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6.1 Metals

Introduction

This chapter focuses mostly on heavy metals, but includes analysis of silicon and the metalloids boron and arsenic.

Heavy metals are of concern for augmentation of drinking water supplies with recycled water due to their toxicity and ubiquitous occurrence in secondary wastewater from both natural and anthropogenic sources. The occurrence of heavy metals in secondary wastewater varies and depends on local factors, such as type of industries, people's way of life, and waste's disposal practices. It is estimated that the discharge of metals to secondary wastewaters from industry and commercial sectors contributed larger loads compared to household sources. Stormwater is not a major contributor to the Perth wastewater treatment plant catchments.

During conventional treatment in secondary wastewater treatment plants (WWTPs), the majority of metals are concentrated in the sewage sludge with about 20% of the concentration present in the secondary wastewater (European Communities, 2001). However, the proportion of metals in the secondary wastewater could be higher for those metals with higher solubility. For example, nickel estimated concentration in secondary wastewater is between 40 to 60% of the concentration in raw wastewater (European Communities, 2001).

Some metals are essential minerals for normal human function. Metals become toxic after ingestion when they are not excreted by the body and accumulate in the tissues interfering with the enzymatic system and metabolism of the body. Symptoms associated with chronic exposure of heavy metals include fatigue, musculoskeletal pain, neurological disorders, depression, failing memory, bone damage, gastrointestinal symptoms and allergic hypersensitivity. In general, heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular, eliminative pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary systems.

A significant component of the toxicity of a number of heavy metals including cadmium, chromium, mercury and the metalloid arsenic, results from the fact that they are pro-oxidant; leading to generation of oxidative stress (Carpenter *et al.*, 2002). Pro-oxidant metals induce expression of a battery of genes whose functions appear to limit oxidation, protect cells from free-radical damage, and prevent

neoplasia. These include, among others, heme oxygenase 1 (HO-1), the ferritin L-gene, expression of NQO1, γ-glutamyltransferases, and γ-glutamylsynthetase. Regulation of these genes occurs through antioxidant response elements in the promoter regions of the genes (Carpenter *et al*. 2002). For example vanadium and chromium in air have been associated with oxidative DNA damage within particulate matter (2.5µm) (Sorensen *et al*. 2005).

Nutritionally, heavy metals are directly antagonistic to essential trace elements and compete with nutrient elements for binding sites on transport and storage proteins, metalloenzymes and receptors. The disruption of the metabolism and the balance of nutrient elements results in alterations in the metabolism of carbohydrate, protein/amino acids, lipids, neurotransmitters and hormones. Metals and metalloids for which epidemiologic studies have suggested a risk associated with their presence in potable water include: mercury, arsenic and lead.

Metals have well documented toxicity and most of them have drinking water guidelines or standards (Table 6.1.1). Cobalt, lithium, tin and vanadium are not currently regulated. However, toxicity data is available to calculate the health values and they were allocated to Tier 2 in the screening health risk assessment.

Table 6.1.1: Drinking water guidelines and standards for metals (mg/L)

		Health Guideline/Standard						**Cancer Classification**		
Symbol	Element	ADWG	WHO	U.S. EPA	Title 22	Canada	EU	IARC	Other	Source
Al	Aluminium	0.1a		0.2 d	1	0.2 b		1	-	
Sb	Antimony	0.003	0.02	0.006	0.006	0.006	0.005	2B*	-	
As	Arsenic	0.007	0.01p	0.01	0.05	0.01	0.01	1	A	IRIS
Ba	Barium	0.7	0.7	2	1	1		NE	D	IRIS
Be	Beryllium			0.004	0.004			1	B1	IRIS
B	Boron	4	0.5 p		7.3 e	5 c	1	NE		
Cd	Cadmium	0.002	0.003	0.005	0.005	0.005	0.005	1	B1	IRIS
Cr	Chromium	0.05	0.05p	0.1	0.05	0.05	0.05	3	A	IRIS
Cr VI	Chromium VI	0.05			0.11 e			1	A	IRIS
Co	Cobalt				0.011 e			2B	-	
Cu	Copper	2	2	1 d	1.3	1	2	NE	D	IRIS
Fe	Iron	0.3 a		0.3 d	0.3 a	0.3 a		1		
Pb	Lead	0.01	0.01	0.015	0.0015	0.01	0.01	2B	B2	RAIS
Li	Lithium				0.073 e			NE		
Mn	Manganese	0.5	0.4	0.002d	0.88 e	0.05 a		NE	D	IRIS
Hg	Mercury	0.001	0.006	0.002	0.002	0.001	1	3**	C**	IRIS
Mo	Molybdenum	0.05			0.18 e			NE	D	RAIS
Ni	Nickel	0.02	0.07	0.1	0.1		0.02	2B	A	IRIS
Se	Selenium	0.01	0.01	0.05	0.05		0.01	3	2***	NTP
Ag	Silver	0.1		0.1 d	0.1			NE	D	IRIS
Tl	Thallium			0.002	0.002			NE	D	RAIS
Sn	Tin				22 e			NE	D	IRIS
U	Uranium	0.02	0.015	0.03	0.11 e	0.02		NE		
V	Vanadium				0.26 e			2B	-	
Zn	Zinc	3 a		5 d	5 a	5 a		NE	D	IRIS

ADWG: Australian Drinking Water Guidelines, WHO: World Health Organisation, U.S. EPA: United States Environmental Protection Agency, Title 22: California Code of regulations, EU: European

Union, IARC: International Agency on Cancer Research, IRIS: Integrated Risk Information System, RAIS: Risk Assessment Information System, NTP: National Toxicology Program

a, aesthetic value; b, operational parameter; c, health value developed as an interim maximum acceptable concentration; p, provisional value; d, secondary drinking water regulation; e, U.S. EPA Region 9 Superfund Preliminary Remediation Goals 2009.
* as antimony trioxide
** as methyl-mercury
*2*** reasonably anticipated to be a human carcinogen (NTP)

Source: (ADWG, 2004, FPTCDW, 2008, WHO, 2006, California DHS, 2008, U.S. EPA, 2006, U.S. EPA, 2008a, U.S. EPA, 2008b, NTP, 2008, U.S. EPA, 2009, European Commission, 1998, IARC, 2008)

Methods

The CCWA metals method covers the measurement of electrical conductivity and the analysis of 28 parameters including silicon and boron in water samples. Samples for electrical conductivity and metals analysis were collected in polyethylene plastic bottles. Samples for mercury analysis were collected in amber glass bottles containing nitric/dichromate preservative.

Electrical conductivity was determined by measurement with an electrical conductivity probe and meter. Samples for metals analysis (except mercury) were diluted, filtered and acidified with nitric acid prior to analysis. Analysis was performed by either inductively coupled plasma atomic emission spectroscopy (ICPAES) or inductively coupled plasma mass spectrometry (ICPMS). Mercury analysis was performed by cold vapour generation atomic absorption (CVAAS). The list of analytes covered by this method, the limits of reporting (LOR) and estimated uncertainties are listed in Table 6.1.2.

NMI used a NATA accredited method where hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red-violet colour was developed and measured by spectrophotometric techniques. For determination of total chromium, NMI used the NATA acredited method for total and dissolved metals by ICPAES.

Quality assurance/ Quality control

The following QA/QC steps were followed for metal analyses:

- Blank with every batch
- 10% duplicates with every batch. Acceptability criteria for duplicates is 20% for samples above 10 times the LOR.
- Recovery from Certified Reference Material, CRM.
- Acceptability criteria for CRM is 80-120% of theoretical value.

Table 6.1.2: Analytes, instrumentation, Limits of Reporting (LOR) and estimation of uncertainty for metals and electrical conductivity in water

Analyte	Instrument	LOR (mg/L)	Standard Relative Uncertainty (%)	Expanded Relative Uncertainty (%) *
Silver	ICPMS	0.0001	2.2	4.4
Aluminium	ICPAES	0.005	5	10
Arsenic	ICPMS	0.001	5.4	11
Boron	ICPAES	0.02	13	26
Barium	ICPAES	0.002	5	10
Beryllium	ICPMS	0.0001	7.9	16
Cadmium	ICPMS	0.0001	2.1	4.2
Cobalt	ICPMS	0.0001	2.1	4.2
Chromium	ICPMS	0.0005	1.6	3.2
Copper	ICPMS	0.0001	2	4
Electrical Conductivity	Electrical conductivity meter	0.2	10	20
Iron	ICPAES	0.005	10	20
Mercury	CVAAS	0.0001	3.5	7
Lithium	ICPMS	0.0001	5.2	10
Magnesium	ICPAES	0.1	5.5	11
Manganese	ICPAES	0.001	5	10
Molybdenum	ICPMS	0.001	1.8	3.6
Nickel	ICPMS	0.001	2	4
Lead	ICPMS	0.0001	4.7	10
Antimony	ICPMS	0.0001	4.3	8.6
Scandium	ICPMS	0.0005	Not Determined	Not Determined
Selenium	ICPMS	0.001	3.5	7
Silicon	ICPAES	0.05	5	10
Tin	ICPMS	0.0001	4.5	9
Strontium	ICPMS	0.0001	1.5	3
Thallium	ICPMS	0.0001	5.3	10.6
Uranium	ICPMS	0.0001	5	10
Vanadium	ICPAES	0.005	5	10
Zinc	ICPMS	0.005	3	6

Results

After excluding trip, field and replicate samples, a total of 2,027 measurements were analysed. The distribution of measurements by event and location is presented in Table 6.1.3. For Events 1 and 2 the majority of samples were grab while for Events 3 to 6 the majority were composite samples. Twenty percent of the samples were analysed in Event 1, in this event samples from a storage dam post-RO water treatment at KWRP were also analysed.

Table 6.1.3: Measurement of metals by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	SWW	Water Reclamation Plant							
				Grab	Comp				Before MF		Post-MF water		Post-RO water		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006	325	25	350	0	0	125	0	25	0	100	0	100	350
2	May/June	6	2007	253	234	487	52	129	0	0	153	0	153	0	0	306
3	September	6	2007	0	348	348	0	0	87	87	0	0	87	87	0	348
4	January	6	2008	56	280	336	56	0	56	84	0	0	56	84	0	280
5	April	5	2008	0	264	264	0	44	44	44	0	22	44	66	0	220
6	June	5	2008	22	220	242	0	0	44	66	0	22	44	66	0	242
Total		32		656	1,371	2027	108	173	356	281	178	44	484	303	100	1746

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

All metals except arsenic, beryllium, cadmium, chromium VI, mercury, selenium, silver, thallium, uranium and vanadium were detected in at least one sample from the secondary wastewater. The percentage of detected metals and the median concentration in secondary wastewater are presented in Figure 6.1.1. Antimony, barium, boron, copper, iron, lead, lithium, magnesium, manganese, strontium, tin and zinc were detected in all samples taken form the secondary wastewater while molybdenum, cobalt and scandium were detected in 19%, 71% and 73% of the samples respectively. Median concentrations ranged from 0.0003 mg/L for antimony, cobalt and tin to 9.35 mg/L for magnesium.

The median concentration of detected metals by WWTP is presented in Figure 6.1.2. There were statistically significant differences in the concentrations of aluminium, antimony, barium, boron, chromium total, cobalt, iron, lead, magnesium, manganese, molybdenum, nickel, silicon and strontium among WWTPs (K-Wallis X^2 $p<0.05$). Higher metals concentrations were expected for KWRP located in an industrial area. However, no specific trend was observed as shown in Figure 6.1.3. Aluminum (0.05

mg/L), nickel (0.004 mg/L), chromium (0.0018 mg/L) and manganese (0.029 mg/L) were higher at KWRP while barium (0.12 mg/L), lead (0.0011 mg/L) and zinc (0,056 mg/L) were higher at Beenyup WWTP. Boron, cobalt and iron were detected in higher concentrations at Subiaco WWTP. However, few samples were taken form this location compared with Beenyup WWTP and influent to KWRP and the results may be affected by sample size.

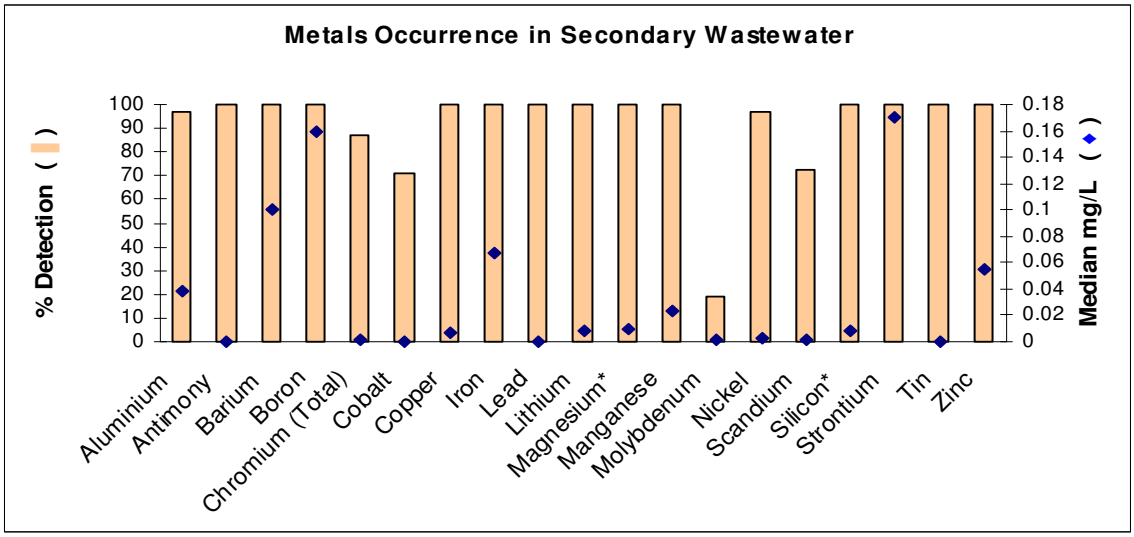

Figure 6.1.1: Metals and metalloids with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (mg/L, diamond). Magnesium* and silicon* in g/L.

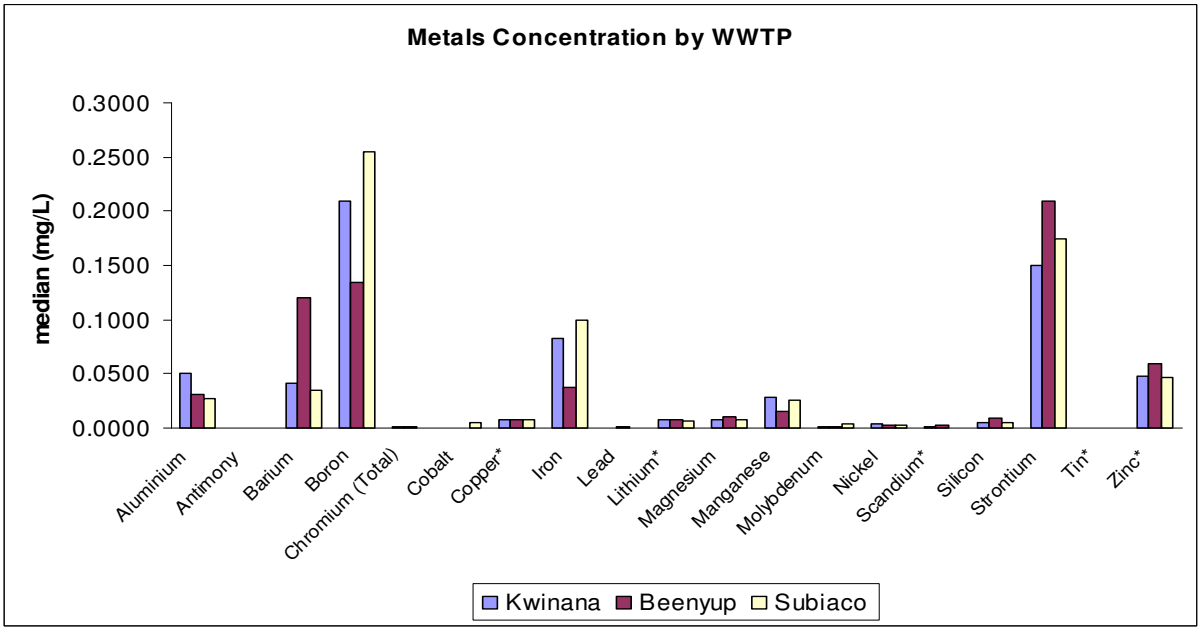

Figure 6.1.2: Median metals and metalloids concentration by WWTP in mg/L. Magnesium and silicon in g/L.

*Metals with statistically significant differences in concentrations among plants.

Of 19 detected metals, 12 have slightly higher concentrations in summer compared with winter. However, the differences were not statistically significant and the higher concentrations during the summer may be due to lower wastewater flow.

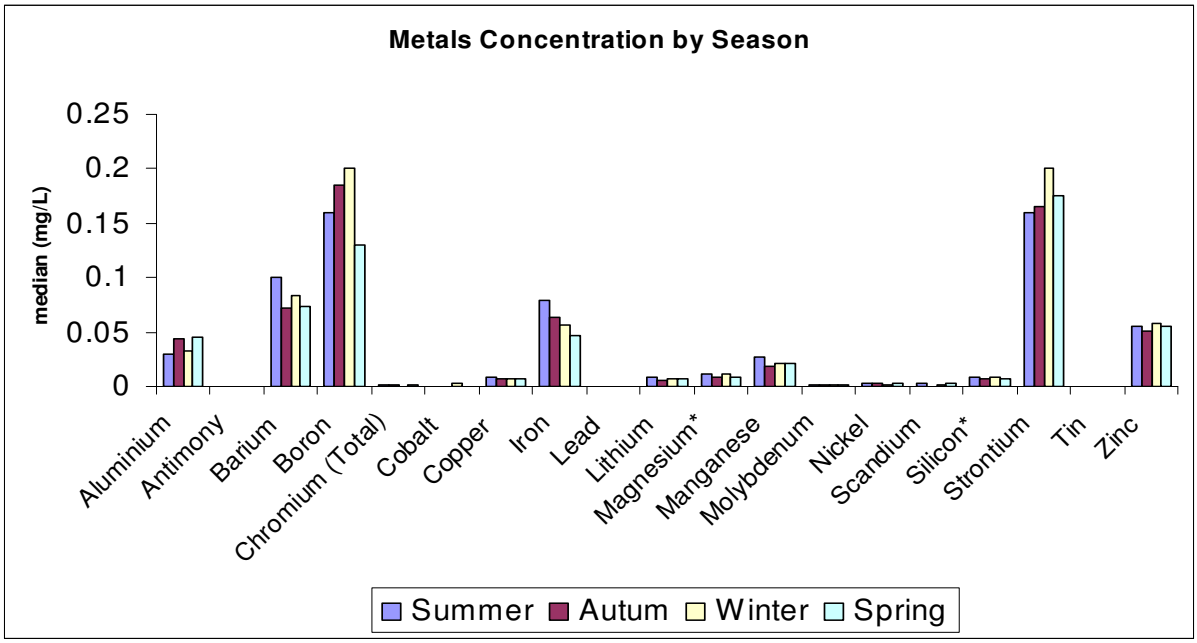

Figure 6.1.3: Median metals and metalloids concentration across all plants by season. Magnesium* and silicon* in g/L

RO Product water characterisation

Twelve of the 19 detected metals in the secondary wastewater were also detected in the post-RO water. Barium, cobalt, magnesium, manganese, molybdenum, scandium and tin were not detected in any of the samples taken post-RO. Boron was detected in 89% of the samples followed by lithium (68%), silicon (61%) and strontium (50%) - Figure 6.1.4. Metals median concentration in the post-RO water ranged from 0.0001 mg/L to 0.01 mg/L for all detected metals except for boron (0.075 mg/L) and silicon (0.12 mg/L).

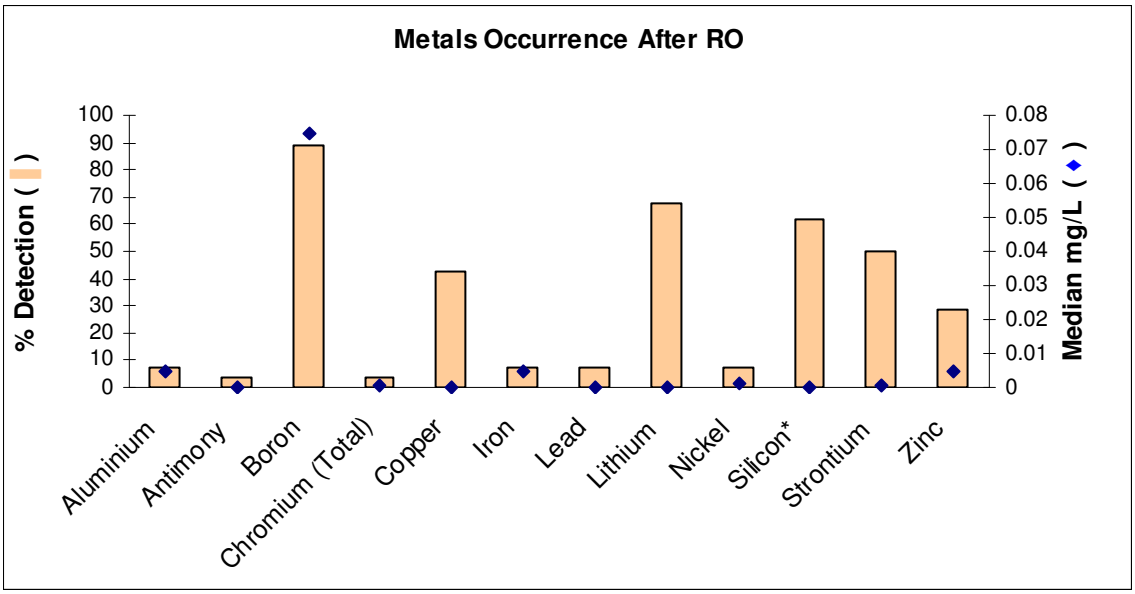

Figure 6.1.4: Metals and metalloids with percentage detections in post-RO water (vertical column) and corresponding median concentrations (mg/L, diamond). Silicon* in g/L

Groundwater characterisation

Of 28 tested metals, 13 (46%) were detected in groundwater and the corresponding median concentration and percentages of detection are presented in Figure 6.1.5. Barium, magnesium, manganese and scandium were detected in Groundwater but not in the post-RO water. Metal concentrations in groundwater were low but higher than in the product water for all metals except boron. Boron median concentration in groundwater was 0.04 mg/L and in the product water was 0.075 mg/L

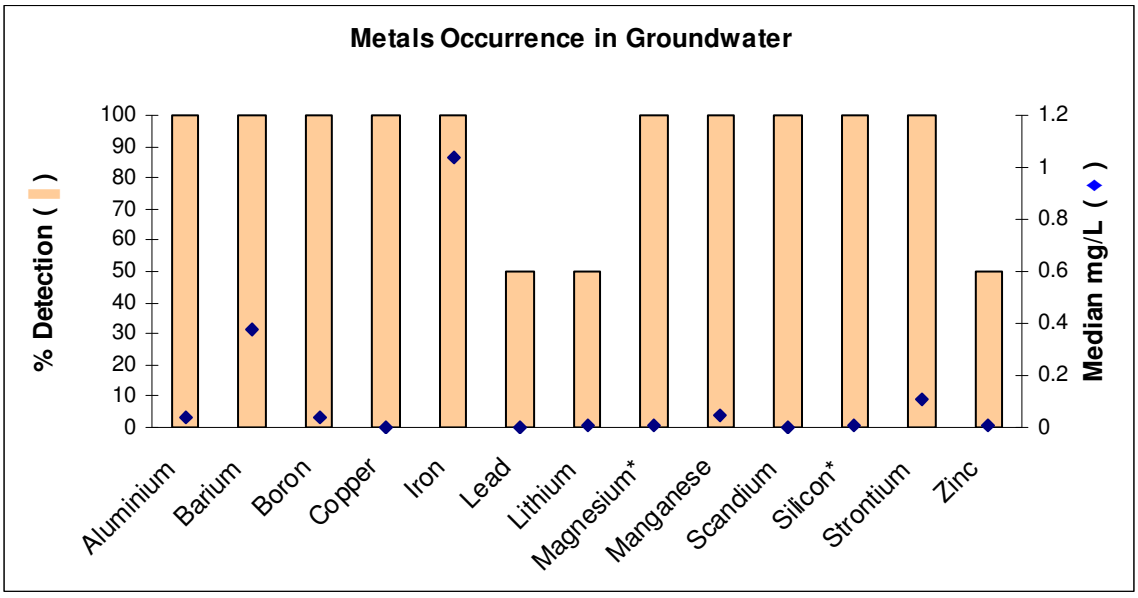

Figure 6.1.5: Metals and metalloids with percentage detections in groundwater (vertical column) and corresponding median concentrations (mg/L, diamond). Magnesium* and silicon* in g/L

Screening health risk assessment

Risk quotients (RQs) for undetected metals were calculated using the LOD as worst case scenario and results are presented in Table 6.1.4. Chromium VI was the only undetected metal with a RQ equal to 1 as the LOR is equal to the health value.

Table 6.1.4: Metals and metalloids without detections in any of the samples and corresponding RQs

Parameter	LOR	n	Tier	Health value	Source	RQ
Arsenic	0.001	32	1	0.007	ADWG, 2004	0.14
Beryllium	0.0001	21	1	0.004	USEPA, 2009	0.025
Cadmium	0.0001	21	1	0.002	ADWG, 2004	0.05
Mercury	0.0001	21	1	0.001	ADWG, 2004	0.1
Selenium	0.001	32	1	0.01	ADWG, 2004	0.1
Silver	0.0001	21	1	0.1	ADWG, 2004	0.001
Thallium	0.0001	21	1	0.002	USEPA, 2009	0.05
Uranium	0.0001	21	1	0.02	ADWG. 20004	0.005
Vanadium	0.005	32	2	0.0147	Cal OEHHA, 2000	0.14
Chromium VI	0.05	10	1	0.05	ADWG, 2004	**1**

n: number of secondary wastewater samples, Mercury LOR lowered in event 2 from 0.0005 to 0.0001 mg/L, silver LOR lowered from 0.005 to 0.0001 mg/L in Event 3. Cal OEHHA Office of Environmental Health Hazard Assessment.

RQs before advanced treatment (secondary wastewater) were calculated using the maximum concentration RQ(max) detected in secondary wastewater - as worst case scenario - and using the median concentration RQ(median). In secondary wastewater, RQ(max) was above 1 for aluminium (RQ(max)=1.1) and iron (RQ(max)=2.4) (Table 6.1.5). The health guidelines for aluminium and iron used to calculate these RQs were based on aesthetic guideline levels (ADWG) and not toxicity values so these RQs greater than 1 are not of health concern. All RQ(median) were below health significance for all detected metals including aluminium (RQ(median)=0.39) and iron (RQ(median)=0.23). All detected metals in the post-RO water have RQ(max) below 1 and RQ(median) 1 to 3 orders of magnitude below 1. This indicates very low health significance at the observed metals concentrations in the post-RO water.

Table 6.1.5: Metals and metalloids detected before MF and post-RO water corresponding RQs

						Before MF		Post-RO water		
parameter	**LOD**	**Tier**	**Health value**	**Source**	**n**	**RQ(median)**	**RQ(max)**	**n**	**RQ(median)**	**RQ(max)**
Aluminium	0.005	1	0.1	ADWG, 2004	31	0.39	**1.1***	28	0.05	0.12
Antimony	0.0001	1	0.003	ADWG, 2004	31	0.1	0.27	28	0.03	0.07
Barium	0.002	1	0.7	ADWG, 2004	31	0.14	0.2		ND	ND
Boron	0.02	1	4	ADWG, 2004	31	0.04	0.10	28	0.019	0.04
Chromium	0.0005	1	0.05	ADWG, 2004	31	0.016	0.08	28	0.01	0.028
Cobalt	0.0001	2	0.011	Cal PRG, 2009	31	0.03	0.45	28	ND	ND
Copper	0.0001	1	2	ADWG, 2004	31	0.004	0.01	28	0.017	0.07
Iron	0.005	1	0.3	ADWG, 2004	31	0.23	**2.4***	28	0.01	0.05
Lead	0.0001	1	0.01	ADWG, 2004	31	0.06	0.25	28	0.0003	0.007
Lithium	0.0001	2	0.073	Cal PRG, 2009	31	0.1	0.14		ND	ND
Magnesium	0.1	2	18	EC, 2001	22	0.52	0.56		ND	ND
Manganese	0.001	1	0.5	ADWG, 2004	31	0.05	0.08		ND	ND
Molybdenum	0.001	1	0.05	ADWG, 2004	31	0.02	0.1		ND	ND
Nickel	0.001	1	0.02	ADWG, 2004	31	0.15	0.3	28	0.05	0.1
Scandium	0.0005	2	0.7	TTC	22	0.003	0.006		ND	ND
Silicon	0.05	2	350	LTD	22	0.03	0.03	21	0.0003	0.0007
Strontium	0.0001	2	4	IRIS, 1996	31	0.04	0.06	28	0.0001	0.0005
Tin	0.0001	2	14	WHO, 2004	31	0.00002	0.0002		ND	ND
Zinc	0.005	2	3	ADWG, 2004	31	0.02	0.04	28	0.0017	0.004

Cal PRG: California Preliminary Remediation Goals; EC European Commission Tolerable Upper Intake Level of Magnesium (2001): LTD: lowest therapeutic dose; TTC: Threshold of Toxicological Concern; IRIS Integrated Risk Information System

**RQ(max) above 1 for aluminium and iron were calculated using aesthetic water guidelines (ADWG) and therefore do not pose a health concern*

Figure 6.1.6 illustrates the percentage of metals' detections in post-RO water and the corresponding risk quotients. Boron was the more commonly detected metalloid (almost 90% of samples) followed by lithium and silicon. RQ median was higher for aluminium and nickel with RQ(median)=0.05 respectively (Figure 6.1.6). However, for all metals the RQ(median) was on order of magnitude below 1 indicating negligible health risk.

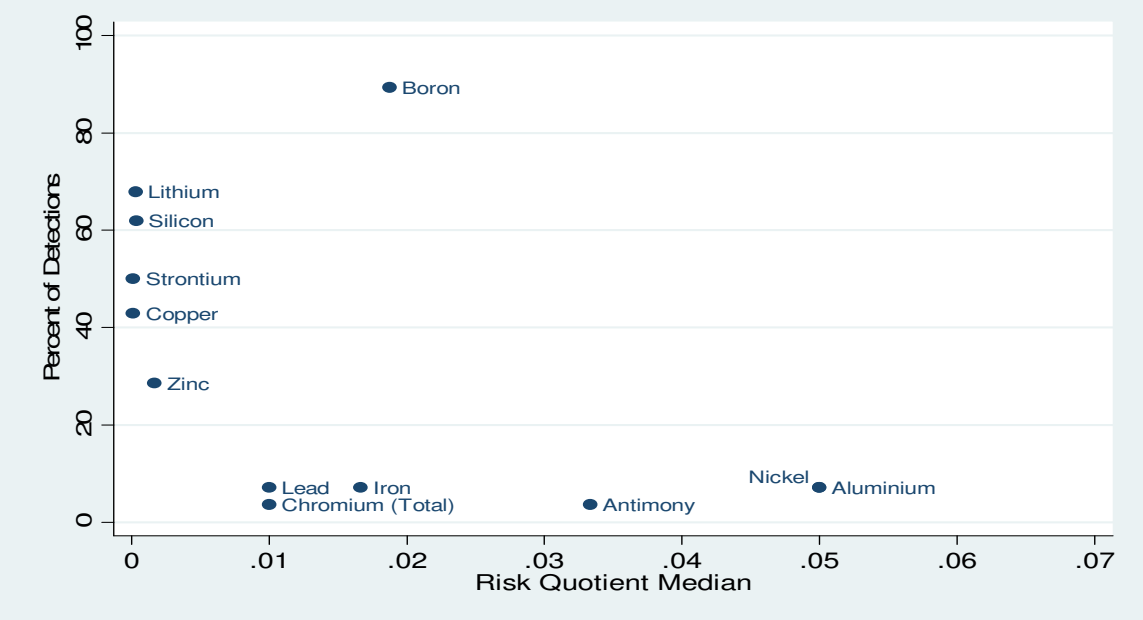

Figure 6.1.6: Detected metals and metalloids in the post-RO water and corresponding median concentrations.

Treatment performance

RO treatment was very effective to remove metals and metalloids. Removal was high (more than 90%) for aluminium, barium, copper, iron, lithium, magnesium, manganese, silicon, strontium and zinc (Figure 6.1.7). For all other metals the percentage of removal ranged from 75% to 90% except boron (59%). The number of paired samples (concentration before and after MF/RO) for the analysis of the efficiency was 25 for the majority of metals. Treatment performance variability tends to decrease when more paired samples were available except for boron for which the percentages of removal ranged from 30% to 90%. No outliers were observed as shown in Figure 6.1.7 which indicates a consistent removal of metals during the advanced treatment and the absence of sample contamination during the analysis.

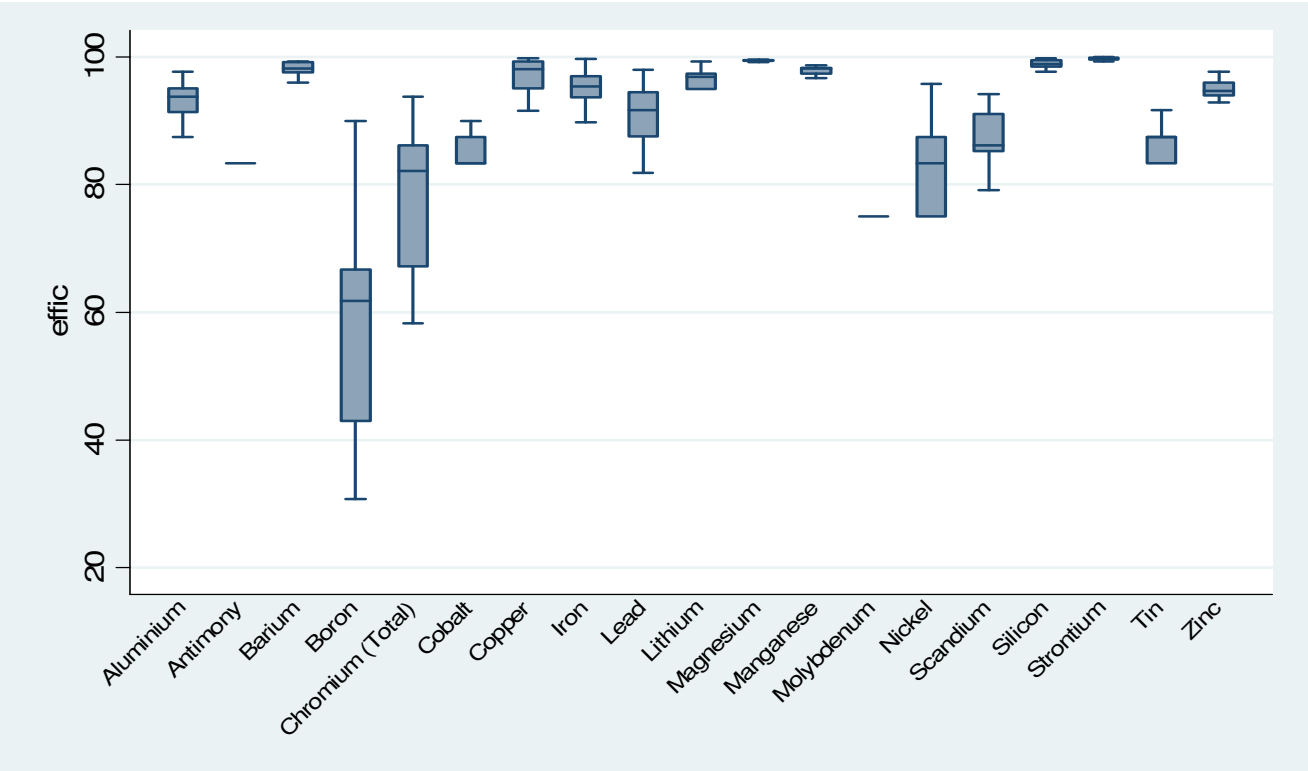

Figure 6.1.7: MF/RO removal efficiency of detected metals and metalloids in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Grab and composite samples analysis

There is a good agreement for metals between grab and composite samples as illustrated in Figure 6.1.8 in which a random scatter of points between the upper and lower confidence intervals distribution is observed. The plot also indicates that grab and composite results are measuring the same in an unbiased way. Differences tend to be higher for higher measured concentrations and only one outlier corresponding to a grab sample was observed. There were no differences between grab and composite samples for 33% of the pairs while composite samples were higher than grab samples for 34% of the pairs.

