were determined by estrogen receptor (ER)- and androgen receptor (AR)-GeneBLAzer assays (Huang *et al*. 2011). The GeneBLAzer assay relies on a genetically-engineered cell line transfected with a beta-lactamase reporter gene to monitor the cellular response to a sample. In brief, a duplicate 3-point geometric serial dilution of each sample was incubated with 10,000 (ER) or 20,000 (AR) cells per well in a poly-D-lysine-coated 384-well plate for 18 h at 37ºC and 5% CO_2, followed by incubation with 1 µM LiveBLAzer-FRET solution for 2 h at room temperature. Fluorescence, a direct measure of estrogenic (ER) or androgenic (AR) stimulation, was then measured at 460 and 520 nm after excitation at 410 nm. The response of each sample dilution curve is compared to a 17β-estradiol (ER) or 5α-dihydrotestosterone (AR) standard curve in agonist mode; tamoxifen (ER) or flutamide (AR) are used in antagonist mode. Results are expressed as biological equivalent concentration of the corresponding analyte. Several quality assurance/quality control (QA/QC) samples are present on each plate and each assay run has to pass strict acceptability criteria against control charts for positive and negative (solvent) controls, concentration-effect curve of the reference standard and minimum induction. The results presented here are from at least two independent assays performed on different days.

2.3. Chemical analysis

The trimethylsilyl ethers of the target EDCs were analysed by high resolution gas chromatography mass spectrometry (HRGC-MS) using an Agilent 6890N gas chromatograph (GC) coupled to an Agilent 5975A inert XL mass spectrometer (MS) and CTC autosampler. Target analytes and isotopically labeled analogues were detected using single ion monitoring of compound specific mass ions. Calibration standards were prepared and derivitised as previously described. Eight-point calibration curves from 1-1000 ng/mL were prepared to estimate analyte concentrations in the sample extracts. Target analytes and surrogate recovery compounds were quantitated by internal standard quantitation using Agilent Enhanced Chemstation data analysis software.The total mass of target compounds in each sample extract was calculated, divided by the volume of sample extracted, and reported as a final concentration in ng/L, or parts per trillion (ppt).

A selection of EDCs were analysed by HRGC-MS. The specific compounds analysed included:

- the estrogenic steroids: 17α- and 17β-estradiol, estrone, estriol, 17α-ethynylestradiol, and mestranol
- the androgenic steroids: testosterone, androstenedione, androstenediol, dihydrotestosterone, hydroxytestosterone, ketotestosterone, 19-nortestosterone
- industrial and domestic derived endocrine disrupting (EDCs) and antimicrobial chemicals including: alkylphenols (nonyl- and octylphenols), bisphenol-A, parabens (methyl-, ethyl-, propyl-, butyl-, benzyl-), triclosan and methyltriclosan, o-phenylphenol, and chloroxylenol.

2.3.1. Method detection limits

Method detection limits (MDLs) for the target analytes ranged from 0.01 to 10.0 ng/L (also see Table 3). The MDLs were estimated as the minimum mass of target compound required to produce a peak height exceeding a signal to noise ratio of three to one. Typical MDLs were equivalent to 0.01 ng/L, based on extracting a sample volume of 10 L. As trace levels of technical nonylphenol (TNP), methyl- and butyl parabens, and chloroxylenol and triclosan were measured in the method blank sample, the MDL for these compounds was adjusted to their corresponding concentration in the method blank sample.

The increased MDLs necessary for adequately detecting the estrogenic steroid hormones,17α-ethynylestradiol and mestranol, and the androgenic steroids reflect the decreased response of these compounds arising from the coelution of numerous compounds within the same region of the chromatogram.

3. RESULTS

3.1. Bioassay analysis

Estrogenic activity was detected in both the MBR and the Bardenpho outlets (2.60 and 0.79 ng/L estradiol equivalents, respectively; Table 1). Androgenic activity was detectable in these samples but below the quantification limit of the assay (< 70 ng/L dihydrotestosterone equivalent). There was no detectable estrogenic or androgenic activity in the samples from Puarenga Stream and the S-10 reference site (Table 1).

