Appendix D: Population, Flows & Climate Change Memo

Memo

To:	Ilze Rautenbach Stantec Wellington Office	From:	Ron Haverland Connect Water
File:	Porirua WWTP Consent	Date:	January 31, 2020

Reference: **Porirua WWTP Consent – Population and Flows and Climate Change**

1 INTRODUCTION

1.1 BACKGROUND

On June 25, 2019 a multi-criteria analysis (MCA) was undertaken on nine combined wastewater treatment plant (WWTP) and wastewater network options. These options were assessed using projected population and flows for the year 2057, on the basis of a consent application for the WWTP discharge being for a term of 35-years.

As new key information has arisen since the evaluation of the short list commenced it has been decided to re-evaluate the WWTP components of the short list as stand alone options. The network component of the Porirua wastewater programme is being reframed to align with outcomes sought under the Te Awarua-o-Porirua Whaitua Implementation Plan.

Porirua City Council staff have indicated agreement that Wellington Water seek a shorter term consent, given the uncertainty regarding the affordability of the network upgrades and long outfall option that the collaborative group assessed as the preferred option, and the need to make dry weather water quality improvements to meet the Whaitua targets by 2040. The consent term application is likely to be 20 years.

1.2 PURPOSE

This memo presents the population and flows for the WWTP for the 20-year consent, to 2043 allowing for the timing for the consent to be granted. In addition, it includes a discussion on the potential effects of climate change on the network flows.

1.3 INFORMATION USED

The following information has been used:

- WWTP flow data supplied by Wellington Water, email dated 25 July 2019.
- *Porirua Growth Strategy 2048* by Porirua City Council, 'Dwelling supply and population projection by area', 4 June 2019
- *Forecast ID for Wellington City and Porirua City,* July 2019
- Our Future Climate New Zealand, NIWA 2016, Version: 1.03.002
- High Intensity Rainfall Design System V4, NIWA
- *Porirua Wastewater Catchment Alternatives Optimisation and Prioritisation,* WCS Engineering Pty Ltd, September 2019

2 POPULATION

The population projections from Forecast ID were used in conjunction with 'Dwelling supply and population projection by area' provided by Porirua City to determine the growth in the Porirua catchment.

Wellington city's northern suburbs contribute to the Porirua WWTP catchment. Population projections from Forecast ID were used to determine the growth in Wellington northern suburbs.

Current and future populations for the catchment are provided in Table 2-1.

Table 2-1 Population data for the Porirua WWTP catchment

	Year 2018	Year 2043
Porirua City	56,600	80,100
Wellington northern suburbs	27,400	40,900
Total	84,000	121,000

Notes;

1. Population extracted from the Porirua Wastewater Network Model

The data shows that the population is forecast to increase by 44% over the period to the year 2043.

For the consent application, the WWTP contributing population is assessed to be 121,000.

2 FLOWS

Influent flow data to the WWTP is provided from the flow meters at the Tangare Drive and Rukutane Point pump stations. Daily total flow data was provided by Wellington Water in an Excel format. The average daily flows for the four year period are shown in Table 2.2.

Table 2-2 Influent flow data for the Porirua WWTP

Year	Average Daily Flow (m^3/day)	Average Daily Flow (L/s)	Rainfall[1] (mm)
2016	24,400	282	1197
2017	26,050	301	1147
2018	26,500	306	1382
2019 (Jan to June)	21,200	246	409

Notes;

1. Data from Greater Wellington Regional Council website for the Taupo Stream Whenua Tapu monitoring site

The previously adopted base year flow used in the nine shortlisted options was 300 L/s. Flows show an increasing trend over a three-year period and then a reduction in 2018/19 to 246 L/s, however this represents only a half year of data. Rainfall would be expected to influence the average daily flows however does not appear to be well correlated, apart from 2018 having the highest daily average flow of 306 L/s and the highest annual rainfall. For the purpose of the 20 year consent, we proposed to use a flow of 306 L/s as the current average daily flow. With an allowance for 44% population growth in the period to 2043, the average daily flow is predicted to be 440 L/s. This assumes that per capita water use remains constant over the period which is considered to be a conservative approach. Wellington Water has the objective of reducing water consumption which will directly impact on wastewater flows. Porirua's catchment is mostly domestic so industries that rely on water use and discharge to the sewer are not significant contributors to the flow.

During storm conditions, the peak flow delivered to the WWTP is approximately 1275 L/s and is limited by the capacity of the Tangare Drive and Rukutane Point pump stations. Network modelling carried out by Wellington Water and WCS Engineering includes scenarios for the optimised masterplan solution for the year 2057 and a 6 month ARI for constructed overflows, which limits the peak flow to the WWTP capacity of 1500 L/s.

3 CLIMATE CHANGE AND NETWORK FLOWS

3.1 CLIMATE CHANGE

Climate change will have impacts on the amount and intensity of rainfall in New Zealand in the future which is likely to increase flows in the sewer network to some degree.

NIWAs Our Future Climate New Zealand, presents a graphical display of the data from global climate models (GCM) which are widely used for projecting climate change. Data from the six GCM's presented by NIWA were used in the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5). The outputs are presented for various representative concentration pathways (RCP) which are different plausible pathways of green house gas concentrations in the atmosphere over the next 100 years or so. NIWA presents four of RCP's (RCP2.6 (low), RCP4.5, RCP6.0 and RCP8.5 (high)), which are the full range of greenhouse gas pathways however represent a good span of RCPs and have been used by all the global modelling groups as inputs to the global climate models.

NIWA present a six model average which show that for all RCP's, the mean annual rainfall for the Porirua region in the period from 1995 to 2055 increases in the range of 0 to 5%. Also from the six model average the change in the annual number of wet days with rain equal to or greater than 25mm for all the RCP's increases in the range of 0 to 2% (refer to attached maps).

Impacts on extreme rainfalls are more significant. NIWA's High Intensity Rainfall Design System V4, was used to determine the impact on rainfall intensity in the year 2050 compared to historical data (based on the Titahi Bay location). For a 50 year average recurrence interval (ARI), the outputs show that for a 10 minute storm duration the rain intensity is predicted to increase in the range of 8 to 11% for RCP2.6 and RCP8.5 respectively and that 5 day storm durations increase in the range of 4 to 6% for the same RCP's.

3.2 NETWORK MODELLING

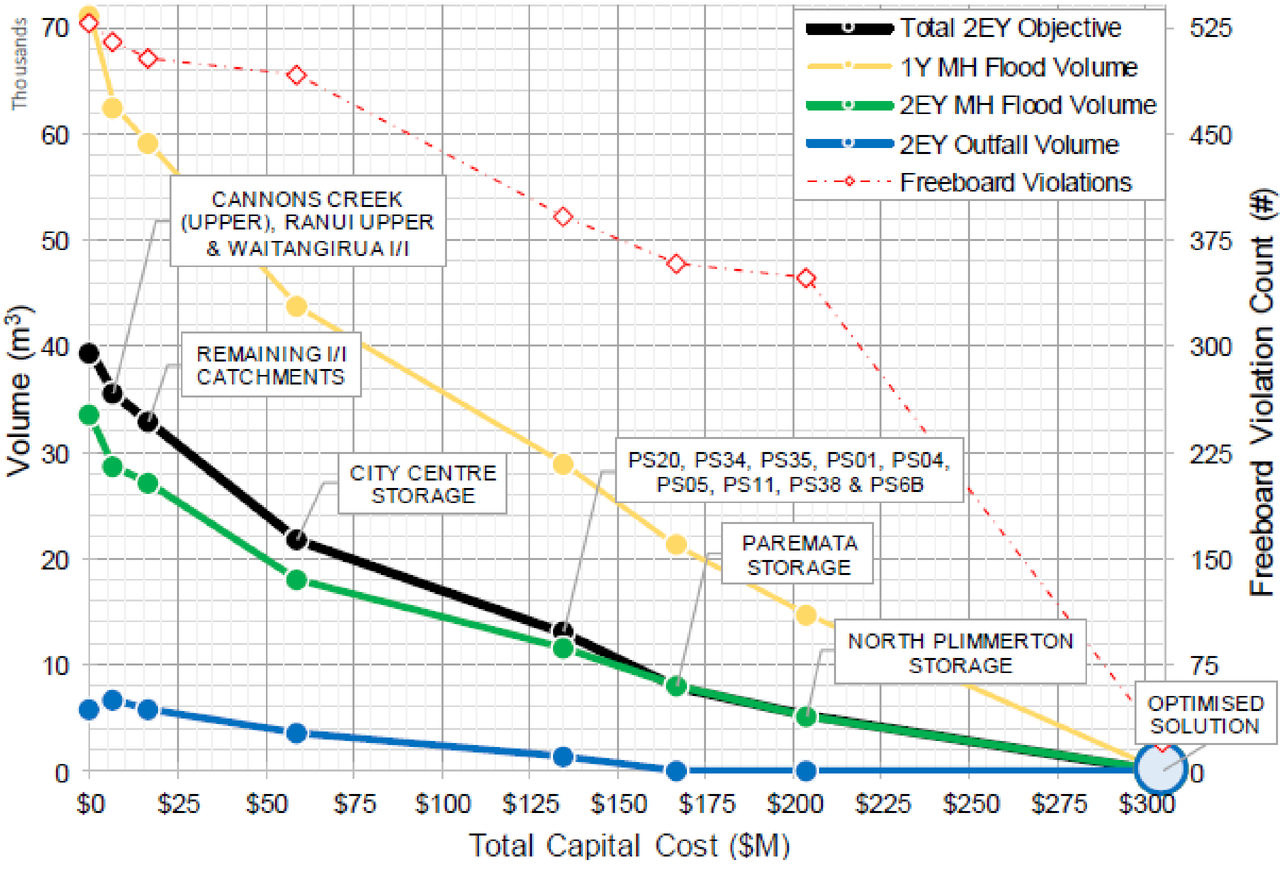
For the network modelling, WCS Engineering prepared a model simulation of historic rainfall from January 2008 to January 2018 and performed a statistical analyses of the overflow results. Design events were selected based on the best balance of overflow volume and overflow peak discharge ARI and after several trials to determine whether peak or volume is most critical, preference was given to peak. Selecting design storms based on continuous model simulation of historic rainfall is an imperfect science. There are inherent complexities associated with critical duration and varying responses depending on whether event volume or peak flow rate is considered. Furthermore, for statistically valid results and to have a greater chance of finding storms that provide similar return periods for both peak and volume, a much larger data set than the 10-year rainfall considered in this analysis is necessary. The design storms selected are intended to provide a reasonable indication of respective return periods for the portion of the catchment to which they are relevant and tend to be slightly conservative. The design storm identified by evaluating the last ten years of rainfall data produces volumes and peaks greater than 11% higher than the synthetic events for the Wellington region (WCS, 2019). Therefore the design storms are considered to be sufficiently conservative to allow for the increase in rainfall intensities of up to 11% due to climate change (email Joel Wilson: 22.01.2020).

The sewer network model also allows for sewer Inflow/Infiltration (I/I) to be increased by 17% to account for asset deterioration (increasing defects in the sewer network). This was provided by Wellington Water as a generally accepted industry figure and was included as a baseline value in the network model.

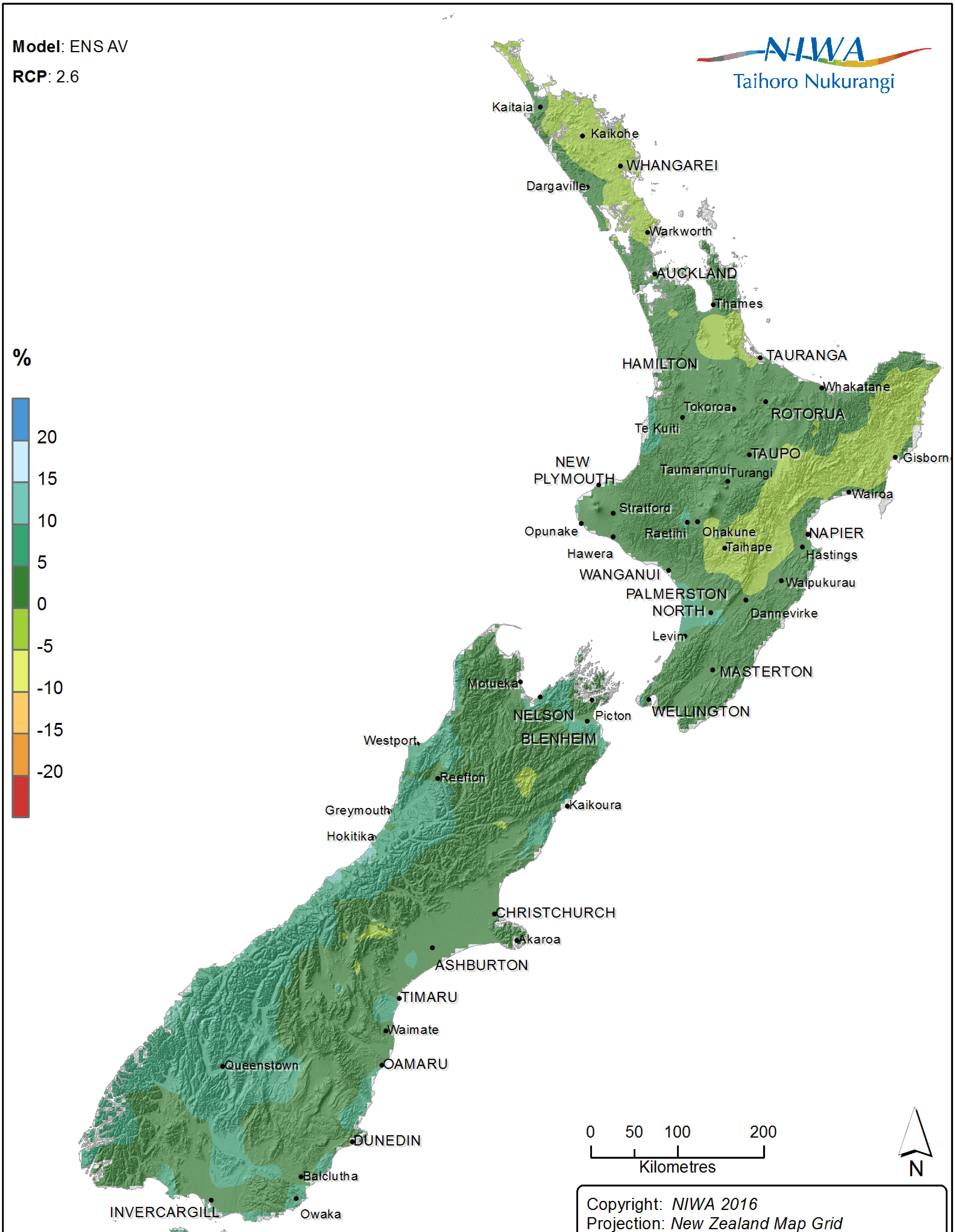
Its important to note that the future flows that will be pumped to the WWTP will be limited to the 1500 L/s capacity of the plant for the duration of the consent and therefore factors such as increasing rainfall intensities due to climate change, I/I deterioration and actual storm intensity versus that modelled, will not result in the flow to the plant exceeding 1500 L/s.