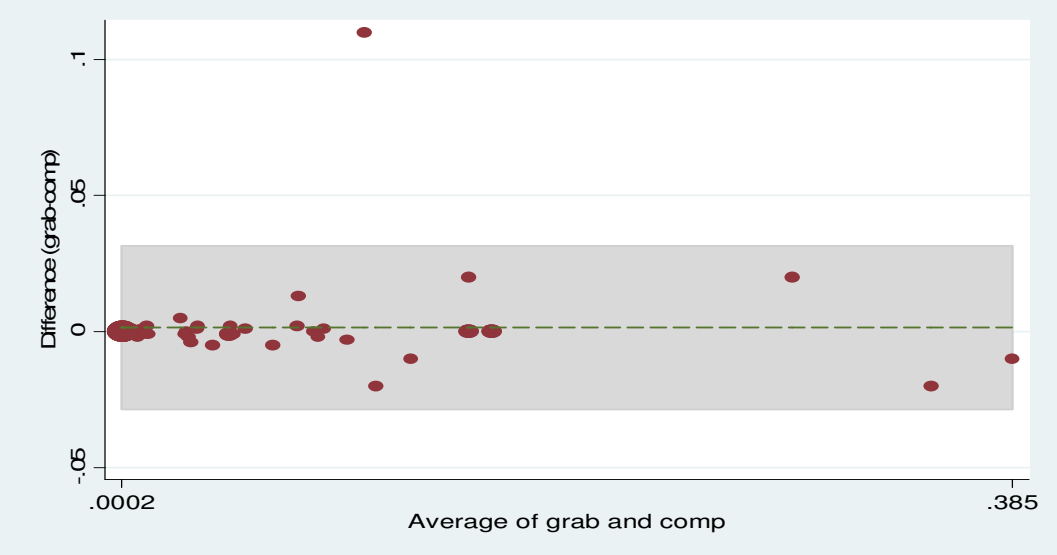

Figure 6.1.8: Bland-Altman plot comparing grab and composite (comp) samples for metals and metalloids.

Correlation of metals and electrical conductivity

Correlation coefficient of the sum of detected metals and conductivity in the post-RO water was low (r=0.1). The correlation between boron concentration and conductivity in the post-RO water was better (r=0.39).

Discussion

Some metals have well documented toxicity and most of them have drinking water guidelines or standards. Moreover, all tested metals have standard analytical methods and the laboratory limit of reporting was better than (below) 10% of the regulatory value. Boron was the only metalloid with a reported standard relative uncertainty of less than 10%.

The results indicate that the majority of the tested metals are always present in the secondary wastewater of the three WWTPs. However, arsenic, cobalt, cadmium, mercury and beryllium were not detected in any of the samples analysed. Concentrations of heavy metals (As, Cd, Cr, Hg, Ni, Pb, Cu, Zn) in secondary wastewater if detected, were at the lower end of the range measured in a range of European secondary wastewaters (Busetti *et al.*, 2005).

There were statistically significant differences for most of the detected metals by location, however, no clear pattern was observed. Aluminum, boron and iron were found in higher concentrations at KWRP while barium, strontium and zinc were found in higher concentrations at Beenyup WWTP. For most of the detected metals, concentrations tend to be slightly higher in summer compared to winter. However, no

statistically significant seasonal differences were observed. Only aluminum and iron have occasionally been detected at levels above the health standards (RQ(max) above 1) in the secondary wastewater. Nevertheless, the advanced treatment was able to constantly remove metals concentrations below health significance and all RQ(median) in the post-RO water were below 1.

Metal concentrations in groundwater were below health values but median concentrations were higher for all detected metals compared to concentrations detected in the product water except boron. Boron median concentration in groundwater was 0.04 mg/L and in the product water was 0.075 mg/L

The results presented are consistent with other IPR projects using MF/RO in which high metals rejection is achieved during the advanced treatment (Daugherty *et al.*, 2005, OCWD, 2006, WBMWD, 2006, Singapore Government, 2002)

Boron was detected in all samples from the secondary wastewater (median IQR 0.13 mg/L to 0.24 mg/L) and it has the lowest rejection during the treatment (average rejection of metals=90%, average rejection boron=59%). Boron mean ratio of measured secondary wastewater concentration/LOD was on average 9.4 times above 1. In addition boron was the only metal with median concentration in product water higher than in groundwater (0.075 mg/L and 0.04 mg/L) respectively. The correlation between boron and conductivity was greater than for other metals in the post-RO water, however the correlation was still low. These particular characteristics point to the selection of boron as the best chemical indicator for operational monitoring. However, more data is required for boron to better characterise the treatment variability given that the treatment efficiency to remove this metal ranged from 30% to 90%.

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6.2 Pesticides

Introduction

Pesticides comprises all substances or compounds used for use for agricultural, pastoral, horticultural, domestic, or industrial purposes for controlling, destroying or preventing the growth and development of, any fungus, virus, insect, mite, mollusk, nematode, plant or animals Pesticides include all preparations and mixtures containing any proportion of any one or more of them.

Pesticides had been detected in all water matrices including secondary wastewater and consequently use of recycled water for augmentation of drinking water supplies may increase the potential for human exposure. Pesticides had been detected at varying frequencies and concentrations in water and the improvement of analytical tools allows the detection of parent compounds and their degradation products at low levels (µg/L or ng/L) (Singh *et al.*, 2004, Duranceau, 2001, Nicholson, 2006, CRCWQT, 2007, Environment ACT, 1999, Davis, 2000, Kolpin *et al.*, 2005, AGWR, 2008).

Pesticides are biologically active substances and the major toxicological concern as contaminants of recycled water for drinking is the potential exposure to low concentrations over long periods of time. Of particular concern are pesticides that may be carcinogenic, those with endocrine activity and those that have a tendency to bioaccumulate. Ecotoxicological studies have revealed potential long-term environmental health risks associated with pesticides at low concentrations (orders of ppb and ppt), particularly those with endocrine-disrupting activity (Kookana *et al.*, 2007). However, no impact on human health has been convincingly demonstrated so far and research in this area remains as a high priority (Damstra *et al.*, 2002).

Pesticides collected during the Project and reported by Chemistry Centre of Western Australia (CCWA) are analysed in this section. The main objectives were to characterise the occurrence of pesticides in secondary wastewater and post-RO; to determine the treatment efficiency to remove these contaminants and; to determine the potential health impacts by calculating RQs after the advanced treatment.

Methods

CCWA methods for pesticides cover the analysis of 117 pesticides at low levels in secondary wastewater and post-RO water.

Pesticide samples were collected in amber glass bottles. Samples were filtered and diluted or extracted by liquid/liquid extraction. Analysis was performed by either Gas Chromatography-Mass Spectrometry (GC/MS), direct injection Liquid Chromatography-Mass Spectrometry (LC/MS) or online Solid Phase Extraction Liquid Chromatography-Mass Spectrometry (SPE LC/MS).

Target analytes covered by these methods and their limits of reporting (LOR) are listed in Table 6.2.1

Table 6.2.1: Pesticide methods and limits of reporting

Analyte	Usage	CAS Number	LOR (mg/L)	Method	Standard Relative Uncertainty (%)	Expanded Relative Uncertainty (%)
2,4,5-T (2,4,5-trichlorophenoxyacetic acid)						
2,4-D (2,4-dichlorophenoxyacetic acid)						
3-Hydroxycarbofuran	Carbamate degradate	16655-82-6	0.4	5Carbamates	29	57
Acephate	Organophoshorous-Insecticide	30560-19-1	1.0	LCOPmix2	11	22
a-Chlordane	Organochlorine-Insecticide	5103-71-9	0.01	OCPYR	14	29
a-Endosulfan	Organochlorine-Insecticide	959-98-8	0.01	OCPYR	14	29
Alachlor	Herbicide	15972-60-8	0.02	wwm2	16	32
Aldrin	Organochlorine-Insecticide	309-00-2	0.01	OCPYR	19	37
Ametryn	Triazine Herbicide	834-12-8	0.10	Method 5Atrazin	28	56
Amitrole	Herbicide	61-82-5	1	Amitrole	19	42
Atrazine	Triazine Herbicide	1912-24-9	0.1	Lcmix		
Atrazine	Triazine Herbicide	1912-24-9	0.10	Method 5Atrazin	26	51
b-Chlordane	Organochlorine-Insecticide	5103-74-2	0.01	OCPYR	9	18
b-Endosulfan	Organochlorine-Insecticide	115-29-7	0.01	OCPYR	14	27
Bentazone	Herbicide	25057-89-0	5	6A	18	32
Bifenthrin	Synthetic Pyrethroid-Insecticide	82657-04-3	0.05	OCPYR	11	23
Bioresmethrin	Synthetic Pyrethroid-Insecticide	28434-01-7	0.02	wwm2	30	60
Bromacil	Herbicide	314-40-9	5	6A	17	33
Butylate	Herbicide	2008-41-5	0.5	NPmix	23	46
Captan	Fungicide	133-06-2	0.50	FV2	13	25
Carbaryl	Carbamate	63-25-2	0.4	5Carbamates	22	43
Carbendazim	Carbamate	10605-21-7	0.4	5Carbamates	48	96
Carbofuran	Carbamate	1563-66-2	0.4	5Carbamates	21	42
Carbophenothion	Insecticide & Acarcide	786-19-6	0.02	wwm2	11	22
Carboxin	Fungicide	5234-68-4	0.02	wwm2	17	35
Chlorfenviphos	Insecticide	470-90-6	0.1	wwm3	13	26
Chlorothalonil	Fungicide	1897-45-6	0.02	wwm2	12	25
Chlorpyrifos	Organophoshorous-Insecticide	2921-88-2	0.05	OP	34	68
Chlorpyrifos ethyl	Insecticide	39475-55-3	0.06	GCmix1	14	28
Chlorpyrifos-methyl	Insecticide & Acarcide	5598-13-0	0.02	wwm2	15	30
Cycloate	Herbicide	1134-23-2	0.5	NPmix	13	25
Cyfluthrin	Synthetic Pyrethroid-Insecticide	68359-37-5	0.05	OCPYR	10	20

Cypermethrin	Synthetic Pyrethroid-Insecticide	52315-07-8	0.05	OCPYR	10	20
Deltamethrin	Synthetic Pyrethroid-Insecticide	52918-63-5	0.05	OCPYR	9	18
Demeton-S-methyl	Organophoshorous-Insecticide	919-86-8	0.05	OP	18	35
Diazinon	Organophoshorous-Insecticide	333-41-5	0.05	OP	9	19
Dichlobenil	Herbicide	1194-65-6	0.1	wwm3	27	55
Diclofop-methyl	Herbicide	51338-27-3	0.1	wwm3	18	37
Dicofol	Organochlorine-Insecticide	115-32-2	0.01	OCPYR	11	22
Dieldrin	Organochlorine-Insecticide	60-57-1	0.01	OCPYR	10	20
Dimethoate	Organophoshorous-Insecticide	60-51-5	0.05	OP	31	31
Diphenamid	Herbicide	957-51-7	0.1	NPmix	11	22
Disulfoton	Insecticide & Acarcide	6/07/2497	0.02	wwm2	12	25
Diuron	Urea Herbicide	330-54-1	10	6B	35	70
Endosulfan Sulphate	Organochlorine-Insecticide	1031-07-8	0.01	OCPYR	9	17
Endrin	Organochlorine-Insecticide	72-20-8	0.01	OCPYR	9	18
EPTC (S-ethyl dipropylthiocarbamate)						
Ethoprophos	Nematicide & Insecticide	13194-48-4	0.02	wwm2	12	24
Fenamiphos	Organophoshorous-Insecticide	22224-92-6	0.05	OP	15	30
Fenarimol	Fungicide	60168-88-9	1	NPmix	16	32
Fenchlorphos	Insecticide	299-84-3	0.1	wwm3	11	23
Fenitrothion	Organophoshorous-Insecticide	122-14-5	0.05	OP	13	26
Fensulfothion	Nematicide	115-90-2	0.1	wwm3	13	25
Fenthion	Organophoshorous-Insecticide	55-38-9	0.05	OP	32	64
Fenvalerate	Synthetic Pyrethroid-Insecticide	51630-58-1	0.05	OCPYR	10	20
Fipronil	Insecticide	120068-37-3	0.50	FV1	15	31
Fluometuron	Urea Herbicide	2164-17-2	10	6B	21	43
Fluvalinate	Synthetic Pyrethroid-Insecticide	69409-94-5	0.05	OCPYR	10	21
Formothion	Insecticide	2540-82-1	0.1	wwm3	13	25
Heptachlor	Organochlorine-Insecticide	76-44-8	0.01	OCPYR	13	26
Heptachlor Epoxide	Organochlorine-Insecticide	1024-57-3	0.01	OCPYR	14	27
Hexachlorobenzene	Organochlorine-Insecticide	118-74-1	0.01	OCPYR	19	37
Hexazinone	Herbicide	51235-04-2	0.10	Method 5Atrazin	17	34
Iprodione	Fungicide	36734-19-7	0.50	FV1	17	33
Lindane	Organochlorine-Insecticide	58-89-9	0.01	OCPYR	11	23
Linuron	Urea Herbicide	330-55-2	10	6B	107	215
Maldison/Malathion	Organophoshorous-Insecticide	121-75-5	0.05	OP	11	22
MCPA (4-chloro-2methylphenoxy) acetic acid)						

MCPB (4-chloro-2-methylphenoxy)butanoic acid)						
Methamidophos	Organophoshorous-Insecticide	10265-92-6	1.0	LCOPmix2	10	20
Methidathion	Organophoshorous-Insecticide	950-37-8	0.05	OP	19	38
Methomyl	Carbamate	16752-77-5	0.4	5Carbamates	38	76
Methoxychlor	Organochlorine-Insecticide	72-43-5	0.01	OCPYR	20	40
Metolachlor	Herbicide	51218-45-2	0.05	NPmix	9	18
Metribuzin	Herbicide	21087-64-9	0.10	Method 5Atrazin	22	43
Metsulfuron-methyl	Urea Herbicide	79510-48-8	10	6B	9	18
Mevinphos	Insecticide & Acarcide	26718-65-0	0.05	OP	20	40
Molinate	Herbicide	2212-67-1	0.5	NPmix	15	30
Napropamide	Herbicide	15299-99-7	0.1	NPmix	10	20
Norflurazon	Herbicide	27314-13-2	0.1	NPmix	20	40
p,p-DDD	Organochlorine-Insecticide		0.01	OCPYR	11	23
p,p-DDE	Organochlorine-Insecticide		0.01	OCPYR	11	22
p,p-DDT	Organochlorine-Insecticide	50-29-3	0.01	OCPYR	16	31
Parathion-Ethyl	Organophoshorous-Insecticide	56-38-2	0.05	OP	10	21
Pebulate	Herbicide	1114-71-2	0.5	NPmix	16	33
Pendimethalin	Herbicide	40487-42-1	0.1	wwm3	12	23
Permethrin	Synthetic Pyrethroid-Insecticide	52645-53-1	0.05	OCPYR	12	24
Phorate	Organophoshorous-Insecticide	298-02-2	0.05	OP	23	46
Piperonyl butoxide	Insecticide	51-03-6	0.1	wwm3	13	26
Pirimicarb	Carbamate	23103-98-2	0.4	5Carbamates	29	57
Pirimiphos-ethyl	Insecticide	23505-41-1	0.02	wwm2	16	31
Procymidone	Organochlorine-Insecticide	32809-16-8	0.01	OCPYR	10	20
Profenofos	Insecticide & Acarcide	41198-08-7	0.02	wwm2	15	31
Promecarb	Carbamate	2631-37-0	5	6A	17	35
Propanil	Herbicide	709-98-8	1	5B	43	85
Propargite	Acarcide	2312-35-8	0.1	wwm3	12	24
Propazine	Herbicide	139-40-2	0.5	NPmix	11	23
Propiconazole	Fungicide	60207-90-1	0.1	5B	39	78
Propiconazole	Fungicide	60207-90-1	0.1	Lcmix		
Propoxur	Insecticide	114-26-1	0.4	5Carbamates	22	44
Propyzamide	Herbicide	23950-58-5	1	5B	15	30
Pyrazophos	Organophoshorous-Insecticide	13457-18-6	0.05	OP	11	23
Quintozene	Fungicide	82-68-8	0.1	wwm3	19	37
Simazine	Triazine Herbicide	122-34-9	0.1	Lcmix		
Simazine	Triazine Herbicide	122-34-9	0.10	Method 5Atrazin	22	42
Sulprofos	Insecticide	35400-43-2	0.1	wwm3	15	29
Tebuthiuron	Herbicide	34014-18-1	10	6B	13	26
Temephos	Insecticide	3383-96-8	0.1	wwm3	9	18
Terbacil	Herbicide	5902-51-2	5	6A	16	32

Terbufos	Nematicide & Insecticide	13071-79-9	0.02	wwm2	13	25
Terbutryn	Herbicide	886-50-0	0.10	Method 5Atrazin	31	61
Tetrachlorvinphos	Insecticide & Acarcide	22248-79-9	0.02	wwm2	18	35
Thiometon	Insecticide & Acarcide	640-15-3	0.02	wwm2	13	27
Triadimefon	Fungicide	43121-43-3	0.1	NPmix	13	27
Triclopyr	Acidic Herbicide	55335-06-3	1	AcidicHerb	21	42
Trifluralin	Herbicide	1582-09-8	0.02	wwm2	11	22
Vernolate	Herbicide	1929-77-7	0.5	NPmix	18	36

Results

Analytical methods were available for 117 of the 162 pesticides selected for analysis. The list of pesticides or degradate compounds for which no analytical methods were developed during the project are presented in Appendix 2.

A total of 4,982 measurements were analysed after excluding QA/QC samples. Grab samples were more common in Events 0, 1 and 2 while 24-hours composite samples were more common for Events 3 to 6. Pesticides were tested in secondary wastewater and post-RO water at KWRP in all events and at BPP during Events 3 to 6 (Table 6.2.2). Samples were also taken from the secondary wastewater at Subiaco WWTP (June 2007 & April 2008) and from groundwater (June 2007 and January 2008). The majority of samples were collected during week days (91.8%). Due to negative results in Events 1 to 3 the number of pesticides analysed in Events 4 to 6 were decreased.

Table 6.2.2: Measurement of pesticides by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	SWW	Water Reclamation Plant							
				Grab	Comp				Before MF		Post-MF water		Post-RO water		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006	1,300	199	1,499	0	0	599	0	100	0	400	0	400	1,499
2	May/June	6	2007	744	930	1,674	186	372	0	0	558	0	558	0	0	1,116
3	September	6	2007	0	828	828	0	0	207	207	0	0	207	207	0	828
4	January	6	2008	58	290	348	58	0	58	87	0	0	58	87	0	290
5	April	5	2008	0	336	336	0	56	56	56	0	28	56	84	0	280
6	June	5	2008	0	297	297	0	0	81	54	0	27	54	81	0	297
Total		32		2,102	2,880	4,982	244	428	1,429	404	658	55	1,333	459	400	4310

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup.

Tested pesticides were classified by main use following the WHO classification (WHO, 2005). Almost half of the pesticides correspond to insecticides (48.8%),

followed by herbicides (38.4%) but few fungicides (9%) and other types of pesticides (acaricides and synergistic pesticides) were analysed. The most frequently analysed pesticides were organophosphorus compounds (31.4%) followed by organochlorine compounds (11%) and phenoxy compounds (6.8%)

Secondary wastewater characterisation

Of 117 pesticides analysed, 10 (7% of the total) were detected in the secondary wastewater. The number of pesticides detected per sample ranged from 1 to 7. Trifluralin was detected in 29 of 32 secondary wastewater samples (91%) followed by metolachlor (72%) and propiconazole (46%). The percentage of detections and median concentrations are presented in Figure 6.2.1. These findings are consistent with historical chemical usage data for the urban area, which use predominantly herbicides for weed control.

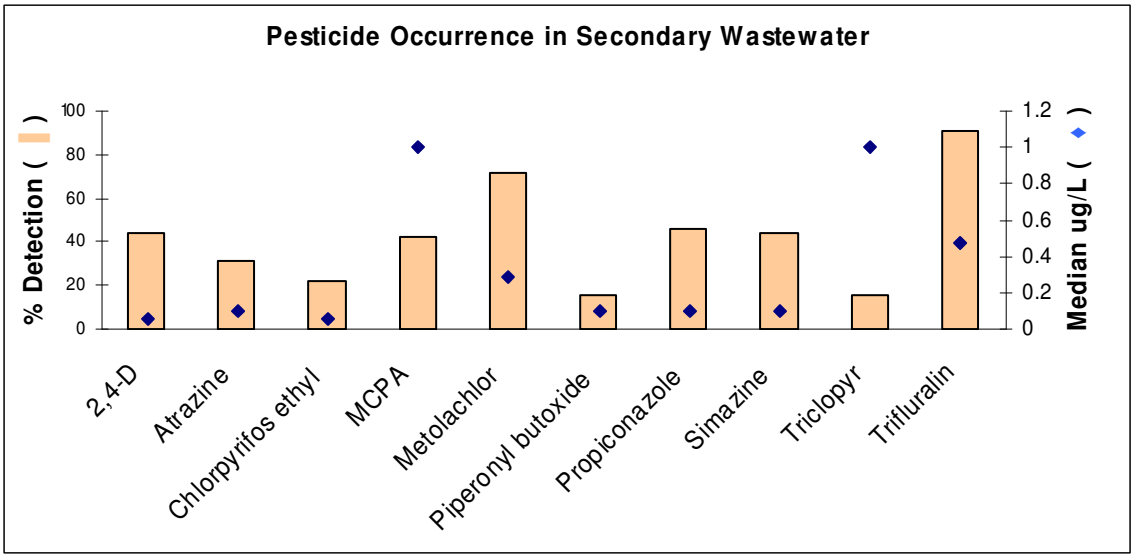

Figure 6.2.1: Pesticides with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (µg/L, diamond).

Seven herbicides, one insecticide (chlorpyrifos ethyl), one fungicide (propiconazole) and one synergistic compound (piperonyl butoxide) were the pesticides detected in the secondary wastewater. Median concentration ranged from 0.05 µg/L for 2,4-D to 1 µg/L for triclopyr.

All detected pesticides except propiconazole and trifluralin have significant differences in the median concentrations among plants (Figure 6.2.2). Geographical variability in the frequency and concentration of pesticides was observed between influent to KWRP and Beenyup WWTP secondary wastewaters. At KWRP, the number of detected pesticides per sample ranged from 4 to 7 while at BPP ranged from 1 to 6. A total of 2,177 samples were taken in KWRP and BPP plant influents for pesticides. Of 1,532 measurements at KWRP there were 109 (7.1%) positive

samples; meanwhile, out of 645 measurements at BPP there were 42 positive samples (6.5%) The differences in the proportion of detections between plants was not statistically significant (X^2 p<0.61). Pesticide concentrations were significantly higher at KWRP compared to BPP (overall K-Wallis X^2 p=0.0005). All pesticides were detected at KWRP and Beenyup except triclopyr detected only at Beenyup.

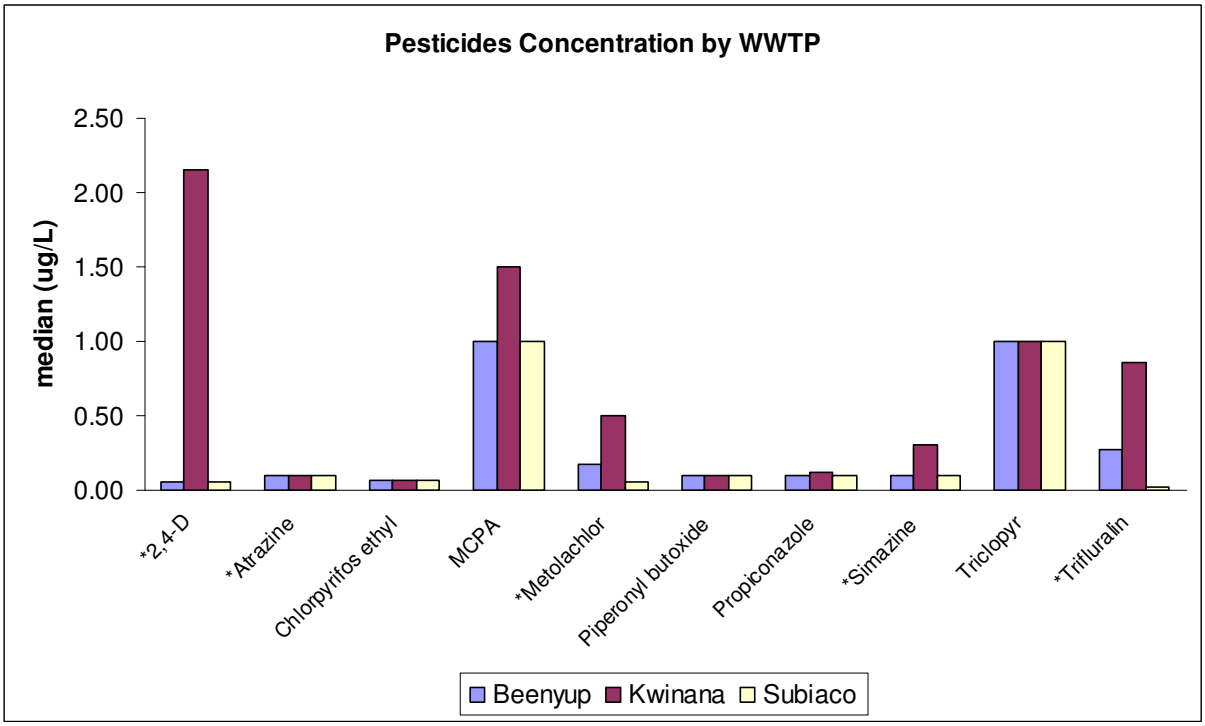

Figure 6.2.2: Median pesticides concentration by WWTP in µg/L

** Pesticides with statistically significant differences in concentrations among plants.*

Pesticides were detected in all seasons and no significant differences in pesticides concentrations were observed between summer and winter sampling events (Figure 6.2.3). Median concentrations were slightly higher in summer for 4-chloro-2-methylphenoxy acetic acid (MCPA) and simazine while median concentrations were higher in autumn for trifluralin and metolachlor.

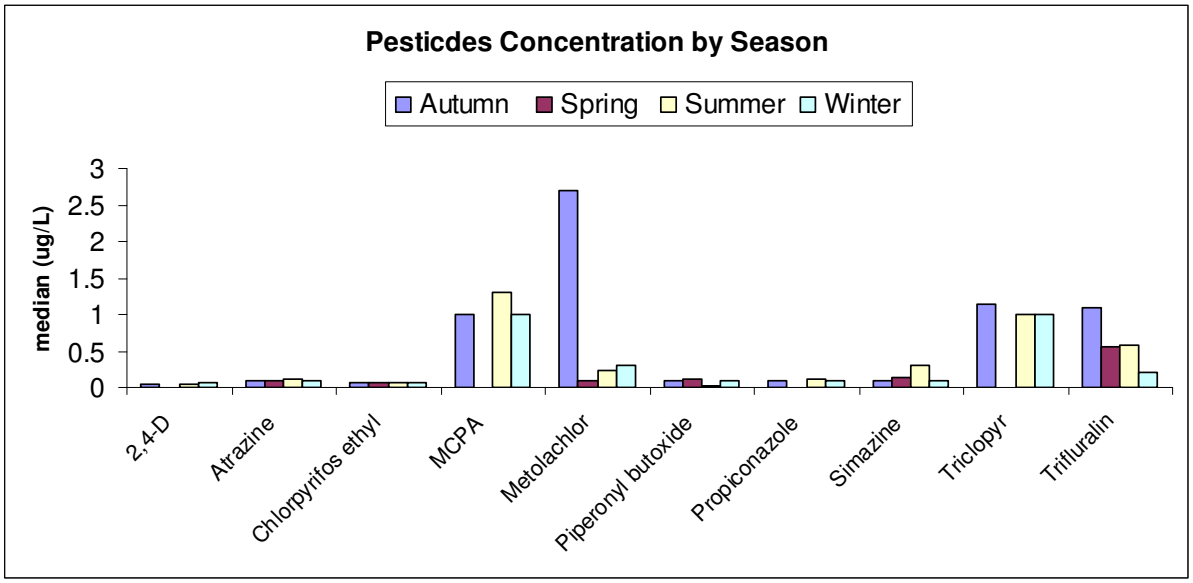

Figure 6.2.3: Median pesticides concentration by season in µg/L

RO Product water characterisation

MF/RO treatment was able to consistently remove the detected pesticides in secondary wastewater to levels below LOR. Only metolachlor was detected in one sample post-RO water (0.08 µg/L, LOR=0.05 µg/L). The replicate sample taken was reported below LOR.

Groundwater characterisation

None of the tested pesticides was detected in the groundwater that is a raw drinking water source.

Screening health risk assessment

For the majority of pesticides, it is believed that there is a threshold dose below which no adverse health effects will occur (i.e. they are not carcinogenic). Drinking water guidelines are calculated based on acceptable daily intakes (ADIs) values mainly derived from animal studies. The ADWG have two types of values for pesticides one is a guideline based on the limit of determination which is based on the precautionary principle that pesticides should not be detected in water at all. The other value is a health-based standard, based on ADIs (ADWG, 2004). This screening health risk assessment used the health values.

For the detected pesticides in secondary wastewater, the RQ(median) was equal or above 1 for atrazine, propiconazole and trifluralin (Table 6.2.3). However, the RQ(median) post-RO water, calculated using the health values, of these three

pesticides were all two orders of magnitude below 1. (RQ=0.0025, RQ=0.001 and RQ=0.0095 for atrazine, propiconazole and trifluralin respectively).

Metolachlor was the only pesticide detected in product water in one occasion. However, the detected concentration (0.08 µg/L) was below the guideline value of 2 µg/L (RQ=0.04) and the health value of 300 µg/L (RQ=0.0003). RQ(max) were calculated in the post-RO for those pesticides detected before MF but undetected in the post-RO water by assuming a concentration equal to the LOR as worst-case scenario. Atrazine and propiconazole have RQ(max) equal to 1 using the guideline value. However, none of the pesticides have a RQ above 1 in the post-RO water when health values were used to calculate the risk quotients. The results indicate very low health significance of pesticides after the advanced treatment (Table 6.2.3).

Pesticides below LOR in all samples are listed in Table 6.2.4. A minimum of 20 negative samples were considered sufficient to estimate that the pesticide was unlikely to be present in further samples analysed. Risk quotients (RQ) were estimated by assuming the concentration in the product water was equal to the LOR as a worst-case scenario. For some pesticides listed in Table 6.2.4, the calculated RQ is equal to 1 using the ADWG guideline value. However, when the health value is used instead all pesticides have RQ values one order of magnitude below 1 except molinate (RQ=0.1) and thiophanate-methyl (RQ=1).

Table 6.2.3: Pesticides detected before MF and post-RO water and corresponding RQs

							Before MF		Post-RO water	
Parameter	**n**	**LOR**	**Tier**	**Guideline value (µg/L)**	**Health value (µg/L)**	**Source**	**RQ(max)**	**RQ(median)**	**RQ Guideline Value**	**RQ Health Value**
2,4-D	16	0.05	1	0.1	30	ADWG, 2004	180	0.5	0.5	0.002
Atrazine	32	0.1	1	0.1	40	ADWG, 2004	3.6	1	1	0.0025
Chlorpyrifos ethyl	32	0.06	2	10	10	ADWG, 2004	0.035	0.006	0.006	0.006
MCPA	26	1	2	2	35	WHO, 2004	4.75	0.5	0.5	0.029
Metolachlor	32	0.05	1	2	300	ADWG, 2004	5	0.145	0.025	0.0002
Piperonyl butoxide	32	0.1	2	100	100	ADWG, 2004	0.0062	0.001	0.001	0.001
Propiconazole	26	0.1	1	0.1	100	ADWG, 2004	4.4	1	1	0.001
Simazine	32	0.1	1	0.5	20	ADWG, 2004	7.2	0.2	0.2	0.005
Triclopyr	26	1	2	10	10	ADWG, 2004	0.51	0.1	0.1	0.1
Trifluralin	32	0.02	1	0.1	50	ADWG, 2004	24	4.75	0.2	0.0004

LOR, limit of reporting; RQ(max), risk quotient calculated using maximum pesticide concentration; RQ(median), risk quotient calculated using median pesticide concentration

Table 6.2.4: Pesticides without detections in any of the samples and corresponding RQs

Pesticide	LOR	No samples	Tier	Health value	Source	RQ	ADWG HV	RQ(HV)
2,4,5-T	0.05	41	1	0.05	ADWG, 2004	1	100	0.0005
2,4-DB acid	1	25	1	90	WHO, 2004	0.01		
Acephate	0.1	18	2	10	ADWG, 2004	0.01		
Alachlor	0.02	24	1	20	WHO, 2004	0.001		
Aldicarb	0.1	6	1	1	ADWG, 2004	0.1		
Aldrin	0.01	19	1	0.01	ADWG, 2004	1	0.3	0.03
Ametryn	0.1	43	1	5	ADWG, 2004	0.02		
Azinphos-methyl	0.06	19	1	2	ADWG, 2004	0.03		
Amitrole	1	18	1	1	ADWG, 2004	1	10	0.1
Bentazon	5	31	2	30	ADWG, 2004	0.17		
Bioresmethrin	0.02	24	2	100	ADWG, 2004	0.0002		
Bromacil	5	31	1	10	ADWG, 2004	0.5		
Bromophos-ethyl	0.06	43	2	10	ADWG, 2004	0.006		
Captan	0.5	59	2	350	TGA, 2008	0.001		
Carbaryl (Sevin)	0.4	43	1	5	ADWG, 2004	0.08		
Carbendazim	0.4	31	2	100	ADWG, 2004	0.004		
Carbofuran	0.4	43	1	5	ADWG, 2004	0.08		
Carbophenothion	0.02	43	2	0.5	ADWG, 2004	0.04		
Carboxin	0.02	43	1	2	ADWG, 2004	0.01		
Chlordane	0.01	19	1	0.01	ADWG, 2004	1	1	0.01
Chlorfenvinphos	0.1	43	2	5	ADWG, 2004	0.02		
Chlorothalonil	0.02	24	1	0.1	ADWG, 2004	0.2		
Chlorpyrifos-methyl	0.02	24	2	35	TGA, 2008	0.001		
Chlorsulfuron	10	31	2	100	ADWG, 2004	0.1		
Clopyralid	1	31	1	1000	ADWG, 2004	0.001		
Cyfluthrin	0.5	35	2	70	TGA, 2008	0.004		
DDT (total isomers)	0.03	19	1	0.06	ADWG, 2004	0.5		
Demeton-S-methyl	0.06	43	2	1.05	TGA, 2008	0.06		
Diazinon	0.06	43	1	1	ADWG, 2004	0.06		
Dicamba	1	31	2	100	ADWG, 2004	0.01		
Dichlobenil	0.1	43	2	10	ADWG, 2004	0.01		
Dichlorvos	0.06	43	1	1	ADWG, 2004	0.06		
Diclofop-methyl	0.1	43	2	5	ADWG, 2004	0.02		
Dicofol	0.01	13	2	3	ADWG, 2004	0.003		
Dieldrin	0.01	19	1	0.01	ADWG, 2004	1	0.3	0.03
Dimethoate	0.06	43	2	50	ADWG, 2004	0.0012		
Diphenamid	0.1	43	1	2	ADWG, 2004	0.05		
Disulfoton	0.02	43	1	1	ADWG, 2004	0.02		
Diuron	10	31	2	30	ADWG, 2004	0.33		
Endosulfan I (alpha)	0.01	19	1	0.05	ADWG, 2004	0.2		
Endosulfan II (beta)	0.01	19	1	0.05	ADWG, 2004	0.2		

Endosulfan sulfate	0.01	19	1	0.05	ADWG, 2004	0.2		
Endothall	1	6	1	10	ADWG, 2004	0.1		
Endrin	0.01	19	1	0.6	WHO, 2004	0.02		
EPTC	0.5	43	1	1	ADWG, 2004	0.5		
Ethion	0.06	43	2	3	ADWG, 2004	0.02		
Ethoprophos	0.02	43	2	1	ADWG, 2004	0.02		
Fenamiphos	0.06	43	2	0.3	ADWG, 2004	0.2		
Fenarimol	1	43	1	1	ADWG, 2004	1	30	0.03
Fenchlorphos	0.1	43	2	30	ADWG, 2004	0.003		
Fenitrothion	0.06	43	2	10	ADWG, 2004	0.006		
Fenoprop (Silvex)	1	31	2	10	ADWG, 2004	0.1		
Fensulfothion	0.1	43	1	10	ADWG, 2004	0.01		
Fenthion	0.06	43	2	7	TGA, 2008	0.009		
Fenvalerate	0.1	6	2	50	ADWG, 2004	0.002		
Fipronil	0.5	36	2	0.7	TGA, 2008	0.7		
Flamprop-methyl	1	25	2	3	ADWG, 2004	0.33		
Fluometuron	10	31	2	50	ADWG, 2004	0.2		
Formothion	0.1	43	2	50	ADWG, 2004	0.002		
Heptachlor	0.02	19	1	0.05	ADWG, 2004	0.4		
Hexazinone	0.1	43	1	2	ADWG, 2004	0.05		
Iprodione	0.5	47	2	140	TGA, 2008	0.004		
Lindane	0.01	19	1	0.05	ADWG, 2004	0.2		
Linuron	10	31	2	35	TGA, 2008	0.29		
Malathion	0.06	43	2	50	ADWG, 2004	0.0012		
MCPB	1	60	2	35	TGA, 2008	0.03		
Mecoprop (MCPP)	1	25	2	10	WHO, 2004	0.1		
Methabenzthiazuron	10	31	2	14	TGA, 2008	0.71		
Methamidophos	1	18	2	1.05	TGA, 2008	0.95		
Methidathion	0.06	43	2	30	ADWG, 2004	0.002		
Methiocarb	0.2	6	1	5	ADWG, 2004	0.04		
Methomyl	0.4	37	1	5	ADWG, 2004	0.08		
Methoxychlor	0.01	18	1	0.2	ADWG, 2004	0.05		
Metribuzin	0.1	43	1	1	ADWG, 2004	0.1		
Metsulfuron-methyl	10	31	2	30	ADWG, 2004	0.33		
Mevinphos	0.06	43	1	5	ADWG, 2004	0.012		
Molinate	0.5	43	1	0.5	ADWG, 2004	1	5	0.1
Napropamide	0.1	43	1	1	ADWG, 2004	0.1		
Norflurazon	0.1	43	1	2	ADWG, 2004	0.05		
Oryzalin	8	6	2	300	ADWG, 2004	0.03		
p,p-DDE	0.01	6	2	0.1	IRIS, 1988	0.1		
Parathion-ethyl	0.06	43	2	10	ADWG, 2004	0.006		
Parathion-methyl	0.06	43	1	0.3	ADWG, 2004	0.2		
Pebulate	0.5	43	1	0.5	ADWG, 2004	1	30	0.02

Pendimethalin	0.1	43	2	300	ADWG, 2004	0.000		
Permethrin	0.1	6	1	1	ADWG, 2004	0.1		
Phorate	0.06	43	2	1.75	TGA, 2008	0.03		
Picloram	1	31	2	300	ADWG, 2004	0.003		
Pirimicarb	0.4	43	2	5	ADWG, 2004	0.08		
Pirimiphos-ethyl	0.02	43	2	0.5	ADWG, 2004	0.04		
Pirimiphos-methyl	0.06	43	2	50	ADWG, 2004	0.0012		
Profenofos	0.02	43	2	0.3	ADWG, 2004	0.07		
Promecarb	5	31	2	30	ADWG, 2004	0.17		
Propachlor	1	6	1	1	ADWG, 2004	1	50	0.02
Propanil	1	55	1	0.1	ADWG, 2004	10		
Propargite	0.1	43	2	50	ADWG, 2004	0.002		
Propazine	0.5	43	2	50	ADWG, 2004	0.01		
Propoxur (Baygon)	0.4	43	2	70	TGA, 2008	0.006		
Propyzamide	1	55	1	2	ADWG, 2004	0.5		
Pyrazophos	0.06	43	2	30	ADWG, 2004	0.002		
Quintozene	0.1	43	2	30	ADWG, 2004	0.003		
Sulprofos	0.1	43	2	10	ADWG, 2004	0.01		
Tau- Fluvalinate*	0.5	35	2	17.5	TGA, 2008	0.01		
Tebuthiuron	10	31	2	245	TGA, 2008	0.04		
Temephos	0.1	43	1	300	ADWG, 2004	0.0003		
Terbacil	5	31	1	10	ADWG, 2004	0.5		
Terbufos	0.02	43	1	0.5	ADWG, 2004	0.04		
Terbutryn	0.1	43	1	1	ADWG, 2004	0.1		
Tetrachlorvinphos	0.02	30	1	2	ADWG, 2004	0.01		
Thiophanate-methyl	5	31	2		ADWG, 2004	5	5	1
Thiometon	0.02	31	2	3	ADWG, 2004	0.007		
Triadimefon	0.1	43	1	100	ADWG, 2004	0.001		
Trichlorfon	1	6	2	5	ADWG, 2004	0.2		
Vernolate	0.5	43	1	0.5	ADWG, 2004	1	30	0.02

* as Fluvalinate

Source (ADWG, 2004, WHO, 2006, Therapeutic Goods Administration, 2008, U.S. EPA, 2008)

Treatment performance

Treatment efficiency was calculated for analytes detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOR after RO, the efficiency was calculated assuming a concentration equal to half the LOR as a worst-case scenario. The average removal of pesticides was high for trifluralin (96%) and metolachlor (94%). For these pesticides the removal variability was also lower as indicated in Figure 6.2.4. Average removal for other pesticides ranged from 62% for atrazine to 87% for

2,4-D. Removal was more variable for simazine (from 50% to 98%) and 2,4-D (from 58% to 99%). It is important to point out that efficiency removal could be even higher. None of the pesticides except metolachlor were detected in the product water therefore half the LOR was used to calculate a conservative treatment removal efficiency. When using the single post-RO detection for metolachlor, the calculated removal efficiency was 99.6%.

