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Analysis of Waikato river water samples for selected endocrine disrupting chemicals and hormonal activity

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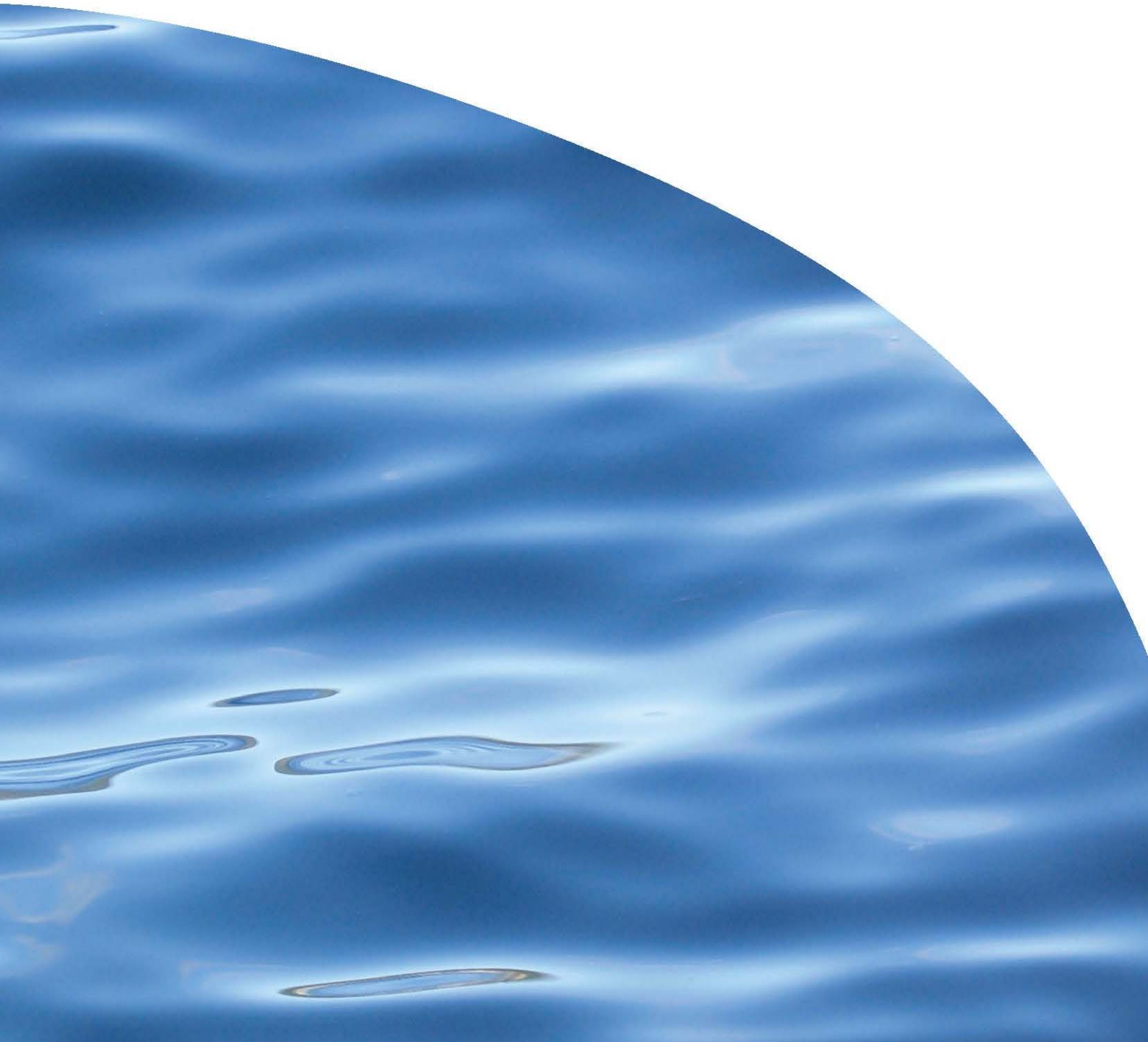

ANALYSIS OF WAIKATO RIVER WATER SAMPLES FOR SELECTED ENDOCRINE DISRUPTING CHEMICALS AND HORMONAL ACTIVITY

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1. INTRODUCTION

Endocrine disrupting chemicals (EDCs) are a class of contaminants that are of significant concern due to their potential to disrupt endocrine functions in wildlife and human populations. Endocrine disrupting chemicals have been defined as "exogenous substances that cause adverse health effects in an intact organism, or its progeny, secondary to endocrine function" (European Commission 1996). Two significant sources of EDCs are from urban wastewater treatment plants (WWTPs) and dairy farming waste, which are released into the environment (Sarmah *et al*. 2006).