The sewer network modelling includes overflow storage and I/I reduction in order to limit flow to the WWTP to 1500 L/s and to reduce overflows from the network. The network masterplan includes I/I reduction programmes for the eastern Porirua area with a 25% reduction in flows applied as a baseline assumption. Figure 1 below shows the relative cost of network improvements and the volume of network overflows. The graph shows that with I/I reduction work, and storage within the network, overflow volumes from the network are reduced. Note that costs in Figure 1 are only for the purpose of comparing options and are not construction cost estimates as they do not include all the relevant uplifts.

Figure 1 Total Capital Cost versus Volume of Overflows from the Porirua Network



Annual Mean Rainfall Change Between 1995 and 2055

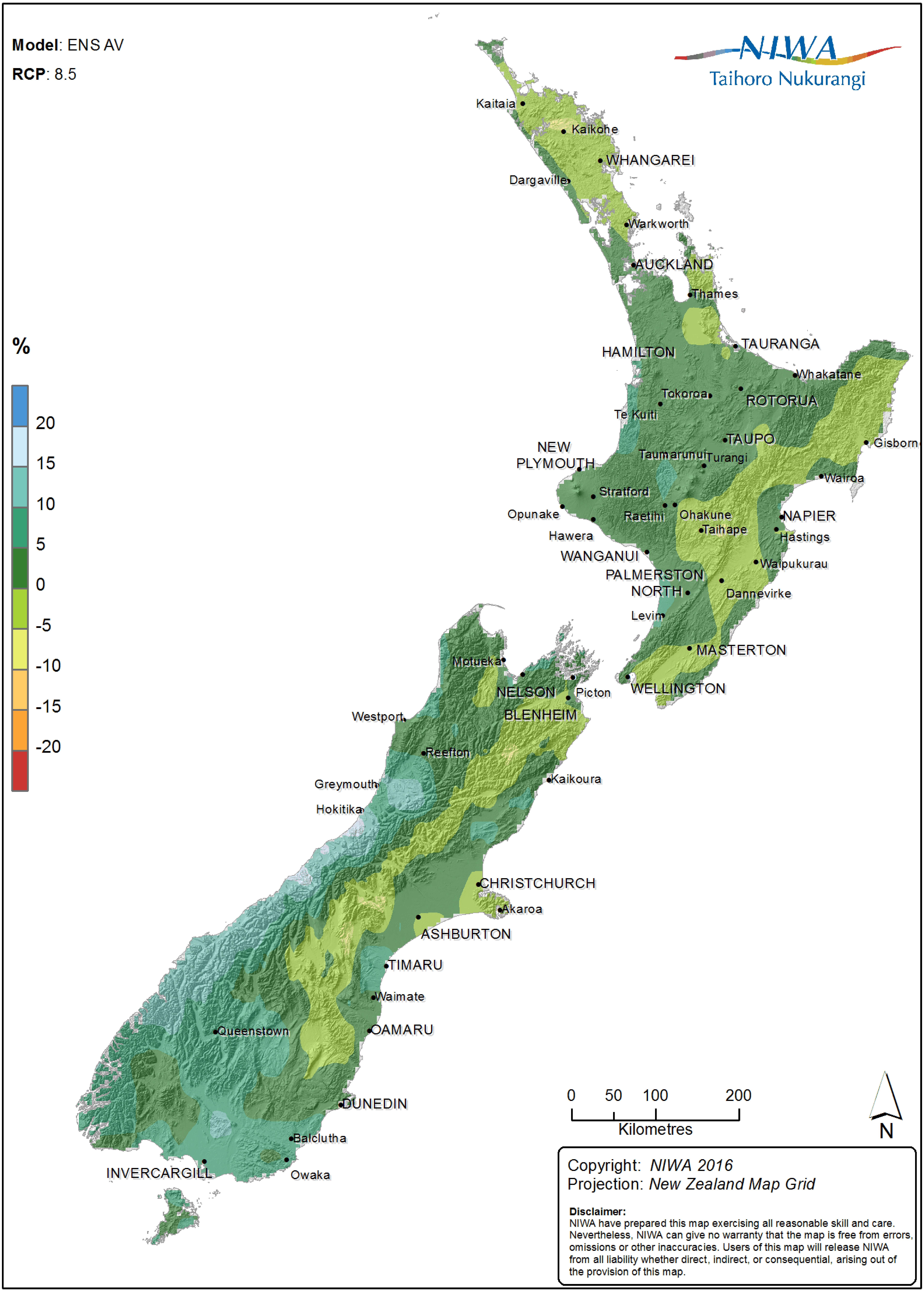


Model: ENS AV

RCP: 2.6

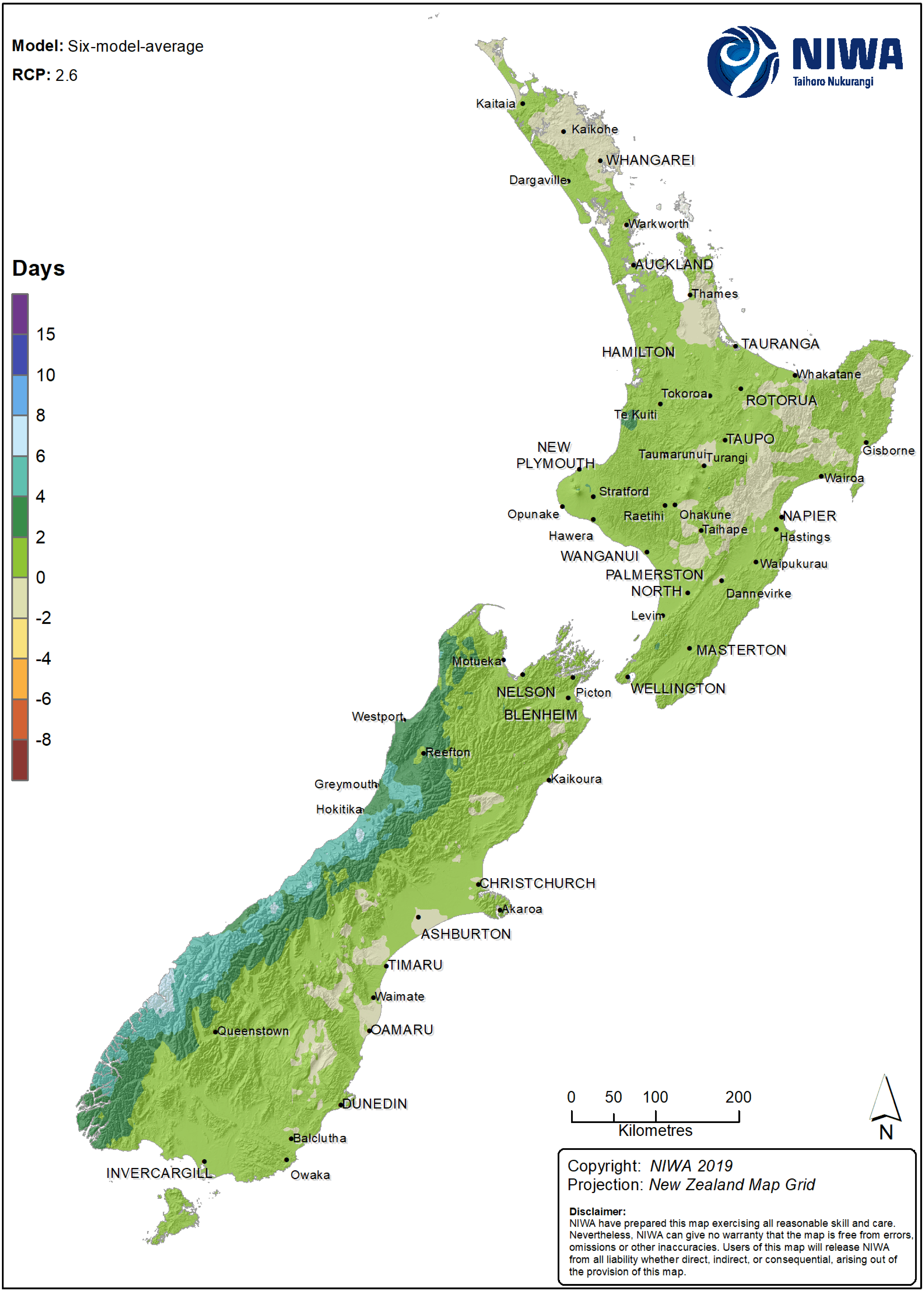
Projection: *New Zealand Map Grid*

Annual Mean Rainfall Change Between 1995 and 2055



Projection: *New Zealand Map Grid*

Change in Number of Annual Wet Days (25 mm) Between 1995 and 2055

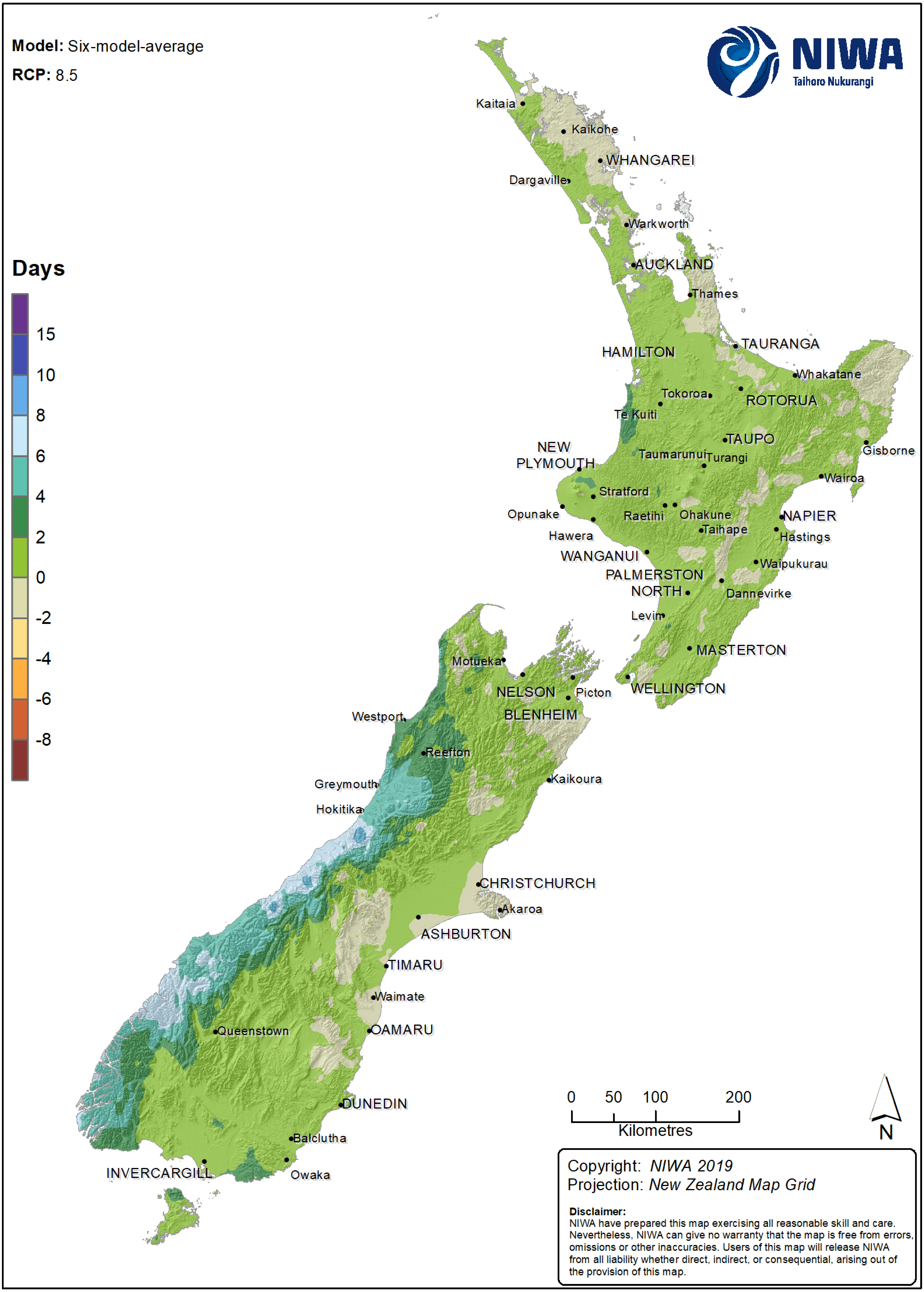


Projection: *New Zealand Map Grid*

Change in Number of Annual Wet Days (25 mm) Between 1995 and 2055

Model: Six-model-average

RCP: 8.5



Projection: *New Zealand Map Grid*

Appendix E: **Analysis of Selected Emerging Contaminants & Direct Toxicity Assessment**

ANALYSIS OF SELECTED EMERGING ORGANIC CONTAMINANTS AND DIRECT TOXICITY ASSESSMENT OF WASTEWATER FROM THE PORIRUA WASTEWATER TREATMENT PLANT

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December 2019

EXECUTIVE SUMARY

Stantec New Zealand contracted Northcott Research Consultants Limited (NRC Ltd) to contribute to a program of work supporting a resource consent application for the Porirua City Wastewater Treatment Plant to discharge treated effluent to the marine coastal environment. The contribution of NRC Ltd was analyse a selection of representative emerging organic contaminants (EOCs) in three samples of influent and effluent from the Porirua Wastewater Treatment Plant (WWTP). In addition, the three effluent samples were subjected to Direct Toxicity Assessment to determine the level of risk the treated and discharged effluent represents to marine organisms following discharge to the ocean.