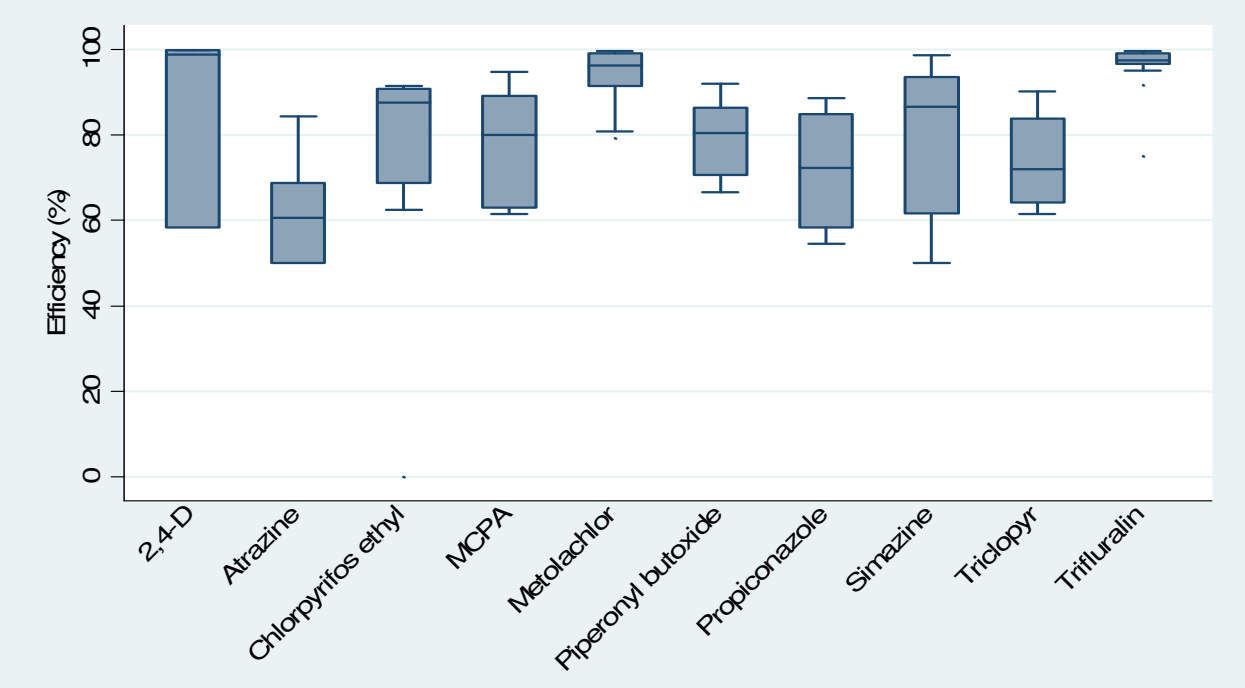

Figure 6.2.4: MF/RO removal efficiency of detected pesticides in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Grab and composite samples

There was a good agreement between grab and composite samples as illustrated in Figure 6.2.5. Pesticide concentrations were higher in grab samples compared with composite samples given that most of the points are located in the upper confidence interval. The plot also indicates that differences tend to be higher for pesticides detected in higher concentrations measured concentrations and two outliers were observed.

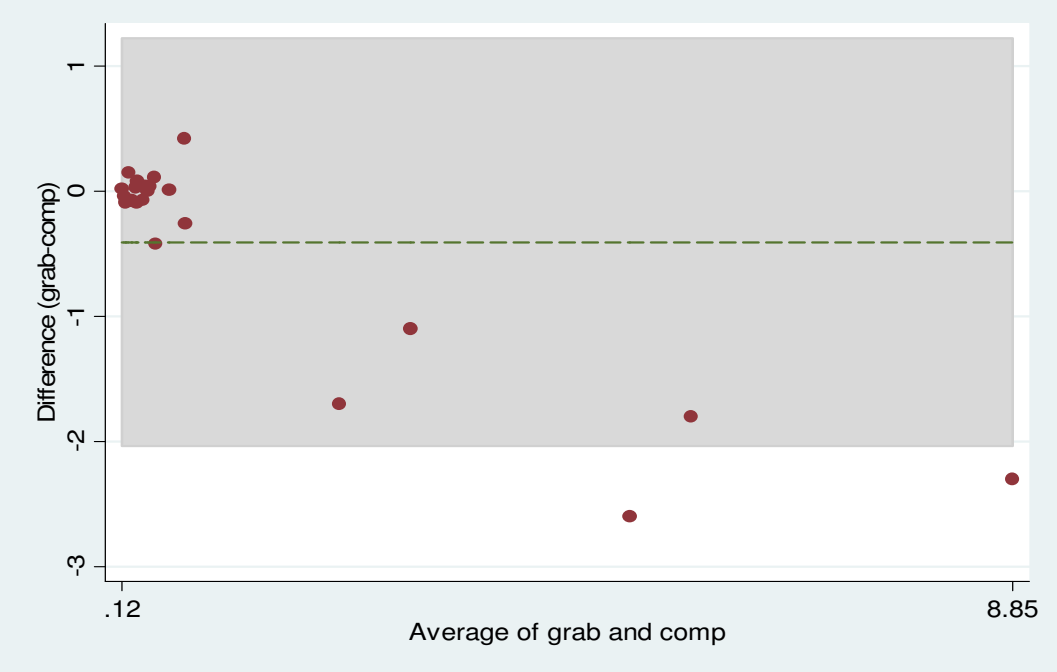

Figure 6.2.5: Bland-Altman plot assessing agreement between grab and composite (comp) samples for detected pesticides (section 3.8).

Discussion

A comprehensive characterisation of pesticides in secondary wastewater and a screening health risk assessment were conducted to evaluate potential health impacts of recycled water for indirect potable reuse. Ten pesticides (7% of the total tested) were detected in secondary wastewater. The majority of pesticides correspond to herbicides commonly used in the urban environment.

Metolachlor was the only detected pesticide in post-RO water in one grab sample (0.08 µg/L). However, the composite sample taken in the same date and location was below the LOR (0.05 µg/L). Data from projects conducting indirect potable reuse indicates that pesticides are below LOR after RO treatment. For example, none of the 24 pesticides tested at Orange County Water District or the 36 pesticides tested at West Basin Water Recycling Facility have been detected in the post-RO water (OCWD, 2006, WBMWD, 2006). Atrazine, simazine and diuron were detected in the secondary wastewater of the Intermunicipal Water Company of the Veurne Region - Torrele (Wulpen) in Belgium- in May, 2003 but RO removed these pesticides by around 98% (AQUAREC, 2006). In the NeWater project, of 50 pesticides tested three were occasionally detected in the plant influent 1,2-dibromo-3-chloropropane, chlordane and toxaphene). The frequency distribution was 4.3%, 16.7% and 18.2% respectively. The maximum concentrations for chlordane (0.02 µg/L) and toxaphene (0.1 µg/L) were within the USEPA and WHO guidelines. Only one of 23 samples of

1,2-dibromo-3-chloropropane was detected above health standards in the secondary wastewater but all samples were below LOR in the product water (Singapore Government, 2002).

Metolachlor, 2,4-D, trifluralin and propiconazole were detected during the sampling event conducted before the project started and were also detected during the project. Thiophanate-methyl was also detected in the preliminary sampling event in one sample at Beenyup WWTP (12 µg/L, LOR=8 µg/L, RQ=1.6). This pesticide was tested during sampling Events 1, 2 and 6 but none of the samples was above the LOR=5 µg/L. Further analysis is recommended for thiophanate-methyl given that it was detected in the past and the calculated RQ in the product water using the health value from the ADWG is equal to 1.

Reverse osmosis was able to reduce the concentration of all pesticides below LOR and RQs calculated the health value from the ADWG were all below 1 in the post-RO water. The calculated treatment removal efficiency average ranged from 62.4% for atrazine to 96.7% for trifluralin, but these values are likely to under-represent the potential removal efficiency as there were no detections after RO. This under-estimate of actual removal is corroborated by the high removal seen for the single metolachlor detection after RO treatment of 99.6%.

None of the field and trip blanks taken as part of the QA/QC process showed any positive results indicating no contamination of samples analysed during the project occurred. No outliers or peaks in the concentration of pesticides in secondary wastewater were found during the time of the project indicating that non point sources for runoff and households were the more likely sources of pollution. Concentration of pesticides in grab samples tend to be higher compared with concentrations in composite samples indicating that composite samples are a better representation of the average pesticide concentration in secondary wastewater.

Despite the fact that pesticide concentrations in secondary wastewater may be very variable and intermittent according to pesticide use, trifluralin was constantly detected in the secondary wastewater in both BPP and KWRP. Trifluralin was detected in 91% of the secondary wastewater samples (mean=0.63 µg/L, std dev=0.57 µg/L, median=0.48 µg/L, max=2.4 µg/L). Trifluralin mean ratio of measured secondary wastewater concentration/LOR was on average 31.3 times above 1. Trifluralin is almost insoluble in water and therefore would be expected to have largely been partitioned into the biosolids produced by the WWTP, but clearly this partitioning is incomplete. Trifluralin is large, has a molecular weight of 335.5 g/mol, and therefore is expected to be effectively removed during the MF/RO treatment, as was observed with a mean percentage removal of 97%. Based on the results trifluralin is considered the best chemical indicator for pesticides during the operational monitoring as long as their patterns of use remain unchanged.

The results indicate that despite the release and detection of pesticides in secondary wastewater due to urban pesticide use practices, the impact on potable supplies

through augmentation using recycled water is likely to be negligible due to the low concentrations detected in secondary wastewater and the removal during the MF/RO treatment.

Pesticides as listed in Appendix 1 were not able to be analysed during this project. Secondary wastewater data should be gathered on some of these, in particular glyphosate that is commonly used in Western Australia.

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6.3 Halogenated disinfection by-products (DBPs)

Introduction

Halogenated DBPs result from chemical reactions between organic precursors such as natural organic matter (NOM) or effluent organic matter (EOM) and disinfectants such as chlorine or chloramine. Factors such as residence time, temperature, pH, organic content, including humic and fulvic acid, and bromide levels affect the composition and concentration of DBPs formed (Nieuwenhuijsen *et al*., 2000) More than 600 DBPs, representing a variety of chemical classes, and produced under a wide range of disinfection conditions and in different waters, have been identified (Richardson *et al*., 2002, 2007). DBPs may be categorised into 3 major classes: inorganic by-products, organic oxidation by-products, and halogenated organic by-products (Clark & Boutin, 2002). This chapter will focus on halogenated organic by-products, which we refer to in this chapter as DBPs. Other disinfection by-products such as inorganic anions and *N*-nitrosamines are discussed in sections 6.13 and 6.4 respectively.

Chemical disinfection of water is a major public health triumph, leading to dramatic decreases in both morbidity and mortality through prevention of waterborne diseases (Hrudey, 2009). It is accepted that the benefits of disinfection significantly outweigh the harm from DBPs. However, the potential human health effects from exposure to DBPs in water have been a concern since the 1970s, when trihalomethanes (THMs) were identified in chlorinated water (Rook, 1974). A considerable body of toxicological data has been developed on DBPs that occur in drinking water, mainly on THMs. Some DBPs are of little health concern, but some are suspected to be carcinogenic or have other developmental or reproductive health effects. Epidemiological studies looking for an association between DBPs and cancer are mainly limited to THM analysis and remain inconclusive because epidemiological studies often poorly assess the exposure (Graves *et al*., 2001) and because risk estimations can be biased through exposure misclassification and unmeasured confounding factors (Reif *et al*., 1996). Nevertheless, long-term THM exposure was associated with a twofold bladder cancer risk, with an odds ratio of 2.10 (95% confidence interval: 1.09, 4.02) for average household THM levels of >49 versus 8 µg/L (Villanueva *et al*., 2007).

Associations have been found between exposure to DBPs, including THMs and haloacetic acids (HAAs), and adverse growth-related birth outcomes, such as low birth weight, intrauterine growth retardation, and small body length or head circumference (Hinckley *et al*., 2005, Tardiff *et al*., 2006). Although there appears to be very good epidemiological evidence for a relationship between chlorination by-products, as measured by trihalomethanes (THMs) in drinking water and bladder cancer, the evidence for other cancers, including colorectal cancer appears to be inconclusive and inconsistent (Nieuwenhuijsen *et al*. 2009). There appears to be some evidence for an association between exposure to DBPs, specifically THMs,

and intrauterine growth retardation and, to a lesser extent, pre-term delivery, but evidence for relationships with other outcomes such as low birth weight (Hoffman *et al*. 2008), stillbirth, congenital anomalies and semen quality (Luben *et al*. 2007) is inconclusive and inconsistent.

The presence of DBPs can impair the safety of recycled water for augmentation of drinking water supplies. DBPs in secondary wastewater may be related to water exposed to household products that contain chlorine, such as bleach (European Communities, 2001) or due to chlorine introduction during wastewater treatment. It is standard practice to chloraminate wastewater before MF to minimise RO membrane fouling, and this occurs at both BPP and KWRP. Both pH and chlorine dose are controlled so that chlorine reacts with ammonia present in influent water to form monochloramine. Treating wastewater for non-potable reuse by chlorine dosing is a common practice and results in increased chloroform release into the environment (Zogorski *et al.*, 2006). THM and HAA formation is significantly lower with chloramination compared to chlorination, although it still occurs at measurable levels (Peterson *et al.*, 1993, Lu *et al*., 2009, Liu *et al.*, 2006). Formation of other emerging DBPs may be increased with chloramination compared to chlorination (Krasner *et al.*, 2006), and generally increased formation of all DBPs is expected with increased contact time (Carlson & Hardy, 1998).

The two dihalomethanes analysed in this section (dibromomethane and chlorobromomethane) have limited evidence for formation during disinfection, but they have been found to be toxic and included in a review of disinfection byproducts by Richardson *et al.* (2007).

In this section a characterisation of DBPs in secondary wastewater and post-RO water is presented for 6 halomethanes, including 4 trihalomethanes (THMs), and 2 others (dibromomethane and chlorobromomethane), 9 haloacetic acids (HAAs), 6 haloacetonitriles (HANs), 6 haloaldehydes (HALs), 4 haloketones (HAKs) and chloropicrin.

Methods

All DBPs were measured by gas chromatography mass spectrometry (GC-MS), although different sample preparation methods were used for different groups. Halomethanes were measured by purge and trap GC-MS. Samples (40 mL) were treated with Na_2SO_4, before a 25 mL aliquot was injected into a thermal desorption purge and trap system. Volatile analytes were ‘purged’ by bubbling helium through the sample, and collected on an activated carbon trap. After the purging was complete, the trap was heated and the analytes released and delivered to the GC for separation using an ultra inert 5% phenyl 95% dimethylpolysiloxane capillary column. Quantification was performed by MS with electron ionisation (EI), with peak

identification and calculation of recovery was aided by inclusion of surrogate standards.

HAAs were preconcentrated by liquid-liquid extraction (LLE) and derivitised before GC-MS. Samples (50 mL) were mixed with methyl *tert*-butyl ether (MtBE, 3mL) at an acidic pH to extract analytes to the organic phase. Derivitisation of analytes to methyl esters improved chromatographic performance. Samples were injected into the GC and separated using a 5% phenyl 95% dimethylpolysiloxane capillary column. Quantification was performed by MS with EI, with peak identification based on retention time and both quantifying and qualifying ions, where possible, and calculation of recovery was aided by inclusion of deuterated internal standards.

HANs, HALs, HAKs and chloropicrin were all measured in one method, which incorporated preconcentration by liquid-liquid extraction (LLE) before GC-MS analysis. Samples (50 mL) were mixed with methyl *tert*-butyl ether (MTBE, 2mL) at an acidic pH to extract analytes to the organic phase. Samples were injected into the GC and separated using a 5% phenyl 95% dimethylpolysiloxane capillary column. Quantification was performed by MS with EI, with peak identification based on retention time and both quantifying and qualifying ions, where possible, and calculation of recovery was aided by inclusion of deuterated internal standards.

All methods were verified for the analytes of interest in both secondary wastewater and post-RO water. The limits of detection (LOD) and estimated uncertainties for each method are listed in Table 6.3.1

Table 6.3.1: Health values, limits of detection (LOD) and estimation of uncertainty for DBPs. Standard relative uncertainty for calculated at 0.5 µg/L for halomethanes, 10 µg/L for all other DBPs

Analyte	Health value (µg/L)	Source	Average LOD (µg/L)	Standard Relative Uncertainty (%)
Halomethane				
Bromochloromethane	70	USEPA, 2006	0.03	14.1%
Chloroform	200	AGWR, 2008	0.06	14.8%
Dibromomethane	0.7	TTC	0.03	13.6%
Bromodichloromethane	60	WHO, 2006	0.02	13.2%
Dibromochloromethane	100	WHO, 2006	0.1	35.5%
Bromoform	100	WHO, 2006	0.09	67.5%
HAA				
Chloroacetic acid	150	ADWG, 2004	3.1	21.3%
Bromoacetic acid	60	USEPA, 2006	0.7	21.8%
Dichloroacetic acid	100	ADWG, 2004	0.9	20.2%
Trichloroacetic acid	100	ADWG, 2004	1.3	18.7%
Bromochloroacetic acid	0.7	TTC	1.1	9.5%
Dibromoacetic acid	60	USEPA, 2008	1.1	9.9%

Dichlorobromoacetic acid	0.7	TTC	2.2	23.3%
Dibromochloroacetic acid	0.7	TTC	2.7	48.2%
Tribromoacetic acid	0.7	TTC	34	68.6%
HAN				
Trichloroacetonitrile	0.7	TTC	0.05	20.5%
Chloroacetonitrile	0.7	TTC	0.05	17.9%
Dichloroacetonitrile	2	WHO, 2006	0.05	9.8%
Bromoacetonitrile	0.7	TTC	0.05	21.1%
Bromochloroacetonitrile	0.7	TTC	0.07	19.6%
Dibromoacetonitrile	70	WHO, 2006	0.06	31.0%
HAL				
Trichloroacetaldehyde	20	ADWG, 2004	0.11	19.3%
Bromochloroacetaldehyde	0.7	TTC	0.12	29.2%
Bromodichloroacetaldehyde	0.7	TTC	0.28	33.5%
Dibromoacetaldehyde	0.7	TTC	0.24	43.2%
Chlorodibromoacetaldehyde	0.7	TTC	0.17	30.7%
Tribromoacetaldehyde	0.7	TTC	0.26	58.0%
HAK				
Chloroacetone	0.7	TTC	0.08	31.6%
1,1-Dichloro-2-propanone	0.7	TTC	0.07	14.8%
Trichloropropanone	0.7	TTC	0.15	13.5%
1,3-Dichloropropanone	0.7	TTC	0.07	18.9%
Other				
Chloropicrin	0.7	TTC	0.12	18.0%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R and quantifying and qualifying ions), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear

regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

A total of 2,401 measurements were analysed after excluding trip, field, and replicate samples. The largest number of samples were analysed in Event 3 (490) followed by Event 6 (404). All samples taken were grab and the distribution of sampling by event and location is presented in Table 6.3.2.

Table 6.3.2: Measurement of disinfection by products by event and location for secondary wastewater

Event	Month	No days	Year	Total	Location									
					GW	SWW	Water Reclamation Plant							
							Before MF		Post-MF water		Post-RO water		Storage dam	Total
							K	B	K	B	K	B	K	
1	November	4	2006	150	0	0	45	0	15	0	45	0	45	150
2	May/June	6	2007	551	70	166	105	0	105	0	105	0	0	315
3	September	6	2007	490	0	0	105	105	35	35	105	105	0	490
4	January	6	2008	386	53	0	53	105	0	0	70	105	0	333
5	April	5	2008	420	0	35	70	105	0	35	70	105	0	385
6	June	5	2008	404	0	0	70	89	35	35	70	105	0	404
Total		32		2,401	123	201	448	404	190	105	465	420	45	2077

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

Of the 32 DBPs tested, 18 (56%) were detected in secondary wastewater. The most commonly detected group of DBPs were the halomethanes in 84% of samples followed by HAAs (14%) and HAKs (6%). The percentage detection for individual analytes ranged from 94% for bromochloromethane and dibromomethane to 3% for bromoacetic acid, chlorodibromoacetic acid and dibromoacetic acid (Figure 6.3.1). While HAAs were the group with the highest median concentrations, they were detected in less than 15% of the samples except trichloroacetic acid (56%). Chloroacetic acid was the DBP in secondary wastewater with the highest median concentration (3.5 µg/L) followed by trichloroacetic acid (2.1 µg/L) and dichlorobromoacetic acid (1.3 µg/L). For other classes of DBPs, chloroform showed

the highest median concentration (0.36 µg/L) followed by dibromomethane (0.26 µg/L) and bromochloromethane (0.22 µg/L).

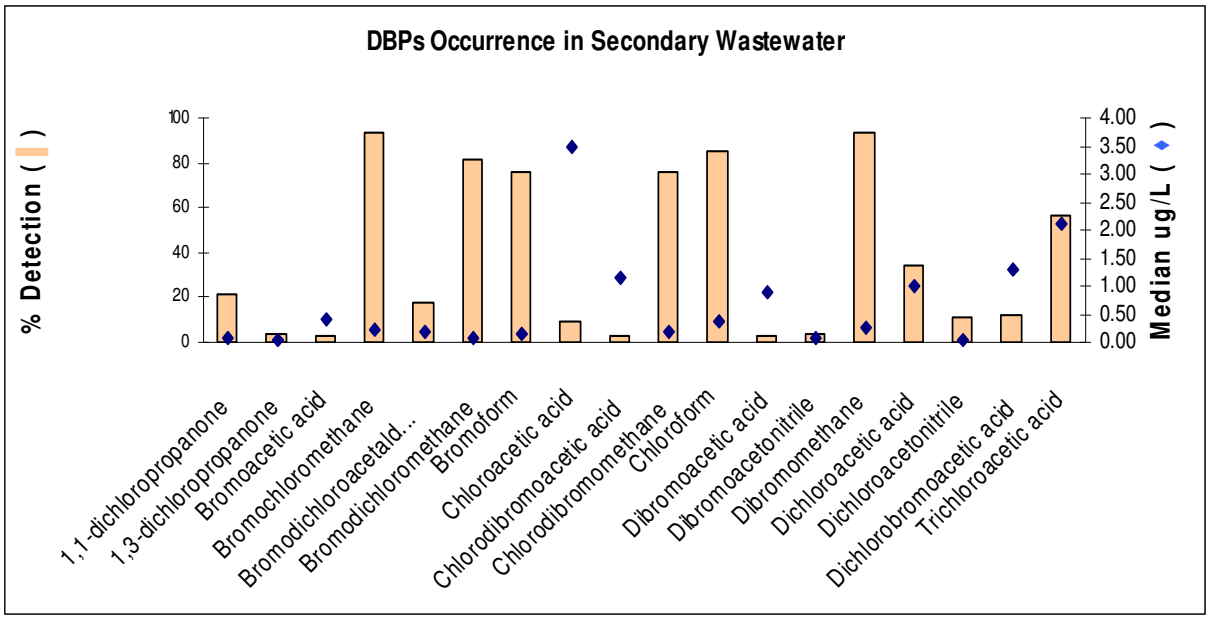

Figure 6.3.1: Detected DBPs in secondary wastewater and corresponding median concentrations (µg/L)

A comparison of median concentrations at each WWTP showed that, overall, there was no statistically significant difference between each WWTP (K-Wallis p=0.082). However individual DBPs did show statistically significant variation. For the halomethanes, median concentrations of chloroform, chlorodibromomethane, and bromodichloromethane were highest at Subiaco WWTP, while bromoform was highest at Beenyup WWTP and bromochloromethane and dibromomethane were highest at KWRP. For the HAAs, the median concentration of trichloroacetic acid was highest at Subiaco, while chlorodibromoacetic acid and dibromoacetic acid were highest at Beenyup WWTP, and bromoacetic acid, dichloroacetic acid and dichlorobromoacetic acid were highest at KWRP, however the differences for these HAAs were not statistically significant.

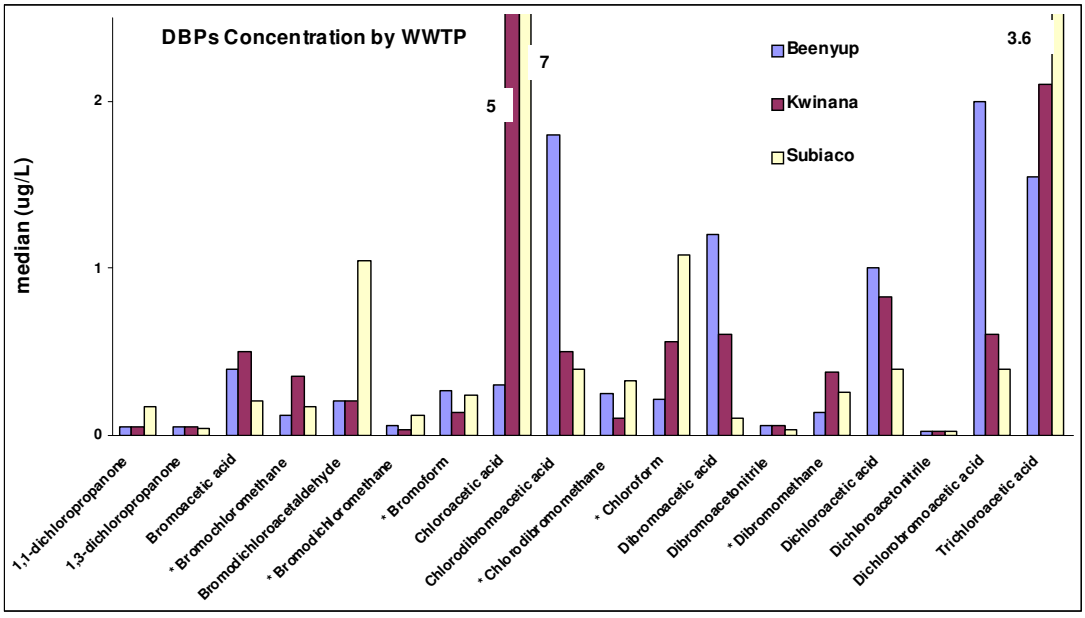

Figure 6.3.2: Median DBPs concentration by WWTP in µg/L.

**DBPs with statistically significant differences in concentrations between plants.*

Significant differences were also observed in the median concentrations of DBPs by season (Figure 6.3.3). Overall, there were statistically significant differences in the median concentration of DBPs by season (K-Wallis p=0.016), with the highest concentrations seen in winter. However, there was no clear seasonal trend observed within each class of DBPs. Winter showed maxima for 7 DBPs, with 1,1-dichloropropanone, bromochloromethane, bromodichloroacetaldehyde, bromoform, chlorodibromomethane, chloroform, and dibromomethane, although the difference for chloroform and dibromomethane was not significant. In spring there was maxima for 8 DBPs (1,3-dichloropropanone, bromoacetic acid, bromochloromethane, chloroacetic acid, dibromoacetic acid, dibromoacetonitrile, dichloroacetonitrile and trichloroacetic acid), and only the maxima for bromochloromethane was not significant. Median concentrations of chlorodibromoacetic acid, dichloroacetic acid and dichlorobromoacetic acid were all significantly higher in summer. None of the DBPs showed maximum concentrations in autumn.

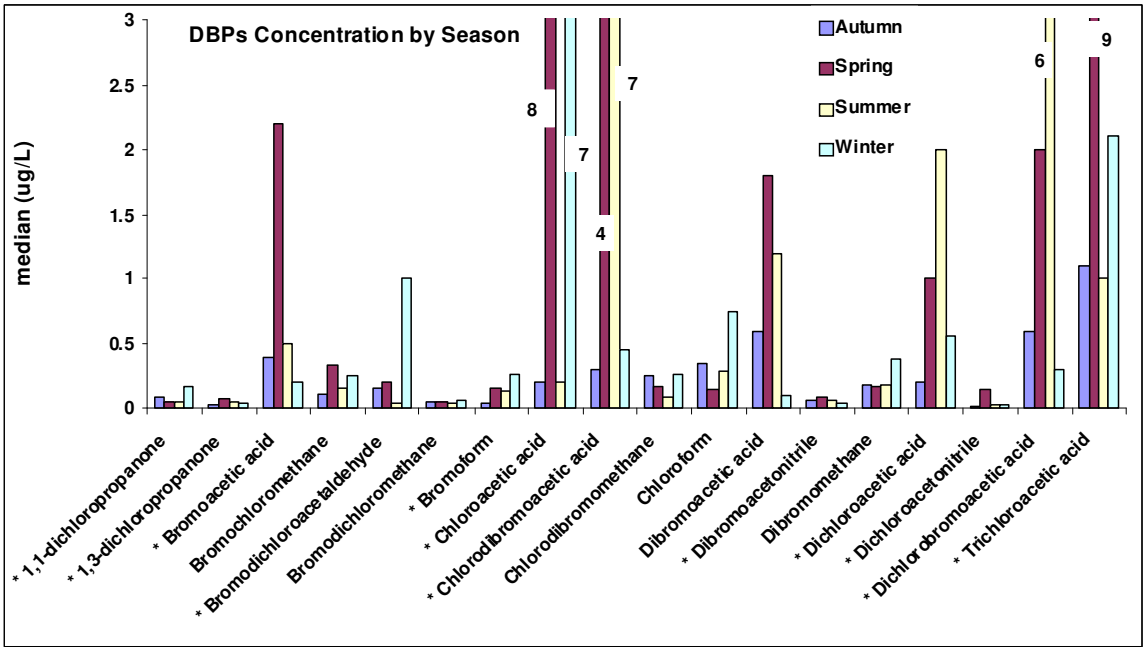

Figure 6.3.3: Median DBPs concentration by season in µg/L

** DBPs with statistically significant differences in concentrations between seasons.*

RO Product water characterisation

A total of 23 of 32 DBPs (72%) were detected in post-RO water. The greatest percentage of detections was for bromochloromethane (100%), followed by dibromomethane (96%) and bromodichloromethane (70%). Median concentrations were highest for dichloroacetic acid (1 µg/L), and for three HAL; bromodichloroacetaldehyde (0.20 µg/L), dibromoacetaldehyde (0.18 µg/L) and tribromoacetaldehyde (0.15 µg/L), all higher than the median concentration measured in secondary wastewater. Chloroform, which showed the highest median concentration in secondary wastewater, was detected in 56% of post-RO water samples with a median concentration of 0.14 µg/L.

While a greater number of DBPs were measured in post-RO water compared to secondary wastewater, six HAAs present in secondary wastewater (bromoacetic acid, chloroacetic acid, chlorodibromoacetic acid, dibromoacetic acid, dichlorobromoacetic acid and trichloroacetic acid) were not measured in post-RO water. In contrast, 11 DBPs not detected in the secondary wastewater were detected in post-RO water. Bromochloroacetonitrile, chloroacetone and trichloroacetaldehyde were detected in the post-RO water in 17%, 13% and 8% of the samples. The other eight DBPs (1,1,3-trichloropropanone, bromoacetonitrile, bromodichloroacetaldehyde, chloropicrin, dibromoacetaldehyde, dibromochloroacetaldehyde, monochloroacetonitrile and tribromoacetaldehyde) were detected in one post-RO water sample (Beenyup 220108).