The Waikato Regional Council (WRC) contracted Cawthron Institute (Cawthron) to assess the endocrine disruption potential of eight water samples collected from the Waikato River between Taupo and Tuakau. Potential sources of EDCs to the Waikato River include wasterwater discharge from a number of WWTPs along its length and the prevalence of intensive dairy farming in the region.The concentration of selected EDCs and the total endocrine activity was assessed using trace chemical analysis and bioassay methods.

2. METHODOLOGIES

2.1. Overview

The approach taken in this work combined the use of trace chemical analysis and biological assays, or bioassays, to measure both the concentration of selected EDCs and the total endocrine disrupting activity in concentrated sample extracts. Bioassays using reporter gene technologies are 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 commonly used because they are easy to use, quick and relatively cheap, making them a good choice for large-scale hormonal activity screening (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 endocrine-active chemicals. This approach has previously been used in New Zealand to assess the endocrine disruption potential of wastewater (Leusch *et al*. 2006) as well as dairy shed and dairy oxidation pond effluent (Sarmah *et al*. 2006; Gadd *et al*. 2010).

2.2. Sample collection and extraction

Water samples were collected from the Waikato River under low flow conditions on 6–7 March 2012 at the Taupo Gates, Ohaaki Bridge, Waipapa, Hamilton Narrows, Horotui Bridge, Huntly Tainui, Mercer Bridge, and Tuakau Bridge by WRC staff. A volume of 16 L was obtained for each sample by filling four replicate 4 L amber glass Winchesters. The samples were stored 4˚C overnight, prior to being transferred to Plant & Food Research Ruakura on 8 March 2012 by WRC staff. On arrival, the samples were acidified (pH = 2.5) and filtered through a glass microfiber filter (47 mm, Labservice) to remove particulate material.

2.2.1. Sample preparation for bioassay

Five litres of filtered sample was extracted using Oasis hydrophilic-lipophilic-balanced (HLB) 1 g 20 mL solid phase extraction (SPE) cartridges. The sample bottle was rinsed three times with MilliQ water and each rinse wash passed through the SPE cartridge. The SPE cartridges were dried under full vacuum and the EDCs eluted with a binary solvent mixture of dichloromethane / methanol (95:5). The SPE solvent extracts were purified by passing through a sequential florisil cartridge (IST, 2 g 12 mL) into a collection vial. The solvent extract was blow dried under a gentle stream of nitrogen gas, redissolved in 0.5 mL of dimethyl sulfoxide (DMSO) and transferred to 2 mL amber glass vials. With a sample volume of 5 L and final extract volume of 0.5 mL, the four samples were concentrated by a factor of 10,000 (relative enrichment factor).

2.2.2. 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 Oasis HLB 1 g 20 mL SPE cartridges and purified as described above (Section 2.2.1). The solvent extract obtained from the florisil purification step was concentrated and exchanged into dichloromethane and further purified using gel permeation chromatography (GPC).

The GPC solvent extract was blow dried under a gentle stream of nitrogen gas. A mixture of isotopically labeled internal standards were added, before the steroid hormones and other polar chemical residues were derivitised to their respective trimethylsilyl ethers.

2.3. MELN and PALM bioassays

The MELN cell line measures estrogenic activity and the principles of the assay have been described by Balaguer *et al*. (1999). The PALM cells measure androgenic activity and have been described by Terouanne *et al*. (2000). Briefly, the MELN and PALM cells were plated and left to adhere in 96-well tissue culture plates (Nunc) at

concentrations of 2.5×10^5 and 1.5×10^5 cells/ml, respectively. The cells were incubated with serially diluted sample extracts for 24 h. The response was measured by addition of a medium containing the light-emitting biological pigment luciferin in the Microbeta Trilux luminometer (Wallac). The estrogenic and androgenic activities were determined by comparing the response of samples to that of the reference chemicals, *i.e.* 17β-estradiol for MELN and the synthetic androgen methyltrienolone (R1881) for the PALM cells.

For the antagonistic assays, cells were incubated with a fixed amount of the natural ligands, either 17β-estradiol or R1881 (0.1 nM), which produced a 50% response in luciferase activity that remained constant even in the presence of increasing amounts of environmental extracts.The synthetic anti-estrogen ICI 182 780 (Sigma) and the anti-androgen bicalutamide were reference compounds used to calibrate the bioassays.