A total of eighty-five individual EOCs from nine different classes of EOCs were analysed of which between 42 to 45 and 38 to 39 were detected respectively in the influent and effluent samples, over the three sampling occasions.

The profile and relative concentration of EOCs in the three Porirua effluent samples, with the exception of a few, was largely similar to that observed in influent and treated effluent from other waste water treatment plants in NZ. While the concentration of three EOCs in the Porirua WWTP effluent, namely galaxolide, TCPP and TBEP were relatively high, the measured concentrations of TCPP and TBEP are of a similar magnitude to that determined in other WWTP effluents in New Zealand. In comparison the measured concentration of galaxolide in the three Porirua WWTP effluent samples was one to two orders of magnitude greater than that measured in other New Zealand WWTPs. This could indicate a specific and constant source within the catchment the WWTP serves.

The risk of twenty-three EOCs measured in the Porirua effluent and for which Predicted No Effect Concentration (PNEC) values were available was assessed. The concentration of all but six of these EOCs fell below their respective PNEC values, indicating they present no risk to aquatic organisms exposed to the undiluted effluent. Of the remaining six EOCs the concentration of three (technical nonylphenol, tris-(2-butoxyethyl)phosphate and triclosan) in the Porirua effluent samples is the same order of magnitude as their respective PNEC/NOEC values, and they therefore represent a low level of risk to aquatic organisms exposed to undiluted effluent from the Porirua WWTP.

In comparison, the concentration of bisphenol-A, 17β-estradiol and estrone exceeded their respective PNEC/NOEC values by 1 to 2 orders of magnitude and represent a moderate risk to aquatic organisms exposed to the undiluted effluent. However, a minimum 36-fold dilution of the Porirua WWTP effluent in the receiving environment (or defined mixing zone) would be sufficient to reduce the concentration of these potent endocrine disrupting chemicals such that they would present no risk to biota in the receiving environment.

The Direct Toxicity Assessment of Porirua WWTP effluent demonstrated the toxicity of the effluent was comparable to that measured in other WWTP effluents in New Zealand. The Porirua WWTP effluent exhibited residual toxicity in the blue mussel embryo test, but this would be effectively eliminated by a 182-fold dilution of the effluent in the receiving environment.

INTRODUCTION

Northcott Research Consultants Limited (NRC Ltd) was engaged by Stantec New Zealand to analyse residues of emerging organic contaminants (EOCs) and the toxicity of wastewater from the Porirua Wastewater Treatment Plant in support of their resource consent application to discharge treated wastewater into the marine coastal environment.

Emerging organic contaminants (EOCs) include a vast number of chemicals used in industrial and domestic cleaning products, paints, inks and surface treatments, kitchen and laundry detergents, personal care products, cosmetics, pharmaceuticals and medicines. Products and medicines containing EOCs are used daily by human population and enter domestic waste water from bathing, laundry and toileting activities.

Treated urban wastewater is one of the major sources of EOCs to the environment in New Zealand which are largely discharged into freshwater or marine ecosystems. Despite being a major source of EOCs very little data is available on the concentrations entering and being discharged from WWTPs into the environment in New Zealand.

Three 24-hour composite paired samples of influent and effluent from the Porirua City Wastewater Treatment Plant were provided for the analysis of EOCs and Direct Toxicity Assessment (DTA) (effluent only) by staff of Veolia Water who manage the Porirua WWTP on behalf of Wellington Water.

A total of eighty-five individual Emerging Organic Chemicals (EOCs) representing nine different classes of EOCs were recommended for analysis. These included:

- Alkylphosphate flame retardants (11 compounds)
- Industrial alkylphenols (7 compounds)
- Insect repellents (3 compounds)
- Nitro- and polycyclic musk fragrances (11 compounds)
- Paraben preservatives (11 compounds)
- Pharmaceuticals (10 compounds)
- Phenolic antimicrobials (8 compounds)
- Phthalate esters and plasticisers (13 compounds)
- Steroid hormones (11 compounds)

This report contains:

- a description of the storage and preparation of samples provided for analysis
- a description of the methods employed for the analysis of EOCs and DTA in the samples
- a summary of the quality assurance data and outcomes
- tabulated results of residues of EOCs measured in the analysed samples
- a comparison of the concentration of EOCs in the Porirua WWTP effluent against other contemporary New Zealand data
- a risk assessment of EOCs in the discharged effluent and DTA outcomes to organisms exposed to the effluent in the receiving environment

METHODOLOGY

SAMPLE DESCRIPTION AND STORAGE

Samples of influent and effluent (24-hour composites) were collected in pre-cleaned 20L glass carboys by the staff of Veolia Water who manage the operation of the Porirua WWTP. Subsamples of composite effluent and influent were transferred into 4L amber glass carboy bottles and couriered to NRC Ltd at the Plant and Food Research Ruakura Laboratories in Hamilton. A further 1L subsample of effluent was transferred into a polycarbonate bottle and couriered to Cawthron Institute in Nelson for Direct Toxicity Assessment (DTA). The samples were couriered in polyfoam boxes (DTA) or large chilly bins (EOCs) containing frozen chiller pads.

The first set of samples were planned to be taken over 24 hours from the 7-8th October but due to complications encountered during setting up this sampling event was abandoned as the samples could not be delivered to Cawthron Institute within the time frame required to initiate DTA. Following discussion with the Veolia Water staff at the Porirua WWTP the decision was made to postpone the initiation of sampling until the following week.

The date of sampling and delivery of samples to the testing laboratories is displayed in table 2. Samples for DTA and EOC analysis were provided on three consecutive weeks (1 to 3). The chilly bin of samples received at Plant and Food Research Ruakura on 23/10/2019 were delivered late in the day by the courier and mistakenly transferred into a walk-in freezer for storage. Inspection of the samples the following day demonstrated the glass bottles were broken and cracked due to expansion of the freezing samples, resulting in the loss of the samples. An additional set of paired influent and effluent samples were therefore obtained on 05/11/2019 (week 4) for the analysis of EOCs only.

Table 1. Dates for sampling and receipt of samples at testing laboratories

Event	Week 1	Week 2[A]	Week 3	Week 4[B]
Date sampled	15/10/2019	22/10/2019	30/10/2019	05/11/2019
Date received	16/10/2019	23/10/2019	31/10/2019	06/11/2019

[A] the sample bottles for chemical analysis were accidentally frozen and the samples lost

[B] additional set of samples to replace those lost on week 2 for chemical analysis

Upon receipt in Hamilton the bottles of influent and effluent were adjusted to <2.5 by the addition of hydrochloric acid and immediately transferred into a walk-in chiller and stored at 4°C overnight, or no longer than 16 hours.

SAMPLE EXTRACTION, PURIFICATION AND DERIVATISATION FOR ANALYSIS OF EOCs

Sample preparation

The samples were removed from storage at 4°C and the pH measured to confirm it remained <2.5. The aqueous samples were filtered through a glass microfiber filter (47 mm, LabServe) topped with diatomaceous earth filter aid media (Hyflo Supercell) to remove particulate material. The sample filtrate was collected in pre-cleaned glass Schott bottles.

The filtered samples were spiked with a solution of deuterated and carbon-13 labelled EOC analogues as surrogate standards at a concentration of 25 parts per trillion (ng/L), and the acidic herbicides surrogate chemicals dichlorprop, flamprop and MCPB, and the plant growth regulator naphthalene acetic acid, at a concentration of 50 parts per trillion (ng/L) to assess their recovery from each analysed sample.

Sample extraction and purification

Emerging organic contaminants (EOCs) were extracted from the filtered samples by passing through an Oasis HLB 1 g 20 mL SPE cartridge. The retained EOCs were eluted with a mixed solvent of dichloromethane and methanol and purified by a combination of florosil and aminopropyl adsorption chromatography. The sample extracts were concentrated and subjected to gel permeation chromatography to remove residual fats and lipids.

The pharmaceutical and mono-phthalic acid esters were extracted using Oasis MCX 60mg 3mL SPE cartridges and eluted with a mixture of ethyl acetate, acetone and ammonium hydroxide. The pharmaceutical solvent extracts were concentrated under a stream of nitrogen gas and stored in acetone prior to derivatisation.

The purified EOC sample extract was split into two equal portions- one for analysis of non-polar semi-volatile EOCs and the other for polar EOCs requiring chemical derivatisation prior to analysis by gas chromatography mass-spectrometry (GCMS).

The fraction of sample extract destined for the analysis of non-polar EOCs was exchanged into isooctane, internal standards (deuterated polycyclic aromatic compounds) added, and transferred into GC vials for the analysis of nitro and polycyclic musk fragrances, phthalate esters, alkylphosphate flame retardants and insect repellents.

Sample extract derivatisation

A solution of deuterated polar internal standards was added to the second fraction of the EOC sample extracts and the polar EOCs (steroid hormones, phenolic antimicrobials, paraben preservatives, and industrial alkylphenols) were derivatised to their respective trimethylsilyl ethers.

An internal standard mixed solution containing deuterated (-d4) monocarboxylic phthalate acid esters and ibuprofen-d3 was added to the pharmaceutical solvent extracts which were evaporated to dryness and derivatised to their respective tertiary-butyl-dimethylsilyl esters by reaction with N-tert-butyldimethyl- silyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% t-butyldimethylsilyl chloride (TBDMSCl).

INSTRUMENTAL ANALYSIS OF EOCs

The analysis of the different classes of EOCs required the use of different GCMS instruments and instrumental analysis methods. Alkylphosphate flame retardants, musk fragrances, insect repellents, industrial alkylphenols, paraben preservatives, phenolic antimicrobials and steroid hormones were analysed using an Agilent 6890N gas chromatograph coupled to a 5975 mass spectrometer operating in single ion monitoring mode. Quantitation of target EOCs was achieved by internal standard quantitation using Agilent ChemStation MS software. Phthalate esters, monocaboxylate phthalate esters and pharmaceuticals were analysed using an Agilent an Agilent 7000 series triple quadrupole GCMS operating in MS/MS mode. Quantitation of target EOCs was achieved by internal standard quantitation using Agilent MassHunter MS/MS software.

QUALITY ASSURANCE PROCEDURES

Quality Assurance (QA) blank SPE cartridges were included with each set of extractions to account for background contaminants present in the laboratory and/or sourced from laboratory personal. Each individual sample was spiked with a solution of deuterated and carbon-13 labelled analogues of target EOCs to monitor and assess the recovery from each analysed sample.

Comparative standards, comprising the same volume of each individual QA surrogate solution incorporated into each batch of extracted samples, were prepared by dispensing aliquots of the individual QA spike solutions into labelled vials at the same time they were added to each batch of samples. The percentage recovery of surrogate compounds spiked into each sample was determined by directly comparing the concentration of analytes measured in the samples against that measured in the corresponding comparative standard(s).

Method detection limits (MDLs) for the eighty-four individual EOCs were calculated using a signal-to-noise ratio of 3:1 and by assessment of the mean concentration of EOCs detected in the QA blank samples. The higher of these two values was adopted as the MDL for each individual compound. The method detection limits (MDL) obtained for each of the eighty Five individual analysed are listed in Appendix Three.

The mass of EOCs measured in the SPE QA blank sample were subtracted from the measured mass in each sample before calculating the final concentration of EOCs in the sample extracts.

DIRECT TOXICITY ASSESSMENT

The Direct Toxicity Assessments (DTA) of the Porirua WWTP effluent samples was completed by the Cawthron Institute using a green algae, a burrowing amphipod and the blue mussels embryos, providing a range of taxa representing those found in the marine receiving environment to which WWTP effluents are often discharged in New Zealand. The particular species, their sources, and the test protocols employed for DTA are listed in table 2 and described more fully in the laboratory analysis report in Appendix 2.

Table 2. Species and testing protocols employed for Direct Toxicity Assessment

Description	Algae	Amphipod	Bivalve[A]
Test Standard	ASTM E1218-04 (2012)	ASTM E1192-97 (2014)	ASTM E724-98 (2012)
Test Species	*Dunaliella tertiolecta*	*Paracorophium excavatum*	*Mytilus galloprovincialis*
Source	Laboratory Culture (CS-175)	Delaware Bay	Tennyson Inlet

[A] embryo larval development

To prepare the effluent samples for testing their salinity was measured and adjusted by the addition of a standard brine solution to reach the optimal salinity for the three test species.

RESULTS

QUALITY ASSURANCE OUTCOMES

The results obtained from quality assurance procedures met or exceeded accepted standards for laboratories undertaking trace analysis of organic contaminants and pesticides, confirming the robustness of the extraction, purification and analysis protocols.

The recovery of isotopic labelled and other surrogate spike standards fell within the accepted range of 70% to 130 % (Table 3), with an overall mean recovery of 96% obtained at a relatively low spiked concentration of 25 to 50 parts per trillion. The 95% confidence intervals for the mean recovery of the surrogate compounds obtained from the analysed samples combined with relatively small 95% confidence intervals provides further demonstration of the robustness of the analytical method.

Table 3. Recovery of surrogate EOCs spiked into influent and effluent samples.