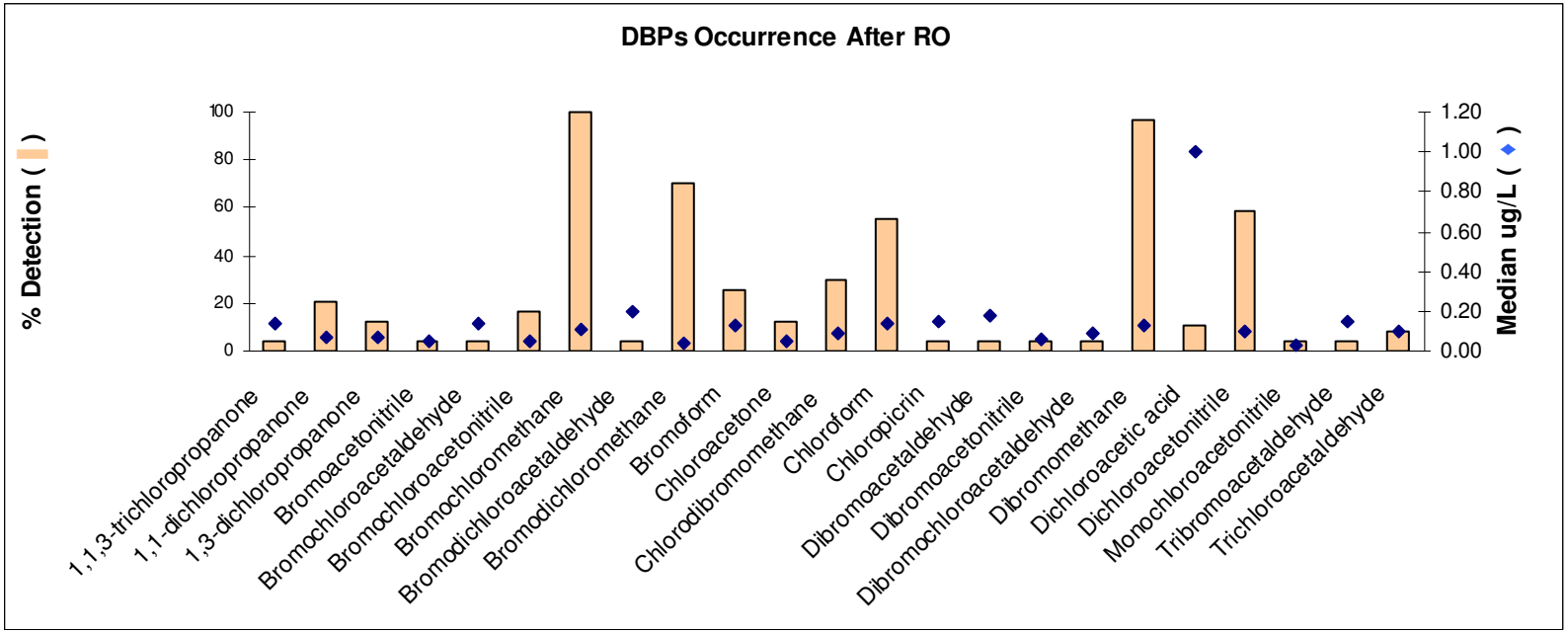

Figure 6.3.4: Detected DBPs in post-RO water and corresponding median concentrations (µg/L)

Groundwater characterisation

Three DBPs (dichloroacetic acid, 1,1-dichloropropanone, dichloroacetonitrile) were detected in groundwater at low concentrations (Table 6.3.3). All detections occurred during the Event 2 (May 2007). All 3 DBPs were detected in the Wanneroo bore line, while dichloroacetonitrile was also detected in the Pinjar bore line.

Table 6.3.3: Measurement of disinfection by products by event and location for groundwater

DBP	LOD (µg/L)	Concentration (µg/L)	Field Blank	Location	Date
Dichloroacetic acid	0.15	0.32	<0.15	Wanneroo	24/05/2007
1,1-dichloropropanone	0.17	0.34	<0.17	Wanneroo	24/05/2007
Dichloroacetonitrile	0.01	0.011	<0.01	Wanneroo	24/05/2007
Dichloroacetonitrile	0.01	0.05	<0.01	Pinjar	24/05/2007

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. For 25 of the DBPs studied, there is no toxicity data available and therefore health values for these DBPs were calculated using the TTC approach, as described in Chapter 3. All DBPs were classified in Cramer class III because they contain chemical structures that permit no strong initial impression of safety and may even suggest a significant toxicity. DBPs whose health values were calculated using the TTC approach have very low health values compared with those DBPs with health values calculated using toxicity data.

Three DBPs were not detected in any of the samples taken and RQs were calculated using the average LOD (Table 6.3.4). The health values for all 3 undetected DBPs were calculated using the TTC approach. Bromochloroacetic acid and tribromoacetic acid have RQs above 1 (RQ=1.5 and RQ=41 respectively), indicating that the average LOD achieved was above the allocated TTC health value. Health values for regulated HAAs ranged from 60 µg/L (dibromoacetic acid & bromoacetic acid) to 150 µg/L (chloroacetic acid), two orders of magnitude above the calculated TTC value of 0.7 µg/L. The TTC approach is very conservative and it may be expected that unregulated HAAs could have comparable toxic effects to regulated HAAs based on similar chemical structures. A less conservative approach for unregulated DBPs could be to divide the health value of the lowest regulated DBP (i.e. dibromoacetic acid for the HAAs) by a safety factor of 10.

Table 6.3.4: DBPs without detections in any of the samples and corresponding RQs

Parameter	Mean LOD (µg/L)	Tier	Health value (µg/L)	Source	RQ
Bromochloroacetic acid	1.02	3	0.7	TTC	**1.5**
Tribromoacetic acid	29	3	0.7	TTC	**41.4**
Trichloroacetonitrile	0.05	3	0.7	TTC	0.07

Table 6.3.5 presents the RQs for the 29 DBPs detected in the secondary wastewater and/or in post-RO water. RQ(max) used the maximum concentration measured for each analyte, while RQ(median) uses the median concentration measured for each analyte, including all non-detects, which were reported as the LOD. In secondary wastewater RQ(max) was above 1 for 1,1-dichloropropanone, chlorodibromoacetic acid, dibromomethane and dichlorobromoacetic acid, which all had health guidelines calculated using the TTC approach. RQ(median) in secondary wastewater was above 1 for chlorodibromoacetic acid (RQmedian=1.6) and dichlorobromoacetic acid (RQmedian=1.9). In post-RO water RQ(max) was above 1 for bromodichloroacetaldehyde (RQmax=1.4) and for dibromoacetaldehyde (RQmax=1.3). Again health values for both compounds were calculated using the TTC approach. RQ(median) were all below 1 for all detected DBPs.

Table 6.3.5: DBPs detected before MF and/or in post-RO water and corresponding RQs

					Secondary Wastewater			After RO		
Parameter	Mean LOD (µg/L)	Tier	Health value (µg/L)	Source	n	RQ* (med)	RQ (max)	n	RQ (med)	RQ (max)
1,1,3-trichloropropanone	0.14	3	0.7	TTC		0.2*	n/a	24	0.2	0.6
1,1-dichloropropanone	0.08	3	0.7	TTC	28	0.1	**1.4**	24	0.1	0.8
1,3-dichloropropanone	0.08	3	0.7	TTC	28	0.07	0.3	24	0.1	0.5
Bromoacetic acid	0.64	1	60	USEPA, 2006	32	0.007	0.04		n/a	n/a
Bromoacetonitrile	0.05	3	0.7	TTC		0.07*	n/a	24	0.1	0.6
Bromochloroacetaldehyde	0.17	3	0.7	TTC		0.2*	n/a	24	0.2	0.7
Bromochloroacetonitrile	0.07	3	0.7	TTC		0.1*	n/a	24	0.1	0.4
Bromochloromethane	0.03	2	40	USEPA, 2006	33	0.01	0.3	27	0.003	0.01
Bromodichloroacetaldehyde	0.34	3	0.7	TTC	28	0.3	5.3	24	0.3	**1.4**
Bromodichloromethane	0.02	1	60	WHO, 2006	33	0.001	0.005	27	0.001	0.01
Bromoform	0.09	1	100	WHO, 2006	33	0.002	0.01	27	0.001	0.003
Chloroacetic acid	3.45	1	150	ADWG, 2004	32	0.02	0.1		n/a	n/a
Chlorodibromoacetic acid	2.33	3	0.7	TTC	32	**1.6**	**10**		n/a	n/a
Chloroacetone	0.07	3	0.7	TTC		0.1*	n/a	24	0.1	0.4
Chlorodibromomethane	0.1	1	100	WHO, 2006	33	0.002	0.009	27	0.001	0.0005
Chloroform	0.07	1	200	AGWR, 2008	33	0.002	0.027	27	0.001	0.004
Chloropicrin	0.12	3	0.7	TTC		0.2*	n/a	24	0.2	0.5
Dibromoacetaldehyde	0.23	3	0.7	TTC		0.3*	n/a	24	0.3	**1.3**
Dibromoacetic acid	1.02	1	60	USEPA, 2008	32	0.02	0.03		n/a	n/a
Dibromoacetonitrile	0.05	1	70	WHO, 2006	28	0.0009	0.002	24	0.001	0.005
Dibromochloroacetaldehyde	0.17	3	0.7	TTC		0.2*	n/a	24	0.1	0.9
Dibromomethane	0.04	3	0.7	TTC	33	0.4	**1.7**	27	0.2	0.7
Dichloroacetic acid	0.81	1	100	ADWG, 2004	32	0.01	0.02	27	0.01	0.08
Dichloroacetonitrile	0.04	1	20	WHO, 2006	28	0.001	0.01	24	0.005	0.03
Dichlorobromoacetic acid	1.88	3	0.7	TTC	32	**1.9**	**8.6**		n/a	n/a
Monochloroacetonitrile	0.05	3	0.7	TTC		0.07*	n/a	24	0.04	0.4
Tribromoacetaldehyde	0.25	3	0.7	TTC		0.4*	n/a	24	0.2	0.9
Trichloroacetaldehyde	0.11	1	20	ADWG, 2004	32	0.006*	n/a	24	0.01	0.7
Trichloroacetic acid	1.17	1	100	ADWG, 2004	32	0.02	0.3		n/a	n/a

LOD, limit of detection; TTC, threshold of toxicological concern, n/a not applicable. RQ of undetected DBPs, value calculated using the average LOD*

The effect of MF/RO treatment on DBP *formation*

In both the BPP and KWRP, wastewater undergoes chloramination before MF to prevent RO membrane fouling. Over the course of the sampling period, a small number of post-MF samples were collected from within both plants in addition to the normal secondary wastewater and post-RO samples to determine the effect of chloramination during the MF/RO process. Paired wastewater, post-MF and post-RO samples were taken on 5 occasions at KWRP (Event 1: 29th November 2006, Event 2: 30th May 2007, 4th June 2007, 7th June 2007, Event 3: 21st September 2007, and

Event 6: 6th June 2008) and on 3 occasions at BPP (Event 3: 26th September 2007, Event 4: 1st April 2008 and Event 6: 5th June 2008). Figure 6.3.5 presents median DBP concentrations of these paired wastewater, post-MF and post-RO samples. For KWRP, the highest concentrations measured are at the post-MF sample point, after chloramination. Indeed some DBPs were measured in post-MF samples that were not detected in either the secondary wastewater or post-RO water samples (e.g., chloroacetic acid, dichloroacetonitrile). The apparent production of DBPs measured in post-MF samples even meant that some compounds not detected in wastewater were detected in post-RO water (e.g. bromochloroacteonitrile and trichloroacetaldehyde). While some DBPs were also elevated in the post-MF samples taken at BPP (e.g. dichloroacetic acid, bromoacetic acid, 1,3-dichloropropanone), the effect was much less pronounced.

There are significant differences in the chloramination process at BPP and KWRP. At BPP, aqueous ammonia and chlorine (sodium hypochlorite) is dosed constantly to maintain consistent chloramine concentrations of 1-1.5 mg/L (winter) and 1.5-2 mg/L (summer) and an ORP reading between 470-510 mV. There is about 20 seconds between the dosing point and the post-MF sample point and a further several minutes in storage prior to RO treatment, and so the time available for DBP formation is short. At KWRP, sodium hypochlorite is also added to achieve a final chloramine concentration of 1.65 mg/L, but ammonia is only added if the concentration in the influent is less than 1 mg/L, a rare occurrence. In addition, there is a much longer residence time between the sodium hypochlorite dosing point and the post-MF sampling point, about 25 minutes when the plant is running at full capacity and longer if the plant is not running at full capacity. It is most likely that the elevated concentrations of DBPs seen post-MF at KWRP are mostly related to the longer residence time in the plant before RO treatment and this is confirmed by comparing plant flow rate to DBP concentrations for each sample day (Table 6.3.6). For Event 3 in particular, KWRP plant flows were almost half of full plant capacity because of reduced demand for product water. Halomethanes, which were not elevated in BPP show a clear trend of greater concentrations at the post-MF sample point when average plant flow was low. This suggests that longer plant residence times can significantly increase halomethane formation. In contrast, HAAs concentrations did not show the same dependence on flow rate, suggesting they form at a faster rate and within the RO feed tank residence time. HAAs were also seen to form within BPP, where time available for formation was shorter.

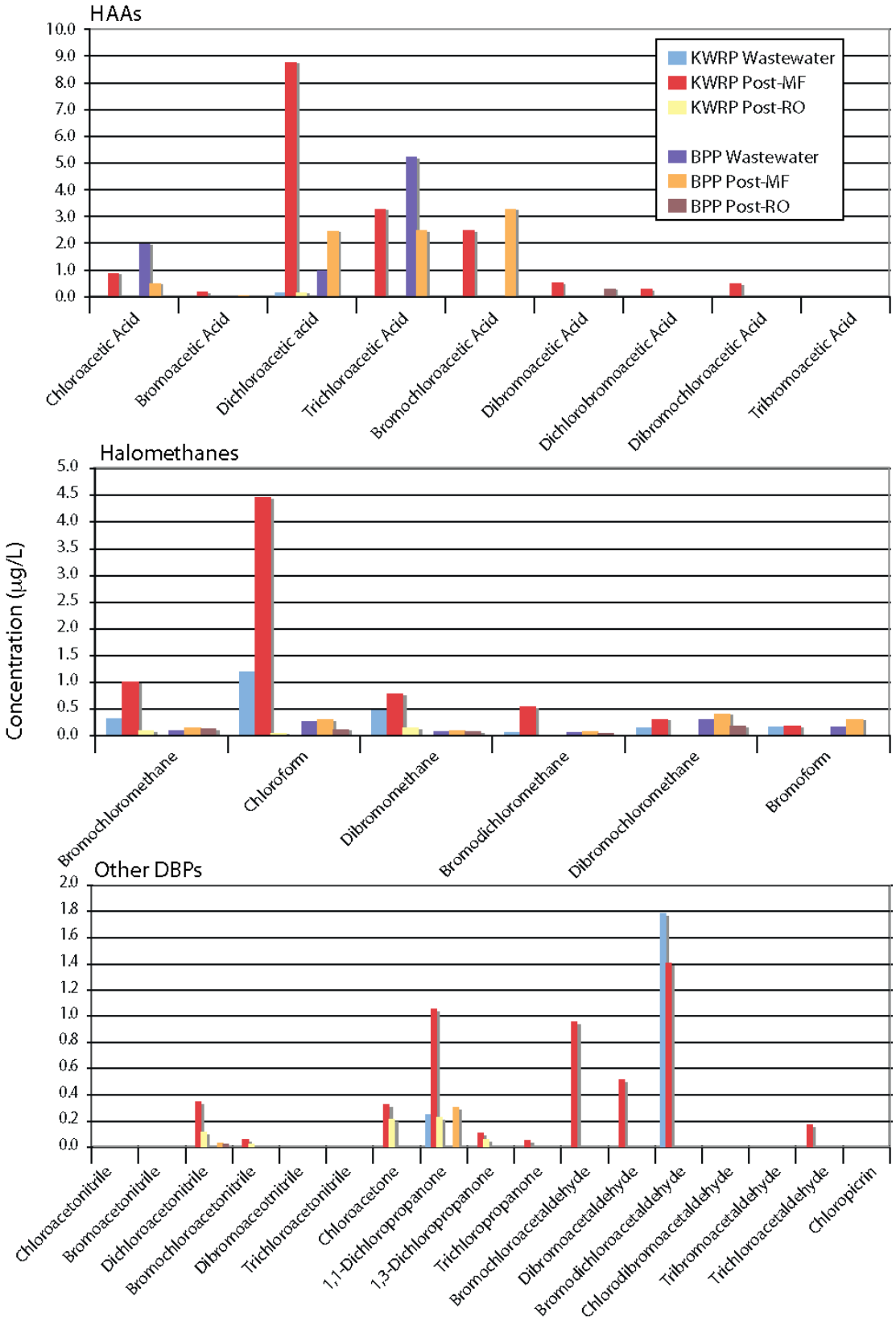

Figure 6.3.5: Median concentrations of HAAs, Halomethanes and other DBPs in paired secondary wastewater, post-MF water and post-RO water samples for both KWRP (n=5) and BPP (n=3).

Table 6.3.6: Comparison of KWRP Plant Flow to post-MF DBP concentration

Sample Date	Average Flow (kL/hr)	Chloroform (µg/L)	Dichloroacetic acid (µg/L)	1,1-Dichloropropane (µg/L)
29 /11/06 11.30am	~719	0.7	3.4	Not measured
30/05/07 9.15am	~285	8.9	9.4	1.1
04/06/07 9.45am	~426	7.1	8.3	1.2
07/06/07 9.54am	~286	7.0	7.1	1.2
21/09/07 11.07am	~574	1.6	13	1.0
06/06/08 9.23am	~500	1.9	9.2	0.41

Treatment performance

Treatment efficiency was calculated for analytes detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD as a worst-case scenario. In general, high variability was observed in the removal of DBPs in particular for halomethanes (Figure 6.3.6). Median treatment performance for DBPs ranged from 33% for bromodichloromethane to 93% for trichloroacetic acid. For some DBPs concentrations post-RO were higher than in secondary wastewater: bromochloromethane, bromodichloromethane, dibromomethane, bromoform and chloroform.

Given the potential for formation of halogenated DBPs by chloramination, as discussed previously, the treatment efficiency of RO alone cannot be determined using secondary wastewater as the starting concentration. The efficiency of RO to remove DBPs is important as it is the only barrier designed to remove dissolved chemicals and calculation of RO treatment efficiency using the post-MF concentration as the starting concentration may be more appropriate in this case. Calculations confirmed that the RO treatment efficiency was higher using paired post-MF and post-RO water samples, compared to when it was calculated using paired secondary wastewater and post-RO water samples for all DBPs except for bromodichloroacetaldehyde, bromoform and trichloroacetic acid (see Figure 6.3.7). Furthermore, by using post-MF data, treatment efficiency could be calculated for 9 additional DBPs, which were measured in post-MF samples but not present in secondary wastewater. Variability in efficiency calculated using post-MF and post-RO data (as represented by standard deviation) remained high, although this might be related to the lower number of paired samples available.

Because of the differences seen in DBP formation in KWRP and BPP, as discussed above, treatment efficiency between post-MF and post-RO samples was also calculated for each plant, although this data is not plotted because of the low number

of paired samples available. Halomethane treatment efficiency at KWRP was calculated to be higher than at BPP, except for bromoform, which was comparable at each plant. This difference is attributed to the higher halomethane concentrations seen in post-MF samples at KWRP. The higher DBP formation in KWRP compared to BPP was also the reason for the wider range of DBP treatment efficiency data calculated using post-MF samples compared to secondary wastewater.

DBPs were also measured in the storage dam at KWRP during Event 1 (November 2006). The concentrations of DBPs in the dam were lower than the samples taken after the RO treatment (data not shown). This may be due to volatilisation given the warm temperatures in the vicinity of the summer period.

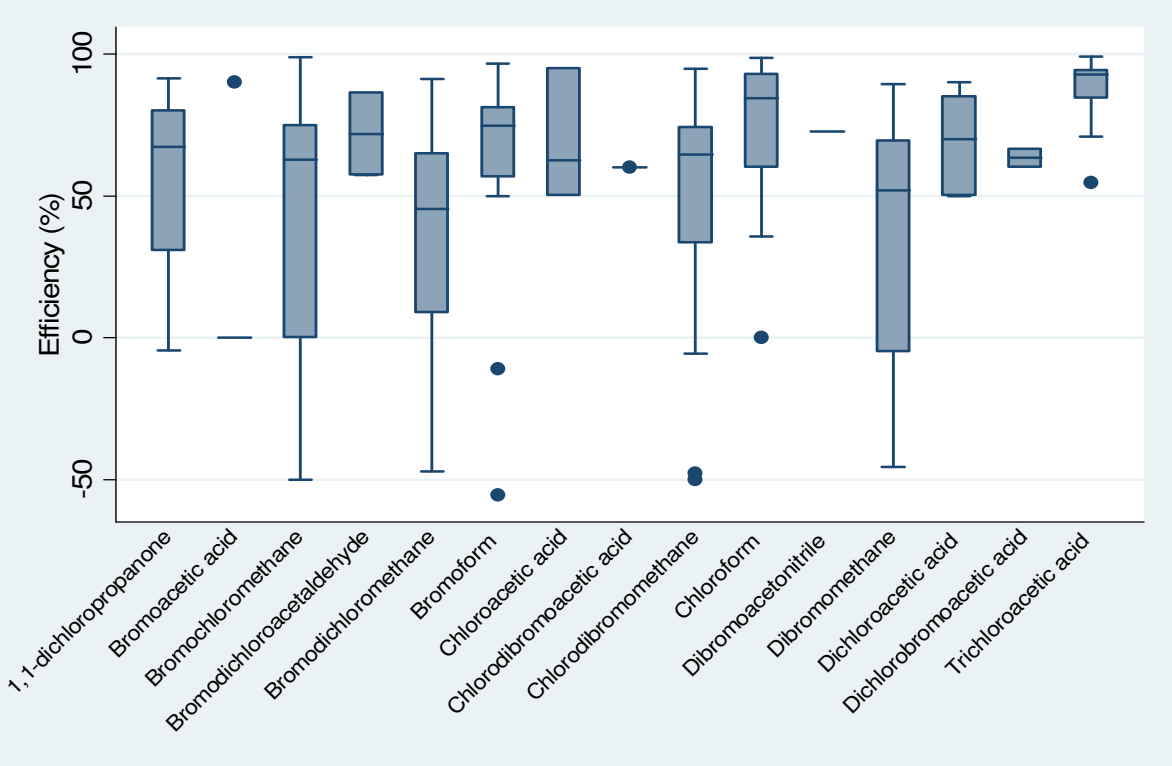

Figure 6.3.6: MF/RO removal efficiency of detected DBPs in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

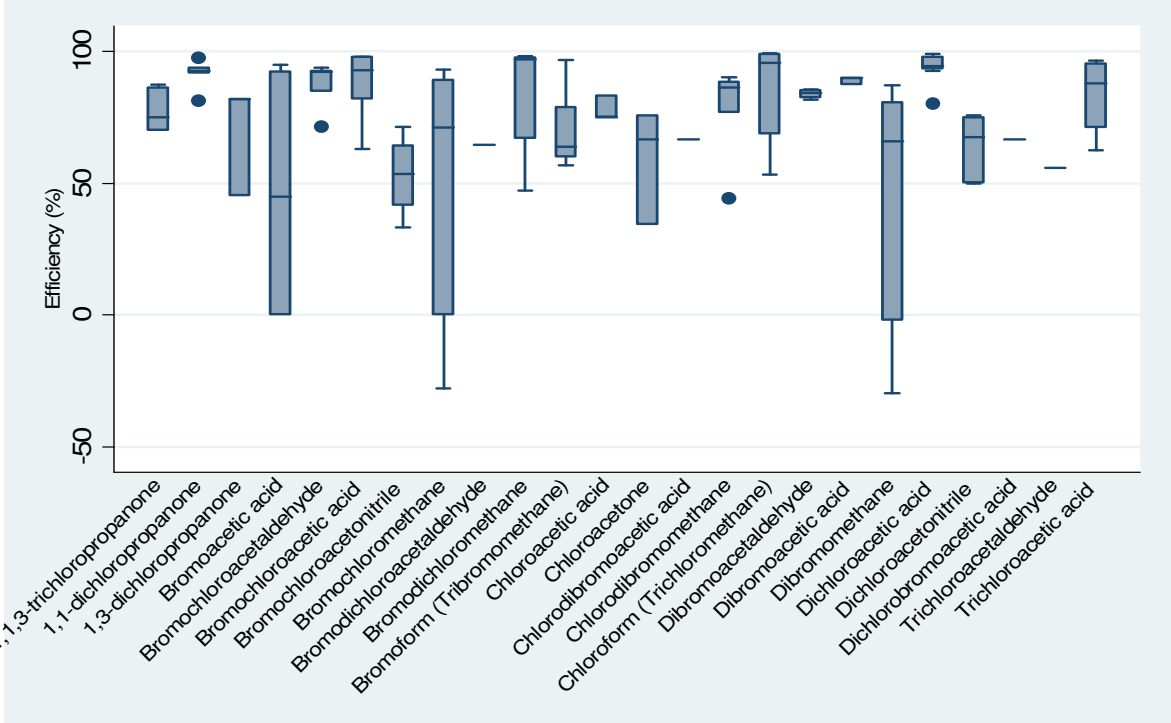

Figure 6.3.7: MF/RO removal efficiency of detected DBPs in post-MF water. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Discussion

More than half of the DBPs tested were found in the secondary wastewater, and seven were detected in more than 50% of the samples. As well as chlorine products collected by the wastewater system, a small amount of chlorine (3-5 mg/L) is added to the return activated sludge in all three WWTPs and this may be a source of halogenated DBPs within each plant. Halomethanes were detected in 84% of the secondary wastewater samples followed by HAAs (14%) and HAKs (6%). This order of abundance corresponds with general abundances reported in the literature, where the majority of detections correspond to halomethanes followed by HAAs, and fewer detections from other organic DBP groups (Krasner *et al.*, 2006). In this study, HAKs were the third most commonly found group of DBPs, whereas HALs have been found to be the third major class formed in many drinking waters (Krasner *et al.*, 2006). There were no significant differences in DBP concentrations observed at each WWTP. While Subiaco WWTP tended to have the highest values, the lack of statistical significance may be because fewer wastewater samples were analysed from Subiaco WWTP compared to BPP or KWRP. Median DBP concentrations were higher in winter compared with summer and DBPs are more likely to be stable and detectable in colder weather because warm temperatures can cause DBPs to volatilise (Metz *et al.*, 2007). In Perth there is not a wide variability between winter

and summer flows (due to segregation of stormwater) and therefore differences in retention time are not expected to have affected concentrations.

Detections of a few DBPs in groundwater are surprising as any DBPs that made their way into groundwater would be expected to be dehalogenated by reducing conditions. All detections were very close to LOD and with a relatively high level of uncertainty so it is difficult to draw any conclusions. As the groundwater samples were actually collected at the influent to the Wanneroo drinking water treatment plant, after passing through several kilometres of pipeline, there is potential for disinfectant to have been introduced for cleaning purposes upstream (e.g. for cleaning bore screens). As detections were in both the Pinjar and Wanneroo lines on the same day this could only have been the result of multiple bores being cleaned.

More DBPs were detected in post-RO water (n=23) than in secondary wastewater (n=18), and the total number of detections was greater in post-RO water than in the secondary wastewater. This suggested that DBPs may form during MF/RO treatment, specifically after chloramination, and this hypothesis is supported by analyses of post-MF samples which show elevated DBP concentrations, particularly for HAAs and halomethanes with longer plant residence times. While the formation of halomethanes and HAAs is generally considered to be insignificant after chloramination, the concentrations formed in post-MF samples are consistent with other studies comparing THM and HAA formation in chlorinated and chloraminated drinking water (Liu *et al.*, 2006, Lu *et al.*, 2009, Peterson *et al.*, 1993). Comparison of data from KWRP and BPP appears to indicate that this formation can be minimised by reducing the time between chloramination and final RO treatment, but further sampling would be required to completely understand the controlling factors.

Calculated treatment removal was variable, with some concentrations in post-RO water higher than the concentration in secondary wastewater. For some DBPs this may be due to uncertainty in the analytical method. However, for most, calculating treatment efficiency using post-MF and post-RO data produced higher values than using secondary wastewater and post-RO water, again supporting the hypothesis that DBPs formed during the MF/RO treatment. More analysis of DBPs before and after RO treatment is recommended to better characterise the treatment variability particularly for halomethanes.

For halomethanes, post-MF to post-RO treatment efficiency at KWRP was generally higher than at BPP. This difference is attributed to the higher halomethane concentrations seen in post-MF samples at KWRP. A greater number of DBPs were measured in KWRP post-MF samples, indicating that more DBP formed during MF/RO at KWRP than at BPP.

The reduction in DBP concentrations seen in the KWRP post-RO storage dam, measured in Event 1 suggest that storage of post-RO water may further reduce final DBP concentrations through volatilisation.

Chloroform is the smallest of the halomethanes so it is more likely to partition through RO. Using post-MF data, chloroform treatment efficiency was greater than 95%, which is significantly higher than the removal estimates of 25-50% seen in other studies (Drewes *et al*., 2008). Removal efficiency of chloroform is highly dependent on the fouling stage of the membrane with higher removal by more fouled membranes (Drewes *et al*. 2005). The general trend of RO removal of chloroform (i.e. post-MF to post-RO treatment efficiency) in this project was for greater removal by the KWRP than the BPP RO membranes. There was only one directly comparable calculation using a positive detection in RO from each site that was during Event 6 that followed the trend (KWRP 97%, BPP 53%). This may be due to the improved rejection of older, more fouled membranes at KWRP compared to the nine-month old BPP membranes (by Event 6). Rejection of chloroform by BPP was more similar to reported values (Drewes *et al*. 2008, Bellona *et al*., 2008).

Reported correlations between THMs and total DBPs, as well as THMs and HAAs, in chlorinated drinking water have indicated that THMs may serve as a potential surrogate for other DBPs (CRCWQT, 2001). Although MF/RO uses chloramination rather than chlorination, the results from this study support the use of halomethanes as the best indicators for total halogenated DBPs in secondary wastewater, although this is predominantly because other DBP classes were not as consistently detected as the halomethanes. Chloroform has previously been suggested as a chemical indicator for membrane treatment efficiency (Drewes *et al*., 2008). However, in this study it was detected only in 85% of the secondary wastewater samples and in 56% of the post-RO water samples. In comparison, bromochloromethane was detected in 94% of secondary wastewater and in all post-RO water samples and therefore would appear to be a more appropriate indicator in this instance. More data is required to identify the best chemical indicator for this group.

DBPs concentrations in post-RO water in this study were low and all RQ(median) were below health significance (RQ(median) less than 0.3). While two DBPs, bromodichloroacetaldehyde and dibromoacetaldehyde, did have RQ(max) greater than 1, both had health values calculated using the TTC approach and it is expected that these health values will be refined as more toxicity data becomes available. While it is difficult to estimate the effect of DBP mixtures, it has previously been shown that exposure to mixtures at the observed individual low (non-toxic) doses is of no health concern (Cassee *et al*., 1998, Vighi *et al*., 2003). It is worth noting that the concentrations of THMs observed in the post-RO water are approximately 10 to 100 times lower than typical concentrations in Perth drinking water (WCWA, 2008) that are considered safe to drink as they meet ADWG guideline levels.

A wide array of halogenated DBPs have been considered in this study, however additional compounds could also be studied in order to monitor DBPs of potential health concern not yet routinely considered. In particular, cyanogen bromide, tribromoacetonitrile, bromoacetaldehyde, chloroacetaldehyde, and

dichloroacetaldehyde were all included in the initial PCRP parameter list, but were not able to be monitored during this study because of method development limitations and/or standard availability. Numerous iodinated DBPs have also been identified in chlorinated and chloraminated drinking water however they are not yet widely measured or regulated. Some iodinated DBPs have been shown to be more toxic than their brominated and chlorinated equivalents (Richardson *et al*., 2007).

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6.4 *N*-Nitrosamines

Introduction

The *N*-nitrosamines are a class of disinfection by-products (DBPs), recently identified, which vary in toxicity and most are probable human carcinogens. *N*-nitrosodimethylamine (NDMA) is the most extensively studied and most commonly formed *N*-nitrosamine and it has been detected as a disinfection byproduct (DBP) in drinking water and wastewater (Zhao *et al.*, 2006, Charrois & Hrudey, 2007). Concentrations of NDMA in drinking-water up to 40 ng/L have been measured, although lower concentrations are more common. NDMA has been used in the production of 1,1-dimethylhydrazine for liquid rocket fuel, and has been reported in foods, beverages, drugs, and tobacco smoke and to be an air and water contaminant (NTP, 2004). NDMA is classified as a probable human carcinogen by the IARC, with a strong likelihood that the mode of action for the induction of tumours involves direct interaction with genetic material (WHO, 2006).

N-nitrosamines form by the reaction of precursors in wastewater with free chlorine in the presence of ammonia and also with monochloramine (Choi & Valentine, 2002, Schreiber & Mitch, 2006). High concentrations of NDMA can form during wastewater chlorination, and therefore the intentional and unintentional reuse of municipal wastewater is a particular area of concern (Mitch *et al.*, 2003). For example up to 460 ng/L of NDMA was produced in full-scale systems when wastewater effluent for irrigation was disinfected with chlorine in the presence of ammonia (Pehlivanoglu-Mantas & Sedlak, 2006). Chloramination of wastewater results in significant NDMA formation with a direct relationship observed between NDMA concentration and chloramine dose (Najm & Trussell, 2001). These mechanisms have particular significance for *N*-nitrosamine formation during MF/RO treatment, as chloramine is used to minimise membrane fouling. While the predominant formation pathway was proposed to be nitrosation of secondary amines (e.g. dimethylamine (DMA) in the case of NDMA) (Mitch *et al.*, 2003), alternate mechanisms involving chloramine have been developed to explain NDMA formation in chlorinated drinking water where nitrite is absent (Mitch & Sedlak, 2002, Choi & Valentine, 2002). Enhanced NDMA formation has been demonstrated from reactions between DMA and dichloramine ($NHCl_2$) (Schreiber & Mitch, 2005) .

In addition to possible formation, *N*-nitrosamines are not typically removed during conventional wastewater treatment. NDMA is very soluble in water and is not likely to bioaccumulate or adsorb to particulate matter. It has been demonstrated that the sorption of NDMA to soils is negligible and reversible (Gunnison *et al.*, 2000). There is evidence for bioremediation of several *N*-nitrosamines in groundwater (Drewes *et al.*, 2006), although others have reported significant variability in evidence for biodegradation of NDMA (West Basin Municipal Water District, 2006).

In this report a total of nine *N*-nitrosamine compounds were analysed: *N*-nitrosodimethylamine (NDMA), *N*-nitrosoethylmethylamine (NEMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-n-propylamine (NDPA), *N*-nitrosodi-n-bultyldiamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitroso-pyrrolidine (NPYR), *N*-nitroso-morpholine (NMOR), *N*-Nitrosodiphenylamine (NDPhA). The *N*-nitrosamines have been analysed separately to the other DBPs because of their significant health risk and because additional studies were conducted during the project to better characterise their occurrence, formation, degradation/removal during the advanced treatment.

Methods

Samples (1 L) were extracted using in-house SPE cartridges packed with LiChrolut EN and Ambersorb 572 resins and concentrated to ~300 µL. Extracts were then separated with gas chromatography (GC) using a wax polyethylene glycol capillary column. Quantification was performed by mass spectrometry (MS) with electron ionization (EI), with peak identification aided by inclusion of deuterated internal standards.

The method is applicable to the determination of these compounds in both secondary treated wastewater and RO treated wastewater (post-RO water) and has been verified for the analysis of the nine *N*-nitrosamine compounds in this project. The limits of detection (LOD) and estimated uncertainties for the method are listed in Table 6.4.1.

Table 6.4.1: Health values, limits of detection (LOD) and estimation of uncertainty for *N*-nitrosamines

Analyte	Health Value (ng/L)	Source	Average LOD Event 2 and 3 (ng/L)	Average LOD Event 4 onwards (ng/L)	Standard Relative Uncertainty (10 ng/L) (%)	Standard Relative Uncertainty (50-100 ng/L) (%)
N-nitrosodimethylamine (NDMA)	10	AGWR, 2008	1.9	1.0	39.7%	21.0%
N-nitrosoethylmethylamine (NEMA)	2	IRIS, 1993	4.0	1.1	34.4%	12.8%
N-nitrosodiethylamine (NDEA)	10	AGWR, 2008	3.5	1.5	55.0%	10.6%
N-nitrosodi-n-propylamine (NDPA)	5	Cal DPH, 2007	4.0	0.9	29.4%	7.5%
N-nitrosodi-n-bultyldiamine (NDBA)	6	IRIS, 1993	10	1.0	43.9%	13.6%
N-nitrosopiperidine (NPIP)	4	OEHHA, 2008	6.0	0.6	45.8%	19.2%
N-nitroso-pyrrolidine (NPYR)	20	IRIS, 1994	4.8	0.9	32.8%	12.0%
N-Nitroso-morpholine (NMOR)	5	OEHHA, 2008	7.8	1.5	32.1%	19.4%
N-Nitrosodiphenylamine (NDPhA)	7000	IRIS, 1993	8.6	15.6	ND*	41.2%

** The relative standard uncertainty for NDPhA at 10 ng/L could not be determined because LOD was often greater than 10 ng/L*

Quality assurance/ Quality control

Limits of detection were deteremined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test. Limits of detection were poorer for Events 2 and Event 3 because only a few deuterated standards were available during these events. From Event 4 onwards, a deuterated internal standard was available for every analyte except for NEMA and this improved limit of detection, precision and accuracy.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty. Precision was significantly better when concentrations >50 ng/L and therefore 2 separate uncertainties were calculated – 1 at 10 ng/L and 1 at 50-100 ng/L.

Two proficiency tests were undertaken during the sampling period and the full details of these are available in Chapter 4. In summary, a NATA-accredited proficiency test for three *N*-nitrosamines was undertaken through Proficiency Testing Australia (PTA) in March 2008. All of Curtin's results fell within acceptance limits determined by the New York Department of Health who supplied the sample, and there was acceptable agreement with the median value determined for each analyte (as determined by z-scores less than 3, see Chapter 4). This test was limited, however, because the sample supplied had a deionised water matrix and analyte concentrations about 3 orders of magnitude greater than those measured during sampling. An additional *N*-nitrosamine inter-laboratory test was therefore organised by Curtin and undertaken during PCRP Sampling Event 6 (June 2008) for measurement of realistic concentrations in wastewater and post-RO water, with participation of Queensland Health Scientific Services, and the Australian Water Quality Research Centre (SA). There was general agreement between laboratories for unspiked wastewater and MF/RO water, although the variation in measured values highlighted the challenge to analytical laboratories required to measure *N*-nitrosamines below guideline values determined for recycled water (AGWR, 2008). The range of NDMA results reported for a sample spiked to 114-120 ng/L was very similar to the range reported in the PTA proficiency test in which NDMA concentrations were approximately one thousand times higher.