2.4. Chemical analysis

The trimethylsilylethers 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. Eight calibration standards (1–1000 ng/mL) were prepared and derivitised (described in Section 2.2.2). Calibration curves were prepared for quantitating the detected target analytes and surrogate recovery compounds 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).

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 EDCs and antimicrobial chemicals including alkylphenols (nonyl- and octylphenols), bisphenol-A, parabens (methyl-, ethyl-, propyl-, butyl-, benzyl-), triclosan and methyl-triclosan, o-phenylphenol, and chloroxylenol.

2.4.1. Method detection limits

The method detection limits (MDLs) for the target analytes ranged from 0.01 to 10.0 ng/L (refer to 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. For alkylphenols, paraben preservatives and phenolic antimcrobial compounds with MDLs greater than 0.01 ng/L, the MDL was determined from the equivalent concentration of compound detected in the method blank sample.

The increased MDLs for the estrogenic steroid hormones 17α-ethynylestradiol and mestranol, and androgenic and progestogenic steroids, reflect the decreased response of these compounds arising from the coelution of multiple steroid hormones within a common region of the chromatogram.

3. RESULTS

3.1. Bioassay analyses

The results of the bioassay analyses are summarised in Table 1, but key findings were:

- There was very low levels of estrogenicity at the Taupo Gates and Ohaaki Bridge sites.
- None of the sites had detectable androgenic activities.
- Some sites showed estrogen and androgen antagonistic activities.

Table 1. Bioassay results for estrogenic and androgenic activities in the eight samples from the Waikato River catchment as determined by the MELN and PALM bioassays.

	Estrogenic activity		**Androgenic activity**	
	Agonist (17β-estradiol equivalent, ng/L)	Antagonist (ICI 182 780 equivalent, ng/L)	Agonist (R1881 equivalent, ng/L)	Antagonist (bicalutamide equivalent, µg/L)
Site				
Taupo Gates	0.13	BDL (< 17.4)	BDL (< 7.1)	BDL (< 2.3)
Ohaaki Bridge	0.17	BDL (< 17.4)	BDL (< 7.1)	BDL (< 2.3)
Waipapa	BDL (< 0.072)	176.2	BDL (< 6.9)	10.1
Hamilton Narrows	BDL (< 0.068)	40.1	BDL (< 7.1)	10.8
Horotui Bridge	BDL (< 0.072)	16.1	BDL (< 6.9)	BDL (< 1.5)
Huntly Tainui	BDL (< 0.072)	30.2	BDL (< 6.9)	4.6
Mercer Bridge	BDL (<0.068)	31.5	BDL (< 7.1)	15.4
Tuakau Bridge	BDL (< 0.072)	28.5	BDL (< 6.9)	2.4

BDL = Below Detection Limit.

3.2. Chemical analyses

3.2.1. Recovery of surrogate standard compounds

The mean recovery of individual carbon-13 labeled 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=8).

Recovery compound	Calculated mean percentage recovery
^{13}C-methylparaben	72.2
^{13}C-ortho-phenylphenol	77.4
^{13}C-butylparaben	79.2
^{13}C-methyltriclosan	72.4
^{13}C-triclosan	98.7
^{13}C-bisphenol-A	95.0
^{13}C-estrone	83.8
^{13}C-17β-estradiol	72.5
^{13}C-17α-ethynylestradiol	91.0
Mean recovery	**82.5**

The level of surrogate standard recovery (> 70% for all 13C-labelled surrogates) meets the acceptance requirements of quality assurance criteria. The level of surrogate compound recovery obtained from the samples spiked at the low concentration of 10 ppt validated the performance of the analytical methodology.

3.2.2. Residues of endocrine disrupting chemicals

The results of the chemical analyses are summarised in Table 3. The only industrial alkylphenol detected in the Waikato River samples was technical nonylphenol (t-NP). Technical nonylphenol was not detected in Waikato River samples upstream from the Narrows Bridge. Traces of t-NP were detected at Narrows Bridge and increased to a maximum concentration of 33.9 ng/L at Horotiu Bridge, downstream from Hamilton City. These traces decreased to a concentration of 10.9 ng/L at the Huntley Tainui Bridge and to trace level concentations further downstream. .

Paraben preservatives and phenolic antimicrobial chemicals were not detected in the Waikato River samples obtained upstream from the Narrows Bridge sample site, which is located downstream from the township of Cambridge and upstream of the city of Hamilton.