Recovery compound	95% confidence interval for mean % recovery [A]
^{13}C6-methylparaben	94 ± 10
^{13}C6-butylparaben	102 ± 9
^{13}C6-4-n-nonylphenol	85 ± 8
^{13}C6-o-phenylphenol	93 ± 6
^{13}C12-triclosan	111 ± 16
^{13}C12-bisphenol-A	93 ± 11
^{13}C6-17α-estradiol	90 ± 7
^{13}C6-estrone	86 ± 5
Musk Xylene-d15	88 ± 8
Tonalide-d_3	92 ± 9
Dichlorprop[B]	85 ± 9
Flamprop[B]	109 ± 8
MCPB[B]	91 ± 7
NAA[BC]	89 ± 6
Mean recovery	**93**

[A] N=6; [B]surrogate for acidic pharmaceuticals; [C]napthalene acetic acid

CONCENTRATION OF EOCs IN WWTP INFLUENT AND EFFLUENT SAMPLES

The concentration of EOCS detected in the three paired samples of influent and effluent and the corresponding % reduction achieved by the wastewater treatment process are listed in full in Appendix 1.

Between forty-two to forty-five individual EOCs were detected in the influent samples over the course of the three sampling occasions and a corresponding thirty-eight to thirty-nine in the treated final effluent. A total of thirty-eight individual EOCs representative of eight of the nine classes of EOCs, were not detected in any of the analysed influent or effluent samples.

Not all of the analysed compounds within each class of EOC were detected in all samples and the following presentation and discussion only includes those EOCs that were detected in the samples of influent and effluent.

Alkylphosphate flame retardants (APFRs)

A total of eleven individual APFRs were analysed in the Porirua wastewater samples but only seven of these were regularly detected in the influent and effluent samples (Table 4). The profile of APFRs in the paired influent and effluent samples was dominated by TBEP and TCPP. The concentration of the seven APFRs detected was reasonably consistent between the three sampling occasions with the exception of Tri-butylphosphate (TnBP) and Tris-(2-butoxyethyl) phosphate (TBEP) which varied two-fold and six-fold respectively. This variation in the concentration of TBP and TBEP reflects variability in their concentration in influent entering WWTPs arising from the secondary use of TBP a plasticiser and additive in some oil-based lubricants, and TBEP as a plasticiser and viscosity regulator in various types of polymers.

Table 4. Concentration of EOCs in Porirua WWTP influent and effluent samples in ng/L (ppt)

	15-October			30-October			5-December		
APFR	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**
TBEP	7965	1316	83.5	27324	4849	82.3	40920	5710	86.0
TCEP	443	526	+18.7	500	487	2.60	368	452	+22.8
TCPP	3476	3678	+5.80	3640	3798	+4.30	3937	4038	+2.60
TiBP	186	182	2.20	187	173	7.50	182	161	11.5
TnBP	237	195	17.7	426	302	29.1	261	214	18.0
TDCP	636	499	21.5	718	491	31.6	666	602	9.60
TPP	134	60.6	54.8	137	42.0	69.3	136	34.9	74.3

Analyte key:
TBEP = Tris-(2-butoxyethyl) phosphate
TCEP= Tris(2-chloroethyl) phosphate
TCPP = Tris (1-chloro-2-propyl) phosphate
TiBP = Tri-isobutyl-phosphate
TnBP = Tributyl-phosphate
TDCP = Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
TPP = Triphenylphosphate

The % removal of the seven APFRs achieved during the wastewater treatment process varied widely and concentration of two compounds (TCEP and TCPP) was higher in the effluent than corresponding influent samples.

Phenolic antimicrobials, parabens and alkylphenols

Six of the eight analysed phenolic antimicrobial chemicals were detected in the influent or effluent samples over each of the three sampling occasions, indicating a consistent and ongoing source into the wastewater reticulation system. No residues of tetrabromocresol or dichlorphen were detected in any of the influent and effluent wastewater samples (table 4).

The removal/degradation of phenolic antimicrobials during wastewater treatment was varied. The antimicrobial chemical chlorophesin which was present in all influent samples was absent in the corresponding effluent samples, indicating it was removed/degraded by the wastewater treatment process. Chloroxylenol, a common ingredient in commercial and domestic disinfectants, was present at relatively high concentrations in the influent samples but exhibited a correspondingly high rate of removal/degradation (>98). In comparison, o-phenylphenol was irregularly detected in both influent and effluent samples at low concentrations (<50 ppt) and exhibited variable removal/degradation. The antimicrobial/preservative chlorophene was present in all influent and effluent samples but was removed/degraded (84 to 94%) to a low residual concentration (13 to 16 ppt).

Triclosan was the more persistent of the phenolic antimicrobial chemicals to wastewater treatment exhibiting reductions of 25 to 70% and residual concentrations of 179 to 371 ppt. Methyl-triclosan, the principle metabolite or transformation product of triclosan, was not detected in influent samples but was present in all effluent samples at a low concentration (4.4 to 16.6 ppt).

The principal sources of triclosan entering WWTPs in New Zealand are residues of antimicrobial active plaque controlling toothpaste, antimicrobial soaps, and antimicrobial products used in medical facilities. Triclosan is subsequently metabolised by microorganisms during wastewater treatment to produce methyl-triclosan. The increased hydrophobicity and Kow of methyl-triclosan (compared to triclosan) results in it partitioning to solids and being subsequently removed during solids separation processes within the wastewater treatment process.

Table 5. Concentration of phenolic antimicrobials, parabens and industrial alkylphenols in Porirua WWTP influent and effluent samples in ng/L (ppt)

	15-October			**30-October**			**5-December**		
Antimicrobials	**Influent**	**Effluent**	**% red**	**Influent**	**Effluent**	**% red**	**Influent**	**Effluent**	**% red**
Chlorophene	218	12.8	94.2	87.5	13.7	84.4	156	15.8	89.9
Chlorophenesin	895	N.D	100	1322	N.D	100	1585	N.D	100
Chloroxylenol	7813	111	98.6	8447	127	98.5	10212	124	98.8
o-phenylphenol	N.D	33.7	none	N.D	N.D	NA	33.5	N.D	100
Methyl triclosan	N.D	4.75	none	N.D	4.41	none	N.D	16.6	none
Triclosan	197	116	41.1	165	122	26.1	210	115	42.8
Parabens									
Butylparaben	N.D	8.76	none	N.D	8.68	none	50.3	13.1	74
Methyl paraben	43.4	N.D	100	389	N.D	100	25.4	N.D	100
Propylparaben	709	N.D	100	904	N.D	100	760	N.D	100
APEs									
4-tert-amylphenol	6.06	1.69	72.1	6.17	1.39	77.5	6.50	1.19	81.7
Tech-NP EQ[A]	573	179	68.8	494	371	24.9	470	186	60.4

[A] Technical nonylphenol equivalents = the sum of the 15 main peaks present in a technical mixture of nonylphenol used for quantitation

N.D = not detected. NA = not appropriate due to absence in influent and effluent

Residues of three of the eleven analysed parabens were detected in the influent and effluent samples (Appendix 1). The three detected parabens followed a decreasing order of concentration in the influent with propyl-paraben > methyl-paraben > butyl-paraben. Methyl- and propyl-paraben were subsequently fully removed/degraded (100%) during wastewater treatment. In comparison, residues of butyl-paraben were not detected in the influent samples whilst a consistently low residual concentration remained in the effluent after treatment.

Only two of the seven analysed alkylphenols, namely 4-tert-amylphenol and technical nonylphenol (Tech-NP), were detected in the influent and effluent samples. Tech-NP was by far the dominant, being present at two orders of magnitude concentration than 4n-NP (Table 5). Tech-NP is the oxidation and microbial degradation product of parent isomeric mixtures of non-ionic nonylphenol ethoxylate surfactants which are used in detergents, paints, pesticides, personal care products, and plastics. Tech-NP is also used as an ingredient in antioxidants, lubricating oil additives, laundry and dish detergents, emulsifiers, and solubilisers. Because of its widespread use Tech-NP is a common contaminant in WWTP influent, effluent and sewage sludges.

Insect repellents, musks and fragrances, and plasticisers

Residues of the insect repellents DEET, Picaradin and Benzylbenzoate were detected in all influent samples with DEET and Picaridin being present at significantly higher concentrations, reflecting their use in many biting insect formulations. While picaridin and benzylbenzoate were fully removed/degraded by the wastewater treatment process, DEET was only partially removed/degraded (84 to 87% removal) and residues of DEET remained in the effluent (239 to 326 ppt).

Table 6. Concentration of insect repellents, musk fragrances and plasticisers in Porirua WWTP influent and effluent samples in ng/L (ppt)

	15-October			**30-October**			**5-December**		
Insect repellents	**Influent**	**Effluent**	**% red**	**Influent**	**Effluent**	**% red**	**Influent**	**Effluent**	**% red**
Benzylbenzoate	60.6	N.D.	100	135	N.D.	100	31.6	N.D.	100
DEET	1878	239	87.3	1758	289	83.6	2079	326	84.3
Picaridin	1379	N.D.	100	1622	N.D.	100	1953	N.D.	100
Musks/Fragrances									
Cashmeran (DPMI)	215	121	43.5	215	154	28.2	263	179	32.1
Celestolide (ADBI)	N.D	4.25	none	4.36	4.18	4.10	4.27	3.99	6.6
Galaxolide (HHCB)	3227	5510	+70.8	3317	6160	+85.6	4002	5727	+43.1
Musk ketone	36.7	27.3	25.5	42.1	32.1	23.8	39.9	29.8	25.4
Tonalide(AHTN)	95.5	123	+28.9	92.9	137	+47.8	110	125	+14.2
Plasticisers									
Bisphenol A	2167	182	91.6	800	247	69.1	1446	127	91.2
Benzyl butyl phthalate	288	17.8	93.8	329	31.0	90.6	227	12.7	94.4
Di-n-butyl phthalate	890	318	64.2	735	94.6	87.1	513	66.4	87.1
Diethyl phthalate	7356	115	98.4	7322	817	88.8	6549	234	96.4
Diethylhexyl phthalate	284	112	60.4	177	140	21.0	176	124	29.8
Dimethyl phthalate	317	8.99	97.2	287	226	21.2	210	25.1	88.0
Mono-butyl-PAE^{A}	63.7	15.3	76.0	151	11.6	92.3	45.3	10.3	77.3
Mono-ethylhexyl-PAE	169	18.0	89.3	439	29.3	93.3	204	15.2	92.5
Mono-methyl-PAE	1.27	2.63	none	1.97	3.04	none	0.58	1.20	none

^{A}PAE = phthalate acid ester

N.D = not detected.   NA = not appropriate due to absence in influent and effluent

Five of the eleven analysed musk and polycyclic musk fragrances were detected in the influent and effluent samples (Appendix 2 and Table 6), four of these being polycyclic musks and one single nitromusk. No residues of the polycyclic musks phantolide and traseolide, or the nitro-musks musk ambrette, mosken, tibetene and xylene were detected. The five detected musk fragrances were present in the influent of all three samples at similar concentrations, indicating a common and constant source into the wastewater reticulation network. The concentration of the five detected musk fragrances is dominated by the polycyclic musk galaxolide followed by the polycyclic musks cashmeran and tonalide with lesser contributions from the polycyclic musk celestolide (Table 6). Musk ketone was the only nitro-musk detected. The dominance of the polycyclic musks galaxolide, cashmeran and tonalide reflects their preferential use in personal care products compared to nitro-musk chemicals which have been progressively phased out and replaced by polycyclic musks. Galaxolide clearly dominates the profile of musk chemicals, reflecting the greater use of this particular musk fragrance in personal care products.

Overall the polycylic and nitromusk chemicals displayed a low rate of removal/degradation during the wastewater treatment process, with the highest removal of 43.5% occurring for cashmeran. The concentration of the polycyclic musks galaxolide and tonalide increased in the treated effluent compared to the influent. The hydrophobicity of these two polycyclic musks results in them partitioning to the particulate phase of wastewater effluents and accumulating within, and being removed from the waste stream, with sewage sludge. Residues of galaxolide and tonalide retained on particulate matter that is not removed by solids separation can subsequently be released into the dissolved phase of the waste stream as the particulate matter continues to be transformed and degraded during subsequent treatment steps. These processes release additional galaxolide and tonalide into the dissolved phase of the wastewater stream, and ultimately the effluent.

The phthalate esters 4-bromophenyl phenyl ether, chloro-ethoxymethane, 4-chlorophenyl phenyl ether and di-n-octylphthalate were not detected in any of the influent and effluent samples (Appendix 1). The profile of phthalate esters within influent was dominated by diethylphthalate which was present at an order of magnitude greater concentration than benzyl butyl-, di-n-butyl-, diethylhexyl-, and dimethyl phthalate (Table 6). The corresponding concentrations of phthalate esters in the effluent samples were significantly reduced with relatively high degrees of removal/degradation being attained (high 80s to 90s % removal). Similarly, residues of three mono-phthalate acid esters, the principal metabolites of phthalate esters, were detected in all influent and effluent samples, and displayed a similar degree of removal/degradation to the parent phthalate esters.

The concentration of bisphenol-A (BPA) in the three influent samples was relatively high (800 to 2167 ppt) but this was reduced by an order of magnitude in the effluent samples (127 to 247 ppt).

Pharmaceuticals and steroid hormones

No residues of the pharmaceuticals aspirin, clofibric acid or meclofenamic acid were detected in any of the influent and effluent samples (Appendix 1). The profile of pharmaceuticals in the influent samples was dominated by the non-steroidal anti-inflammatory drugs (NSAIDs) acetaminophen, ibuprofen and naproxen. These three pharmaceuticals were either fully removed (acetaminophen) or significantly removed (ibuprofen and naproxen) during wastewater treatment.

The concentration of salicylic acid in the influent samples was moderately high (100's of ppt) and that of ketoprofen moderately low (10's of ppt), but the high degree of removal of both during wastewater treatment resulted in relatively low concentrations in the final treated effluent (table 7)

Residues of the anti-convulsant carbamazepine and NSAID diclofenac were measured in the 100's of ppt in all of the influent and effluent samples and were relatively resistant to removal/degradation (table 7). While carbamazepine was subject to some degree of removal (28 to 37%) the concentration of diclofenac increased in the effluent relative to that in the corresponding influent samples (+34 to +72%).