Results

A total of 648 measurements were analysed for *N*-nitrosamines. No samples were analysed during Event 1; the maximum number of samples were analysed in Event 3 (Table 6.4.2). In addition to the 6 sampling events carried out for all analytes, an extra sampling event was conducted in October 2008 for *N*-nitrosamines only. The majority of the samples were grab samples with some composite samples collected during sample Events 2 and 3. Wastewater and post-RO water characterisation is based on grab samples because analysis indicated that DBP formation continued over the course of the composite sampling period, as discussed below.

Table 6.4.2: Frequency of *N*-nitrosamine analyses by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	WW	Water Reclamation Plant							
				Grab	Comp				Before MF		After MF		After RO		Storage dam	Total
									K	B	K	B	K	B		
1	November	4	2006	0	0	0	0	0	0	0	0	0	0	0	0	0
2	May/June	6	2007	72	81	153	18	36	0	0	45	0	54	0	0	99
3	September	6	2007	126	9	135	0	0	27	36	9	9	27	27	0	135
4	January	6	2008	108	0	108	18	0	18	27	0	0	18	27	0	90
5	April	5	2008	108	0	108	0	9	18	27	0	9	18	27	0	99
6	June	5	2008	81	0	81	0	0	9	9	9	9	18	27	0	81
7	October	2	2008	54	0	54	0	0	0	18	0	18	0	18	0	54
Total		34		549	90	639	36	45	72	117	63	45	135	126	0	558

Comp, composite; GW, groundwater, WW, wastewater; MF; microfiltration, RO, reverse osmosis; K, Kwinana, B, Beenyup

Wastewater characterisation

All *N*-nitrosamines measured were detected in wastewater. The percentage of detection in wastewater ranged from 24% for NDPhA to 96% for NDMA. The highest median concentration was for NDMA (16 ng/L) which was between 1.8 and 8 times higher than the median concentration of other measured *N*-nitrosamines. NDMA was also the most commonly detected compound of the group with 24 of 25 wastewater samples above the LOD (Figure 6.4.1).

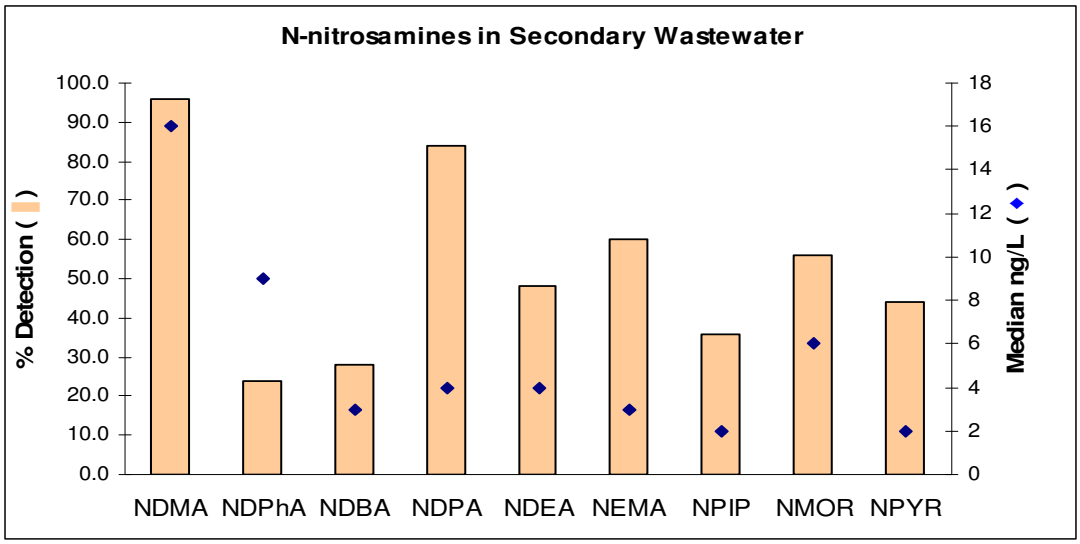

Figure 6.4.1: *N*-nitrosamines with percentage detections in secondary treated wastewater (vertical bar) and corresponding median concentrations (ng/L, diamond).

In the secondary treated wastewater, the median NDMA concentration was significantly higher at influent to KWRP (24 ng/L), compared with Beenyup WWTP (15.5 ng/L) and Subiaco (8 ng/L); K Wallis p=0.07 (Figure 6.4.2). Median NMOR and NDPhA concentrations were also highest at KWRP but the differences were not statistically significant. Median concentrations of NDBA, NDPA, NDEA, NEMA and NPYR were higher at Subiaco WWTP but, again, the differences were not statistically significant. None of the *N*-nitrosamines analysed were highest at Beenyup WWTP.

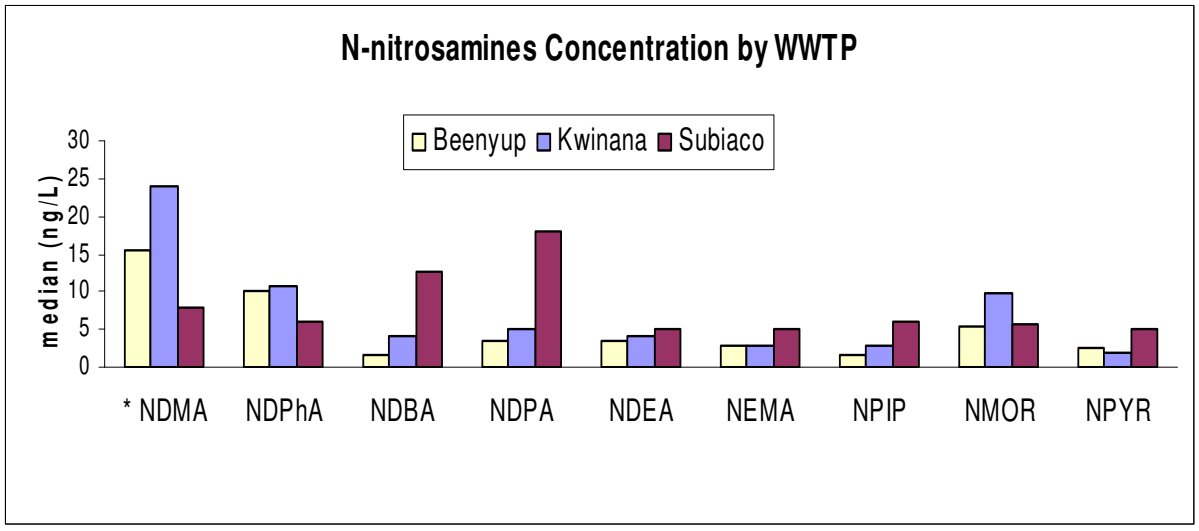

Figure 6.4.2: Median *N*-nitrosamines concentrations by WWTP (ng/L)

**N-nitrosamines with statistically significant differences in concentrations among plants.*

Significant differences were observed in the concentrations of all N-nitrosamines by season except for NDPA, NPIP and NMOR (Figure 6.4.3). NDMA and NDEA were higher in autumn while NEMA, NPIP, NMOR and NDPhA were higher in spring. While the results may be affected by the different LOD achieved in each sampling event, analysis using only analytes with more than 50% of detections showed the same results.

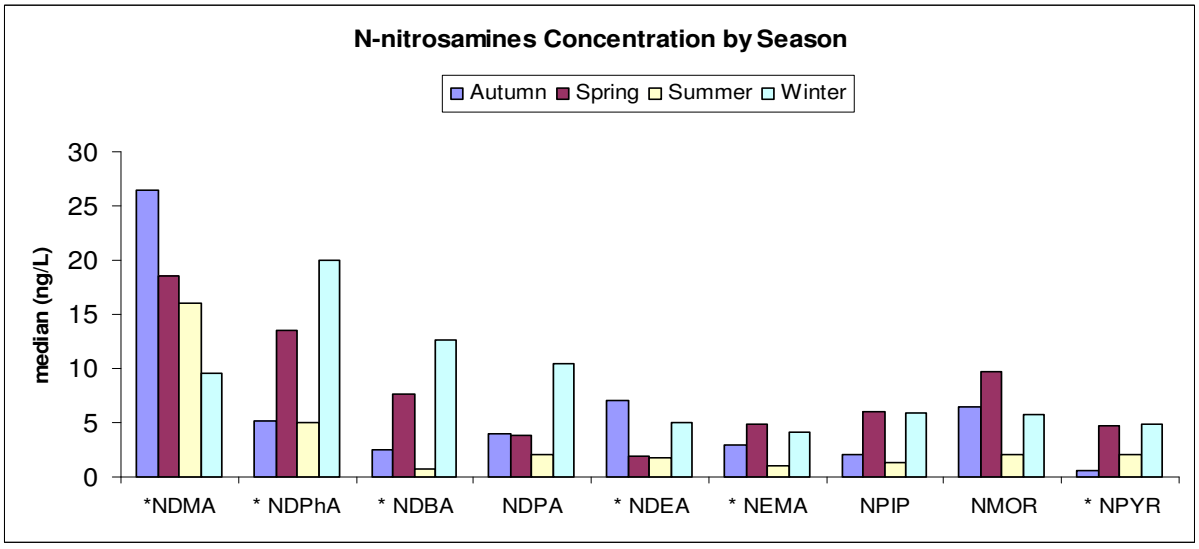

Figure 6.4.3: Median *N*-nitrosamines concentrations by season (ng/L).

**N-nitrosamines with statistically significant differences in concentrations by season.*

Post-RO water characterisation

All *N*-nitrosamines, except NEMA were also detected in post-RO water. The percentage of detections ranged from 3.8% for NMOR and NPIP to 92.3% for NDMA (Figure 6.4.4). NDPA was the second most commonly detected *N*-nitrosamine (23.1%) followed by NDEA (15.4%), NDPhA (11.5%) and NDBA (11.5%). Median concentration was highest for NDPhA (11.3 ng/L) followed by NDMA (4.5 ng/L). NDMA concentrations in post-RO water was higher at KWRP (mean=8.5 ng/L, std dev=7.8 ng/L, median=6 ng/L, max=30 ng/L) compared with BPP (mean=4.8 ng/L, std dev=2.9 ng/L, median=4.8 ng/L, max=9 ng/L) and there was also greater variability in the post-RO water at KWRP, as demonstrated by the std dev at each site.

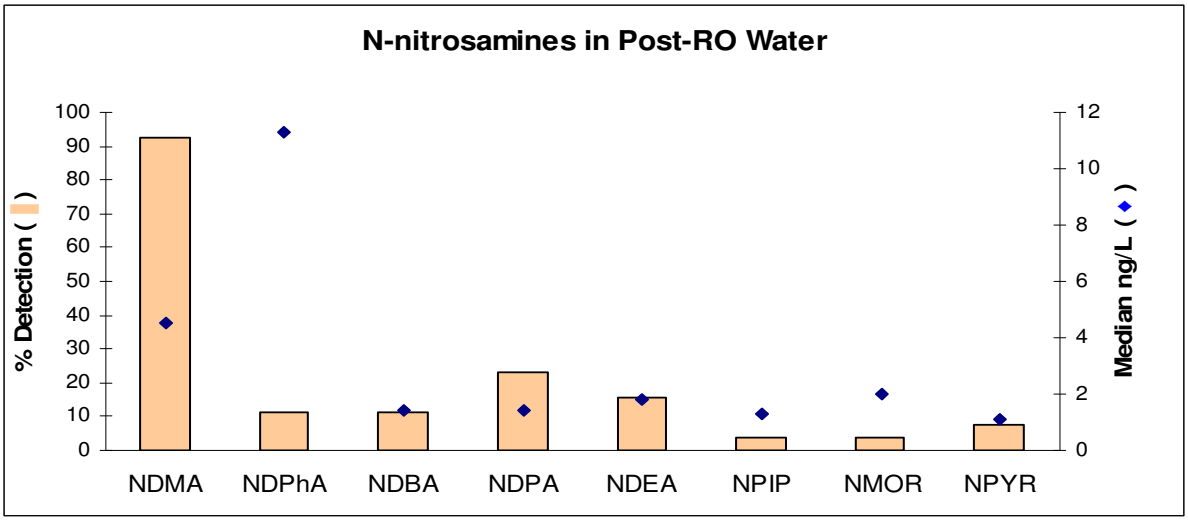

Figure 6.4.4: *N*-nitrosamines with percentage detections in post-RO water (columns) and corresponding median concentrations (diamonds, ng/L)

Groundwater characterisation

None of the *N*-nitrosamine compounds were detected in any of the bulk groundwater samples analysed.

Screening health risk assessment

Risk quotients calculated for secondary wastewater and post-RO water are shown in Table 6.4.3. RQ(max) used the maximum concentration measured for each analyte, while RQ(median) uses the median concentration measured for each analyte, including all non-detects which were reported as the LOD. Only NEMA was never detected in post-RO water and in this case to calculate the RQ(median) results below LOD were assumed equal to the median LOD. RQ(max) in wastewater was greater than 1 for all *N*-nitrosamines except for NDPhA. RQ(median) in wastewater was also greater than 1 for NDMA, NEMA and NMOR. RQ(max) in post-RO water was greater than 1 for all *N*-nitrosamines except NDPhA and NDEA, however all *N*-nitrosamine RQ(median) were below 1 for post-RO water (Table 6.4.3).

Table 6.4.3: *N*-nitrosamines detected in wastewater and post-RO water and corresponding RQs.

					Wastewater		Post-RO Water	
Parameter	**mean LOD**	**Tier**	**Health value (ng/L)**	**Source**	**RQ(median)**	**RQ(max)**	**RQ(median)**	**RQ(max)**
NDMA	1.3	1	10	AGWR, 2008	1.6	4.3	0.45	3
NDPhA	13.2	2	7000	IRIS, 1993	0.001	0.03	0.002	0.007
NDBA	4.03	2	6	IRIS, 1993	0.5	2.1	0.23	2.1
NDPA	1.88	2	5	Cal DPH, 2007	0.8	17.8	0.28	1.4
NDEA	2.12	2	10	AGWR, 2008	0.4	1.5	0.18	0.7
^NEMA	2.08	2	2	IRIS, 1993	1.5	12	0.88^	n/a
NPIP	2.43	2	4	OEHHA, 2008	0.5	2.5	0.33	1.5
NMOR	3.58	2	5	OEHHA, 2008	1.2	6.4	0.4	2.2
NPYR	2.17	2	20	IRIS, 1994	0.1	1.5	0.055	0.45

LOD, limit of detection (ng/L); RQ(max), risk quotient calculated using maximum analyte concentration; RQ(median), risk quotient calculated using median analyte concentration. Mean LOD is average from all sampling events. ^NEMA below LOD in post-RO water so RQ calculated using the median LOD and RQ(max) not applicable

Hazard quotients (HQs) were calculated for post-RO water for each sampling day at BPP (Figure 6.4.5) and KWRP (Figure 6.4.6) by adding the RQ of individual compounds. Two hazard values were calculated, HQ(detected) summed the RQ of detected compounds only, while the worst case scenario HQ(wcs) also included a contribution for undetected *N*-nitrosamines. HQ(wcs) was calculated for the 9 analysed *N*-nitrosamines by adding the RQs of detected *N*-nitrosamines in post-RO water plus the RQ based on half the LOD of each undetected *N*-nitrosamine during that day as worst-case scenario.

HQs were generally higher at KWRP and HQ(wcs) at KWRP was always above one. At BPP HQs were higher during Sampling Event 3, which was the first sampling even undertaken after commissioning the BPP. However, in general HQs calculated under both detected and worst case assumptions were close to 1.

At BPP the contribution of NDMA RQ to the HQ(detected) ranged from 16% to 100% while for HQ(wcs) ranged from 11% to 60%. At KWRP NDMA RQ contribution to the HQ(detected) ranged from 14% to 100% while for HQ(wcs) ranged from 4.6% to 72.5% indicating that the NDMA RQ does not provide a good estimate for the total effect of all *N*-nitrosamines.

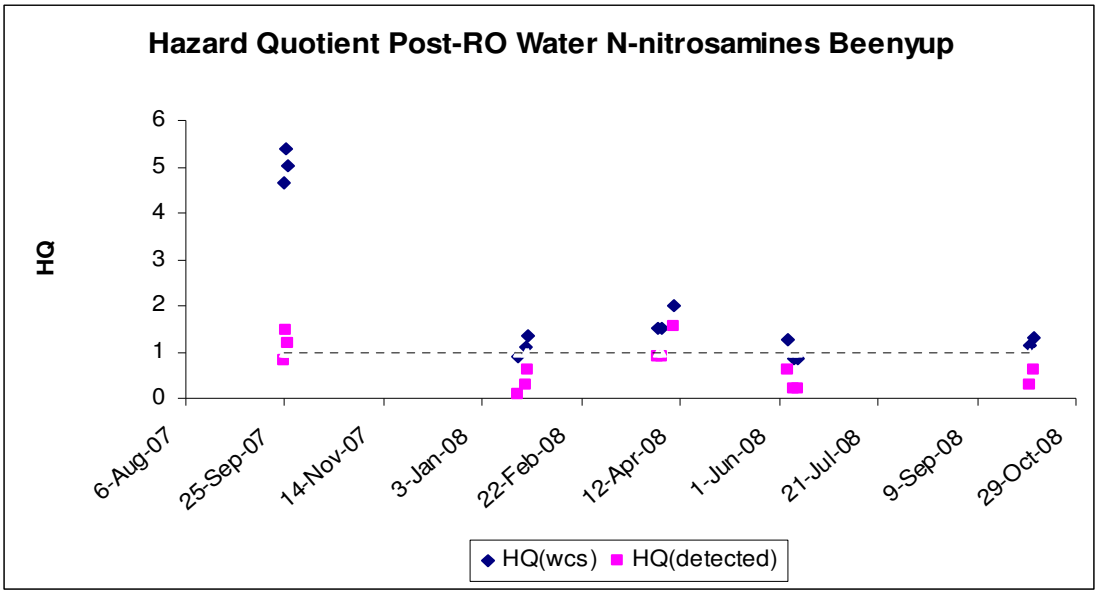

Figure 6.4.5: *N*-nitrosamines hazard quotients in post-RO water at BPP. HQ(wcs) is a 'worst case scenario' that used half the LOD for undetected *N*-nitrosamines. HQ(detected) summed only the RQ of detected compounds.

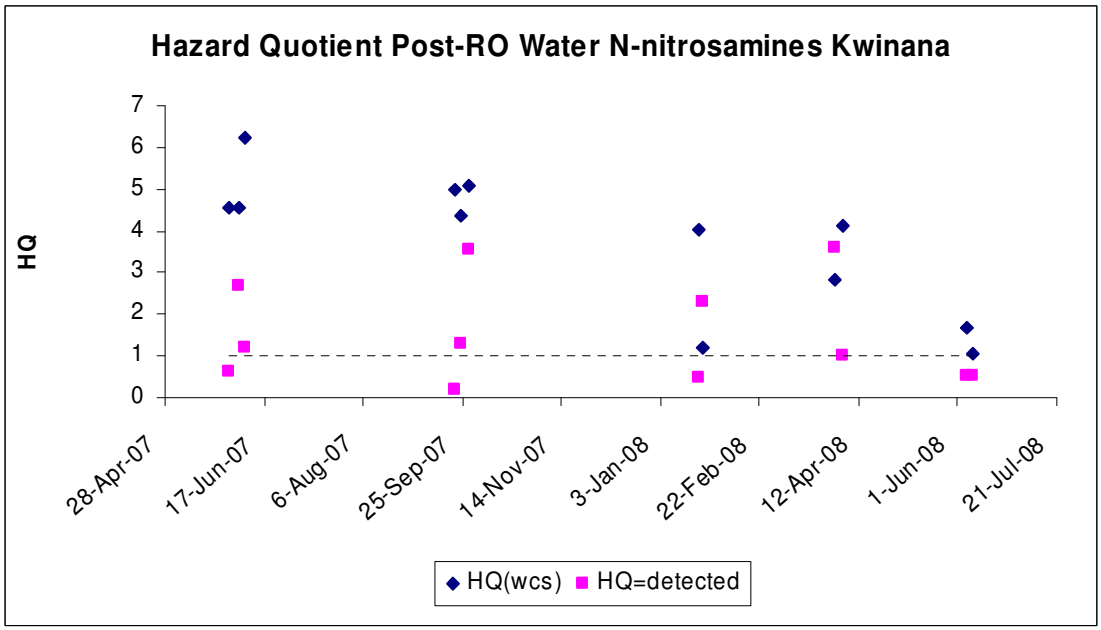

Figure 6.4.6: *N*-nitrosamines hazard quotients in post-RO water at KWRP. HQ(wcs) is a 'worst case scenario' that used half the LOD for undetected *N*-nitrosamines. HQ(detected) summed only the RQ of detected compounds

The effect of MF/RO treatment on N-nitrosamine formation

In both the BPP and KWRP, wastewater undergoes chloramination before MF to minimise RO membrane fouling. Chlorine dose is controlled at a target pH of about 6 so that chlorine reacts with ammonia present in influent water to form

monochloramine. Given the large volume of literature demonstrating *N*-nitrosamine formation through chloramination, a small number of post-MF samples were collected from within both plants in addition to the normal secondary wastewater and post-RO samples. Grab and 24 hour composite samples were also taken for all sample points, although more grab samples were taken.

Figure 6.4.7 presents average concentrations of all secondary wastewater, post-MF and post-RO samples collected during the project (note different scales). While there was a high degree of variation, the highest *N*-nitrosamine concentrations were measured post-MF, attributed to formation within the MF/RO plant after chloramination.

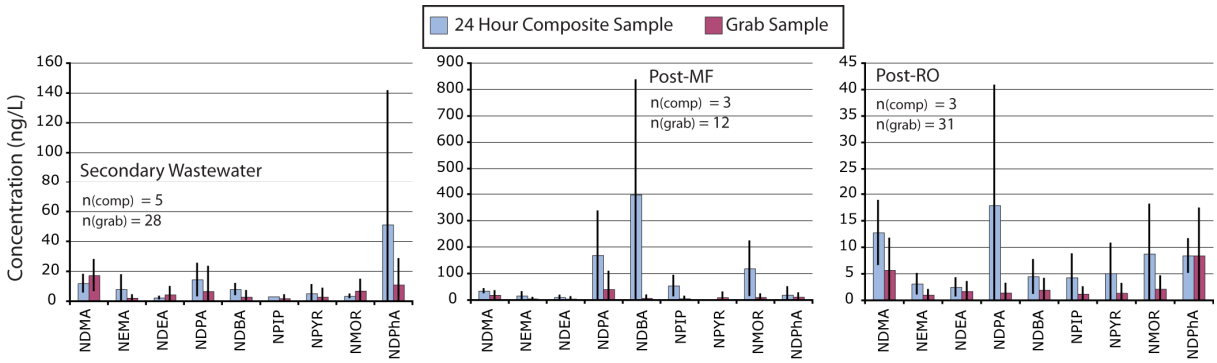

Figure 6.4.7: Concentrations of *N*-nitrosamines in secondary wastewater, post-MF water and post-RO water, averaged over all collected grab or composite samples.

NB different concentration axis scales.

Error bars represent standard deviation. n(comp) and n(grab) = the total number of composite or grab samples for each water type.

Comparison of composite sample and grab sample data indicates that on average, composite samples are higher in concentration than corresponding grab samples for both post-MF and post-RO sample locations. While grab samples were quenched immediately, the composite samples were collected over 24 hours and then quenched upon sub-sampling the composite sample. It is therefore possible that *N*-nitrosamine formation continued over the course of the composite sampling period. There is less difference between concentrations for grab and composite secondary wastewater samples. However, these samples have not undergone chloramination and therefore it is reasonable that there is less DBP formation during collection of composite secondary wastewater samples.

N-nitrosamine concentrations were higher in composite samples compared with grab samples in 92% of paired samples as illustrated in Figure 6.4.8. The plot also indicates that the difference between grab and composite samples tends to be higher for *N*-nitrosamines detected in higher concentrations. Four paired samples with high *N*-nitrosamine concentrations were outside the 95% confidence interval for the data. For 3 of these 4 paired samples composite concentrations were significantly higher than the grab samples, either indicating formation of *N*-nitrosamines in unpreserved samples or very high variability at high concentrations. Differences between grab and

composite samples were higher post-MF than post-RO water (Figure 6.4.7). All paired samples outside the 95% confidence interval were recorded at the post-MF sample point. The 3 pairs with the higher composite samples were all from the 1 post-MF sample on 7 June 2007 at KWRP, which significantly biased any further analysis.

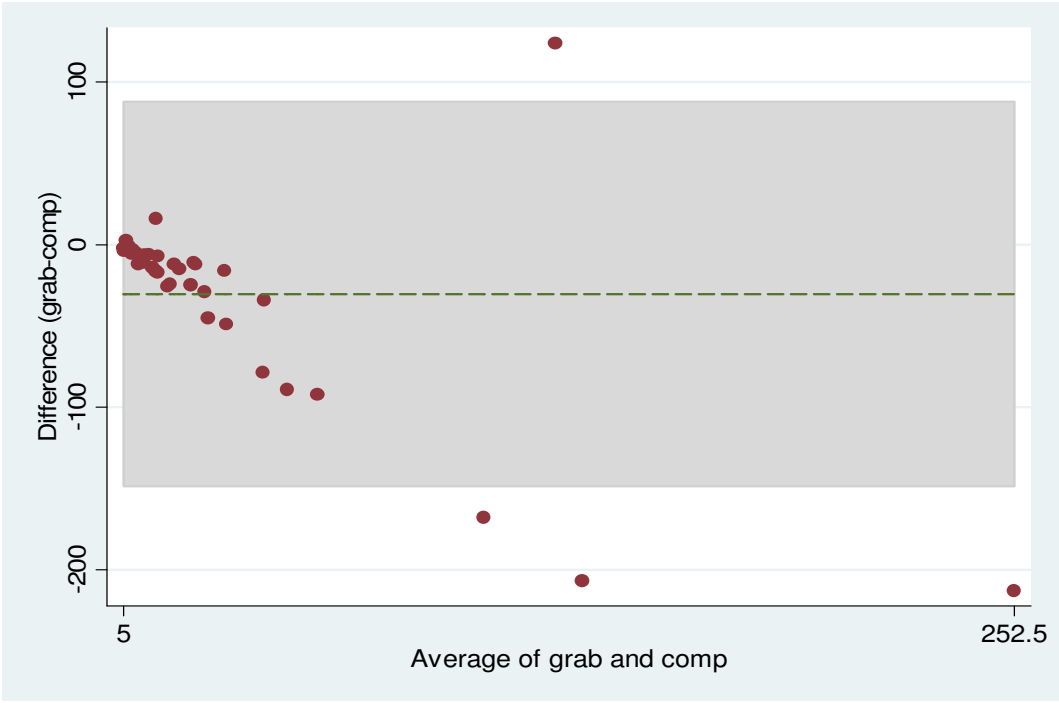

Figure 6.4.8: Bland-Altman plot comparing grab and composite (comp) samples for detected *N*-nitrosamines concentration.

Although *N*-nitrosodimethylamine (NDMA) was most frequently measured in all water types, the highest concentrations were measured for the larger *N*-nitrosamine compounds (e.g., NPROP, NPIP, NMOR and NDBA). These high concentrations occurred much less frequently, however, suggesting that specific precursors for these *N*-nitrosamines may only be present intermittently in secondary wastewater.

During sample Event 3, *N*-nitrosamine formation was investigated at both BPP and KWRP by analysing duplicate grab samples, 1 quenched immediately and the replicate quenched after 24 hours (Figure 6.4.9). Only NDMA and NPYR show consistent concentrations above detection for all samples. NDMA results from KWRP were lower in samples preserved immediately compared to those with delayed preservation, consistent with a hypothesis that chloramination causes *N*-nitrosamine formation and that it continues until quenching. The NDMA results measured at BPP were essentially identical in both samples. However, this still supports the hypothesis that chlorine (chloramine) is required for *N*-nitrosamine formation. In contrast, higher concentrations of NPYR were observed in immediately preserved samples than in samples with delayed preservation at both KWRP and BPP. More data is required to conclusively determine what is actually occurring.

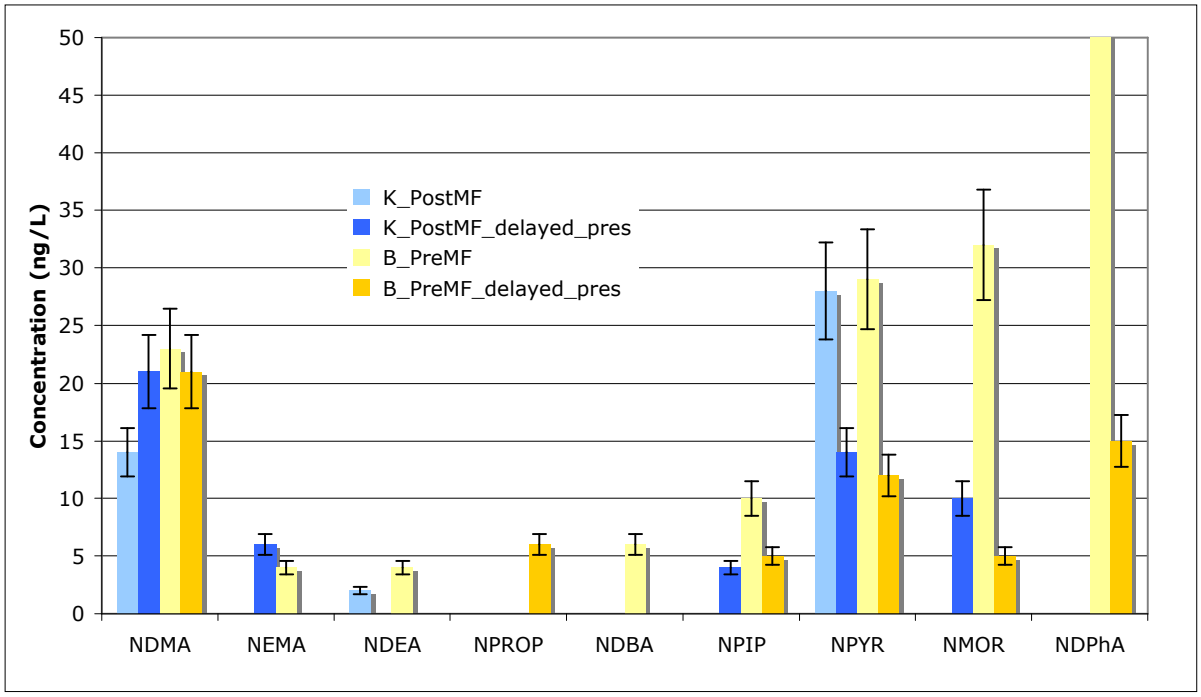

Figure 6.4.9: Comparison of pre-MF and post-MF grab samples preserved immediately and after 24 hours (delayed pres). Concentration of NDPhA in B-PreMF was 93 ng/L. K= KWRP, B= BPP.

Interpretation of results from these quenching experiments is complicated by the fact that, at KWRP, replicate samples were taken Post-MF, after chloramination, while at BPP samples were taken Pre-MF and before chloramination. Therefore, further tests on the effect of delayed preservation were undertaken during an extra *N*-nitrosamines-only sampling event in October 2008, as discussed below.

Treatment performance

Treatment efficiency was calculated for analytes detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD after RO, a conservative estimate of efficiency was calculated assuming a concentration equal to half the LOD as a worst case scenario. Average treatment performance ranged from 40.1% for NDPhA to 79.6% for NDBA (Figure 6.4.10). The mean removal percentage for NDMA was 74%, and ranged from 30% to 94%. On one occasion each, the concentration for NDPhA, NDEA and NMOR was higher after RO than in secondary wastewater. In all cases, however, the difference between wastewater and post-RO water concentrations was within the calculated expanded uncertainty of the analytical method. It is also important to note that sampling was not designed to specifically account for the

residence time in the MF/RO plant, therefore the flows sampled pre and post treatment were not necessarily identical. Temporal variation in influent water quality (of secondary treated wastewater) may have rendered the samples before and after treatment not directly comparable.

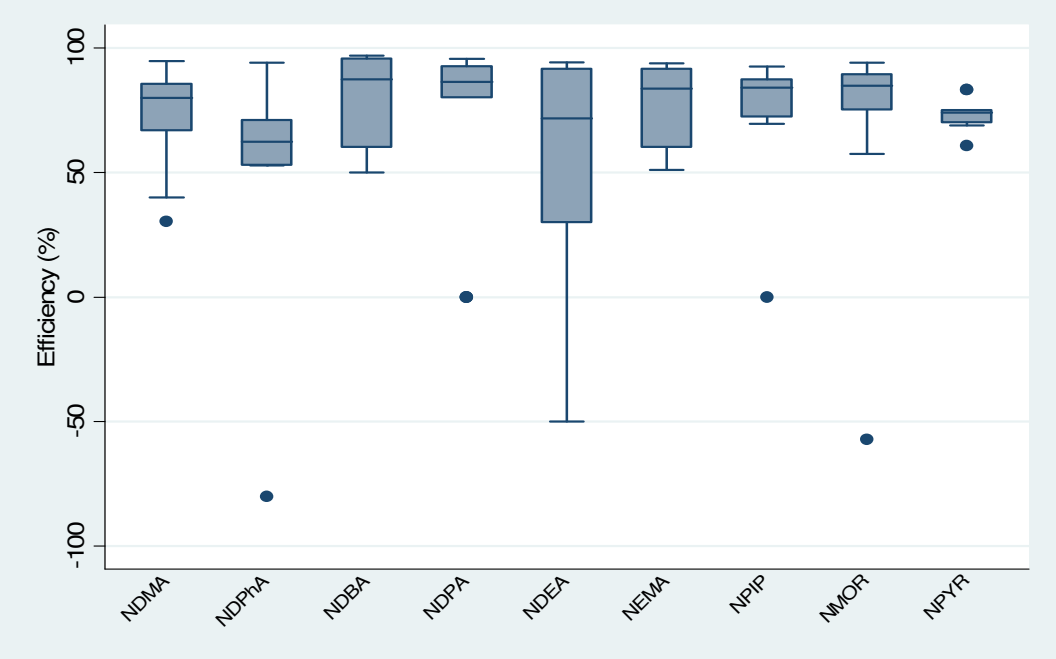

Figure 6.4.10: Microfiltration/Reverse osmosis removal efficiency of detected *N*-nitrosamines in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Given the potential for formation of *N*-nitrosamines by chloramination, as discussed previously, the treatment efficiency of RO alone cannot be determined using secondary wastewater as the pre-treatment concentration. Calculation of RO treatment efficiency for sample days for which post-MF and post-RO water data was available demonstrated that the RO treatment efficiency was higher than those calculated between secondary wastewater and post-RO water for NEMA, NDEA, NDPA, NPIP, NPYR and NMOR (see Figure 6.4.11). Calculated RO treatment efficiency for NDMA and NDBA using post-MF to post-RO water was lower than the efficiency calculated between secondary wastewater and post-RO water. Conclusive results for NDPhA could not be determined because there was only one post-MF result above LOD. Furthermore, the variability in efficiency calculated using post-MF and post-RO data (as represented by standard deviation) was lower for all *N*-nitrosamines except NPYR and NDMA despite the lower number of paired samples.