Table 1. Bioassay results for estrogenic and androgenic activities in sample extracts and laboratory blanks.

	Estrogenic activity		**Androgenic activity**	
	Agonist (17β-estradiol equivalent, ng/L)	Antagonist (tamoxifen equivalent, µg/L)	Agonist (5α-dihydrotestosterone equivalent, ng/L)	Antagonist (flutamide equivalent, µg/L)
Samples				
MBR	2.60 ± 0.64	BDL (< 3)	BQL (< 70)	BDL (< 15)
BardenPho	0.79 ± 0.22	BDL (< 3)	BQL (< 70)	BDL (< 15)
Puarenga Stream	BDL (< 0.05)	BDL (< 3)	BDL (< 45)	BDL (< 15)
S-10 reference site	BDL (< 0.05)	BDL (< 3)	BDL (< 45)	BDL (< 15)
Laboratory blanks				
Solvent (DMSO)	BDL (< 0.05)	BDL (< 3)	BDL (< 45)	BDL (< 15)
SPE blank	BDL (< 0.05)	BDL (< 3)	BDL (< 45)	BDL (< 15)

Notes: BQL = Below Quantification Limit; BDL = Below Detection Limit. Numbers are average ± standard deviation (n = 3 replicate analyses of the sample extracts).

3.2. Chemical analysis

3.2.1. Recovery of surrogate standard compounds

The mean recovery of individual carbon-13 labelled surrogate standards spiked into each sample prior to extraction and the overall mean recovery of all surrogate compounds is displayed in Table 2. The surrogate standard compounds were spiked into 10 L of prefiltered sample at an equivalent concentration of 10 ng/L (ppt). This represents a low level rate of spiking for quality assurance (QA) determinations.

Table 2. Recovery of surrogate standards spiked into individual samples (n=4).

Recovery compound	Calculated mean percentage recovery
^{13}C-methylparaben	90.8
^{13}C-ortho-phenylphenol	109.0
^{13}C-butylparaben	107.3
^{13}C-methyltriclosan	100.8
^{13}C-triclosan	96.1
^{13}C-bisphenol-A	106.7
^{13}C-estrone	97.1
^{13}C-17 β -estradiol	94.9
^{13}C-17 α-ethynylestradiol	91.9
Mean recovery	**99.4**

The results reported in Table 3 have been corrected for any contributions of individual compounds measured in the QA/QC blank sample. The high recovery rates obtained for ^{13}C-surrogate spiked compounds and the consistency of their recovery precluded the additional application of surrogate recovery corrections to the analytical data.

3.2.2. Residues of endocrine disrupting chemicals

A number of EDCs were detected in the MBR and Bardenpho wastewater effluent samples at relatively low concentrations (Table 3). The EDC concentrations were typically higher in the effluent from Bardenpho treatment compared to the MBR treatment effluent. The concentration of EDCs detected in the Bardenpho and MBR treated effluents generally followed the order of alkylphenols> paraben preservatives> phenolic antimicrobials> steroid hormones>others. This pattern reflects the relative amounts of these chemicals within personal care products (PCPs) and in urban waste water.

The principal alkylphenol detected in the wastewater effluents was TNP, a complex mixture of branched alkyl chain isomers. Single alkylphenol components detected in these effluents were present at relatively low concentrations (below 3 ng/L). Residues of paraben preservatives, phenolic antimicrobial chemicals, and the industrial plasticiser bisphenol-A (BPA), did not exceed 10 ng/L in effluent samples and were not detected in the waters of the Puarenga Stream or Waipa Forest reference site, S10 (Table 3). Estrone (the principal oxidative metabolite of the estrogenic steroid 17β-estradiol) was detected in all samples, including the Waipa Forest reference site. The principal man-made active ingredient in oral contraceptive pills, 17α-ethynylestradiol, was only detected in the effluent from the Bardenpho treatment stage (Table 3). 17β- and 17α-estradiol, the principal estrogenic steroids naturally excreted by humans and livestock, respectively, were not detected in effluent or stream water samples. Similarly, estriol (the secondary metabolite of 17β-estradiol) and mestranol (another common active ingredient in oral contraceptive pills) were not detected in any

samples.The principal metabolite of testosterone, androstenedione, was the only androgenic steroid hormone detected in both effluent samples but at relatively low concentrations (Table 3).