A possible explanation for the increased concentration of diclofenac in the effluent samples is that it enters the WWTP in both a free and conjugated form (glucuronide or sulphate). The conjugated form is subsequently deconjugated by wastewater microorganisms to produce the free acidic form of diclofenac that is specifically targeted by the extraction and analysis procedure.

Table 7. Concentration of pharmaceuticals and steroid hormones in Porirua WWTP influent and effluent samples in ng/L (ppt)

	15-October			30-October			5-December		
Pharmaceuticals	Influent	Effluent	% red	Influent	Effluent	% red	Influent	Effluent	% red
Acetaminophen	1675	N.D	100	1598	N.D	100	1406	N.D	100
Carbamazepine	626	451	28.0	684	493	27.9	846	536	36.6
Diclofenac	382	657	+72	556	913	+64.2	502	674	+34.2
Ibuprofen	9323	30.7	99.7	5538	54.5	99.0	7146	62	99.1
Ketoprofen	54.4	8.12	85.1	59.1	7.63	87.1	58.3	9.82	83.2
Naproxen	2620	86.7	96.7	2953	182	93.8	45.3	10.3	77.3
Salicylic acid	515	20.2	96.1	1151	36.3	96.8	204	15.2	92.5
Steroid hormones									
testosterone	275	4.00	98.5	244	3.62	98.5	178	1.94	98.9
17α-estradiol	N.D	6.15	none	N.D	6.45	none	N.D	N.D	NA
17β-estradiol	28.3	40.8	+44	N.D	49.8	none	34.5	7.96	76.9
Estriol	518	N.D	100	186	N.D	100	463	N.D	100
Estrone	79.0	177	+124	68.9	214	+210	83.0	51.5	37.9

N.D = not detected.

Steroid hormones

No residues of the androgenic steroid hormones androstenediol and 19-nortestosterone, the estrogenic steroid hormones 17α-ethynylestradiol and mestranol (active ingredients in synthetic contraceptive medicines), or the progestins 19-norethindrone and norgestrel were detected in any of the influent or effluents samples (Appendix 1).

Testosterone, present in the influent samples, was removed/degraded to a high degree (>98%) by wastewater treatment to low residual concentrations in the effluent (<4ppt). Estriol, a metabolite of 17β-estradiol and estrone, exhibited the highest concentration for estrogenic steroid hormones in the influent samples but was effectively removed/degraded during wastewater treatment (100%) (table 7).

17β-estradiol and its principal metabolite estrone were detected in all influent and effluent samples and displayed either no, or a relatively low extent of removal (maximum removal of 77% and 38% respectively for 5th December sample) by the wastewater treatment process. The concentration of 17β-estradiol and estrone increased in the effluent relative to influent for the samples taken on 15th and 30th October.

Residues of 17α-estradiol were only detected in the effluent of samples from 15th and 30th October at relatively low concentrations (<10ppt). The biological potency of 17α-estradiol is significantly lower than that of 17β-estradiol, but it is transformed by aerobic microorganisms to estrone which is biologically more potent.

Direct Toxicity Assessment

The results of the DTA of three samples of Porirua WWTP effluent, obtained over three consecutive weeks, are summarised in table 8 and reported in full in the laboratory test report (Appendix Two).

The test species applied to assess the toxicity of the treated effluent samples include some of the most sensitive to assess potential impacts in the receiving environment. The algal test incorporates several consecutive generations over its 96-hour duration and therefore provides an indication of potential chronic (long-term) toxicity. The blue mussel assay utilises the early life stage embryo and is therefore the most sensitive of the three toxicity assays used in the DTA procedure.

Table 8. Results of the Direct Toxicity Assessment of effluent from the Porirua WWTP.

	15-October			22-October			30-October		
Parameter	**Algae**	**Amphipods**	**Blue**	**Algae**	**Amphipods**	**Blue**	**Algae**	**Amphipods**	**Blue**
EC_{10}[A] (%) (95%CI)	>81	>84	0.78	>81	>84	1.27	>80	>84	0.38
EC_{50}[B] (%) (95%CI)	>81	>84	1.42	>81	>84	1.49	>80	>84	0.60
NEC[C] (%) (± SE)	n/c[D]	n/c	1.22	n/c[D]	n/c	1.21	n/c	n/c	0.40
NOEC[E] (%)	81	84	0.78	81	84	0.78	80	84	0.39
LOEC[F] (%)	>81	>84	1.56	>81	>84	1.56	>80	>84	0.78
TEC[G] (%)	>81	>84	1.1	>81	>84	1.1	>80	>84	0.55

[A] median effective concentration substance at which 10% of the test population was affected. [B] median effective concentration substance at which 50% of the test population was affected. [C] no effect concentration. [D] not calculated. [E] no observable effect concentration. [F] lowest observable effect concentration. [G] threshold effect concentration, or the lowest concentration that should not cause any effect of the related measured endpoint.

Prior to testing the effluent samples are first adjusted to a specific salinity that is optimal for the three marine test species. The addition of the required volume of standard brine solution dilutes the effluent samples and this salinity adjusted sample becomes the lowest dilution test solution of effluent (highest % effluent composition). This corresponded to 80% or 81% effluent composition for the algal tests and 84% effluent composition for the amphipod tests.

The usual endpoint for the algal toxicity test is growth inhibition, but a growth stimulation was observed for all three tested effluent samples. This effect is typically observed during algal toxicity assessments of WWTP effluents and results from the presence of available nutrients (phosphate and nitrates) in the effluent sample. As such growth inhibition of the algae was not observed for any of the tested Porirua WWTP effluent samples, even at the highest test concentrations, corresponding to an effluent composition of 80% and 81%. Similarly, the tested Porirua WWTP effluents did not produce a toxic response in the amphipod test that reduced their survival, even at the highest concentration that corresponded to an effluent composition of 84%.

Therefore, if the Porirua WWTP effluent was to inhibit the growth of the algal test species the composition of effluent would need to be greater than 80 or 81%. Similarly, the composition of effluent would have to be greater than 84% before it could begin to reduce the survival of the test amphipod species.

These thresholds of effluent composition (80, 81 and 84%) are a product of the way in which these standardised tests are required to be completed and reported. If the algae and amphipod test species could be exposed to Porirua WWTP effluent at effluent compositions exceeding these threshold compositions (80, 81 and 84%) it does not mean they will begin to exhibit a negative or toxic response.

The increased sensitivity of the blue mussel embryo toxicity test is aptly demonstrated by the significantly higher rates of dilution that were necessary to ensure no detrimental effect upon their survival over the course of the 48 hour exposure test. The % composition of Porirua WWTP effluent necessary to ensure zero toxic response to the embryos of blue mussel were 1.1, 1.1, and 0.55% for the week 1,2 and 3 samples respectively. This corresponds to dilution factors for the Porirua WWTP effluent of 91-, 91-, and 182-fold respectively for effluents sampled on the 15th, 22nd, and 30th October.

Regional Councils in New Zealand typically require WWTP effluent discharges to meet a standard of no detectable toxicity at a dilution rate of 200-fold and the Porirua WWTP effluent meets this criteria. While the analysed effluent from the Porirua WWTP and other WWTPs in New Zealand typically meet this requirement there is a seasonal variation in the toxicity of WWTP effluents in New Zealand and they are commonly more toxic in autumn than other seasons (Champeau, pers comm).

Comparison with other WWTPs in New Zealand

The most comprehensive set of data with which to compare the concentration of EOCs measured in the Porirua WWTP effluent samples against is that obtained from a national survey of EOCs in the influent and effluent of 13 WWTPs in New Zealand (Northcott et al, 2013). The WWTPs selected in this national survey represented a broad range of treatment technologies, catchment population, balance of domestic to industrial inputs, and geographic distribution throughout New Zealand (table 9).

Table 9. Characteristics of WWTPs included in the 2012 National Survey project

WWTP	Description	ADF[A] (m^3)	Population	Industrial	Domestic
1	Milli-screened	20,000	55,000	25	75
2	BTF (domestic)	51,000	60,000	50	50
3	Primary sedimentation	2,330	4,000	40	60
4	Primary sedimentation	940	1,900	25	75
5	Primary sedimentation	1300	7000	5	95
6	Sedimentation and UVB	1,170	3,330	0	100
7	Sedimentation, activated sludge	45,000	140,000	10	90
8	Sedimentation, BTF, sedimentation	16,000	20,000	20	80
9	Sedimentation, BTF, sedimentation	25,000	48,000	20	80
10	Primary sedimentation	900	4,000	0	100
11	Primary sedimentation, UV	No Data	700	0	100
12	Sedimentation, BTF, sedimentation	180,000	360,000	10	90
13	Sedimentation, activated sludge	300,000	1,000,000	40	60

[A] ADF = average daily flow, [B] UV = UV treatment of final effluent

The concentration of EOCs measured in the Porirua WWTP influent and effluent samples (expressed as the range of min to max) are compared against the minimum to maximum concentration measured in the influent and effluent from these thirteen WWTPs in Table 10.

The values highlighted in green in table ten represent the concentration range of EOCs in the Porirua influent and effluent samples that fall within the range of concentration obtained from thirteen WWTPs across New Zealand. The values highlighted in red in table ten represent the average concentration of EOCs in the Porirua influent and effluent samples that exceed the maximum concentration measured in thirteen WWTPs across New Zealand.

The concentration of fourteen of the EOCs in the Porirua WWTP influent exceeded the concentration range determined in the previous study of New Zealand WWTPs (table 10). Two of these EOCs (propyl-paraben and bisphenol-A) were fully removed/degraded by the Porirua wastewater treatment to non-detectable concentrations in the effluent. The concentration of a further six of the fourteen EOCs was reduced by the wastewater treatment process to fall within the range of concentrations obtained from the thirteen WWTPs.

The concentration range for eight EOCs in the Porirua WWTP effluent samples exceeded the maximum concentration previously measured in the effluent of thirteen WWTPs in New Zealand (table 10). These included two polycyclic musks (cashmeran and galaxolide), four alkylphosphate flame retardants (TiBP, TCEP, TCPP, and TBEP), and two phenolic antimicrobials (chlorophene and methyl-triclosan, the metabolite of triclosan). While the concentration of these eight EOCs in the Porirua WWTP effluent exceeds the maximum concentrations previously determined in the effluent of thirteen WWTPs in New Zealand, they represent one-third of the twenty-four EOCs common to both assessments (table 10). The remaining two-thirds of the common EOCs either fall below or within the concentration range obtained from the study of thirteen New Zealand WWTPs.

The concentration of three EOCs in the Porirua WWTP effluent, namely galaxolide, TCPP and TBEP, were relatively high. However, the concentration of TCPP and TBEP were of a similar magnitude to that determined in the previous study. In comparison the concentration of galaxolide in the three Porirua WWTP effluent samples was one to two orders of magnitude greater than that measured in other New Zealand WWTPs. This could indicate a specific and constant source within the catchment the Porirua WWTP serves.

The conclusion of this comparison is that with the exception of galaxolide the concentration of EOCs in the Porirua WWTP effluent is broadly comparable to that in treated effluent from other WWTPs across New Zealand.

The toxicity of the Porirua WWTP effluent, determined by DTA, was also within the range and comparable with, the toxicity of treated effluents from other WWTPs in New Zealand (Champeau pers comm).

Table 10. Comparison of the concentration of EOCs in the influent and effluent of WWTPs in New Zealand and Porirua WWTP.

	Concentration of EOCS in ng/L							
	13 NZ WWTPs						Porirua WWTP	
	Influent			Effluent			Influent	Effluent
Musk	Min	Max	Mean	Min	Max	Mean	Min to Max	Min to max
Cashmeran	N.D[A]	64.4	26.2	N.D.	58.6	14.9	215-263[B]	121-179
Celestolide	N.D	8.63	4.80	N.D.	24.2	6.59	N.D-4.36[C]	4.18-4.27
Galaxolide	9.03	432	81.4	24.4	902	243	3227-4002	5510-6160
Tonalide	5.22	89.5	54.2	9.83	168	60.9	92.3-110	123-137
Musk Ketone	7.36	44.6	20.2	N.D.	36.7	13.8	36.7-42.1	27.3-32.1
Alkylphosphate flame retardants								
TiBP	N.D	283	69.1	N.D.	103	29.2	182-187	161-182
TnBP	N.D	1508	209	26.9	499	128	237-426	195-302
TCEP	15.0	451	101	16.3	303	108	368-500	452-526
TCPP	25.5	1009	383	70.5	1024	321	3476-3937	3678-4038
TDCP	N.D	516	185	1.92	630	222	636-718	494-602
TBEP	N.D	6302	1396	N.D.	3441	783	7965-40920	1316-5710
TPP	N.D	128	59.9	6.10	3277	301	134-136	34.9-60.6
Insect								
DEET	28.2	798	279	15.2	1836	220	1758-2079	239-326
Benzylbenzoate	N.D	220	90.3	N.D	270	30.4	31.6-135	N.D
Antimicrobials								
Chloroxylenol	104	3750	1241	4.07	2633	322	7813-10212	111-127
o-Phenylphenol	N.D	294	55.2	N.D	6825	549	N.D-33.5	N.D-33.7
Chlorophene	N.D	21.4	3.42	N.D	N.D	N.D	87.5-218	12.8-15.8
methyl-	N.D	106	11.4	N.D	5.45	1.38	N.D	4.41-16.6
Triclosan	24.7	100	60.5	4.43	158	38.3	165-210	115-122
Paraben preservatives								
Methyl-	89.5	2670	1147	N.D.	772	81.6	25.4-389	N.D
Ethyl-Paraben	N.D	296	104	N.D.	39	4.11	N.D	N.D.
Propyl-Paraben	43.4	696	328	N.D.	177	29.4	709-904	N.D
Butyl-Paraben	17.1	177	76.9	N.D.	13.0	2.70	N.D-50.3	8.68-13.1
Plasticiser								
Bisphenol-A	5.59	199	40.6	N.D	66.9	17.0	1406-1675	N.D

[A] N.D. = not detected

[B] values in red highlight represent those exceeding the maximum range

[C] values in green highlight represent those falling below the minimum or within the range of minimum to maximum

THE RISK OF PORIRUA WWTP EFFLUENT TO EXPOSED BIOTA

While the treated and discharged effluent from the Porirua WWTP has been demonstrated to contain residues of EOCs this does not necessarily mean they represent a risk to exposed organisms in the receiving environment.