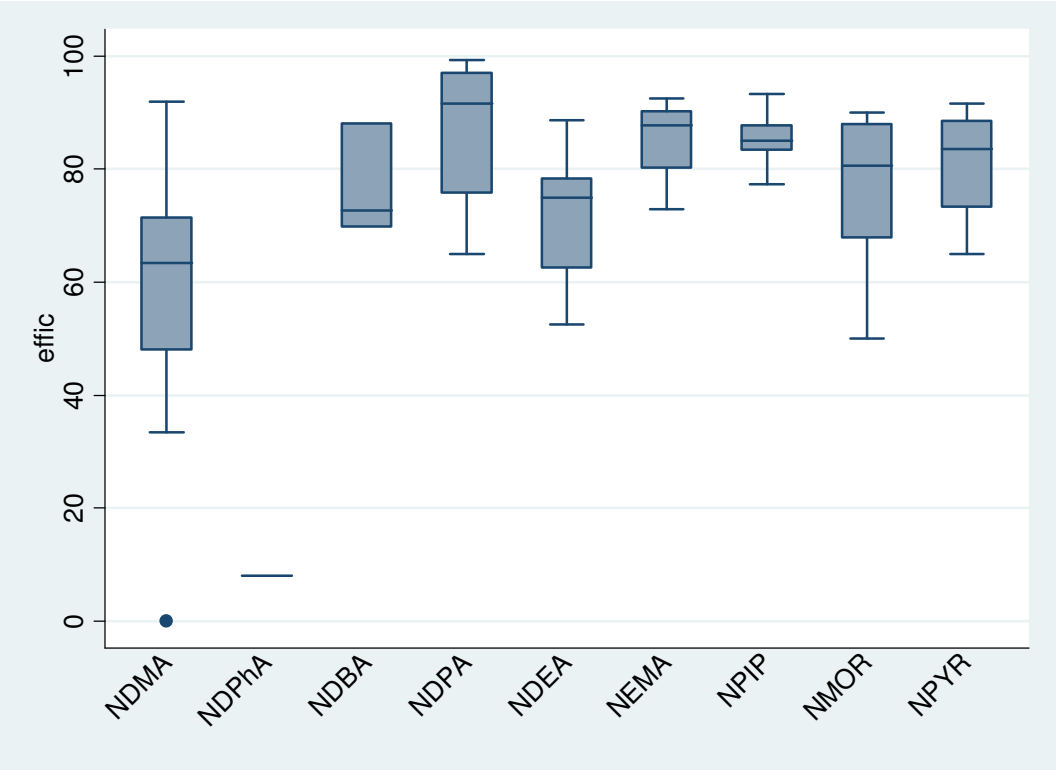

Figure 6.4.11: Reverse osmosis removal efficiency of detected *N*-nitrosamines post-MF. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Event 7: additional *N*-nitrosamine-only sampling

An additional sampling event for *N*-nitrosamines was undertaken at Beenyup WWTP and BPP on the 6th and 8th of October 2008. The aim of the additional sampling event was to monitor *N*-nitrosamine concentrations in both the Beenyup WWTP and BPP more intensively than was conducted in other PCRP sampling events. Two replicate grab samples were taken at each sampling point, as well as field and trip blanks, and additional spiked samples for quality assurance (QA) purposes. In addition, further sampling was undertaken to confirm whether longer contact time during the treatment process resulted in higher concentrations of *N*-nitrosamines by analysing samples which were quenched 2hrs and 24hrs after sampling, as well as immediately. The sampling points measured are listed in Table 6.4.4. Specific objectives included:

- Determining whether *N*-nitrosamines are present in primary treated wastewater and whether secondary treatment affects *N*-nitrosamine levels.
- Confirmation of *N*-nitrosamine formation during MF/RO
- Determining whether the apparent decrease in *N*-nitrosamines concentrations after RO treatment is from removal or degradation.

- Investigating whether NDMA is a valid indicator for other *N*-nitrosamine compounds

Table 6.4.4: Sampling points used during Event 7

Beenyup WWTP	
BWW1	Supernatant from primary sedimentation treatment (primary effluent)
BWW2	After advanced secondary treatment (biological treatment, clarification)
Beenyup Pilot RO Plant	
BSP1	Pre-Cl inlet (analogous to BWW-2)
BSP5	Post-Cl/Pre-MF
BSP6	Post-MF
BSP7	Post-RO
BSP3	Reject water from MF process
BSP4	Reject water from RO process

Results averaged over two replicates are presented in Table 6.4.5. Comparison of BWW1 and BWW2 on 6th October 2008 indicates that *N*-nitrosamines, particularly NDMA and NDPhA, are present in raw wastewater and that the WWTP treatment process does remove or transform a component of the concentration. NDPhA, in particular was very high, even in secondary wastewater.

Table 6.4.5: Results from Event 7. Results are averages of 2 replicates, ± std dev. Results marked with an asterisk denote that one replicate was <LOD and therefore the result above LOD is given without averaging

	NDMA	NEMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR	NDPhA
Sample Date: 06/10/08									
BWW1	58 ± 7.7	3.1*	<1.6	<2.5	<6.1	6.9*	7.2*	6.1*	700 ± 271
BWW2	20.4*	4.2 ± 0.04	<1.6	<2.5	11 ± 3.9	2.2*	<0.7	<5	370 ± 16
BSP1	7.4*	<2.6	2.8*	2.3 ± 0.3	<1.2	0.6 ± 0.1	<0.7	1.2 ± 0.1	<18
BSP5	6 ± 1.0	<2.6	<1.8	2.3 ± 0.1	<1.2	<0.3	<0.7	1.8 ± 0.1	<18
BSP6	11 ± 1.0	4.8 ± 0.1	<1.8	0.8 ± 0.1	1.9 ± 0.5	0.5*	1.7 ± 0.3	1.4 ± 0.01	<18
BSP7	<1.6	<2.6	<1.8	<0.4	<1.2	<0.3	<0.7	<1.1	<18
BSP3	2.8*	3.4 ± 0.6	<1.8	<0.4	<1.2	<0.3	<0.7	1.8*	<18
BSP4	50 ± 55	9.3 ± 2.8	<1.6	<2.5	10.9*	9 ± 1.9	<1	<5	11.1*
Sample Date: 08/10/08									
BWW2	37 ± 0.7	9 ± 0.7	<1.6	3.9*	<6.1	3.0*	<1	6.8*	40 ± 23
BSP1	13 ± 0.4	3 ± 0.4	<1.8	4 ± 0.6	<1.2	0.9 ± 0.03	<0.7	4 ± 0.1	<18
BSP5	8 ± 1.2	<2.6	<1.8	2 ± 0.6	<1.2	0.3 ± 0.05	1.8 ± 0.5	2.7 ± 0.1	<18
BSP6	6.7 ± 0.4	<2.6	<1.8	1.5 ± 0.3	<1.2	0.6 ± 0.4	2.1 ± 0.3	3.3 ± 0.8	<18
BSP7	2.5 ± 0.3	<2.6	<1.8	<0.4	<1.2	<0.3	<0.7	<1.1	<18
BSP3	16 ± 13	5.8*	4.8*	5 ± 3.9	<1.2	<0.3	<0.7	3.9 ± 0.4	<18
BSP4	66 ± 1.6	12.9 ± 0.4	<1.6	3.7 ± 1.1	32.5*	9 ± 1.5	<1	23.2 ± 0.5	15 ± 5.1

The high concentrations of *N*-nitrosamines in BSP4 (RO reject water) confirms that rejection by RO is the major process of *N*-nitrosamine removal during MF/RO treatment. The mass balance (incorporating flux) across RO (data not shown) suggested that further nitrosamine formation may occur in reject water, however, results were variable and further data would be required to make any firm conclusions. *N*-nitrosamines were also detected in BSP3 (MF reject water), but concentrations are not different within the standard deviation from that at BSP5 or BSP6, which suggests there is no significant removal during this step.

There is no evidence of significant post-chloramination formation of *N*-nitrosamines on either day of sampling, as evidenced by comparison of both SP5 and SP6 with SP1. This is in contrast with results averaged over the entire study (e.g. Figure 6.4.7, which includes data from Event 7). However overall average post-MF concentrations were significantly influenced by KWRP data and BPP results from Event 3, in which high *N*-nitrosamine concentrations were measured. Event 3 was the first sampling event after BPP began operation and therefore results from Event 7 may better represent normal Pilot Plant operation. Again, it should be remembered that because of residence time in the plant, the flows sampled at each sampling point may be influenced by temporal variation and therefore not necessarily directly comparable.

The effect of contact times during the treatment process was tested using duplicate samples from BSP1, BSP5, BSP6, and BSP7 that were quenched immediately, after 2 hours, or after 24 hours. Two hours is representative of contact times generally expected in MF/RO plants, while studies of NDMA formation potential have demonstrated that the majority of formation occurs within the first 20 hours (Mitch *et al.*, 2003). The results for NDMA only are shown in Figure 6.4.12. There was considerable variation between results from the two sampling days, with the only consistent pattern seen in BSP7 where 24 hour delayed preservation led to increased NDMA concentrations.

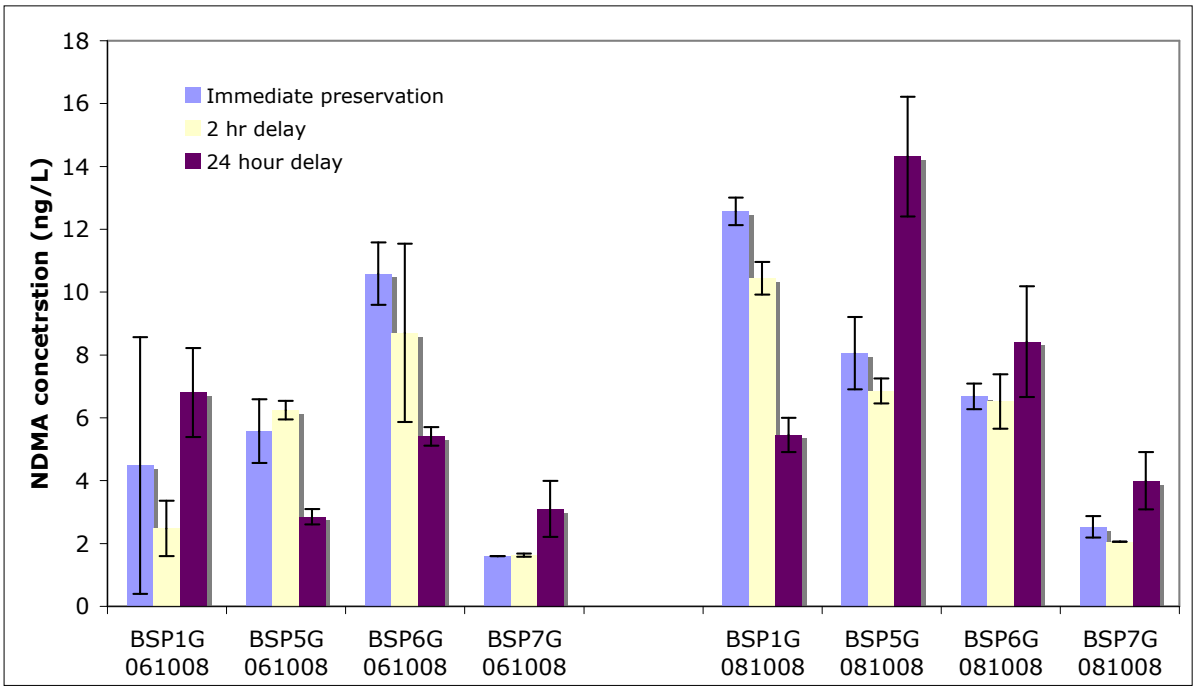

Figure 6.4.12: Effect of delaying preservation on NDMA concentration. Each bar is the average of duplicate analysis, while error bars represent std dev.

Discussion

All *N*-nitrosamines measured were detected in secondary treated wastewater and the results are consistent with other studies reporting abundant *N*-nitrosamine compounds or precursors in treated wastewater (Mitch *et al.*, 2003, Boorman, 1999, Cheng *et al.*, 2005, Pehlivanoglu-Mantas & Sedlak, 2006, Pehlivanoglu-Mantas *et al.*, 2006). The results support previous findings in which conventional wastewater treatment does not effectively remove NDMA (Kavanaugh & Sedlak, 2006). Median concentration of *N*-nitrosamines including NDMA were higher in wastewater at influent to KWRP than Beenyup WWTP, which is consistent with other studies reporting higher *N*-nitrosamine concentrations entering wastewater treatment plants in industrial areas than in residental areas (Kavanaugh & Sedlak, 2006).

The high percentage of detection in wastewater samples and high median concentrations for NDMA demonstrate that it is the best chemical indicator of the group and this is consistent with other IPR projects (OCWD, 2004, OCWD, 2006, WBMWD, 2006). However, NDMA concentrations do not predict the presence of other *N*-nitrosamines, and NDMA alone does not represent the potential toxic effects of all *N*-nitrosamines. The observation of infrequent but high concentrations of the larger *N*-nitrosamines suggests that specific precursors for these *N*-nitrosamines are only present intermittently in secondary wastewater, and from a different source to NDMA precursors.

Our results for *N*-nitrosamine rejection are consistent with other studies reporting *N*-nitrosamine rejections by RO membranes between 54% to 97% (Miyashita, 2007). While treatment efficiency calculated using post-MF samples was generally better than efficiency calculated using secondary wastewater, it is still evident that *N*-nitrosamines, in particular those of low molecular weight, are not efficiently rejected by the RO membrane and can be detected in post-RO water. NDMA remains an excellent indicator for the efficacy of RO rejection, because of the frequent detection post-RO relative to other *N*-nitrosamines.

While there is some evidence that *N*-nitrosamines form during MF/RO treatment, the difference between results from BPP immediately post-commissioning and subsequently suggest that formation is minimal after a start-up period of treatment operation. Studies by other research groups of NDMA formation potential indicate that formation potential of secondary wastewater and post-MF water is typically hundreds or thousands of ng/L (Pehlivanoglu-Mantas *et al.*, 2006, Mitch *et al.*, 2003) and, while NDMA formation potential has not been measured for Beenyup WWTP wastewater, it would appear that the current chloramination regime at BPP does not cause significant formation compared to expected formation potential. Continued formation of *N*-nitrosamines in post-RO water is also possible, and while concentrations measured after 24 hours are relatively low, this may warrant further investigation given NDMA formation potential in post-RO water has been measured at 10-50 ng/L (Mitch *et al.*, 2003).

Despite HQs greater than one calculated for both KWRP and BPP, the potential public health impact is considered low. The proportional oral intake of NDMA attributable to the consumption of drinking water relative to other exogenous sources consumed such as beer or foods, and endogenous sources (created in the digestive system) combined was predicted to be 0.02% (Fristachi & Rice, 2007) indicating that the majority of NDMA exposure for humans is not from drinking water consumption. When only exogenous sources were considered, the proportional oral intake from water was predicted to be 2.7% (Fristachi & Rice, 2007). Therefore the increased risk to human health from NDMA provided by drinking water consumption is relatively minor. Nevertheless, HQs were sometimes above 1 and therefore further study and monitoring is recommended during the groundwater replenishment trial. In particular methods of *N*-nitrosamine minimisation, such as MF/RO plant optimisation or precursor removal, may reduce post-RO *N*-nitrosamine concentrations. The potential for degradation in the local groundwater system also needs to be assessed.

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6.5 VOCs

Introduction

Volatile organic compounds (VOCs) are organic chemicals that have a relatively low boiling point (≤250 ℃ measured at a standard atmospheric pressure of 101.3 kPa) and high vapor pressure relative to its water solubility. VOCs therefore significantly vaporise and enter the atmosphere. VOCs are an important group of environmental contaminants to monitor and manage because of their widespread and long-term use. They are produced in large volumes and are associated with several products, including plastics, adhesives, paints, gasoline, fumigants, refrigerants, dry-cleaning fluids, solvents, deodorisers and fuels (Zogorski *et al.*, 2006).

The presence of VOCs in drinking water is of concern because some are potential carcinogens or have other health effects, and because they can change the taste and odour of drinking water. Some VOCs may adversely affect the liver, kidneys, spleen, and stomach, as well as the nervous, circulatory, reproductive, immune, cardiovascular, and respiratory systems. Some VOCs may affect cognitive abilities, balance, and coordination. At high levels of exposure, many VOCs can cause central nervous system depression (Brouwer *et al.*, 2005 Boyes *et al.*, 2000, Herpin *et al.*, 2009). All can be irritating upon contact with the skin, to mucous membranes of the eyes or to the mucous membranes if inhaled (Toccalino *et al.*, 2006, WHO, 2006)

VOCs have been detected in different types of water including secondary wastewater. While influent concentrations of VOCs can be 1-10 µg/L, atmospheric emissions during treatment generally lead to significantly lower dissolved concentrations in secondary wastewater (Atasoy *et al.*, 2004, Battistoni *et al.*, 2007). The aeration that occurs during wastewater treatment and during many sludge treatment processes removes most of the VOCs at the treatment plant (NRC, 1996). VOCs are also sometimes found in public drinking water supplies. Tetrachloroethylene, trichloroethene, 1,1-dichloroethene and benzene are examples of VOCs that are occasionally detected (Williams *et al.*, 2002). VOCs are also commonly detected in groundwater along with gasoline oxygenates (e.g. MTBE, see section 6.14) as a result of careless industrial practices. For example 18 of 88 VOCs were detected in 28 wells sampled in the San Diego Ground-Water Ambient Monitoring and Assessment study (Wright *et al.*, 2005). Groundwater contamination with non-aqueous phase liquids such as chlorinated solvents and petrol hydrocarbons can be difficult to remediate (Patterson *et al.,* 1993).

This section presents the characterisation of secondary wastewater and post-RO water for 57 VOCs, as listed in Table 6.5.1, as well as an assessment of the health risk associated with VOCs for augmentation of drinking water supplies with post-RO water.

Methods

All VOCs were measured by purge and trap GC-MS. Samples (40 mL) were treated with Na_2SO_4, before a 25 mL aliquot was injected into a thermal desorption purge and trap system. Volatile analytes were 'purged' by bubbling helium through the sample, and collected on an activated carbon trap. After the purging was complete, the trap was heated and the analytes released and delivered to the GC for separation using an ultra inert 5% phenyl 95% dimethylpolysiloxane capillary column. Quantification was performed by MS with electron ionisation (EI), with peak identification and calculation of recovery was aided by inclusion of surrogate standards.

Table 6.5.1: Health values, limits of detection (LOD) and estimation of uncertainty for VOCs

	Health value (µg/L)	Source	Average LOD (µg/L)	Standard Relative Uncertainty (0.5 µg/L)
1,1,1,2-Tetrachloroethane	1	IRIS, 1991	0.07	27.8%
1,1,1-Trichloroethane	200	USEPA, 2009	0.03	14.3%
1,1,2,2-Tetrachloroethane	0.2	IRIS, 1994	0.03	12.2%
1,1,2-Trichloro-1,2,2-trifluoroethane	1200	USEPA, 2009	0.03	28.2%
1,1,2-Trichloroethane	5	USEPA, 1992	0.10	31.2%
1,1-dichloroethane	5	OEHHA, 2003	0.06	12.3%
1,1-dichloroethene	30	ADWG, 2004	0.05	20.5%
1,1-dichloropropene	0.7	TTC	0.04	23.7%
1,2,3-Trichlorobenzene	0.7	TTC	0.03	52.6%
1,2,3-Trichloropropane	21	IRIS, 1990	0.06	21.7%
1,2,4-Trichlorobenzene	30	ADWG, 2004	0.03	55.3%
1,2,4-Trimethylbenzene	330	IRIS, 1991	0.04	20.8%
1,2-dibromo-3-chloropropane	1	WHO, 2006	0.05	31.3%
1,2-Dibromoethane (Ethylene Dibromide)	1	ADWG, 2004	0.06	19.1%
1,2-dichlorobenzene	1500	ADWG, 2004	0.03	17.7%
1,2-dichloroethane	3	ADWG, 2004	0.02	8.4%
1,2-dichloroethene, cis	60	ADWG, 2004	0.03	13.2%
1,2-dichloroethene, trans	60	ADWG, 2004	0.04	21.5%
1,2-Dichloropropane	40	WHO, 2006	0.02	16.4%
1,2-dichloropropene	0.7	TTC	0.03	19.1%
1,3,5-trimethylbenzene	330	OEHHA, 2001	0.09	59.0%
1,3-dichlorobenzene	0.7	TTC	0.05	20.9%
1,3-Dichloropropane	0.7	TTC	0.08	23.8%
1,3-Dichloropropene	20	WHO, 2006	0.02	20.1%
1,4-dichlorobenzene	40	ADWG, 2004	0.03	15.9%
2,2-Dichloropropane	0.7	TTC	0.19	31.9%
2-Chlorotoluene	70	IRIS, 1990	0.15	41.3%
2-propyltoluene	7	TTC	0.04	26.7%
4-Chlorotoluene	100	USEPA, 2008	0.23	83.6%
Benzene	1	ADWG, 2004	0.04	30.4%

Bromobenzene	0.7	TTC	0.12	38.3%
Bromomethane	25	IRIS, 1991	0.07	20.7%
Carbon disulfide	700	IRIS, 1990	0.04	53.4%
Carbon tetrachloride	3	ADWG, 2004	0.05	24.3%
Chlorobenzene	300	ADWG, 2004	0.03	13.2%
Chloroethane	0.7	TTC	0.03	23.2%
Chloromethane	14	USEPA OW, 1996	0.06	20.1%
Dichlorodifluoromethane	700	IRIS, 1995	0.25	21.0%
Dichloromethane	4	ADWG, 2004	0.10	32.6%
Ethyl benzene	300	ADWG, 2004	0.07	37.0%
Hexachlorobutadiene	0.7	ADWG, 2004	0.16	46.1%
Isopropyl benzene	350	IRIS, 1997	0.13	43.8%
m-xylene	600	ADWG, 2004	0.08	48.0%
Naphthalene	70	IRIS, 1998	0.04	41.7%
n-butyl benzene	7	TTC	0.05	53.7%
n-propyl benzene	7	TTC	0.18	64.2%
o-xylene	600	ADWG, 2004	0.04	18.2%
p-Isopropyltoluene	7	TTC	0.05	34.6%
p-xylene	600	ADWG, 2004	0.09	40.2%
sec-butyl benzene	7	TTC	0.03	not determined
Styrene	30	ADWG, 2004	0.12	37.4%
tert butyl benzene	7	TTC	0.07	31.7%
Tetrachloroethene	50	ADWG, 2004	0.11	27.9%
Toluene	800	ADWG, 2004	0.10	30.2%
Trichloroethene	50	ADWG, 2004	0.03	17.0%
Trichlorofluoromethane (Freon 11)	150	CalDPH, 1997	0.09	50.4%
Vinyl Chloride	0.3	ADWG, 2004	0.07	22.8%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R and quantifying and qualifying ions), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear

regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

A total of 57 VOCs were analysed in at least one sampling event. A total of 4,390 measurements were analysed for VOCs excluding field, trip and replicate samples. The distribution of sampling by event and location is presented in Table 6.5.2. All samples were grab samples and a few measurements were excluded from the statistical analysis due to clear evidence of sample contamination. Groundwater samples were taken in Events 2 and 4.

Table 6.5.2: Measurement of VOCs by event and location

Event	Month	No days	Year	Total	Location								
					GW	SWW	Water Reclamation Plant						
							Before MF		Post-MF water		Post-RO water		Storage dam	Total
							K	B	K	B	K	B	K	
1	November	4	2006	528	0	0	159	0	53	0	158	0	158	528
2	May/June	6	2007	848	106	265	159	0	159	0	159	0	0	477
3	September	6	2007	742	0	0	159	159	53	53	159	159	0	742
4	January	6	2008	784	112	0	112	224	0	0	112	224	0	672
5	April	5	2008	770	0	55	110	220	0	55	110	220	0	715
6	June	5	2008	718	0	0	159	196	0	106	106	151	0	718
Total		32		4,390	218	320	858	799	265	214	804	754	158	3,852

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

A total of 19 VOCs (34%) were detected in secondary wastewater (Figure 6.5.1). The most commonly detected VOC was 1,4-dichlorobenzene (94.6%), followed by tetrachloroethene (83.8%), carbon disulfide (80.0%) and chloromethane (59.5%). Of all the detected VOCs, 11 (58%) were detected in less than 20% of the samples analysed, indicating an inconsistent occurrence in secondary wastewater. Median concentrations for these compounds were dominated by non-detects, reported as LOD. Median concentrations for compounds with greater than 50% detection ranged from 0.81 µg/L for 1,4-dichlorobenzene to 0.02 µg/L for carbon disulphide.

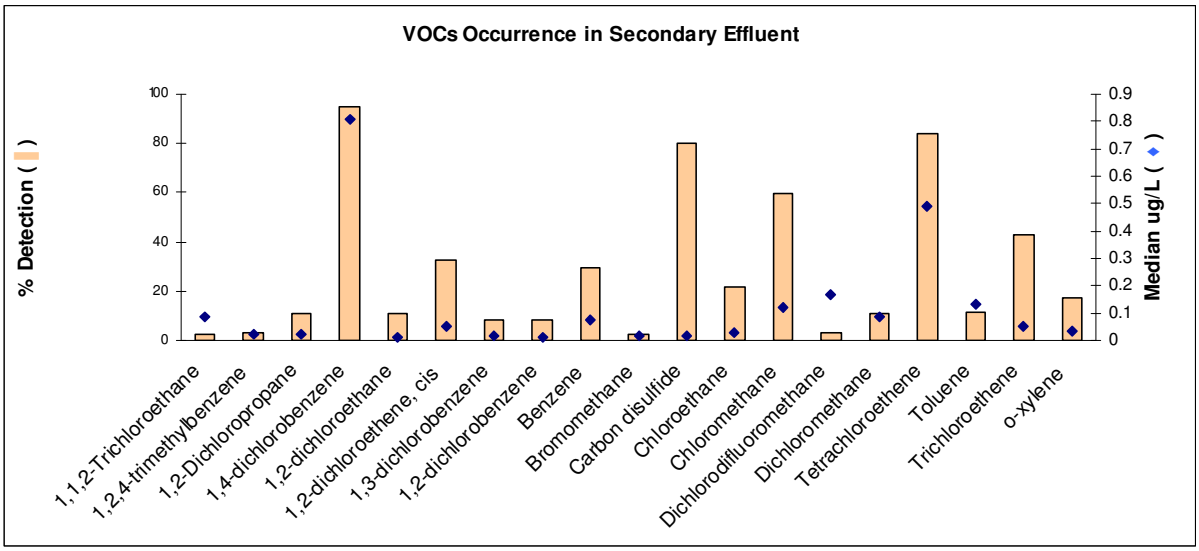


Figure 6.5.1: VOCs with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (µg/L, diamond).

Comparison of median concentrations of VOCs for Beenyup WWTP, Subiaco WWTP and KWRP influent is presented in Figure 6.5.2. Comparison is only made for 1,4-dichlorobenzene, cis-1,2-dichloroethene, carbon disulfide, chloromethane, tetrachloroethene and trichloroethene, as these compounds had percentage detections greater than 30%. For all other analytes, comparisons of median concentrations were dominated by non-detects, reported as LOD.

Overall analysis for the 6 VOCs with percentage detections greater than 30% showed significant differences in the median concentration by WWTP (K-Wallis X^2 p=0.0001). The median concentration of 1,4-dichlorobenzene was highest at Subiaco WWTP, however fewer samples were taken at Subiaco compared with the other sites and therefore there is more variability and less confidence in the results from this location. Comparison of Beenyup and KWRP shows that median concentrations of VOCs tended to be higher at KWRP. For example, the median concentration of chloromethane at KWRP was 2 times the median concentration at Beenyup (KWRP median=0.12 µg/L; Beenyup median=0.06 µg/L). The median concentration of tetrachloroethene at KWRP was 6.4 times higher than at Beenyup (KWRP median=2.4 µg/L; Beenyup median=0.37 µg/L). However, the percentage detection of each compound at each plant was similar.

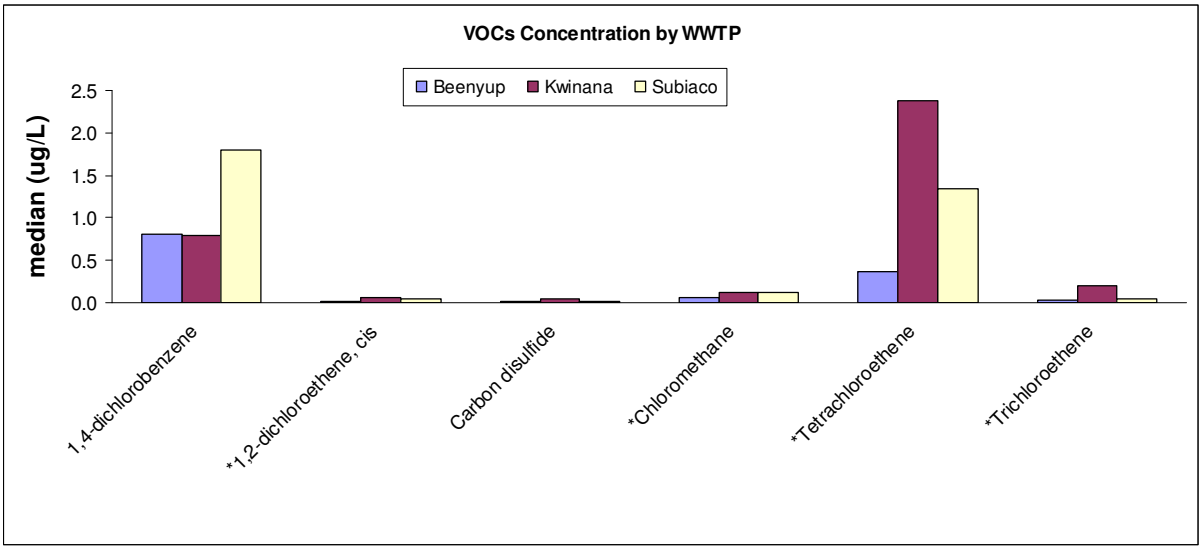


Figure 6.5.2: Median VOCs concentration by WWTP in µg/L

*VOCs with statistically significant differences in concentrations among plants.

Seasonal comparison of median VOC concentrations is presented in Figure 6.5.3. Again comparison is only made for 1,4-dichlorobenzene, cis-1,2-dichloroethene, carbon disulfide, chloromethane, tetrachloroethene and trichloroethene, as these compounds had percentage detections greater than 30%. For all other analytes, comparisons of median concentrations were dominated by non-detects, reported as LOD.

Overall median VOC concentrations were higher in spring (0.125 µg/L) and winter (0.12 µg/L) than in summer (0.025 µg/L) and autumn (0.022 µg/L), and the differences were statistically significant (K-Wallis X^2 p=0.0001). 1,4-dichlorobenzene, tetrachloroethene, and trichloroethene all had highest median concentrations in spring, while highest median concentrations for cis-1,2-dichloroethene and chloromethane were equal in spring and winter, and the highest median concentration of carbon disulphide was in winter.

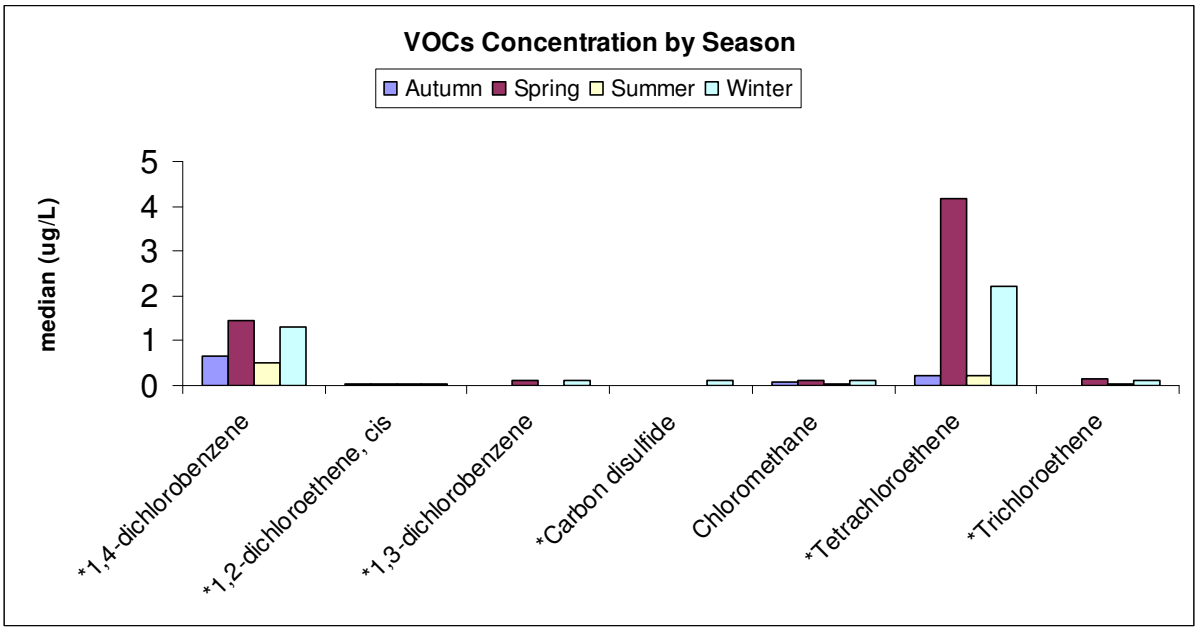


Figure 6.5.3: Median VOCs concentration by season in µg/L

* *VOCs with statistically significant differences.*

RO Product water characterisation

A total of 24 VOCs (42%) were detected in post-RO water and 16 of these VOCs were also detected in secondary wastewater. The most commonly detected VOC was 1,4-dichlorobenzene (89.7% detections) followed by chloromethane (62.1%) and carbon disulfide (47.1%), with respective median concentrations of 0.2 µg/L, 0.09 µg/L, and 0.02 µg/L.

Eight VOCs were detected in post-RO water but not in the secondary wastewater, including 1,2,3-trichlorobenzene, chlorobenzene, ethyl benzene, naphthalene, m-xylene, n-butyl benzene, p-xylene and tert butyl benzene. The percentage detection for all VOCs detected in post-RO water but not in secondary wastewater was below 10% except for p-xylene (14.3%).

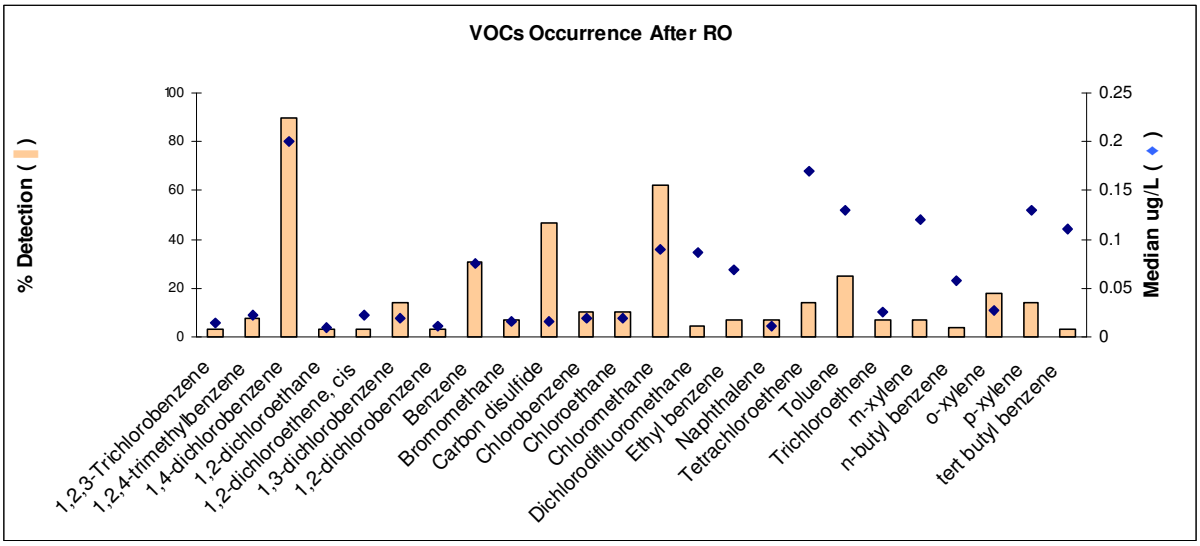


Figure 6.5.4: VOCs with percentage detections in post-RO water (vertical column) and corresponding median concentrations (µg/L, diamond).

Groundwater characterisation

Three VOCs (1,4-dichlorobenzene, benzene and toluene) were detected in groundwater. 1,4-dichlorobenzene (0.005 µg/L) was detected once in a sample taken from the Pinjar bore line during Event 4 (January 2008). A replicate sample from the Pinjar bore line and a sample from the Wanneroo bore line taken the same day were below the LOD (0.003 µg/L). Benzene was also detected above LOD (0.04 µg/L) in all groundwater samples taken during Event 4. Two replicate samples from the Pinjar bore line were 0.08 µg/L and 0.13 µg/L, while the concentration in the single Wanneroo bore line sample was 0.1 µg/L. Toluene (0.54 µg/L) was detected once in a sample from the Wanneroo bore line during Event 2. The toluene concentration of the Pinjar bore line samle taken on the same day was below LOD (0.13 µg/L).

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. Health values were derived directly from guideline values for regulated compounds and calculated from toxicity data or using the TTC approach for unregulated compounds, as described in Chapter 3.

A total of 23 VOCs were not detected in any of the samples taken and RQs were calculated using the average LOD (Table 6.5.3). For 16 of the undetected VOCs the calculated RQs were extremely low, between one and four orders of magnitude below 1. While RQs for the other 5 undetected VOCs were slightly higher, they all remained below 1, ranging between 0.11 for 1,3-dichloropropane to 0.29 for 2,2-

dichloropropane. The human health risk from these VOCs is therefore estimated to be very low.