Bisphenol-A and estrone were the only EDCs detected in the Puarenga Stream. The concentrations for these compounds were relatively low at 2.36 and 0.49 ng/L, respectively. The only compound detected in the reference water sample (Waipa Forest S10) was estrone, also at a relatively low concentration of 0.54 ng/L (Table 3).

Table 3. Concentration of endocrine disrupting chemicals measured in samples (ng/L, or, ppt).

Compound	MBR	BPhos	Puarenga Stream	S-10 reference site	MDL[a]
Alkylphenols					
4-t-Amylphenol	N.D[b]	N.D	N.D	N.D	0.01
4-n-Amylphenol	N.D	N.D	N.D	N.D	0.01
4-t-octylphenol	2.17	0.77	N.D	N.D	0.01
4-t-heptphenol	N.D	0.47	N.D	N.D	0.01
4-n-octylphenol	N.D	0.11	N.D	N.D	0.01
4-n-nonylphenol	N.D	N.D	N.D	N.D	0.01
Technical nonylphenol (TNP) equivalents[c]	33.6	60.4	ND	ND	10.00
Paraben preservatives					
Methylparaben	1.83	41	N.D	N.D	1.00
Ethylparaben	N.D	N.D	N.D	N.D	0.01
Propylparaben	1.63	6.15	N.D	N.D	0.01
Butylparaben	N.D	3.94	N.D	N.D	1.00
Benzylparaben	2.87	4.80	N.D	N.D	0.01
Phenolic antimicrobials					
Chloroxylenol	10.00	2.20	N.D	N.D	1.00
o-phenylphenol	N.D	N.D	N.D	N.D	0.01
methyl triclosan	2.74	3.11	N.D	N.D	0.01
Triclosan	4.15	5.04	N.D	N.D	1.00
Other					
Bisphenol A (BPA)	3.93	0.68	2.36	N.D	
Estrogenic steroid hormones					
17α-estradiol	N.D	N.D	N.D	N.D	0.01
17β-estradiol	N.D	N.D	N.D	N.D	0.01
Estrone	10.53	1.78	0.49	0.54	0.01
Estriol	N.D	N.D	N.D	N.D	0.01
17α-ethynylestradiol	N.D	1.87	N.D	N.D	0.05
Mestranol	N.D	N.D	N.D	N.D	0.05
Androgenic steroid hormones					
Testosterone	N.D	N.D	N.D	N.D	0.05
Androstenedione	1.55	1.42	N.D	N.D	1.00
Adrostenediol	N.D	N.D	N.D	N.D	0.05
Dihydrotestosterone	N.D	N.D	N.D	N.D	0.05
Hydroxytestosterone	N.D	N.D	N.D	N.D	0.05
11-Ketotestosterone	N.D	N.D	N.D	N.D	0.05
19-Nortestosterone	N.D	N.D	N.D	N.D	0.05

[a] Method detection limit in ng/L, determined for 10 L of extracted aqueous sample.

[b] Not detected.

[c] Measured as the sum of the principal nine components of a mixture of branched alkyl chain nonylphenol isomers.

4. DISCUSSION

The dominance of technical nonylphenol in effluent from the MBR and Bardenpho treatment stages is consistent with the widespread use of domestic and industrial products containing technical nonylphenol ethoxylate surfactants and/or TNP mixtures. Technical nonylphenol is an industrial product that is ethoxylated to produce technical mixtures of nonylphenol ethoxylate (TNPE) surfactants, which are similarly comprised of a series of highly branched complex isomers. Both TNPE and TNP are widely used in the processing of wood and metal, and used as emulsifiers and detergents. TNPE entering WWTPs are aerobically degraded to produce TNP. A number of isomers of TNP have estrogenic activity, including the single isomeric compound 4-nonylphenol (Leusch *et al*. 2006b).