The risk of chemical contaminants is determined by assessing their toxicity to exposed organisms, or to selected "model" organisms that are representative of various taxa (i.e Daphnia magna and salmonids for freshwater invertebrates and fish). The parameters that are derived from toxicity assessments and subsequently applied to determine the risk of a specific chemical include the Predicted No Effect Concentration (PNEC) and No Observable Effect Concentration (NOEC).

PNEC values are intended to be conservative and predict the concentration at which a chemical will likely have no adverse or toxic effect to organisms in an ecosystem. If the predicted concentration for an EOC is less than the PNEC value it will not cause an adverse effect on aquatic organisms exposed to the EOC, and therefore it presents no risk to aquatic organisms. For those EOCs where a PNEC is not available, the no observable-effect concentration (NOEC) is applied. The ability to assess the risk that EOCs could present in receiving environments is currently limited by the availability of PNEC and NOEC values.

PNEC/NOEC values were only available for twenty-three of the thirty-nine EOCs detected in the Porirua effluent samples and these are presented in table 11. While these PNEC/NOEC values have been produced for aquatic organisms they have typically been derived for freshwater, and not marine species. Consequently, they can be applied to assess the relative risk the detected EOCs represent to marine aquatic organisms, but not the absolute risk.

A comparison of the measured concentration range of EOCs in the Porirua effluent samples against the corresponding PNEC/NOEC values demonstrates the concentration of the greater proportion of EOCs fall significantly below their respective PNEC/NOEC values, and therefore present no risk to marine organisms that may be exposed to the undiluted effluent (table 11).

The concentration of six EOCs in the Porirua effluent samples were either comparable to, or exceeded, their respective PNEC/NOEC values. These include the industrial alkylphenol, technical nonylphenol; the alkylphosphate flame retardant Tris-(2-butoxyethyl) phosphate (TBEP); the antimicrobial, triclosan; the plasticiser bisphenol-A; and two estrogenic steroids, 17β-estradiol and estrone (table 11).

The concentration of three of these six EOCs in the Porirua effluent samples is the same order of magnitude as their respective PNEC/NOEC values, and they therefore represent a low level of risk to aquatic organisms exposed to undiluted effluent from the Porirua WWTP.

In comparison, the concentration of the endocrine disrupting EOCs bisphenol-A, 17β-estradiol and estrone in the Porirua WWTP effluent samples exceeded their respective PNEC/NOEC values by 1 to 2 orders of magnitude. Therefore, at the concentrations measured in the Porirua WWTP effluent, these three EOCs represent a moderate risk to aquatic organisms exposed to the undiluted effluent. This outcome is not uncommon for these three EOCs as their impact upon biological organisms is widely studied and their relatively low PNEC values reflect their potency as endocrine disrupting chemicals and its long-term chronic impact upon exposed biota.

The level of risk contaminants present to exposed organisms in the environment is typically quantified by a Risk Quotient (RQ). RQs are calculated by dividing the predicted environmental concentration (PEC) of a contaminant by their PNEC value. RQs less than 1 indicate a contaminant presents no risk to organisms exposed to it at the PEC.

RQs for residues of bisphenol-A, 17β-estradiol and estrone in the Porirua WWTP effluent discharged into the marine coastal environment cannot be determined without first calculating the dilution of the discharged effluent within the defined zone of mixing, and the corresponding PECs. However, the information presented in table 11 demonstrates a minimum 36-fold dilution of the Porirua WWTP effluent in the receiving environment (or defined mixing zone) would produce RQs less than 1 for the endocrine disrupting EOCs.

Table 11. Risk characterisation for EOCs detected in Porirua WWTP effluent

Emerging Organic Chemical	Effluent concentration range (ng/L)	PNEC/NOEC (ng/L)	Risk of EOCs to aquatic organisms (GREEN=no risk; RED=risk)	Dilution required for no risk to aquatic organisms	Source
Industrial alkylphenols					
Technical nonylphenol	179-371	330	= or > PNEC/NOEC	0-fold	European Union 2002
Alkylphosphate Flame Retardants					
TnBP	195-302	660,000	3 orders of magnitude < PNEC/NOEC	0-fold	Verbruggen 2005
TiBP	161-182	150,000	3 orders of magnitude < PNEC/NOEC	0-fold	Verbruggen 2005
TBEP	1316-5710	1,300	= or > PNEC/NOEC	0-fold	Verbruggen 2005
TCEP	452-526	460,000	3 orders of magnitude < PNEC/NOEC	0-fold	Verbruggen 2005
TCPP	3678-4038	160,000	2 orders of magnitude < PNEC/NOEC	0-fold	Verbruggen 2005
TDCP	491-602	1,300	1 order of magnitude < PNEC/NOEC	0-fold	Env Canada 2016
TPP	34.9-60.6	740	1 order of magnitude < PNEC/NOEC	0-fold	Verbruggen 2005
Phenolic Antimicrobials					
Triclosan	115-122	100	= or > PNEC/NOEC	0-fold	WFD-UKTAG 2009
Polycyclic musks					
Galaxolide	5510-6160	68,000	1 order of magnitude < PNEC/NOEC	0-fold	Hera 2004
Tonalide	123-137	3,500	1 order of magnitude < PNEC/NOEC	0-fold	Hera 2004
Pharmaceuticals					
Carbamazepine	451-536	9000	1 order of magnitude < PNEC/NOEC	0-fold	Zhao et al 2017
Diclofenac	657-913	9800	1 order of magnitude < PNEC/NOEC	0-fold	Zhao et al 2017
Ibuprofen	30.7-62.0	13875	3 orders of magnitude < PNEC/NOEC	0-fold	Ortez de Garcia, 2014
Naproxen	10.3-182	14,199	2 to 3 orders of magnitude < PNEC/NOEC	0-fold	Ortez de Garcia, 2014
Salicylic acid	15.2-36.3	118,700	4 orders of magnitude < PNEC/NOEC	0-fold	Ortez de Garcia, 2014
Plasticisers					
Bisphenol-A	127-247	60	1 order of magnitude > PNEC/NOEC	4-fold	Wright-Walters, 2011
Benzyl butyl phthalate	12.7-17.8	51,000	3 orders of magnitude < PNEC/NOEC	0-fold	Staples 2000
Di-n-butyl phthalate	66.4-318	10,000	2 to 3 orders of magnitude < PNEC/NOEC	0-fold	Staples 2011
Diethyl phthalate	112-140	940,000	3 orders of magnitude < PNEC/NOEC	0-fold	Staples 2000
Dimethyl phthalate	8.99-226	3,251,000	4 to 6 orders of magnitude < PNEC/NOEC	0-fold	Staples 2000
Estrogenic steroid hormones					
17β-estradiol	7.96-49.8	2.0	= or 1 order of magnitude > PNEC/NOEC	25-fold	Caldwell et al 2012
Estrone	51.5-214	6.0	1 to 2 orders of magnitude > PNEC/NOEC	36-fold	Caldwell et al 2012

^{A}N.D = not detected; ^{B}NA = not available

Analyte key: **TnBP** = Tributyl-phosphate **TiBP** = Tri-isobutyl-phosphate **TBEP** = Tris-(2-butoxyethyl) phosphate **TCEP**= Tris(2-chloroethyl) phosphate **TCPP** = Tris (1-chloro-2-propyl) phosphate **TDCP** = Tris[2-chloro-1-(chloromethyl)ethyl] phosphate **TPP** = Triphenylphosphate

As previously mentioned, the results of DTA of the three consecutive weekly samples of Porirua WWTP effluent exhibited residual toxicity in the blue mussel embryo test, but this would be effectively eliminated by a 182-fold dilution of the effluent in the receiving environment.

Therefore, the treated effluent samples that were tested over the three-week sampling period would be considered non-toxic to exposed biota in the marine receiving environment if it was subject to a dilution factor equal to or greater than 182-fold.

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APPENDIX ONE. Concentration of EOCs in Porirua WWTP influent and effluent samples in ng/L (ppt)

Chemical class and individual EOCs	**15-October**			**30-October**			**5-December**		
Alkylphenols	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**
4-n-amylphenol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
4-tert-amylphenol	6.06	1.69	72.1	6.17	1.39	77.5	6.50	1.19	81.7
4-tert-heptylphenol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
4-n-nonylphenol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Tech-NP EQ[A]	573	179	68.8	494	371	24.9	470	186	60.4
4-n-Octylphenol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
4-tert-Octylphenol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Alkylphosphate flame retardants									
TBEP	7965	1316	83.5	27324	4849	82.3	40920	5710	86.0
TCEP	443	526	+18.7	500	487	2.60	368	452	+22.8
TCPP	3476	3678	+5.80	3640	3798	+4.30	3937	4038	+2.60
TiBP	186	182	2.20	187	173	7.50	182	161	11.5
TnBP	237	195	17.7	426	302	29.1	261	214	18.0
TDCP	636	499	21.5	718	491	31.6	666	602	9.60
TEHP	N.D	N.D.	NA	N.D.	N.D.	NA	N.D.	N.D.	NA
ToCP	N.D	N.D.	NA	N.D.	N.D.	NA	N.D.	N.D.	NA
TmCP	N.D	N.D.	NA	N.D.	N.D.	NA	N.D.	N.D.	NA
TpCP	N.D	N.D.	NA	N.D.	N.D.	NA	N.D.	N.D.	NA
TPP	134	60.6	54.8	137	42.0	69.3	136	34.9	74.3

N.D = not detected. NA = not appropriate due to absence in influent and effluent. [A] Technical nonylphenol equivalents = the sum of the 15 main peaks present in a technical mixture of nonylphenol used for quantitation

Analyte key

TBEP = Tris-(2-butoxyethyl) phosphate
TCPP = Tris (1-chloro-2-propyl) phosphate
TnBP = Tributyl-phosphate
TEHP = Tris(2-ethylhexyl) phosphate
TmCP = Tri-m-cresyl phosphate
TPP = Triphenylphosphate
TCEP= Tris(2-chloroethyl) phosphate
TiBP = Tri-isobutyl-phosphate
TDCP = Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
ToCP = Tri-o-cresyl phosphate
TpCP = Tri-p-cresyl phosphate

APPENDIX ONE. Concentration of EOCs in Porirua WWTP influent and effluent samples in ng/L (ppt)- continued

Chemical class and individual EOCs	**15-October**			**30-October**			**5-December**		
Antimicrobials/preservatives	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**
3,4,5,6-tetrabromo-o-cresol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Chlorophene	218	12.8	94.2	87.5	13.7	84.4	156	15.8	89.9
Chlorophenesin	895	N.D	100	1322	N.D	100	1585	N.D	100
Chloroxylenol	7813	111	98.6	8447	127	98.5	10212	124	98.8
Dichlorophen	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
o-phenylphenol	N.D	33.7	none	N.D	N.D	NA	33.5	N.D	100
Methyl triclosan	N.D	4.75	none	N.D	4.41	none	N.D	16.6	none
Triclosan	197	116	41.1	165	122	26.1	210	115	42.8
Paraben preservatives									
Benzyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Butylparaben	N.D	8.76	none	N.D	8.68	none	50.3	13.1	74
isobutyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Ethylparaben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Heptyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Hexyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Methyl paraben	43.4	N.D	100	389	N.D	100	25.4	N.D	100
Pentyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Phenyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Propylparaben	709	N.D	100	904	N.D	100	760	N.D	100
isopropyl-paraben	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Insect repellents									
Benzylbenzoate	60.6	N.D.	100	135	N.D.	100	31.6	N.D.	100
DEET	1878	239	87.3	1758	289	83.6	2079	326	84.3
Picaridin	1379	N.D.	100	1622	N.D.	100	1953	N.D.	100

N.D = not detected.   NA = not appropriate due to absence in influent and effluent

APPENDIX ONE. Concentration of EOCs in Porirua WWTP influent and effluent samples in ng/L (ppt)- continued

Chemical class and individual EOCs	**15-October**			**30-October**			**5-December**		
Musks and fragrances	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**
Cashmeran (DPMI)	215	121	43.5	215	154	28.2	263	179	32.1
Celestolide (ADBI)	N.D	4.25	none	4.36	4.18	4.10	4.27	3.99	6.6
Galaxolide (HHCB)	3227	5510	+70.8	3317	6160	+85.6	4002	5727	+43.1
Musk ambrette	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Musk ketone	36.7	27.3	25.5	42.1	32.1	23.8	39.9	29.8	25.4
Musk moskene	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Musk tibetene	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Musk xylene	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Phantolide	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Tonalide(AHTN)	95.5	123	+28.9	92.9	137	+47.8	110	125	+14.2
Traseolide (ATII)	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Pharmaceuticals									
Acetaminophen	1675	N.D	100	1598	N.D	100	1406	N.D	100
Aspirin	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Carbamazepine	626	451	28.0	684	493	27.9	846	536	36.6
Clofibric	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Diclofenac	382	657	+72	556	913	+64.2	502	674	+34.2
Ibuprofen	9323	30.7	99.7	5538	54.5	99.0	7146	62	99.1
Ketoprofen	54.4	8.12	85.1	59.1	7.63	87.1	58.3	9.82	83.2
Meclofenamic acid	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Naproxen	2620	86.7	96.7	2953	182	93.8	45.3	10.3	77.3
Salicylic acid	515	20.2	96.1	1151	36.3	96.8	204	15.2	92.5

N.D = not detected.   NA = not appropriate due to absence in influent and effluent