Low concentrations of methyl-, propyl- and butyl-paraben, chloroxylenol, and methyl-triclosan were detected in waters sampled at the Narrows Bridge and at various sampling sites further downstream. Residues of methyl-paraben, propyl-paraben, chloroxylenol, and methyl-triclosan persisted in the Waikato River as far downstream as the Tuakau Bridge sampling site. Higher concentrations of these chemicals were most often measured in water sampled at Horotiu Bridge, downstream from the city of Hamilton, with the highest concentrations obtained at the Mercer Bridge site.

All of the Waikato River samples contained low concentrations of bisphenol-A with the highest concentration (4.26 ng/L) measured in water sampled downstream from the city of Hamilton at the Horotiu Bridge sample site.

No steroid hormone residues were detected in any of the analysed river water samples.

Table 3. Concentration of endocrine disrupting chemicals measured in Waikato River samples (ng/L or ppt). The results have been corrected for any contributions of individual compounds measured in the QA / QC blank sample.

Compound	Taupo Gates	Ohaaki Bridge	Waipapa	Hamilton Narrows	Horotiu Bridge	Huntley Tainui Bridge	Mercer Bridge	Tuakau Bridge	MDL[a]
Alkylphenols									
4-t-Amylphenol	N.D[b]	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
4-n-Amylphenol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
4-t-octylphenol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
4-t-heptphenol	N.D	0.47	N.D	N.D	0.38	N.D	N.D	N.D	0.01
4-n-octylphenol	N.D	0.11	N.D	N.D	N.D	N.D	N.D	N.D	0.01
4-n-nonylphenol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Technical nonylphenol equivalents[c]	ND	ND	ND	T[d]	33.88	10.87	T	T	10.00
Paraben preservatives									
Methylparaben	N.D	N.D	N.D	0.20	0.29	0.28	0.46	0.17	1.00
Ethylparaben	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Propylparaben	N.D	N.D	N.D	0.12	0.21	0.12	0.26	0.12	0.01
Butylparaben	N.D	N.D	N.D	0.56	0.49	N.D	N.D	N.D	1.00
Benzylparaben	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Phenolic antimicrobials									
Chloroxylenol	N.D	N.D	N.D	0.12	0.21	0.14	0.16	0.08	1.00
o-phenylphenol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Methyl-triclosan	N.D	N.D	N.D	0.20	0.29	0.28	0.46	0.17	0.01
Triclosan	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.00
Other									
Bisphenol A	0.83	1.02	2.76	0.96	4.26	0.90	1.92	0.87	0.05

Table 3. *continued.* Concentration of endocrine disrupting chemicals measured in Waikato River samples (ng/L or ppt).). The results have been corrected for any contributions of individual compounds measured in the QA / QC blank sample.

Compound	Taupo Gates	Ohaaki Bridge	Waipapa	Hamilton Narrows	Horotiu Bridge	Huntley Tainui Bridge	Mercer Bridge	Tuakau Bridge	MDL[a]
Estrogenic steroid hormones									
17β-estradiol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
17-α-estradiol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Estrone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Estriol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.01
17α-ethynylestradiol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Mestranol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Androgenic steroid hormones									
Testosterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Androstenedione	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.00
Adrostenediol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Dihydrotestosterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Hydroxytestosterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
11-Ketotestosterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
19-Nortestosterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Progestogenic steroid hormones									
Progesterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.00
Hydroxyprogesterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.00
Medroxyprogesterone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
19-Norethindrone	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05
Levonorgestral	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.05

[a] Method detection limit in ng/L, determined for 10 litres 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. [d] Trace level detected but below the limit of quantitation

4. DISCUSSION

Technical nonylphenol (t-NP) is used as an industrial product and is ethoxylated to produce corresponding technical mixtures of nonylphenol ethoxylate (t-NPE) surfactants. Technical nonylphenol and t-NPE are comprised of a series of highly branched complex isomers. Both t-NP and t-NPE are widely used in the processing of wood and metal, and as emulsifiers and detergents. Nonylphenol ethoxylate entering wastewater treatment plants (WWTPs) is aerobically degraded to produce t-NP. A number of isomers of t-NP have estrogenic activity, including the single isomeric compound 4-nonylphenol (Leusch *et al*. 2006). The only alkylphenol detected in the waters of the Waikato River was t-NP, the most widely used alkylphenol in domestic and industrial products. The dominance of t-NP in the Waikato River water samples downstream from Hamilton is consistent with their widespread use in this large urban centre and their release into the Waikato River from the Hamilton City Council Pukete WWTP and stormwater drainage system.