Technical nonylphenol

The TNP concentrations measured in the WWTP effluent samples are lower than the 200 ng/L (0.20 μg/L) background concentration of TNP in continental waters of Europe (WHO IPCS 2004). More significantly, the concentrations are an order of magnitude lower than the European Union's predicted no effect concentration (PNEC) of 330 ng/L, or 0.33 μg/L (EU, 2002). The PNEC is the estimated concentration below which exposure to a substance is not expected to cause adverse effects. The risk of TNP in the treated effluent discharged to the Puarenga Stream is negligible as TNP was not detected in the stream water sample. The concentration of TNP entering the Puarenga Stream within treated effluent will be further reduced by dilution from the stream flow, which in turn will reduce any potential risk.

Antimicrobial chemicals

The presence of low residual concentrations of antimicrobial chemicals in the effluents is not surprising as they are added to a variety of domestic and industrial products and formulations to prevent microbial activity. This inherent resistance to microbial degradation means they are more likely to persist during wastewater treatment that uses microbial activity to degrade organic substances. The relatively higher concentrations of methyl- and propyl-paraben reflect the predominance of these chemicals in a wide range of personal care products. Chloroxylenol is an anti-septic chemical in common disinfectant products like Dettol™ so its presence in the MBR and BardenPho effluents is not surprising. Similarly, the antimicrobial chemical triclosan is a common ingredient in toothpaste and is routinely detected in WWTP treated effluents. During treatment, triclosan is microbially transformed to methyl-triclosan. Both triclosan and methyl triclosan are hydrophobic and strongly associate with suspended solids during wastewater treatment. The relatively low levels of these two chemicals in the MBR and Bardenpho effluent samples likely reflect the efficacy of solid removal during these treatment processes.

Bisphenol-A

Bisphenol-A (BPA) is an industrial chemical extensively used in the production of polycarbonate plastic containers. As polycarbonate plastics degrade, they release residues of BPA. Residues of BPA are widespread in the environment and are strongly associated with human activity. The presence of BPA in the Puarenga Stream likely reflects the accumulation of plastic residues within nearby urban environments and the input of urban run-off into the Puarenga watershed. The concentration of BPA measured in the stream is three orders of magnitude less than the PNEC values of 1.5 and 1.6 ug/L set respectively by the European Union (EU, 2008) and Japan (AIST 2007), and two orders of magnitude lower than the PNEC of 0.175 ug/L for Canada (Canada 2008). More significantly, the concentration is an order of magnitude lower than the most up to date PNEC of 0.06 ug/L, obtained from a weight of evidence assessment of data from 61 studies assessing the effects of BPA upon aquatic organisms (Wright-Walters *et al*. 2011).

Estrone

The estrogenic steroid, estrone was detected in all samples, but only the MBR effluent (10.53 ng/L) exceeded the PNEC of 3 ng/L proposed for the protection of aquatic organisms by the England and Wales Environment Agency (Young *et al*, 2002). This level of estrone can account for some of the MBR bioassay estrogenicity results, based on its estimated relative potency that would equate to 1.2 to 3.9 ng/L 17β-estradiol (Table 1; Leusch *et al*. 2006b). The Puarenga Stream and reference site S10 samples had concentrations of estrone below the 3 ng/L predicted no effect concentration (PNEC) for aquatic wildlife (Young *et al.* 2002). In addition, both samples were below the level of detection of the bioassay and therefore, do not currently represent a risk to aquatic organisms within these watersheds. The presence of estrone in the reference site S10 was unexpected. The potential for run-off from the Waipa Forest wastewater effluent irrigation scheme to subsequently enter this waterway should be clarified to confirm if this presents a potential source and pathway for estrone to enter this waterway. The sources of estrone in the Puarenga Stream could be from agricultural activites (Gadd *et al*. 2010), run-off from the Waipa Forest wastewater effluent irrigation scheme into the upper headwaters and leakage from the Rotorua City wastewater reticulation system.