Table 6.5.3: VOCs without detections in any of the samples and corresponding RQs

Parameter	Mean LOD	n	Tier	Health value	Source	RQ
1,1,1,2-tetrachloroethane	0.071	82	2	1	IRIS, 1991	0.07
1,1,1-trichloroethane	0.035	82	1	200	USEPA, 2009	0.0002
1,1,2,2-tetrachloroethane	0.024	82	1	0.2	IRIS, 1994	0.12
1,1,2-trichloro-1,2,2-trifluoroethane	0.029	28	1	1200	USEPA, 2009	0.00002
1,1-dichloroethane	0.056	82	2	5	OEHHA, 2003	0.01
1,1-dichloropropene	0.037	82	3	0.7	TTC	0.05
1,2,3-trichloropropane	0.056	82	2	21	IRIS, 1990	0.003
1,2,4-trichlorobenzene	0.026	82	1	30	ADWG, 2004	0.0009
1,2-dibromo-3-chloropropane	0.049	82	1	1	WHO, 2006	0.05
1,2-dichloroethene, trans	0.037	82	1	60	ADWG, 2004	0.0006
1,2-dichloropropene	0.031	72	3	0.7	TTC	0.04
1,3-dichloropropane	0.077	82	3	0.7	TTC	0.11
2,2-dichloropropane	0.204	82	3	0.7	TTC	0.29
2-chlorotoluene	0.162	82	2	70	IRIS, 1990	0.002
4-chlorotoluene	0.241	82	2	100	USEPA, 2008	0.002
carbon tetrachloride	0.045	82	1	3	ADWG, 2004	0.02
bromobenzene	0.126	82	3	0.7	TCC	0.18
ethylene dibromide	0.058	72	1	1	ADWG, 2004	0.06
hexachlorobutadiene	0.173	82	1	0.7	ADWG, 2004	0.25
isopropyl benzene	0.142	82	2	350	IRIS, 1997	0.0004
vinyl chloride	0.070	82	1	0.3	ADWG, 2004	0.23
n-propyl benzene	0.191	82	3	7	TTC	0.03
sec-butyl benzene	0.025	10	3	7	TTC	0.004

LOD, limit of detection; n, total number of samples; values in µg/L; RQ calculated using mean LOR as measured concentration post-RO water

A total of 27 VOCs were detected in either secondary wastewater or post-RO water and RQs are presented in Table 6.5.4. RQ(max) used the maximum concentration measured for each analyte, while RQ(median) uses the median concentration measured for each analyte. In secondary wastewater, both RQ(max) and RQ(median) were always below 1. While RQ(max) was only slightly below 1 for 1,3-dichorobenzene (0.95), chloroethane (0.8), and tetrachloroethene (0.64), most were between 1 and 4 orders of magnitude below 1. All RQ(median) were between 1 and 4 orders of magnitude below 1. In post-RO water, both RQ(max) and RQ(median) were again always below 1. The highest RQ(max) was for 1,3-dichlorobenzene (0.17), followed by 1,2,3-trichlorobenzene (0.15) and benzene (0.14), with all other values between 1 and 4 orders of magnitude below 1. For RQ(median), all values were between 1 and 4 orders of magnitude below 1. The results indicate that measured concentrations in the post-RO water are not of potential human-health concern.

Unlike some other classes of compounds (e.g. dioxins and PAHs) there is no common mechanism causing toxic effects for VOCs for most compounds, and

therefore the RQs cannot be summed to give a combined assessment of risk of toxicity.

Table 6.5.4: VOCs detected in secondary wastewater and/or post-RO water and corresponding RQ

Parameter	Mean LOD	Tier	Health value	Source	Wastewater			Post-RO Water		
					n	RQ(median)	RQ(max)	n	RQ(median)	RQ(max)
1,1,2-trichloroethane	0.098	1	5	USEPA, 1992	37	0.02	0.04			
1,2,4-trimethylbenzene	0.037	2	175	IRIS, 1991	32	0.0001	0.0004	25	0.0001	0.0004
1,2-dichloropropane	0.018	1	40	WHO, 2006	37	0.001	0.005			
1,2-dichlorobenzene	0.027	1	1500	ADWG, 2004	37	0.00001	0.0001			
1,2-dichloroethane	0.019	1	3	ADWG, 2004	37	0.003	0.02	29	0.003	0.02
1,2-dichloroethene, cis	0.028	1	60	ADWG, 2004	37	0.001	0.002	29	0.0004	0.001
1,3-dichlorobenzene	0.053	3	0.7	TTC	37	0.03	0.95	29	0.03	0.17
1,4-dichlorobenzene	0.032	1	40	ADWG, 2004	37	0.02	0.08	29	0.005	0.02
benzene	0.044	1	1	ADWG, 2004	37	0.08	0.11	29	0.08	0.14
bromomethane	0.234	2	25	IRIS, 1991	37	0.001	0.01	29	0.001	0.01
carbon disulfide	0.042	2	700	IRIS, 1990	20	0.00001	0.001	17	0.00002	0.0002
chloroethane	0.031	3	0.7	TTC	37	0.04	0.80	29	0.03	0.10
chloromethane	0.065	2	14	USEPA OW, 1996	37	0.01	0.04	29	0.01	0.03
dichlorodifluoromethane	0.205	2	700	IRIS, 1995	30	0.0002	0.001	24	0.0001	0.001
dichloromethane	0.092	1	4	ADWG, 2004	9	0.02	0.04	29	0.003	0.01
tetrachloroethene	0.109	1	50	ADWG, 2004	37	0.01	0.64	28	0.0002	0.002
toluene	0.103	1	800	ADWG, 2004	35	0.0002	0.0003	29	0.0004	0.001
trichloroethene	0.027	1	50	ADWG, 2004	37	0.0007	0.01	28	0.0002	0.0003
m-xylene	0.08	1	600	ADWG, 2004	35	0.0001	0.0001	28	0.00004	0.0001
1,2,3-trichlorobenzene	0.031	3	0.7	TTC				29	0.02	0.15
chlorobenzene	0.031	1	300	ADWG, 2004				29	0.0001	0.0002
ethyl benzene	0.071	1	300	ADWG, 2004				28	0.0002	0.0005
naphthalene	0.036	2	70	IRIS, 1998				29	0.0002	0.003
n-butyl benzene	0.049	3	7	TTC				28	0.01	0.01
o-xylene	0.039	1	600	ADWG, 2004				28	0.00004	0.0001
p-xylene	0.089	1	600	ADWG, 2004				28	0.0002	0.0004
tert butyl benzene	0.074	3	7	TTC				29	0.02	0.02

LOR: limit of reporting, n total number of samples, values in µg/L; EPA OW, USEPA Office of Water; IRIS, Integrated Risk Information System of the United States EPA; TTC, threshold of toxicological concern.

Seven VOCs, listed in Table 6.5.5, were not detected in either secondary wastewater or post-RO water but were detected in either post-MF water or in the KWRP storage dam. 1,1-Dichloroethene was detected once in the KWRP storage dam in Event 1 (14th December 2006). Five other VOCs (1,3,5-trimethylbenzene, 2-propyltoluene, styrene, trichlorofluoromethane and p-isopropyl toluene) were detected in KWRP post-MF samples, while 1,3-dichloropropene was detected in one BPP post-MF sample in Event 5 (1st April 2008). For these compounds RQ(maximum) was calculated using maximum measured concentrations, while RQ was calculated using

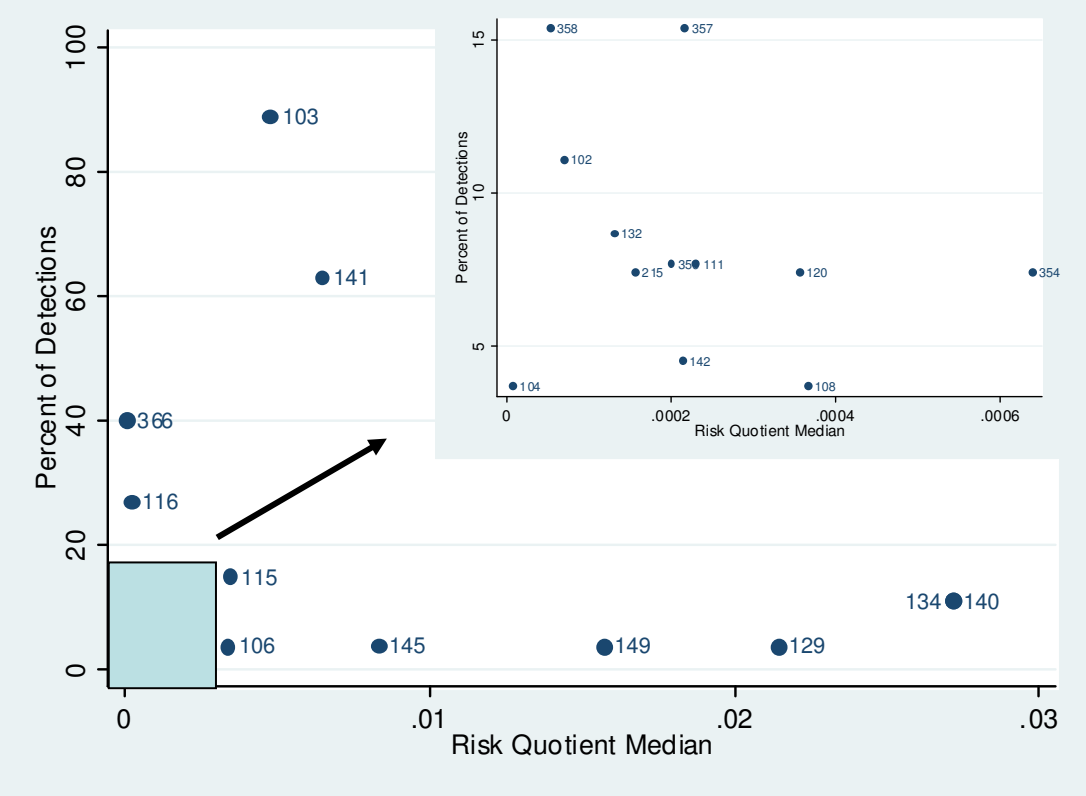
average LOD. All RQ(max) were below 1 with the highest value being 0.17 for 1,3-dichloropropene. All calculated RQs were 1 to 3 orders of magnitude below 1.

Table 6.5.5: VOCs detected only after the KWRP storage dam or post-MF water and corresponding RQ

Parameter	No of Detections		Mean				Maximum concentration		
	After MF	Storage dam	LOR	Tier	Health value	Source	Value	RQ(max)	RQ
1,1-dichloroethene		1	0.056	1	30	ADWG, 2004	0.212	0.007	0.002
1,3,5-trimethylbenzene	5		0.089	2	330	OEHHA, 2001	0.76	0.002	0.0003
1,3-Dichloropropene	1		0.075	1	20	WHO, 2006	0.03	0.02	0.004
2-propyltoluene	2		0.044	3	7	TTC	2.3	0.3	0.01
Styrene	3		0.12	1	30	ADWG, 2004	0.33	0.011	0.004
Trichlorofluoromethane (Freon 11)	1		0.088	1	150	CalDPH, 1997	0.27	0.002	0.001
p-Isopropyltoluene	2		0.054	3	7	TTC	0.15	0.02	0.01

LOR, limit of reporting; n, total number of samples; values in µg/L; RQ calculated using mean LOR as measured concentration post-RO water

The scatter of VOC percentage detections versus RQ in post-RO water is presented in Figure 6.5.5. It demonstrates that, while many compounds were detected in post-RO water, they were all of very low health significance (RQ<0.03). The percentage of detections was also normally less than 20%. Furthermore the four VOCs with percentage of detections greater than 20% all had RQ<0.01.



Number	Parameter	Number	Parameter	Number	Parameter
132	1,2,4-trimethylbenzene	*100*	benzene	*129*	1,2,3-trichlorobenzene
103	1,4-dichlorobenzene	*354*	bromomethane	*102*	chlorobenzene
106	1,2-dichloroethane	*366*	carbon disulfide	*111*	ethyl benzene
108	1,2-dichloroethene, cis	*140*	chloroethane	*215*	naphthalene
134	1,3-dichlorobenzene	*141*	chloromethane	*145*	n-butyl benzene
116	toluene	*142*	dichlorodifluoromethane	*358*	o-xylene
120	trichloroethene	*110*	dichloromethane	*357*	p-xylene
356	m-xylene	*115*	tetrachloroethene	*149*	tert butyl benzene

Figure 6.5.5: Detected VOCs in post-RO water and corresponding RQs for parameters as numbered above.

The effect of MF/RO treatment on VOC concentration

In both the BPP and KWRP, wastewater undergoes chloramination before MF to prevent RO membrane fouling. Over the course of the sampling period, a small number of post-MF samples were collected in addition to the normal secondary wastewater and post-RO samples to determine the effect of chloramination during the MF/RO process. Paired wastewater, post-MF and post-RO samples were taken on 6 occasions at KWRP (Event 1: 29th November 2006, Event 2: 30th May 2007, 4th June 2007, 7th June 2007, Event 3: 21st September 2007, and Event 6: 6th June 2008) and on 3 occasions at Beenyup (Event 3: 26th September 2007, Event 4: 1st April 2008 and Event 6: 5th June 2008). Figure 6.5.7 presents median VOC concentrations of these paired wastewater, post-MF and post-RO samples for all VOCs with at least one detection in post-MF water.

At KWRP, there were 21 analytes for which the highest median concentration was measured in a post-MF sample: chloromethane, trichlorofluoromethane (freon 11), carbon disulphide, cis-1,2-dichloroethene, trichloroethene, benzene, toluene, ethyl benzene, o-xylene, m-xylene, p-xylene, styrene, 1,3,5-trimethylbenzene, tert-butyl benzene, 1,2,4-trimethylbenzene 1,3-dichlorobenzene, 2-propyltoluene, p-isopropyl toluene, 1,2-dichlorobenzene, n-butyl benzene, and naphthalene. While the percentage detections for some of these analytes were low, 11 were present in more than 50% of post-MF samples: chloromethane (100%), cis-1,2-dichloroethene (83%), toluene (83%), ethyl benzene (100%), o-xylene (100%), m-xylene (100%), p-xylene (100%), styrene (67%), 1,3,5-trimethylbenzene (83%), 1,2,4-trimethylbenzene (100%), and naphthalene (83%).

In section 6.3, chloramination was found to increase the concentration of some disinfection by-products (DBPs) at KWRP, particularly the halomethanes. Chloromethane is considered a disinfection byproduct (Krasner *et al.*, 2006) and may also have been formed by chloramination. cis-1,2-dichloroethene is a chlorinated solvent and would not be expected to be formed by disinfection. All of the other nine VOCs frequently detected in post-MF samples are aromatic compounds associated with gasoline or diesel exhaust (Elbir *et al.*, 2007, Liu *et al.*, 2008, Watson *et al.*, 2001) or with oil refinery emissions (Chen *et al.*, 2006, Scheff & Wadden, 1993). The KWRP MF/RO plant is located on the site of an oil refinery that produces petrol, diesel, liquefied petroleum gas (LPG), aviation gasoline, jet fuel and bitumen and it is likely that trace concentrations of associated compounds would be found in water samples from KWRP. The low concentrations measured in post-RO water and field blanks compared to post-MF water suggests that this contamination did not occur during sampling, but most likely occured during the MF treatment where the water is exposed to the atmosphere for about 25 minutes. A number of the tanks also have air vents that may enable some exposure to the atmosphere both before MF (but after Panel 1) and after RO of up to an hour. However the air vents are expected to be a less significant source of exposure than the MF open tanks. Even if atmospheric exposure increased concentrations post-RO, there is carbon dioxide stripping via aeration with in situ air in a degassing tower that may have removed VOCs.

At BPP, there were only 4 VOCs for which the highest median concentration was measured in a post-MF sample (i.e. carbon disulphide (66%), toluene (100%), 1,3-dichloropropene (33%), and 1,2-dichlorobenzene (66%). These compounds are unlikely to form through chlorination or chloramination. However, they do not show the fingerprint of petroleum-based contamination either and the source of these VOCs is not obvious.

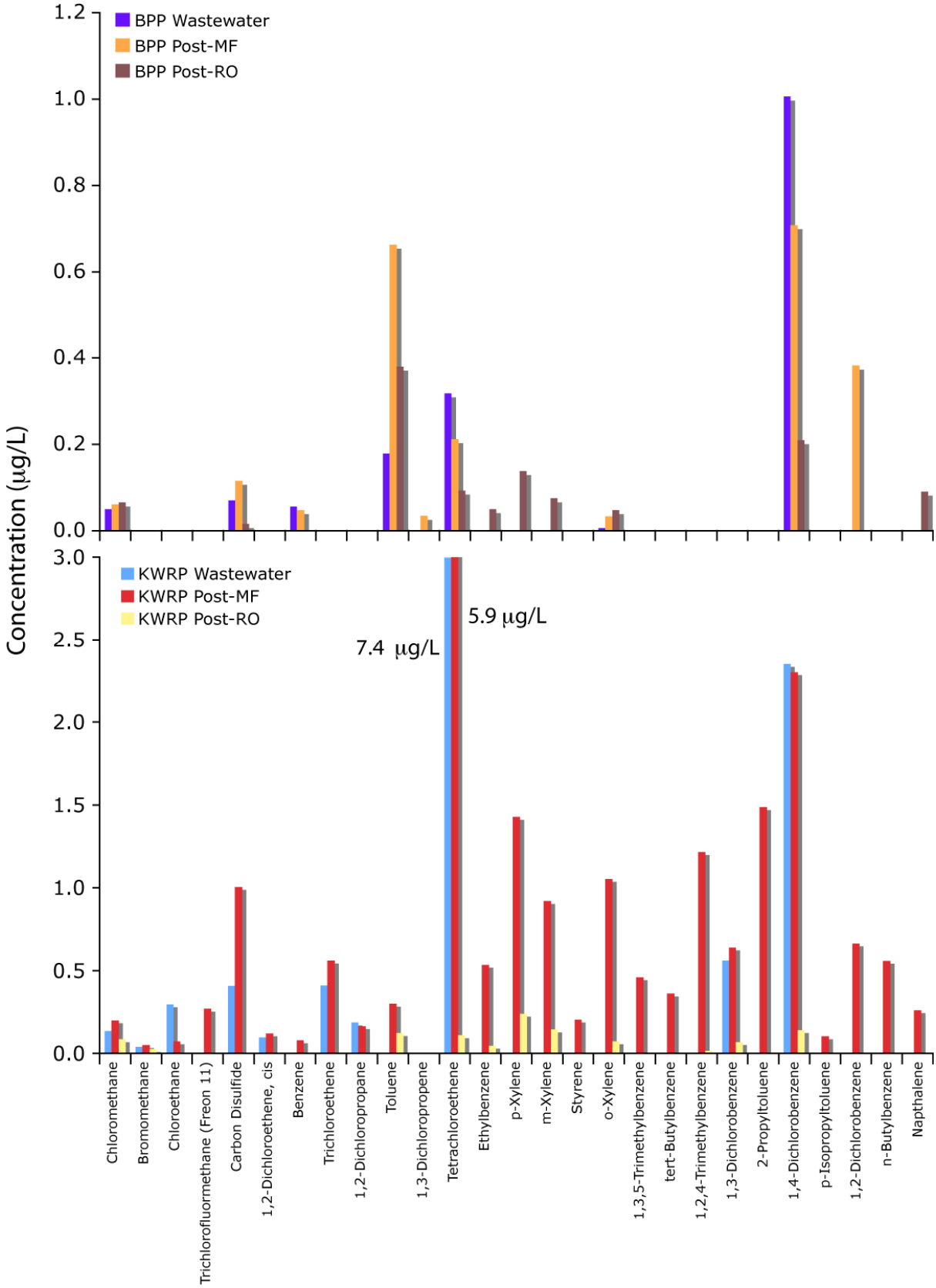


Figure 6.5.6: Median concentrations of VOCs in paired secondary wastewater, post-MF water and post-RO water samples for both KWRP (n=6) and BPP (n=3).

Treatment performance

Treatment efficiency was initially calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD. Very high variability in the removal of VOCs was observed as depicted in Figure 6.5.8. The median removal efficiency ranged from -77% for dichlorodifluoromethane to 96% for tetrachloroethene.

For 17 samples, corresponding to 6 VOCs (1,4-dichlorobenzene=1 sample, benzene=4, carbon disulfide=3, chloromethane=7, dichlorodifluoromethane=1, o-xylene=1), the concentrations in post-RO samples were higher than their paired secondary wastewater samples. For 10 (59%) of these paired samples, the concentration in post-RO water was not statistically different from the secondary wastewater, as the difference was within the uncertainty of the analytical method and therefore calculation of removal efficiency using this data is inconclusive. Differences were seen (outside of uncertainty) for carbon disulphide (1 sample), chloromethane (4 samples), dichlorodifluoromethane (1 sample), and o-xylene (1 sample). Dichlorodifluoromethane was only detected in one sample (KWRP, 29th November 2006) and calculating removal efficiency using one data point is inconclusive. As discussed above, elevated concentrations of carbon disulphide and o-xylene occured in post-MF samples and therefore the increased concentrations in post-RO samples here may be a result of contamination. Chloromethane may be forming after chloramination and was also elevated in post-MF samples.

Given the apparent increase in some VOCs in post-MF samples, the treatment efficiency of RO alone was determined using paired post-MF and post-RO samples (Figure 6.5.9). Calculations confirmed that the RO treatment efficiency was higher using paired post-MF and post-RO water samples, compared to when it was calculated using paired secondary wastewater and post-RO water samples, for all VOCs detected in secondary wastewater except for 1,1,2-trichloroethane, 1,2-dichloroethane, bromomethane, dichloromethane, cis-1,2-dichloroethene, chloroethane, and dichlorodifluoromethane. Generally treatment efficiency was not different for those compounds although it is noted that 1,1,2-trichloroethane, 1,2-dichloroethane were not detected in post-MF samples, while dichloromethane was never measured in post-MF samples. Furthermore, by using post-MF data, treatment efficiency could be calculated for 13 additional VOCs, which were measured in post-MF samples but not present in secondary wastewater. Variability in efficiency calculated using post-MF and post-RO data (as represented by standard deviation) was generally lower but remained high, although this might be related to the fewer paired samples available.

Calculation of treatment efficiency for chloromethane using paired post-MF and post-RO samples gave a median treatment efficiency of 68%, and a standard deviation of 44%. This is significantly greater removal than the treatment efficiency using secondary wastewater (median treatment efficiency=37%, std dev=87%).

Furthermore, if treatment efficiency is only calculated from samples from KWRP, median treatment efficiency improves to 79% with a standard deviation of 13%. This again suggests that chloromethane concentrations increase in KWRP but not in BPP. As described in section 6.3 (Halogenated DBPs), the time between the hypochlorite dosing point and post-MF sample point is 20 seconds in BPP and 25 minutes or longer in KWRP, depending on plant flow. It is most likely that the elevated concentrations of chloromethane seen post-MF at KWRP are related to the longer residence time in the plant after chloramination and this agrees with the trend seen for other halomethanes.

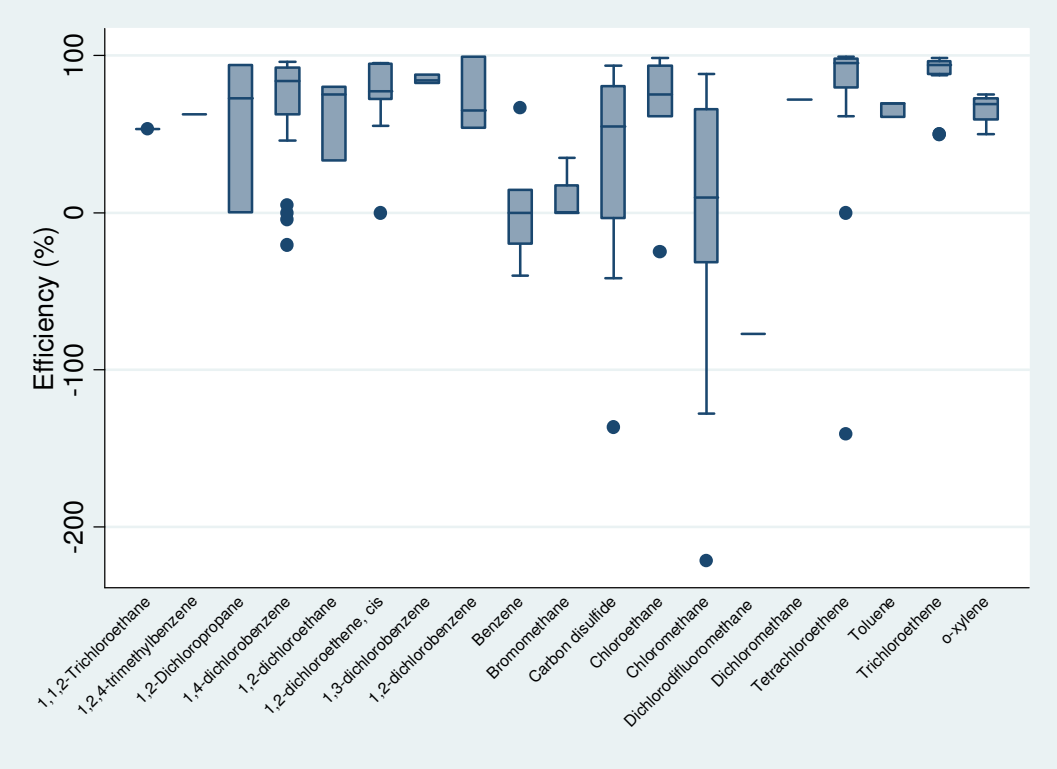


Figure 6.5.7: MF/RO removal efficiency of detected VOCs in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

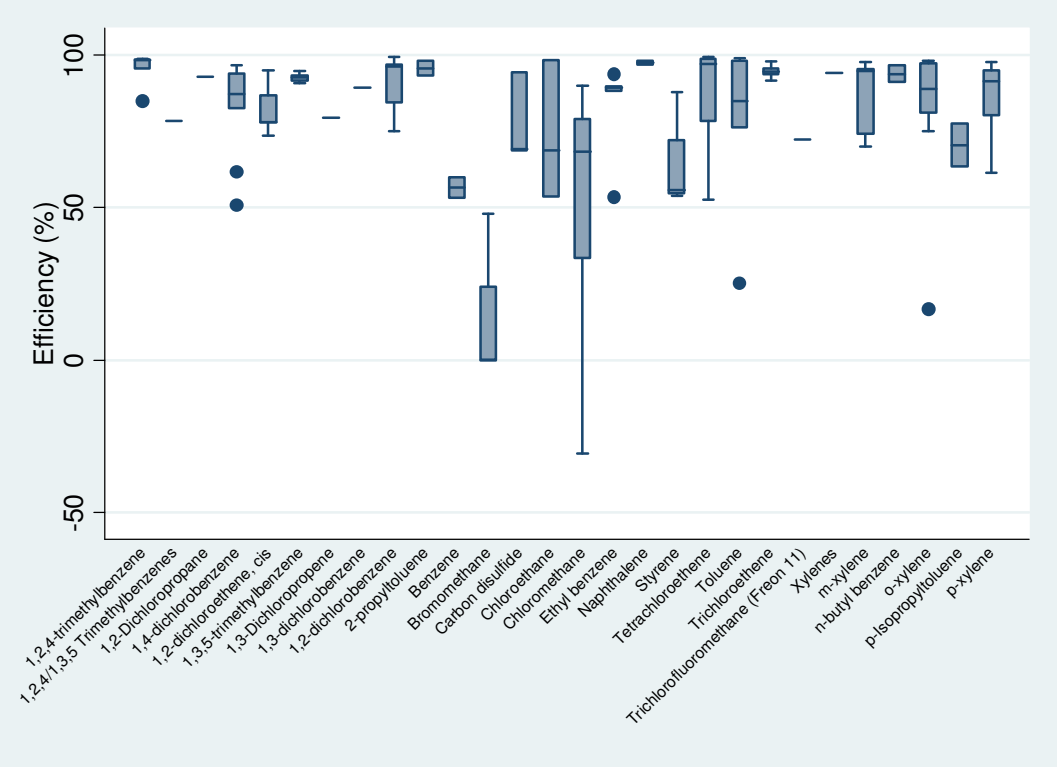


Figure 6.5.8: RO removal efficiency of detected VOCs in post-MF wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

VOCs were also analysed in the storage dam at KWRP during sampling Event 1. For all VOCs median measured concentrations were lower in storage dam samples than in post-RO samples except for chloromethane and 1,1-dichloroethene.

Discussion

A total of 19 VOCs were detected in secondary wastewater of which 1,4-dichlorobenzene was reported in 94.6% of the samples. The differences observed in the concentrations of VOCs by location may be related to the differences in water quality of the wastewater catchments. However, the significantly higher concentrations seen in wastewater influent at KWRP compared to secondary wastewater samples at Beenyup is probably related to the fact that KWRP is on the site of an oil refinery. Seasonal differences in some VOCs concentrations were also observed, with higher concentrations observed in winter and spring compared with summer and autumn. VOCs are more likely to be stable and detectable in cold water because warm temperatures can cause VOCs to volatilise (Metz *et al.*, 2007) and be more readily degraded by the activated sludge process (Martinez *et al*. 2006). This

seasonality was consistent when duplicate seasonal sampling was undertaken in summer and winter.

Three VOCs (1,4-dichlorobenzene, benzene and toluene) were detected in groundwater, although detections were just above LOD. Toluene and benzene may indicate potential VOC contamination of groundwater such as has been reported associated with landfills and leaking underground petrol storage tanks (Zogorski *et al.*, 2006). Given the limited number of samples and low concentrations detected, further investigation is required to confirm the presence of these compounds. VOCs have been frequently detected in shallow ground water beneath urban areas (up to 90% of samples) (Hamilton *et al.*, 2004). Samples taken during this study were a mixture of groundwater from shallow aquifers and deep, confined aquifers. In general, deep aquifers are less vulnerable than shallow aquifers to anthropogenic contaminants that originate on or near the land surface. Low level VOC contamination has been observed elsewhere at higher concentrations in public wells where large withdrawal rates result in greater draw on groundwater from below developed areas (Zogorski *et al.*, 2006). However all drinking water bores in Western Australia are protected by catchment protection reserves.

Twenty four VOCs were detected in post-RO water, a greater number than were detected in secondary wastewater. Eight VOCs were detected in post-RO water but not in secondary wastewater. However, seven of these eight compounds were found in highest concentration in post-MF samples at KWRP, where increases in concentration were linked to contamination from an oil refinery because the KWRP MF treatment is low pressure and occurs in tanks that are open to the atmosphere. Calculated treatment removal was variable, with some concentrations in post-RO water higher than the concentration in secondary wastewater. For some VOCs this may be due to uncertainty in the analytical method. However, for others it is attributed to industrial contamination during the MF/RO process. Contamination is not likely to be associated with antiscalant dosing that occurs immediately prior to RO. For most VOCs calculating treatment efficiency using post-MF and post-RO data produced higher values than using secondary wastewater and post-RO water. In particular, the improvement in chloromethane treatment efficiency using post-MF water at KWRP supports the hypothesis that DBPs formed during the MF/RO treatment, as described in section 6.3. More analysis of VOCs before and after RO treatment is recommended to better characterise the treatment variability.

The rejection of VOCs in a range of different RO membranes has been reported as highly variable (Agenson *et al.*, 2003). For some VOCs (1,1,1-trichloroethane, carbon tetrachloride, p-, m- and o-xylenes, tetrachloroethylene, 1,2-dichlorobenzene) rejection was higher than 90% (Agenson *et al.*, 2003), but for others rejection was much lower (e.g. 6-54% for benzene). Using paired post-MF and post-RO samples, 59% of analytes in this study had a rejection efficiency greater than 80%, while 41% had a rejection efficiency greater than 90%. Some VOCs did show lower median rejection, for example benzene (56%), bromomethane (0%) and styrene (55%). It has been found that rejection of VOCs is influenced by solute size, branching of

functional groups and K_{ow} (Agenson *et al.*, 2003). VOCs with poorer rejection usually have smaller molecular width and length, and lower K_{ow}. While VOCs are not highly hydrophobic ($\log K_{ow}<3$), K_{ow} has been found to influence rejection, which suggests that there is some degree of interaction between the solute and the membrane.

1,4-dichlorobenzene is considered the best chemical indicator of the group. It was detected in almost 95% of the secondary wastewater samples and in almost 90% of the post-RO water samples. 1,4-dichlorobenzene has a long history of domestic use in toilet deoderising products, moth repellants, and mildew control agents (Aronson *et al.*, 2007, NICNAS, 2000). Median treatment efficiency by the MF/RO treatment was 86%.

All calculated RQs were below 1 and the ingestion of water containing VOCs, even those concentrations observed in post-MF samples, is unlikely to result in adverse human health effects. While this risk assessment only takes into account exposure through ingestion, VOC exposure routes may include inhalation, and dermal uptake (van Dijk-Looijaard & van Genderen, 2000). However, VOC concentrations are very low and any potential human risk from inhalation, dermal contact and ingestion will be further minimised by the retention time of the recycled water in the aquifer. No human health risk is anticipated at the VOCs concentrations detected in the post-RO water.

Four additional VOCs, bromoethene, hexachloroethane, 1,2,3-trimethylbenzene and acrylamide, were recommended for monitoring in this project but were not tested. Acrylamide in particular is recommended for future monitoring because of its cargenogenic effects and the fact that it used in the manufacture of some RO membranes (Nasef & Hegazy, 2004).

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6.6 Phenols

Introduction

Phenols are a group of chemical compounds in which a hydroxyl group (-OH) is bonded directly to an aromatic hydrocarbon. Other alkyl or phenyl functional groups may also be bonded to the aromatic hydrocarbon, producing alkylphenols or phenylphenols. Alkylphenols are often used as intermediates in the manufacture of other chemicals, including detergents, fuel additives, polymers, and phenolic resins. Many alkylphenols have shown estrogenic activity, which tends to increase with increasing alkyl chain length (Kochukov *et al.*, 2009, Soto *et al.*, 1995), and nonylphenol and octylphenol have been studied most (Schwaiger *et al.*, 2002, Han *et al.*, 2002, European Commission, 2006, Arnold *et al.*, 1996). Nonylphenol in particular has been identified as priority hazardous substance in water because of its high abundance in the environment (European Commission, 2006, Sharma *et al.*, 2009). Nonylphenol is used in the manufacture of nonylphenol ethoxylates (NPEOs), which have been widely used in industrial, agricultural, and domestic consumer products, and is also a commonly found breakdown product of manufactured materials. Nonylphenol was identified as an endocrine disruptor after it was found to be leaching from laboratory plastic ware (Soto *et al.*, 1991), and its estrogenic activity has been confirmed by *in vitro* and *in vivo* assays and observed reproductive alterations in aquatic organisms (Schwaiger *et al.*, 2002, Han *et al.*, 2002, European Commission, 2006).

Phenols that incorporate a phenyl group include 2-phenylphenol, a commonly used disinfectant and biocide, and bisphenol A, used in the manufacture of polycarbonate plastic, as protective coatings on food containers, and for composites and sealants in dentistry. Bisphenol A was also identified as an endocrine disruptor after leaching from plastic laboratory ware (Krishnan *et al.*, 1993) and also exhibits estrogenic activity in bioassays (Han *et al.*, 2002).

Another common class of phenols are chlorophenols, often formed in drinking water as a result of the chlorination of phenols (Kim *et al.*, 1997, Onodera *et al.*, 1984), They are also used as biocides and are degradation products of phenoxy herbicides and wood preservatives (WHO, 2003b). The most commonly detected chlorophenols in drinking water are 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and all can be detected by taste or odour at 0.1-2 µg/L (WHO, 2006). Pentachlorophenol and 2,4,6 TCP are classified as possibly carcinogenic to humans by IARC 2B (IARC, 2008), and the toxic effects of chlorophenols are directly proportional to the degree of chlorination.

All described phenol classes have been detected in secondary wastewater and there is potential for human exposure through augmentation of drinking water supplies with recycled water because of their widespread use. Octylphenol, nonylphenol and bisphenol A, in particular are often measured because of their potential estrogenic

activity (Tan *et al.*, 2007, Bursch *et al.*, 2004, Sanchez-Avila *et al.*, 2009, Auriol *et al.*, 2006, Clara *et al.*, 2005b). They are well removed during wastewater treatment (Bursch *et al.*, 2004, Sanchez-Avila *et al.*, 2009, Korner *et al.*, 2000, Williams *et al.*, 2007, Clara *et al.*, 2005b), but concentrations of 10-100 ng/L remain in wastewater effluent (Auriol *et al.*, 2006, Tan *et al.*, 2007). Shorter chain alkylphenols have also been detected in wastewater effluent, but generally at lower concentrations (Rudel *et al.*, 1998, Bicchi *et al.*, 2009). Removal of phenolic compounds by WWTPs has been found to increase with increased sludge retention time (Bursch *et al.*, 2004, Clara *et al.*, 2005a, Sanchez-Avila *et al.*, 2009, Clara *et al.*, 2005b). Nonylphenols have been found in high concentrations in WWTP sludge (Pryor *et al.*, 2002), with little difference between sludge from WWTPs treating predominantly domestic waste to that with high proportions of industrial waste.

While some phenols have been found to be carcinogenic to animals (WHO, 2003a), most concern is focused on their possible activity as endocrine disruptors. The level of estrogenic activity of the industrial-sourced alkylphenols: nonylphenol, octylphenol and bisphenol A is considerably lower than natural or synthetic hormones (Williams *et al.*, 2007) and it has been concluded that alkylphenol exposure through drinking water is a minor component of total human exposure (Wenzel *et al.*, 2003, SCHER, 2008, European Commission, 2002). The percentage removal of nonylphenol, octylphenol and bisphenol A in WWTPs is similar to that of steroidal hormones, but can be higher or lower depending on the treatment process (Drewes *et al.*, 2005, Williams *et al.*, 2007).