Concentrations of t-NP measured in Waiakto River water at the Horotiu and Huntley Tainui bridge sample sites were 33.88 and 10.87 ng/L, respectively. These concentrations are below the 0.20 µg/L (200ng/L) concentration of t-NP considered to represent the average background concentration within the continental waters of Europe (WHO IPCS 2004). More significantly, these concentrations are an order of magnitude lower than the European Union predicted-no-effect concentration (PNEC) of 0.33 µg/L, or 330 ng/L for t-NP.

The detection of methyl-, propyl- and butyl-paraben, chloroxylenol, and methyl-triclosan in Waikato River water is not surprising given the predominance of these chemicals in a wide range of personal care products. Methyl-, propyl- and butyl-parabens are specifically designed to extend the lifetime of formulated products by inhibiting microbial activity. Chloroxylenol is an antibacterial chemical used in many antiseptic and disinfectant products. Methyl-triclosan is a bacterial metabolite of the parent chemical triclosan, which is a common ingredient in liquid soaps and toothpastes. A recent report on emerging organic contaminants in the influent and effluent of WWTPs in New Zealand demonstrated that methyl-, propyl- and butyl-paraben, chloroxylenol, and triclosan are not totally removed by wastewater treatment and are released into receiving waterways in New Zealand (Northcott *et al.* 2013).

During wastewater treatment, triclosan can be transformed by bacterial activity to methyl-triclosan. This chemical is more hydrophobic than triclosan and readily adsorbs to particulate material and tends to accumulate in the sewage sludge. Therefore, it is not surprising that methyl-triclosan has not been detected in treated effluent discharged from WWTPs in New Zealand (Northcott *et al*. 2013). The detection of low concentrations of methyl-triclosan in Waikato River water downstream of the township of Cambridge can be attributed to the release of triclosan followed by *in situ* microbial degradation by bacteria within the river.

The concentrations of methyl-, propyl- and butyl-paraben, chloroxylenol, and methyl-triclosan measured in the Waikato River samples are relatively low. Predicted-no-effect concentrations for these chemicals are not readily available, but a useful comparison is triclosan; a chemical demonstrated to bioaccumulate in aquatic organisms and exhibit endocrine disrupting activity. The PNEC for triclosan in fresh water, proposed by the European Commission Water Framework Directive Annex VIII (WFD-UKTAG, 2009), is 0.1 µg/L. This is two-orders of magnitude higher than the concentration of methyl-, propyl- and butyl-paraben, chloroxylenol, and methyl-triclosan detected in the Waikato River water samples.

The only EDC detected in all samples of the Waikato River was bisphenol-A (BPA). Bisphenol-A is an industrial chemical used in the production of polycarbonate plastics that are incorporated into a wide variety of consumer goods including water bottles, sports equipment, the lining of water pipes, the inner coatings of food and beverage cans, and thermal paper used to print sales receipts from cashier machines. The US EPA estimate approximately 0.5 million kg of BPA is released into the environment per annum, principally through the degradation of plastic products that are themselves ubiquitously distributed in the environment and concentrated in urban environments. Urban stormwater discharges are a major source of BPA into waterways.

Bisphenol-A is considered a ubiquitous pollutant and is atmospherically transported and distributed around the world. It is found in all of the world's oceans and in the snow and ice of the Arctic and Antarctica. The detection of BPA in the upper waters of the Waikato River reflect what can be considered background concentrations within Lake Taupo.

Bisphenol-A was detected in the Waikato River at a maximum concentration of 4.26 ng/L at the Horotiu Bridge sample site downstream of Hamilton City. This is three-orders of magnitude less than the PNEC values of 1.5 and 1.6 µg/L for BPA, respectively, set by the European Union (EU, 2008) and Japan (AIST 2007), and two-orders of magnitude lower than the PNEC of 0.175 µg/L for Canada (Canada 2008). More significantly, the concentration of 4.26 ng/L BPA in the Waikato River sampled at Horotiu Bridge is an order of magnitude lower than the most up-to-date PNEC of 0.06 µg/L, obtained by a weight of evidence approach from 61 studies assessing the effects of BPA upon aquatic organisms (Wright-Walters *et al*. 2011).