APPENDIX ONE. Concentration of EOCs in Porirua WWTP influent and effluent samples in ng/L (ppt)- continued

Chemical class and individual EOCs	**15-October**			**30-October**			**5-December**		
Phthalates and plasticisers	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**	**Influent**	**Effluent**	**% removal**
Bisphenol A	2167	182	91.6	800	247	69.1	1446	127	91.2
4-bromophenyl phenyl ether	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Chloro-ethoxymethane	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
4-Chlorophenyl phenyl ether	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Benzyl butyl phthalate	288	17.8	93.8	329	31.0	90.6	227	12.7	94.4
Di-n-buty lphthalate	890	318	64.2	735	94.6	87.1	513	66.4	87.1
Diethyl phthalate	7356	115	98.4	7322	817	88.8	6549	234	96.4
Diethylhexyl phthalate	284	112	60.4	177	140	21.0	176	124	29.8
Dimethyl phthalate	317	8.99	97.2	287	226	21.2	210	25.1	88.0
Di-n-octyl phthalate	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Monobutyl-Phthalate acid ester	63.7	15.3	76.0	151	11.6	92.3	45.3	10.3	77.3
MonoEthylhexyl-Phthalate acid ester	169	18.0	89.3	439	29.3	93.3	204	15.2	92.5
Monomethyl-Phthalate acid ester	1.27	2.63	none	1.97	3.04	none	0.58	1.20	none
Androgenic steroid hormones									
Androstenediol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
19-Nortestosterone	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
testosterone	275	4.00	98.5	244	3.62	98.5	178	1.94	98.9
Estrogenic steroid hormones									
17α-estradiol	N.D	6.15	none	N.D	6.45	none	N.D	N.D	NA
17β-estradiol	28.3	40.8	+44	N.D	49.8	none	34.5	7.96	76.9
Estriol	518	N.D	100	186	N.D	100	463	N.D	100
Estrone	79.0	177	+124	68.9	214	+210	83.0	51.5	37.9
17α-ethynylestradiol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Mestranol	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Progestins									
19-Norethindrone	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA
Norgestrel	N.D	N.D	NA	N.D	N.D	NA	N.D	N.D	NA

N.D = not detected. NA = not appropriate due to absence in influent and effluent

APPENDIX TWO

Direct Toxicity Assessment Laboratory Report

Cawthron Institute, Ecotoxicology

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13 November 2019

CAWTHRON REPORT NO. 3438

RESULTS FROM TOXICITY TESTS CONDUCTED ON PORIRUA WASTEWATER EFFLUENT FOR 3 CONSECUTIVE WEEKS

Project Number: 17397

Client: Northcott Consultants Ltd
Attention: Grant Northcott

Sample details

ID	Week 1	Week 2	Week 3
Collected by	Nico		
Sample type	Wastewater	Wastewater	Wastewater
Date sampled	15/10/2019	22/10/2019	30/10/2019
Date received	16/10/2019	23/10/2019	31/10/2019

Characteristics (at reception)			
Temperature (°C)	9.5	10	8.7
Dissolved oxygen (mg/L)	10.5	11.5	9.8
Salinity (PSU)	0.3	0.2	0.4
pH	7.7	7.6	7.4

Results

	Test species	EC_{10} (%) (95%CI)	EC_{50} (%) (95%CI)	NEC (%) (± SE)	NOEC (%)	LOEC (%)	TEC (%)
Week 1	Algae	> 81	> 81	n/c	81	> 81	> 81
	Amphipods	> 84	> 84	n/c	84	> 84	> 84
	Blue mussel	0.78 (0.72-0.86)	1.42 (1.39-1.46)	1.22 ± 1.17	0.78	1.56	1.1
Week 2	Algae	> 81	> 81	n/c	81	> 81	> 81
	Amphipods	> 84	> 84	n/c	84	> 84	> 84
	Blue mussel	1.27 (0.83-1.71)	1.49 (1.38-1.60)	1.21 ± 1.10	0.78	1.56	1.1
Week 3	Algae	> 80	> 80	n/c	80	> 80	> 80
	Amphipods	> 84	> 84	n/c	84	> 84	> 84
	Blue mussel	0.38 (0.35-0.41)	0.60 (0.59-0.62)	0.40 ± 1.45	0.39	0.78	0.55

n/c: data not calculated

Reviewed by

Olivier Champeau
Ecotoxicologist

Louis Tremblay, PhD
Environmental toxicologist

Methods and exposure parameters: for week 1 sample

	Algae	**Amphipod**	**Bivalve** - embryo larval development
Test start – end dates	17-21/10/2019	17-21/10/2019	01/11-03/11/2019
Standard – Cawthron SOP	ASTM E1218-04 (2012) – ETX 4.33.1	ASTM E1192-97 (2014) – ETX 4.34	ASTM E724-98 (2012) – ETX 4.32.1
Test species	*Dunaliella tertiolecta*	*Paracorophium excavatum*	*Mytilus galloprovincialis*
Source	Laboratory culture (CS-175)	Delaware Bay	Tennyson Inlet
Endpoint	Growth inhibition	Survival	Survival
Density, number per container	12.3 ± 4.1 × 10^3 /mL	10	400
Test containers	96 well sterile microplate	80 mL glass container	20 mL glass vials (28 × 61 mm)
Exposure time	96 hr	96 hr	48 hr
Sample pre-treatment	0.45 µm filtration, ASW brine added	ASW brine added	ASW brine added
Concentrations	0, 0.2 to 81%	0, 0.78 to 84%	0, 0.2 to 75%
Replicates	10 for controls, 5 for treatments	10 for controls, 3 for treatments	10 for controls, 5 for treatments
Light	Continuous 200 µmol/m^2/sec	None	None
Temperature	18.6 ± 0.1°C	17.7 ± 0.1°C	17.7 ± 0.1°C
Dissolved oxygen (beginning/end)	8.3 mg/L	9.9 / 7.7 ± 0.3 mg/L	8.1 mg/L
pH	7.8	7.8	7.9
Aeration	None	None	None
Salinity (beginning / end)	26.3 PSU	20.8 / 20.7 ± 0.1 PSU	33.3 PSU
Sensitivity (EC_{50} with 95%CI)	0.14 (0.09–0.19) mg Cu^{2+}/L	2.15 (1.82 – 2.48) mg Zn^{2+}/L	0.101(0.98–0.104) mg Zn^{2+}/L
Control quality for sensitivity (average EC_{50} with 2×SD)	0.163 (0.11–0.22) mg Cu^{2+}/L (*n* = 20)	1.86 (0.63 – 3.1) mg Zn^{2+}/L (*n*= 26)	0.084(0.0.25–0.143) mg Zn^{2+}/L (*n* = 33)
Test acceptability (in controls)	CV < 20%, 16-fold increase in control	< 10% mortality	> 80% in controls
Test compliance	☑	☑	☒ (> 48h after sampling)
Note	Age of culture: 6 days	Amphipods collection date: 17/10/2019	Mussel collection date: 15/10/2019 Spawning method: thermal stress

For week 2 sample

	Algae	**Amphipod**	**Bivalve** - embryo larval development
Test start – end dates	24-28/10/2019	24-28/10/2019	01/11-03/11/2019
Standard – Cawthron SOP	ASTM E1218-04 (2012) – ETX 4.33.1	ASTM E1192-97 (2014) – ETX 4.34	ASTM E724-98 (2012) – ETX 4.32.1
Test species	*Dunaliella tertiolecta*	*Paracorophium excavatum*	*Mytilus galloprovincialis*
Source	Laboratory culture (CS-175)	Delaware Bay	Tennyson Inlet
Endpoint	Growth inhibition	Survival	Survival
Density, number per container	12.1 ± 1.7 × 10^3 /mL	10	400
Test containers	96 well sterile microplate	80 mL glass container	20 mL glass vials (28 × 61 mm)
Exposure time	96 hr	96 hr	48 hr
Sample pre-treatment	0.45 µm filtration, ASW brine added	ASW brine added	ASW brine added
Concentrations	0, 0.2 to 81%	0, 0.78 to 84%	0, 0.2 to 75%
Replicates	10 for controls, 5 for treatments	10 for controls, 3 for treatments	10 for controls, 5 for treatments
Light	Continuous 200 µmol/m^2/sec	None	None
Temperature	17.8 ± 0.6°C	17.6 ± 0.1°C	17.6 ± 0.1°C
Dissolved oxygen (beginning/end)	8.3 mg/L	9.6 / 7.4 ± 0.4 mg/L	9.2 mg/L
pH	7.8	7.7	7.6
Aeration	None	None	None
Salinity (beginning/end)	26.3 PSU	20.9 / 21.8 ± 0.4 PSU	33.3 PSU
Sensitivity (EC$_{50}$ with 95%CI)	0.143 (0.133–0.153) mg Cu^{2+}/L	1.62 (1.37 – 1.87) mg Zn^{2+}/L	0.101(0.98–0.104) mg Zn^{2+}/L
Control quality for sensitivity (average EC$_{50}$ with 2×SD)	0.162 (0.0.11–0.22) mg Cu^{2+}/L (*n* = 21)	1.85 (0.62 – 3.1) mg Zn^{2+}/L (*n* = 27)	0.084(0.0.25–0.143) mg Zn^{2+}/L (*n*=33)
Test acceptability (in controls)	CV < 20%, 16-fold increase in control	< 10 % mortality	> 80% in controls
Test compliance	☑	☑	☒ (> 48h after sampling)
Note	Age of culture: 6 days	Amphipods collection date: 24/10/2019	Mussel collection date: 22/10/2019 Spawning method: thermal stress

For week 3 sample

	Algae	**Amphipod**	**Bivalve** - embryo larval development
Test start – end dates	31/10-4/11/2019	31/10-4/11/2019	01/11-03/11/2019
Standard – Cawthron SOP	ASTM E1218-04 (2012) – ETX 4.33.1	ASTM E1192-97 (2014) – ETX 4.34	ASTM E724-98 (2012) – ETX 4.32.1
Test species	*Dunaliella tertiolecta*	*Paracorophium excavatum*	*Mytilus galloprovincialis*
Source	Laboratory culture (CS-175)	Delaware Bay	Tennyson Inlet
Endpoint	Growth inhibition	Survival	Survival
Density, number per container	12.1 ± 1.7 × 10^3 /mL	10	400
Test containers	96 well sterile microplate	80 mL glass container	20 mL glass vials (28 × 61 mm)
Exposure time	96 hr	96 hr	48 hr
Sample pre-treatment	0.45 µm filtration, ASW brine added	ASW brine added	ASW brine added
Concentrations	0, 0.2 to 81%	0, 1.56 to 84%	0, 0.2 to 75%
Replicates	10 for controls, 5 for treatments	10 for controls, 3 for treatments	10 for controls, 5 for treatments
Light	Continuous 200 µmol/m^2/sec	None	None
Temperature	18.1 ± 0.1°C	17.6 ± 0.1°C	17.6 ± 0.1°C
Dissolved oxygen (beginning/end)	8.3 mg/L	9.3/ 7.4 ± 0.4 mg/L	8.3 mg/L
pH	7.8	7.6	7.6
Aeration	None	None	None
Salinity (beginning/end)	26 PSU	21.6 / 21.7 ± 0.1PSU	33.2 PSU
Sensitivity (EC_{50} with 95%CI)	0.163 (0.141–0.194) mg Cu^{2+}/L	2.1 (1.7 – 2.5) mg Zn^{2+}/L	0.101(0.98–0.104) mg Zn^{2+}/L
Control quality for sensitivity (average EC_{50} with 2×SD)	0.162 (0.11–0.22) mg Cu^{2+}/L (*n* = 22)	1.86 (0.63 – 3.1) mg Zn^{2+}/L (*n* = 28)	0.084(0.0.25–0.143) mg Zn^{2+}/L (*n* = 33)
Test acceptability (in controls)	CV < 20%, 16-fold increase in control	<10 % mortality	> 80% in controls
Test compliance	☑	☑	☑
Note	Age of culture: 6 days	Amphipods collection date: 31/10/2019	Mussel collection date: 22/10/2019 Spawning method: thermal stress

APPENDICES

Appendix 1. Raw data for the algae growth, amphipod survival and mussel embryo-larval development assays

Table A.1.1a Algal density ($\times 10^3$ algae/mL) in the wastewater sample

Control	Sample (%) – week 1									
0	**0.20**	**0.39**	**0.78**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**81**
485.5	463.9	496.9	448.9	503.2	510.5	516.2	527.5	506.3	518.2	567.6
498.5	504.5	510.5	496.6	511.4	533.4	513.1	523.0	542.4	520.3	592.2
517.4	435.9	464.4	373.0	457.3	371.9	412.0	467.9	552.8	464.8	588.5
504.8	474.7	355.8	378.6	459.0	531.2	420.7	421.5	504.0	436.9	608.7
519.9	389.5	431.2	404.0	457.8	427.4	491.3	538.7	448.5	393.2	529.5
493.6										
441.4										
331.1										
348.5										
382.8										

Table A.1.1b Algal density ($\times 10^3$ algae/mL) in controls in the test and control plates

	n	Average	Standard deviation	Coefficient of variation
Dilution ASW - test plate	10	452.4	72.2	16 %
Dilution ASW - control plate	22	441.1	40.1	9 %
Brine ASW - control plate	17	398.6	25.8	6 %

No statistical difference ($P < 0.05$) was found between controls

Table A.1.2a Algal density ($\times 10^3$ algae/mL) in the wastewater sample

Control	Sample (%) – week 2									
0	**0.20**	**0.39**	**0.78**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**84**
499.3	496.3	494.7	493.6	522.6	518.2	539.9	533.9	639.6	634.1	710.1
504.2	509.6	543.1	517.1	540.9	556.4	595.0	624.8	628.5	639.5	698.7
500.4	534.9	538.8	521.9	550.3	502.1	517.1	579.4	823.9	695.3	772.4
513.9	544.3	550.1	518.0	523.7	569.3	595.9	568.3	623.6	691.0	761.1
474.5	550.6	538.5	561.2	561.1	574.2	603.0	641.7	782.1	886.8	755.6
499.0										
523.2										
385.5										
441.6										
479.0										