Androgens

The concentration of 17α-ethynylestradiol in the Bardenpho effluent (1.87 ng/L) exceeded the recently modified PNEC of 0.10 ng/L (Caldwell *et al*. 2012). The biological activity measured 0.79 ng/L EEQ (Table 1) and is slightly lower than the expected 1.9-2 ng/L EEQ that this concentration of 17α-ethynylestradiol would be expected to elicit. Comparisons between chemical and biological results should always be interpreted with caution. The very complex nature of effluent extracts can interfere in many ways with the biological processes as the effects of all chemicals present are integrated. However, it is important to emphasise that the risk associated with 17α-ethynylestradiol is negligible as it was not measured in the Puarenga Stream

suggesting it has been diluted to below detaction limit and/or attenuated by natural processes of degradation or sorption.

Very low concentrations of androstenedione were measured in the effluent from the MBR and Bardenpho treatment samples but not in the Puarenga Stream. The absence of a positive bioassay response for these samples confirms the negligible risk of androstenedione and other androgens.

5. CONCLUSIONS

Most EDC concentrations detected in the MBR and Bardenpho treatment stage effluents were below the PNECs for aquatic organisms. It is important to note that as these concentrations were determined in WWTP effluents prior to discharge to the environment, the concentration of these compounds will be further reduced by dilution within the Puarenga Stream and are therefore unlikely to cause harm to aquatic organisms in the receiving waterways.

The bioassays provided integrated estimates of estrogenic and androgenic activities. Both MBR and Bardenpho effluent samples contained estrogenic activities, but the levels in the Puarenga and Waipa Forest reference stream waters were all below the detection limits of the bioassays.

Only the concentrations of estrone and 17α-ethynylestradiol in the MBR and Bardenpho treated effluents exceeded the PNECs for the protection of aquatic wildlife. However subsequent dilution within the Puarenga Stream combined with processes of natural attenuation will reduce the concentration of these EDCs to levels that pose negligible risk.

The source of the low concentrations of estrone measured in the waters of the Puarenga and Waipa Forest reference (site S10) streams cannot be determined from this study. Identifying the source of estrone within these watersheds would require desigining and implementing a targeted sampling strategy within each watershed.

Overall, the results suggest that the risk of the RDC WWTP treated effluents to cause endocrine disruption within receiving waterways is negligible. However, it is important to note that the results of this research were from one-off grab samples and extrapolation must therefore be interpreted with care. While the MBR and Bardenpho treatment stages provide some degree of integration and smoothing of the loading of contaminants entering the WWTP, their effect on the temporal and diurnal variability of EDCs activity and concentration within the treated effluents cannot be established from this study. A more robust sampling program incorporating time-integrated or flow proportional sampling over a number of days throughout the year would provide a more representative depiction of the concentration of residual EDCs in the WWTP effluents.

6. AKNOWLEDGEMENTS

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7. REFERENCES

AIST (Japan's National Institute of Advanced Industrial Science and Technology). 2007. AIST Risk Assessment Document Series 4. Bisphenol A.

Balaguer P, Francois F, Comunale F, Fenet H, Boussioux AM, Pons M, Nicolas JC, Casellas C 1999. Reporter cell lines to study the estrogenic effects of xenoestrogens. Science of the Total Environment 233: 47–56.

Caldwell DJ, Mastrocco F, Anderson PD, Lange R,Sumpter JP. 2012. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α ethinylestradiol. Environmental Toxicology and Chemistry 31:1396-1406.

Canada 2008. Environment Canada, Health Canada. Screening Assessment for the Challenge Phenol, 4,4' (1-methylethylidene)bis- (Bisphenol A) CAS 80-05-7. October 2008 http://www.ec.gc.ca/substances/ese/eng/challenge/batch2/batch2_80-05-7_en.pdf

European Commission. 1996. European workshop on the impact of endocrine disruptors on human health and wildlife. Weybridge, UK, Report No. EUR 17549, Environment and Climate Research Programme, DG XXI. Brussels, Belgium: European Commission.

EU 2002: RAR, 2002: European Union Risk Assessment Report: 4-Nonylphenol (branched) and nonylphenol. CAS Nos: 84852-15-3 and 25154-52-3. EINECS Nos: 284-325-5 and 246-672-0. Risk assessment.