Estrogenic activity was found only in the Taupo Gates and Ohaaki Bridge samples at levels close to the detection limit of the bioassay and an order of magnitude below the PNEC of 2 ng/L estimated for 17β-estradiol (Table 1; Caldwell *et al*. 2012).. It is interesting to note that the same sites showed no antagonistic activity while all the other sites showed opposite trends with no estrogenicity but low levels of antagonistic activity. The presence of agonistic and antagonistic activities in WWTP effluent has been previously reported (Conroy *et al*. 2007). It has been proposed that the loss of estrogenic and androgenic activities in treated effluents is not caused solely by the

degradation of agonistic compounds but also by the presence and production of antagonist compounds (Conroy *et al*. 2007). This may explain the presence of estrogenic activity at the more pristine sites of Taupo Gates and Ohaaki Bridge where the presence of antagonistic chemicals is less likely and estrogenicity may be from agricultural activities (Gadd *et al*. 2010). In comparison, androgenic activity was not detected in any of the Waikato River water samples (Table 1). While low levels of anti-estrogenic and androgenic activity was detected in some samples, it is not possible to provide a risk assessment at this stage as the mechanisms are still poorly understood.

Despite the degree of sample concentration (10 L water samples were concentrated 20,000x for bioassays and 30,000x for chemical analysis) prior to analysis and the low method detection limits that were achieved, steroid hormones and a number of other target analytes were not detected in Waikato River samples. This reflects the high flow rate and corresponding level of dilution achieved within the Waikato River. The various point sources of steroid hormones into the Waikato River catchment, treated effluents from WWTPs in particular, are massively diluted even under the low flow rate conditions during which the field sampling was undertaken.

However, previous investigations have demonstrated the Waikato River receives inputs of steroid hormones from diffuse agricultural sources and WWTP effluent point sources (Sarmah *et al.* 2006). The absolute mass of steroid hormone residues entering the greater Waikato catchment each day from these sources will be significant. Aquatic organisms within localised areas, particularly those adjacent to discharges of WWTPs effluent, could potentially be impacted by residues of steroid hormones and other EDCs that are continually released into the Waikato River.

Further assessment and characterisation of EDCs at localised sites considered likely to be impacted by EDCs is required to conclusively determine whether or not the presence of these biologically active chemicals has the potential to negatively impact river biota. As previously mentioned, the identifiable inputs of EDCs to the Waikato River are massively diluted and this affects the detection of these biologically potent chemicals. Future investigations of EDCs and other organic contaminants within the Waikato River would benefit from the deployment of integrative passive sampling devices that accumulate very low concentrations of contaminants over a period of weeks. Calibration of these devices allows the time-weighted average waterbourne concentration of contaminants to be calculated. Devices such as the Polar Organic Contaminant Integrative Sampler (POCIS) are routinely used by the US EPA, US Geological Survey, and European Commission funded NORMAN Network to assess the concentration of EDCs and other organic contaminants in waterways. To assess whether biota is affected by EDCs, it may be advisable to conduct a biomarker-based survey. For instance, the induction of egg-yolk precursor vitellogenin in male fish is a marker of exposure to estrogenic compounds (Jones *et al*. 2000).

5. CONCLUSIONS

Overall, the results obtained from this assessment of EDCs in the Waikato River demonstrate that when specific contaminants are present, it is at relatively low concentrations. The concentrations of chemical residues measured in the Waikato River are one to three-orders of magnitude below their respective predicted-no-effect concentration (PNEC). The bioassay responses were similarly low, suggesting the current concentration of these chemicals in Waikato River water pose negligible risks to aquatic biota.

However, it is important to acknowledge the samples analysed in this study were obtained from a one-off sampling and the results must therefore be interpreted with caution. The absence of many of the target EDCs in the analysed river water samples does not necessarily mean these chemicals are not present in the Waikato River; nor does it mean these chemicals present no risk to biota.

Endocrine disrupting chemicals can elicit effects at very low concentrations and their potency can be additive when they are present as mixtures. The PNECs for EDCs continue to decrease as our understanding of the risks these chemicals pose to wildlife and humans are better defined. This is illustrated by the range of PNEC values for bisphenol-A referred to in this report. The latest proposed bisphenol-A PNEC of 0.060 µg/L, determined by numerous scientific studies, is two-orders of magnitude lower than the existing PNEC values adopted by the EU and Japan. Current PNEC values are determined from single chemical exposures and do not take account of the potential cumulative effects of mixtures of contaminants upon biological receptors.

It is likely the PNEC for EDCs will continue to reduce as risk assessments become more refined and robust. Future assessments of EDCs within the Waikato River will need to acknowledge these modified PNECs and adopt sampling strategies that accommodate the significant level of dilution that occurs within the Waikato River.

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