Table A.1.2b Algal density ($\times 10^3$ algae/mL) in controls in the test and control plates

	n	Average	Standard deviation	Coefficient of variation
Dilution ASW - test plate	10	482.1	41	9 %
Dilution ASW - control plate	20	461.4	23	5 %

No statistical difference ($P < 0.05$) was found between controls

Table A.1.3a Algal density (×10³ algae/mL) in the wastewater sample

Control	Sample (%) – week 3									
0	**0.20**	**0.39**	**0.78**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**80**
262.3	371.2	403.8	428.4	450.9	414.8	345.3	318.6	323.0	383.4	531.0
352.7	361.2	408.8	420.5	425.5	446.4	338	316.7	310.5	444.8	531.0
351.1	427.9	426	425.5	453.4	496.9	342.1	304.4	416.6	392.3	474.6
363.6	369.9	403.4	410.9	435.8	439.2	330.7	299.3	328.7	399.9	514.3
359.0	305.0	437.6	487.6	455.3	554.6	423.7	288.3	393.1	515.6	588.8
349.8										
379.4										
348.9										
339.4										
257.5										

Table A.1.3b Algal density (×10³ algae/mL) in controls in the test and control plates

	n	Average	Standard deviation	Coefficient of variation
Dilution ASW - test plate	10	339.2	16.1	4.4 %
Dilution ASW - control plate	20	306.2	22.5	7.3 %
Brine ASW - control plate	20	313.3	15.6	5.3 %

No statistical difference (P<0.05) was found between controls

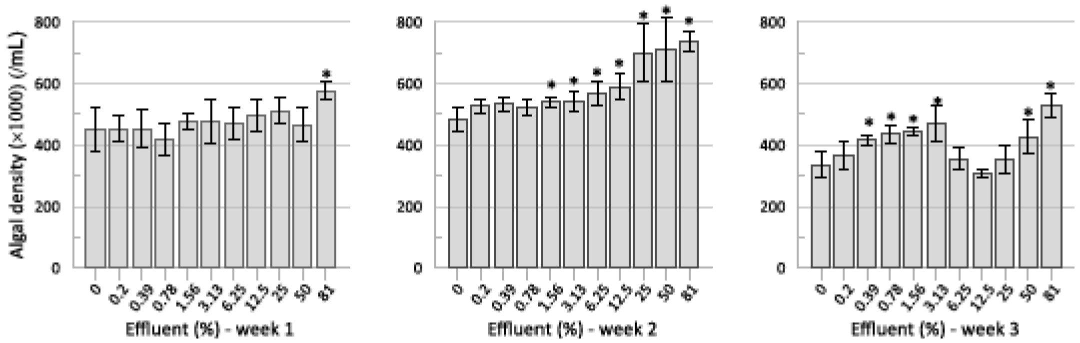

Figure A.1.1 Algal density (×10³ algae/mL) in the wastewater sample at the three sampling times (week 1, week 2 and week 3). Asterisk (*) indicates a significant difference from control (P<0.05)

Table A.1.4. Survival rate (%) of amphipods (*Paracorophium excavatum*) exposed to wastewater samples.

Control	Sample (%) – week 1							
0	**0.78**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**84**
100	100	90	100	100	100	100	100	100
100	90	100	100	100	100	90	100	89
100	90	87.5	87.5	100	100	100	90	100
90								
90								
100								
100								
90								
100								
100								

Control	Sample (%) – week 2							
0	**0.78**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**84**
80	100	100	90	100	100	90	100	89
100	100	100	100	100	100	100	100	90
90	100	89	100	100	100	86	100	100
100								
100								
90								
100								
100								
89								
100								

Control	Sample (%) – week 3							Control
0	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**84**	**Brine water**
100	70	100	100	100	100	100	90	100
100	86	100	90	100	100	100	89	100
100	86	100	89	70	89	89	100	100
100								100
56								
100								
100								
100								
100								
100								

Table A.1.5. Survival rate (in %) of blue mussel (*Mytilus galloprovincialis*) D-larvae (D-yield) exposed to wastewater sample.

	Sample (%) – week 1				
0	**0.20**	**0.39**	**0.78**	**1.56**	**3.13**
76.9	87.1	78.2	74.9	41.8	0.0
77.5	82.9	77.4	61.8	65.1	0.0
69.5	76.2	68.8	57.4	21.3	0.0
90.1	88.3	84.8	78.9	14.7	0.0
87.6	86.3	85.4	82.3	0.0	0.0
87.1					
82.5					
76.4					
94.9					
96.4					

	Sample (%) – week 2			
0	**0.20**	**0.78**	**1.56**	**3.13**
76.9	72.0	74.4	35.7	0.0
77.5	74.8	71.6	42.4	0.0
69.5	76.0	65.5	18.2	0.0
90.1	76.2	56.6	26.4	0.0
87.6	56.0	79.3	2.6	0.0
87.1				
82.5				
76.4				
94.9				
96.4				

	Sample (%) – week 3			
0	**0.20**	**0.39**	**0.78**	**1.56**
77.5	81.4	67.4	10.1	0.0
69.5	86.0	69.0	8.8	0.0
90.1	71.5	62.2	9.5	0.0
87.6	90.9	90.6	22.9	0.0
87.1	91.3	81.6	4.3	0.0
82.5				
76.4				
94.9				
96.4				
76.9				

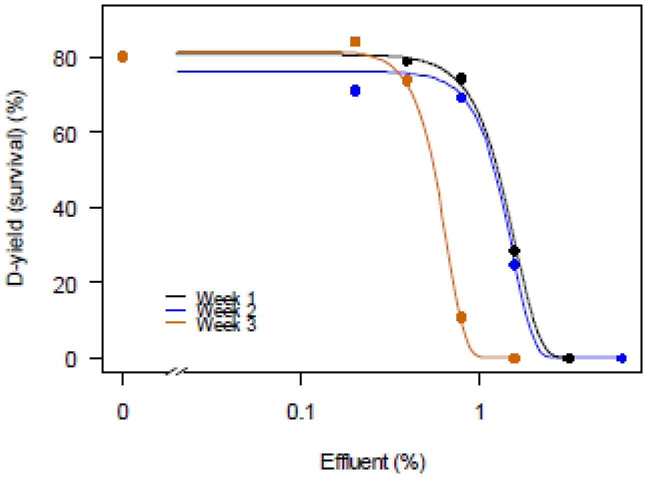

Figure A.1.2 Average of mussel larvae survival (dots) and fitted model (lines) at the three sampling times.

Appendix 2. Statistical analysis

Calculation of the EC_{50} (the concentration which produces an effect on 50% of the test organisms), with associated 95% confidence intervals (CI), were done with R (R Core Team, 2015) using bootstrap resampling with the 'drc' package with a 3-parameter Weibull model applied to determine mussels LC50s (Ritz and Streibig, 2005).

Hypothesis testing (level of statistical significance of $P < 0.05$) was based on the method described in Hall & Golding (1998). Statistical analyses were conducted with Statistica 13.3 (TIBCO Software Inc., Statistica, Tulsa, USA).

Appendix 3 – References

ASTM 1997. E1192-97 2014 - Standard guide for conducting acute toxicity tests on aqueous ambient samples and effluents with fishes, macroinvertebrates, and amphibians. West Conshohocken, PA, ASTM International. Pp. 14.

ASTM 1998. E724-98 2012 - Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. West Conshohocken, PA, ASTM International. Pp. 20.

ASTM 2004. E1218-04 2012 - Standard guide for conducting static toxicity tests with microalgae. West Conshohocken, PA, ASTM International. Pp. 14.

Hall JA, Golding LA 1998. Standard methods for whole effluent toxicity testing: development and application. Report no. MfE80205. NIWA report for the Ministry for the Environment Wellington, New Zealand. pp. 53.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ritz, C, Streibig, JC 2005. Bioassay Analysis using R. Journal of Statistical Software:12(5). Pp. 16.

Appendix 4. Acronyms and definitions

Acronym	Definition
ASTM	American Society for Testing and Materials
ASW	Artificial seawater
CI	Confidence interval (95%)
Cu	Copper (metal)
CV	Coefficient of variation
DO	Dissolved oxygen
DTA	Direct toxicity assessment
EC	Effective concentration
LOEC	Lowest Observed Effect Concentration
NEC	No Effect Concentration
NOEC	No Observed Effect Concentration
PSU	Practical salinity unit
SD	Standard deviation
TEC	Threshold Effect Concentration
Zn	Zinc (metal)

$EC_{x\text{-}t}$	**Effective Concentration** is the generic term for a concentration of substance or material that is estimated to cause some defined effect on a proportion (x%) of the test organisms after a defined period of exposure (t). This kind of endpoint allows the classification and the comparison of the toxic potency or intensity of different chemicals. More terms can be derived to describe specific effects (*e.g.* lethality, inhibition): $LC_{x\text{-}t}$ (Lethal Concentration) is the concentration of substance or material that is estimated to be lethal to a proportion (x%) of the test organisms after a defined period of exposure (t). This is an acute toxicity indicator. $IC_{x\text{-}t}$ (Inhibitory Concentration) is the concentration of substance or material that is estimated to have an inhibitory effect (*e.g.* algal growth) on a proportion (x%) of the test organisms after a defined period of exposure (t). This is a chronic toxicity indicator.
LOEC	The **Lowest Observed Effect Concentration** is the lowest concentration of a test substance or material which is observed to have a statistically significant adverse effect on the test organisms for a defined time of exposure and under the test conditions, relative to the control.
NEC	The **No Effect Concentration** is derived from the dose-response model. It is a threshold below which the response is assumed constant and equal to the control response
NOEC	The **No Observed Effect Concentration** is the highest concentration of a test substance or material which is observed not to have a statistically significant adverse effect on the test organisms for a defined time of exposure and under the test conditions, relative to the control.
Practical salinity unit	It is a unit based on the properties of sea water conductivity to measure salinity. It is equivalent to parts per thousand or (‰) or to g/kg.
Reference toxicant	Chemical used to assess the constancy of response of a given species of test organisms to that chemical. It is assumed that any change in sensitivity to the reference substance will indicate the existence of some similar change in degree of sensitivity to other chemicals/effluents whose toxicity is to be determined.
TEC	Threshold Effect Concentration is the geometric mean of the NOEC and the LOEC. It represents the lowest concentration that should not cause any effect for the related measured endpoint.

APPENDIX THREE

Emerging Organic Chemicals and their calculated Method Detection Limits

Emerging Organic Chemical	MDL (ng/L)
Alkylphosphate Flame Retardants	
TiBP	0.10
TBP	0.10
TCEP	0.10
TCPP	0.10
TDCP	0.10
TPP	0.40
TBEP	0.10
TEHP	0.10
ToCP	10
TmCP	10
TpCP	10
Phenolic Antimicrobials	
Chloroxylenol	0.05
o-phenylphenol	0.10
Chlorophene	0.10
methyl triclosan	0.05
Triclosan	0.10
Tetra-bromocresol	1.0
Parabens	
Methylparaben	0.05
Ethylparaben	10
Propylparaben	0.05
Butylparaben	0.05
Benzylparaben	0.05
Industrial Alkylphenols	
4-t-Amylphenol	0.10
4-n-Amylphenol	0.10
4-t-octylphenol	0.10
4-t-heptphenol	0.10
4-n-octylphenol	0.10
4-n-nonylphenol	0.10
Tech-NP-equivalents	5.0
Insect Repellents	
DEET	1.0
Picaradin	1.0
Benzylbenzoate	1.0

APPENDIX ONE

Emerging Organic Chemicals and their calculated Method Detection Limits- continued

Emerging Organic Chemical	MDL (ng/L)
Nitro- and Polycyclic Musk Fragrances	
Cashmeran	1.0
Celestolide	1.0
Phantolide	1.0
Musk ambrette	1.0
Traseolide	1.0
Galaxolide	2.5
Musk xylene	1.0
Tonalide	5.0
Musk moskene	1.0
Musk tibetene	1.0
Musk ketone	1.0
Acidic Pharmaceuticals	
Acetaminophen	0.20
Aspirin	20.0
Carbamazepine	0.10
Clofibric acid	0.50
Diclofenac	0.10
Ibuprofen	0.10
Ketoprofen	0.10
Meclofenamic acid	0.50
Naproxen	0.10
Salicylic acid	2.0
Phthalate esters and plasticisers	
Chloro-ethoxymethane	5.0
Dimethyl phthalate	1.0
Diethyl phthalate	5.0
4-Chlorophenyl phenyl ether	0.10
4-bromophenyl phenyl ether	0.10
Di-n-butyl phthalate	20
Benzyl butyl phthalate	1.0
Diethyl hexylphthalate	25
Di-n-octyl phthalate	5.0
Monomethyl-PAE	1.0
Monobutyl-PAE	1.0
MonoEH-PAE	1.0
Bisphenol A	1.0

APPENDIX ONE

Emerging Organic Chemicals and their expected Method Detection Limits- continued

Emerging Organic Chemical	MDL (ng/L)
Steroid Hormones	
Estrone	0.02
17α-estradiol	0.02
17β-estradiol	0.02
Estriol	0.05
Mestranol	0.02
17α-ethinylestradiol	0.02
Androstenediol	0.10
19-Nortestosterone	1.0
Androstenedione	0.10
Testosterone	0.10
19-Norethindrone	1.0
Norgestrel	1.0

Analyte key

TiBP = Tri-isobutyl-phosphate
TBP = Tributyl-phosphate
TCEP= Tris(2-chloroethyl) phosphate
TCPP = Tris (1-chloro-2-propyl) phosphate
TDCP = Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
TPP = Triphenylphosphate
TBEP = Tris-(2-butoxyethyl) phosphate
TEHP = Tris(2-ethylhexyl) phosphate
ToCP = Tri-o-cresyl phosphate
TmCP = Tri-m-cresyl phosphate
TpCP = Tri-p-cresyl phosphate