EU 2008. European Union Updated Risk Assessment Report. Bisphenol A, CAS No: 80-05-7. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities.

Gadd JB, Tremblay LA, Northcott GL. 2010. Steroid estrogens, conjugated estrogens and estrogenic activity in farm dairy shed effluents. Environmental Pollution. 158: 730-736.

Huang R, Xia M, Cho M-H, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP 2011. Chemical Genomics Profiling of Environmental Chemical Modulation of Human Nuclear Receptors. Environ Health Perspect 119: 1142-1148.

Leusch FDL, de Jager C, Puijker L, Levi Y, Lim R, Tremblay LA, Wilson VS, Sacher F, Chapman HF 2010. Comparison of five *in vitro* bioassays to measure estrogenic activity in environmental waters. Environmental Science & Technology 44: 3853–3860.

Leusch FDL, Chapman HF, Van den Heuvel MR, Tan BLL, Gooneratne SR and Tremblay LA. 2006a. Bioassay-derived androgenic and estrogenic activity in

municipal sewage in Australia and New Zealand. Ecotoxicology and Environmental Safety. 65:403-411.

Leusch FDL, Van den Heuvel MR, Chapman HF, Gooneratne SR, Eriksson AME and Tremblay LA. 2006b. Development of methods for extraction and in vitro quantification of estrogenic and androgenic activity of wastewater samples. Comparative Physiology and Biochemistry Part C. 143:117-126.

Mnif W, Dagnino S, Escande A, Pillon A, Fenet H, Gomez E, Casellas C, Duchesne M-J, Guillermina Hernandez-Raquet G, Cavailles V, Patrick Balaguer P, Bartegi A 2010. Biological analysis of endocrine-disrupting compounds in Tunisian sewage treatment plants. Archives of Environmental Contamination and Toxicology 59: 1–12.

Muller M, Rabenoelina F, Balaguer P, Patureau D, Lemenach K, Budzinski H, Barcelo D, Lopez de Alda M, Kuster M, Delgenes J-P, Hernandez-Raquet G 2008. Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plant. Environmental Toxicology and Chemistry 27: 1649–1658.

Northcott GL., Strong, J., Tremblay, LA., and Wilkins, A. 2013. Emerging organic contaminants enter New Zealand's aquatic environments with waste water treatment plant effluents. In Proceedings of the 2013 New Zealand Land Treatment Collective annual conference, Gielen, G., Heaphy, M. (ed.). 10-12 April, Blenheim, New Zealand.

Sarmah AK, Northcott GL , Leusch FDL, Tremblay LA. 2006. A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand. Science of the Total Environment. 355:135-144.

Tremblay LA, Fisher P, Leusch FDL, van den Heuvel MR, Nicolas J-C, Pilon A, Balaguer P 2005. Evaluation of the potential of sodium fluoroacetate (1080) and fluorocitrate to interact with androgen and oestrogen receptors. Australasian Journal of Ecotoxicology 11: 155–162.

Tremblay LA, Trought K, Northcott GL, and Tscharntke I. 2010. Evaluation of the efficacy of the Beachlands/Maraetai Sewage Treatment Plant to remove oestrogenic and androgenic activity. A Landcare Research Contract Report: LC56 prepared for Manukau Water Limited. 14 p.

World Health Organisation International Programme on Chemical Safety. 2004. Integrated risk assessment: nonylphenol case study. International Programme on Chemical Safety, WHO, Geneva, Switzerland.

Wright-Walters M, Volz C, Talbott E, Davis D. 2011. An updated weight of evidence approach to the aquatic hazard assessment of Bisphenol-A and the derivation of a new predicted no effect concentraton (PNEC) using a non-parametric methodology. Science of the Total Environment. 409:676-85.

Young WF, Whitehouse P, Johnson I, Sorokin N. 2002. Proposed predicted no effect concentrations (PNECs) for natural and synthetic steroid oestrogens in surface waters. Environment Agency R & D Technical Report P2-T04/1, England and Wales Environment Agency, Bristol; 2002. 172 pp.