The effects of phenols on human fertility and reproductive performance have been investigated in a number of studies, but results are equivocal (Hotchkiss *et al.*, 2008). Although there is clear evidence for the estrogenic activity of nonylphenol and octylphenol in whole animals, particularly during development, it is difficult to use the available data to determine health guidelines for humans with any confidence. Although many studies have shown associations among endocrine disrupting compounds (EDC) and human reproductive alterations, many others have not found such associations and there remains a great deal of uncertainty about the effects of background levels of EDCs on human reproduction (Hotchkiss *et al.*, 2008). More research is needed to elucidate any adverse human health effects of these chemicals.

Brominated phenols have been implicated in taste and odour problems with drinking water (Blythe, 2007), however there is very little toxicity data and limited data on environmental levels (WHO, 2005), so they were not considered in this study.

In this section, secondary wastewater and post-RO water are characterized for seven alkylphenols (4 n-nonylphenol, 4 n-octylphenol, 4 n-pentylphenol, 4 tert-butylphenol, 2-sec-butylphenol, 2,4-dimethylphenol, o-cresol), seven chlorophenols (4-chlorophenol, 2,6-dichlorophenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chloro-3-methylphenol, pentachlorophenol), as well as 2 phenylphenol and bisphenol A. The potential human health impact at the

concentrations found in secondary wastewater and in post-RO water is also evaluated.

Methods

Phenols were preconcentrated by stir bar sorptive extraction (SBSE) before GC-MS analysis. To increase sensitivity, each phenol was derivitized before preconcentration to its corresponding acetate by adding acetic anhydride and sodium carbonate to each sample (60 mL). After derivitisation, a polydimethylsiloxane (PDMS) coated stir bar was placed in each sample and analytes were sorbed onto the PDMS phase during 20 hours of constant stirring. Stir bars were then removed from the sample, dried and introduced directly into the GC using a specially modified thermal desorption inlet. Analytes were thermally desorbed from the stir bar into the GC and separated using a 60m 5% phenyl 95% dimethylpolysiloxane capillary column. Detection was performed by MS with electron ionization (EI), with peak identification and calculation of recovery was aided by inclusion of deuterated surrogate standards.

Table 6.6.1: Health values, limits of detection (LOD) and estimation of uncertainty for phenols.

Parameter	Health value (ng/L)	Source	Average LOD (ng/L)	Standard Relative Uncertainty (%) (50 ng/L)
2 phenylphenol	1000000	WHO, 2006	54	68.2%
4-chlorophenol	10000	RIVM, 2001	12.5	32.8%
2,6-dichlorophenol	10000	RIVM, 2001	8.8	28.4%
2-chlorophenol	300000	ADWG, 2004	16.3	26.5%
2,4-dichlorophenol	200000	ADWG, 2004	8.3	32.6%
2,4,6-trichlorophenol	20000	ADWG, 2004	32	41.0%
Pentachlorophenol	10000	ADWG, 2004	19.5	27.1%
4 n-nonylphenol	50000	EC, 2002	29.3	33.8%
4 n-octylphenol	50000	OECD 1995	38	65.1%
4 n-pentylphenol	7000	TTC	9.8	37.2%
4 tert-butylphenol	2500000	SCHER 2008	5.5	29.0%
bisphenol A	175000	IRIS, 1993	13.3	38.1%
2,4-dimethylphenol	70000	IRIS, 1990	2	Not determined
2-sec-butylphenol	7000	TTC	9.5	40.7%
o-cresol	175000	IRIS, 1990	26.8	44.8%
4-chloro-3-methylphenol	350	TTC	6.8	21.6%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R and quantifying and qualifying

ions), limit of detection, and in-house reproducibility. Additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

Phenols were analysed from Event 3 onwards, and the distribution of sampling by event and location is presented in Table 6.6.2. A total of 651 measurements, corresponding to 16 analytes were analysed for phenols after excluding field blanks, trip blanks, and replicates. Two analytes, 2 phenylphenol and 2,4-dimethylphenol, were only measured in Event 3 (6 wastewater samples and 6 post-RO samples). For all other phenols a total of 22 wastewater samples and 20 post-RO samples from Events 3 to 6 were collected. Grab samples were analysed in Event 3, while composite samples were analysed in Events 4, 5 and 6. Groundwater samples were analysed in Event 4 and Subiaco WWTP samples were analysed in Event 5.

Table 6.6.2: Frequency of Phenols analyses by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	WW	Water Reclamation Plant							
				Grab	Comp	Total			Before MF		After MF		After RO		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006	0	0	0	0	0	0	0	0	0	0	0	0	0
2	May/June	6	2007	0	0	0	0	0	0	0	0	0	0	0	0	0
3	September	6	2007	207	0	207	0	0	48	48	15	0	48	48	0	207
4	January	6	2008	28	140	168	28	0	28	42	0	0	28	42	0	140
5	April	5	2008	0	166	166	0	14	28	42	0	14	26	42	0	304
6	June	5	2008	26	126	152	0	0	28	42	13	13	28	28	0	152
Total		32		261	432	693	28	14	132	174	28	27	130	160	0	651

Comp, composite; GW, groundwater, WW, wastewater; MF; microfiltration, RO, reverse osmosis; K, Kwinana, B, Beenyup

Wastewater characterisation

Of 16 phenols measured, 11 (69%) were detected in secondary wastewater (Figure 6.6.1). The percentage of detection ranged from 5% for 4-n-pentylphenol and 4-nonylphenol to 73% for 4-tert-butylphenol. The highest median concentration was for 2-phenylphenol (69.5 ng/L) while the lowest median concentration was for 2,4-dimethylphenol (2 ng/L).

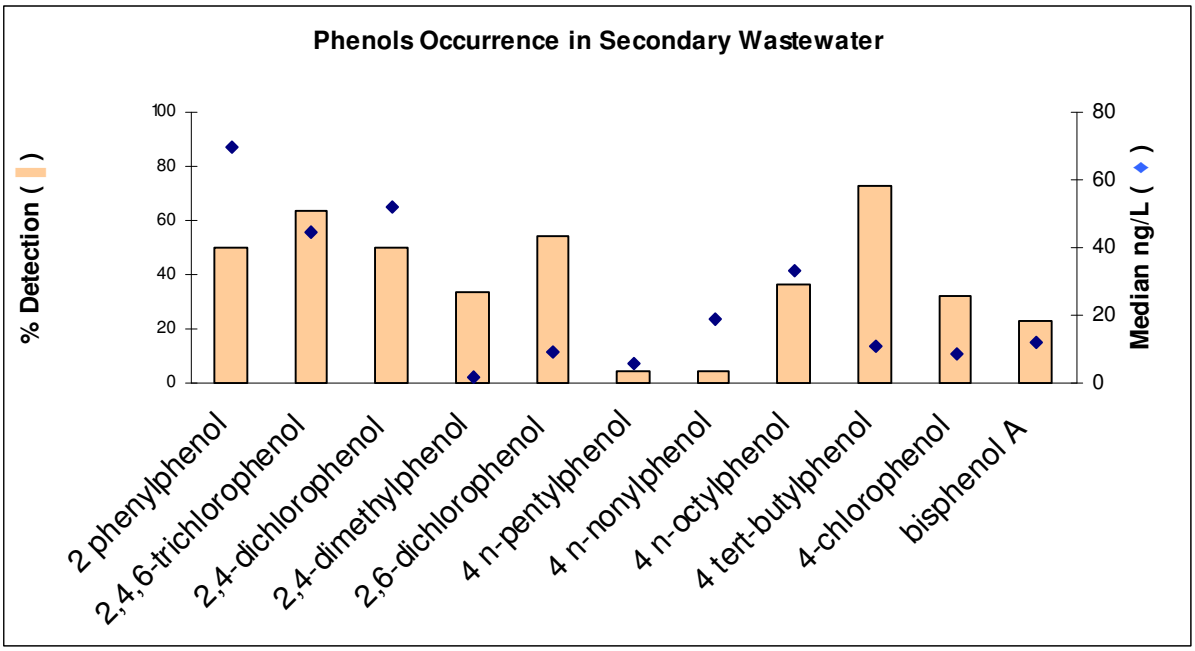

Figure 6.6.1: Phenols with percentage detections in secondary treated wastewater (vertical bar) and corresponding median concentrations (ng/L, diamond).

Given that only one sample was analysed for Subiaco WWTP, comparison was made of secondary wastewater median concentrations at Beenyup WWTP and KWRP influent only (Figure 6.6.2). In the secondary treated wastewater, the median concentration of 2 phenylphenol, 2,4,6-trichlorophenol and 2,6-dichlorophenol were significantly higher at KWRP, compared with Beenyup WWTP (Wilcoxon-Mann-Whitney p=0.04, p=0.002 and p=0.001 respectively). Median concentrations for 2,4-dichlorophenol and 4 tert-butylphenol were also highest at KWRP but the differences were not statistically significant. Median concentrations of 4 n-octylphenol, 4-chlorophenol and bisphenol A were higher at Beenyup WWTP but the differences were not statistically significant.

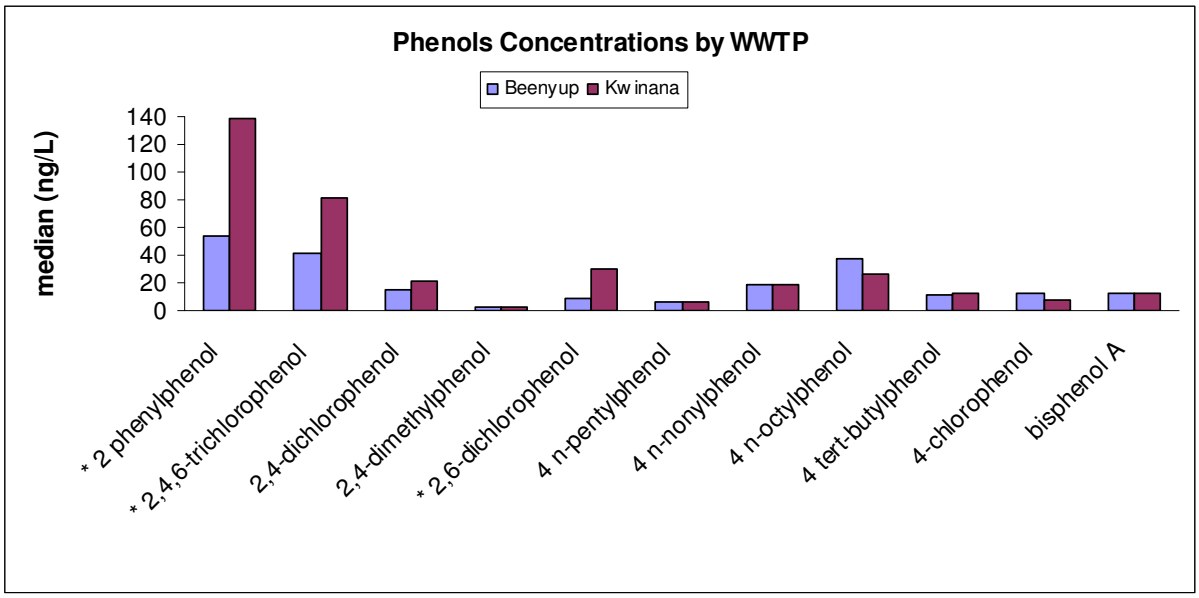

Figure 6.6.2: Median phenol concentrations by WWTP (ng/L)

* *Phenols with statistically significant differences in concentrations among plants.*

Comparison of seasonal trends is only made for 5 analytes with greater than 30% detection (Figure 6.6.3), which were 2,4,6-trichlorophenol, 2,6-dichlorophenol, 4 n-octylphenol, 2,4-dichlorophenol and 4 tert-butylphenol. Median concentrations for all other analytes were dominated by non-detects, reported as LOD. The chemicals 2 phenylphenol and 2,4-dimethylphenol are also not included because they were only analysed in Event 3.

The median concentrations of both 2,4,6-trichlorophenol and 2,6-dichlorophenol were highest in spring, however the difference was not significant. While the median concentration of 4 n-octylphenol was significantly higher in spring, this was caused by the high LOD in Event 3 compared to other events, rather than a true seasonal difference. Median concentrations of both 2,4-dichlorophenol and 4 tert-butylphenol were highest in winter, and the differences were statistically significant.

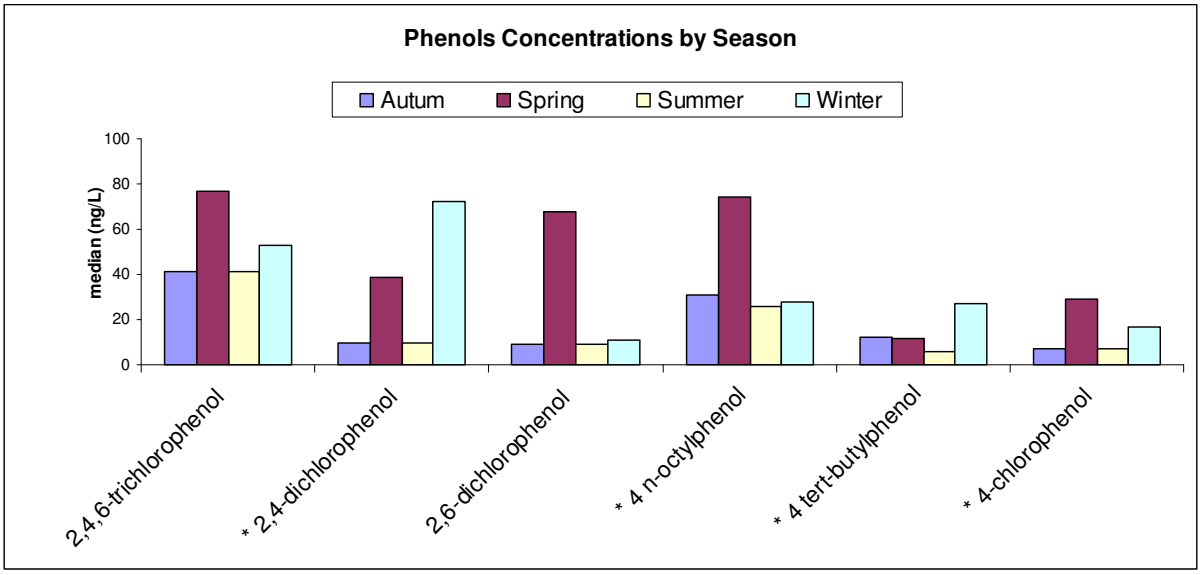

Figure 6.6.3: Median phenols concentrations in secondary wastewater by season (ng/L).

** Phenols with statistically significant differences in concentrations by season.*

Post-RO water characterisation

A total of eight phenols (50%) were detected in post-RO water (Figure 6.6.4). Six of the detected phenols were also detected in secondary wastewater while two phenols (4-chloro-3-methylphenol and o-cresol) were not. Only 2,4-dichlorophenol (48%, median concentration=13 ng/L) and bisphenol A (32%, median concentration=12 ng/L) were detected in greater than 30% of samples. The percentage of detections for other phenols ranged from 5% for o-cresol and 4-chloro-3-methylphenol to 17% for 2-phenylphenol. The median value of less frequently detected phenols was an LOD.

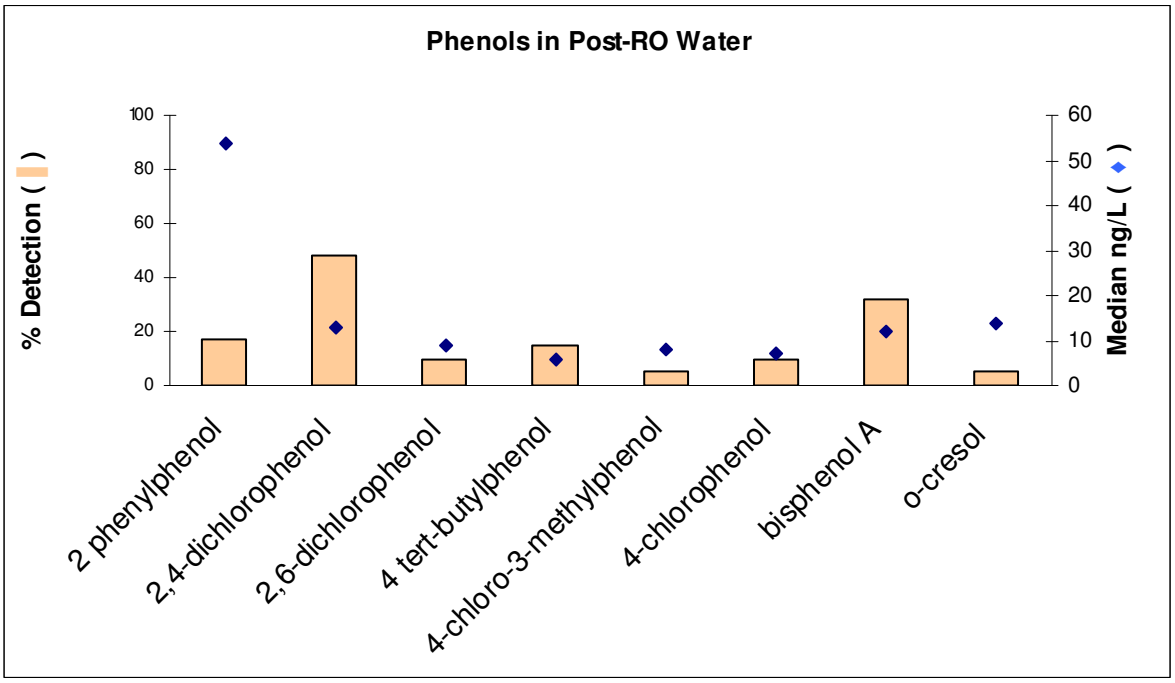

Figure 6.6.4: Phenols with percentage detections in post-RO water (columns) and corresponding median concentrations (diamonds, ng/L)

Groundwater characterisation

None of the phenols investigated were detected in any of the bulk groundwater samples analysed in Event 4.

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. For those phenols that were not detected, the RQ(median) was calculated using the average LOD as the observed concentration. Table 6.6.3 presents the RQs for phenols in secondary wastewater and post-RO water. All RQ values were 1 to 5 orders of magnitude below 1, indicating very low health risk.

Table 6.6.3: Phenols detected in wastewater and post-RO water and corresponding RQs.

					Wastewater		Post-RO Water	
Parameter	**Mean LOD (ng/L)**	**Tier**	**Health value (ng/L)**	**Source**	**RQ(median)**	**RQ(max)**	**RQ(median)**	**RQ(max)**
2-phenylphenol	54	1	1000000	WHO, 2006	0.00007	0.0002	0.00005	0.000081
4-chlorophenol	12.5	2	10000	RIVM(2001)	0.0009	0.003	0.0007	0.003
2,6-dichlorophenol	8.8	2	10000	RIVM(2001)	0.001	0.01	0.0009	0.002
2-chlorophenol*	16.3	1	300000	ADWG, 2004	0.00005	n/a	0.00005	n/a
2,4-dichlorophenol	8.3	1	200000	ADWG, 2004	0.00008	0.001	0.00005	0.0003
2,4,6-trichlorophenol	32	1	20000	ADWG, 2004	0.002	0.006	0.002	n/a
pentachlorophenol*	19.5	1	10000	ADWG, 2004	0.0019	n/a	0.0019	n/a
4-nonylphenol	29.3	2	50000	EC (2002)	0.0004	0.001	0.0006	n/a
4-octylphenol	38	2	50000	OECD(1995)	0.0007	0.001	0.0008	n/a
4-pentylphenol	9.8	3	7000	TTC	0.0009	0.003	0.001	n/a
4-tert-butylphenol	5.5	2	2500000	SCHER (2008)	0.000004	0.00001	0.000002	0.00002
bisphenol A	13.3	2	175000	IRIS, 1993	0.00007	0.0002	0.00007	0.0002
2,4-dimethylphenol	2	2	70000	IRIS, 1990	0.00003	0.00004	0.00003	n/a
2-sec-butylphenol*	9.5	3	7000	TTC	0.001	n/a	0.001	n/a
2-cresol	26.8	2	175000	IRIS, 1990	0.0002	n/a	0.00008	0.0006
4-chloro-3-methylphenol	6.8	3	350	TTC	0.02	n/a	0.02	0.19

** Phenols without detections; LOD, limit of detection (ng/L); RQ(max), risk quotient calculated using maximum analyte concentration; RQ(median), risk quotient calculated using median analyte concentration. Mean LOD is average from all sampling events. OECD, Organization for Economic Cooperation and Development; SCHER, Scientific Committee on Health and Environmental Risk, RIVM, Research for Man and the Environment, National Institute of Public health and the Environment; EC, European Commission.*

Post MF characterisation

In both the Beenyup Pilot Plant and KWRP, wastewater undergoes chloramination before MF to prevent RO membrane fouling. Over the course of the sampling period, a small number of post-MF samples were collected from within both plants in addition to the normal secondary wastewater and post-RO samples to determine the effect of chloramination during the MF/RO process. Paired wastewater, post-MF and post-RO samples were taken on 2 occasions at KWRP (Event 3: 21st September 2007, and Event 6: 6th June 2008) and on 2 occasions at Beenyup (Event 4: 1st April 2008 and Event 6: 5th June 2008). Figure 6.6.5 presents median phenol concentrations of these paired wastewater, post-MF and post-RO samples for all phenols that were detected in post-MF samples at both KWRP and BPP.

The limited number of post-MF samples taken and low percentage detections for some phenols means that interpretation of the data is difficult. The exception is 2,4-dichlorophenol, which shows a consistent increase in concentration in post-MF samples at KWRP, suggesting it is formed within the MF/RO process as a result of chloramination. As described in section 6.3 (Halogenated DBPs), the time between the hypochlorite dosing point and post-MF sample point is 20 seconds in BPP and 25 minutes or longer in KWRP, depending on plant flow. It is most likely that the shorter residence time precluded significant formation in BPP.

While similar conclusions cannot be made for any other chlorophenol, it is noted that the only detections above LOD (10 ng/L) for 2-chlorophenol were in two post-MF samples (29 ng/L, BPP 1st April 2008 and 12 ng/L KWRP, 6th June 2008). It is also noted that even if RQ(max) for 2-chlorophenol and 2,4-dichlorophenol are determined using the maximum concentrations measured in post-MF water, values are still several orders of magnitude less than 1, with 2-chlorophenol RQ(max) =0.0001, and 2,4-dichlorophenol RQ(max) =0.0027.

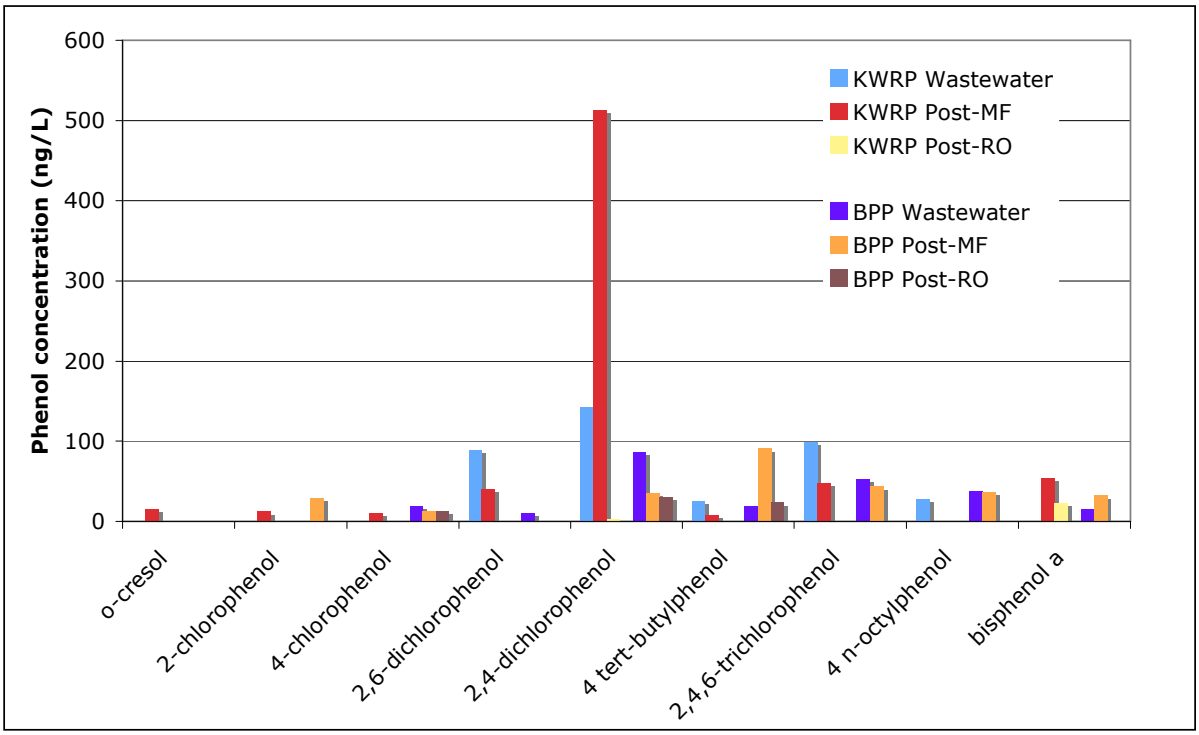

Figure 6.6.5: Median concentrations of select phenols in paired secondary wastewater, post-MF water and post-RO water samples for both KWRP (n=2) and BPP (n=2).

Treatment performance

Treatment efficiency was calculated for analytes detected in secondary wastewater as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD in post-RO samples, the efficiency was calculated assuming a concentration equal to half the LOD. Average treatment performance ranged from 35% for bisphenol A to 84.6% for 4 n-nonylphenol (Figure 6.6.6) and removal was above 50% for all phenols except bisphenol A. In many cases the large variability in results is attributed to the fact that concentrations in secondary wastewater were often close to the LOD measured for post-RO samples, as well as a low number of paired replicates samples.

Bisphenol A was detected in a number of field blanks in Event 3, suggesting trace concentrations of bisphenol A were introduced by the sampling process and this

could have affected post-RO samples as well. No contamination was seen in field blank measurements from Events 4 to 6 and the calculated treatment efficiency for bisphenol A using this data only is much higher (61%).

Because of the differences seen in 2,4-dichlorophenol concentrations in KWRP and BPP, treatment efficiency between post-MF and post-RO samples was also calculated for each plant. Calculation of 2,4-dichlorophenol RO treatment efficiency for KWRP using post-MF samples (median=99%) gave significantly better treatment efficiency than calculated using paired secondary wastewater and post-RO water samples (median=88%). Median treatment efficiency using post-MF samples for Beenyup was much poorer, however, at 19%. It is not anticipated that RO treatment efficiency should differ significantly between plants and this difference is attributed to the higher concentrations of 2,4-dichlorophenol seen in post-MF samples at KWRP.

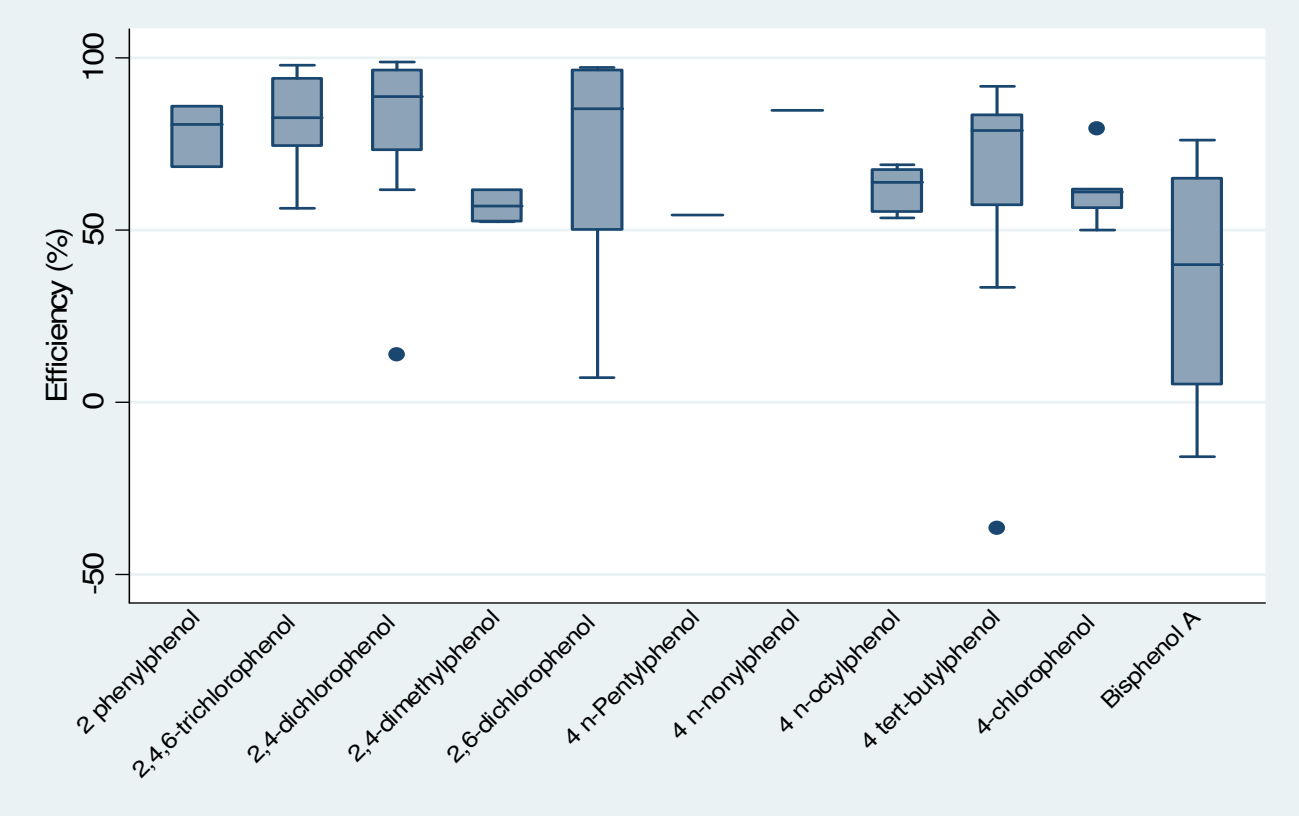

Figure 6.6.6: MF/RO removal efficiency of detected phenols in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Discussion

The majority of phenols studied were detected in secondary treated wastewater and the results are consistent with other studies reporting phenolic compounds in WWTP effluents at ng/L concentration. However, none of the investigated phenols was detected in more than 80% of the wastewater samples and observed concentrations were lower than concentrations reported in other studies, particularly for 4-nonylphenol, 4-octylphenol and bisphenol A (Tan *et al.*, 2007, Rudel *et al.*, 1998, Auriol *et al.*, 2006, Williams *et al.*, 2007). Similarly, a general trend seen in other studies of secondary wastewater, where 4-nonylphenol>bisphenol A>4-octylphenol (Voutsa *et al.*, 2006, Tan *et al.*, 2007, Clara *et al.*, 2005a) was not seen in this study. Instead 4 n-octylphenol has a higher median concentration and greater number of detections than either 4 n-nonylphenol or bisphenol A. It should be noted that in this study the analysis of nonylphenol and octylphenol were for the specific isomers 4 n-nonylphenol and 4 n-octylphenol respectively, whereas other studies have considered alternative isomers or the sum of multiple isomers.

No clear pattern was observed in phenols concentrations by WWTP. While concentrations tended to be higher during spring or winter for those analytes with percentage detections greater than 30%, the trend was not generally significant. While another Australian study showed no seasonal trends, differences in sampling schemes probably means comparison is not possible (Williams *et al.*, 2007).

Eight compounds were detected in post-RO water, including 4-chloro-3-methylphenol and 2-cresol, both of which were not detected in secondary wastewater. However, both these phenols were only detected in 1 sample (BPP, 8th June 2008), and all detected phenols had less than 20% detections except for 2,4-dichlorophenol and bisphenol A.

While the majority of phenols showed median RO removal of better than 50%, removal of bisphenol A was low and variable. However, recalculation without Event 3 data improved median treatment efficiency to 61%, suggesting trace concentrations of bisphenol A were introduced into the sampling process during Event 3. Bisphenol A is a very common water contaminant at ng/L levels sourced from plastics, including potentially from the membranes used in treatment. Other studies have reported removal of bisphenol A between 70% and 100% (Hu *et al.*, 2003, Wintgens *et al.*, 2004, Lee *et al.*, 2008), and we expect that the removal calculated for bisphenol A in this study is an underestimate because all detections in secondary wastewater in Events 4 to 6 were just above LOD. The best treatment efficiency was calculated to be 99% for 2,4-chlorophenol using paired post-MF and post-RO samples. It is expected that this will be the most accurate treatment efficiency calculation because the post-MF concentrations were about 2 orders of magnitude greater than the average LOD, whereas most other phenol detections were less than an order of magnitude greater.

Rejection of phenols by RO is expected to be high. Agenson *et al*. (2004) found greater than 99.8% rejection for semi volatile organics (including bisphenol A and ter-butyl phenol) in NF and RO membranes. Kimura *et al*. (2003) also reported >90% rejection for bisphenol-A, while Drewes *et al*. (2008) reported >90% rejection for nonylphenol in RO systems. Phenols have a molecular size greater than membrane molecular cut-off, and size exclusion is thought to be the most likely mechanism of rejection. Molecular length, molecular width and partitioning coefficient (log K_{ow}) have been shown to best predict rejection for phenols (Agenson *et al.*, 2003). Nevertheless, rejection of phenols can be problematic because these compounds can absorb into the membranes via hydrogen bonding, diffuse across the membrane and partition into the permeate, resulting in variable rejection. This phenomena have been found to be dependent on the solute and type of membrane material (Bellona *et al.*, 2004).

All RQ values for both secondary wastewater and post-RO were 1 to 5 orders of magnitude below 1, indicating very low health risk.

While the concentration of phenols measured in this study were higher than the concentrations measured of estrogenic hormones, their contribution to total sample estrogenicity remains extremely low and this is in agreement with the findings of other studies (Bicchi *et al.*, 2009, Tan *et al.*, 2007). Diet is still considered the major route of human exposure to bisphenol A and other phenols. For example, the European Commission's Scientific Committee on Food estimated bisphenol A exposure to be 0.48–1.6 µg/kg body weight/day from food sources (Vandenberg *et al.*, 2007) compared with an estimate intake from water of 0.34 ng/kg bwt/day from this study.

Of the phenols tested, 4-tert-butylphenol had the highest percentage of detections in secondary wastewater (73%). However 4-tert-butylphenol has a lower toxicity and therefore higher health value (2500 µg/L) than 2,4,6-trichlorophenol (health value 20 µg/L), which was detected in 63% of secondary wastewater samples. While neither chemical is sufficiently present in wastewater to be considered as a treatment performance indicator, 2,4,6-trichlorophenol may be considered the best reference chemical for phenols.

In addition to the 16 phenols analysed in this project, phenol and cyclohexylphenol were analysed in the preliminary sampling event conducted in June 2005. Both analytes were below LOD of 10 ng/L and 20 ng/L respectively. Two nitrophenols (2-nitrophenol and 4-nitrophenol) were recommended for monitoring in this project but were not analysed because of limitations in development of analytical methods. It is recommended that these phenols be evaluated in future.

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6.7 Polycyclic aromatic hydrocarbons (PAHs)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds with 2 or more fused aromatic rings (Wenzl *et al.*, 2006, WHO, 2003). PAHs result from the incomplete combustion of fossil fuels, from both natural and anthropogenic sources, and exist in the environment as complex mixtures. PAHs containing up to four benzene rings are considered 'light', while those containing more than 4 benzene rings are considered 'heavy'. Heavy PAHs are more stable and more toxic than light PAHs. Generally, PAHs are hydrophobic in nature and their water solubility (K_{ow} from 2 to 5) decreases with increasing ring numbers (ATSDR, 1995).

PAHs are of major public health concern because of their ubiquitous occurrence and because they have been identified as carcinogenic, mutagenic, and teratogenic. They comprise the largest group of known cancer causing agents. The International Agency for Research on Cancer (IARC) considers several PAHs and PAH derivatives to be probable (Group 2A) or possible (Group 2B) human carcinogens (IARC, 2008). The mutagenicity risk of well characterised PAHs in a mixture can be estimated as the sum of the risk posed by individual PAHs in the mixture (White, 2002). However, complex mixtures may modify the carcinogenic potency of PAHs (Courter *et al.*, 2007), actually producing lower carcinogenic potency than that estimated based on individual additive effects (Staal *et al.*, 2008). The less than additive effects of a mixture may be due to saturation of metabolic pathways needed to activate mutagenic PAHs (White, 2002).

Substantial volumes of PAH are emitted to the atmosphere and inputs of PAHs to wastewater are mainly from atmospheric deposition onto paved surfaces and runoff (Blanchard *et al.*, 2004). A study of PAH fluxes in 5 sewers leading to a Paris WWTP demonstrated that higher fluxes were seen from sewers from industrial areas than domestic areas (Blanchard *et al.*, 2004), although the 10-20 times higher flux seen in winter was attributed to domestic heating. PAHs from households have been shown to contribute up to 50-60% of the total load of pyrene and phenanthrene in the wastewater collection system (European Communities, 2001). Removal efficiency of PAHs in wastewater treatment is generally high (>98%) (Sanchez-Avila *et al.*, 2009). The hydrophobic nature of PAHs means they partition onto sludge and solid particles rather than remaining in the aqueous phase (Blanchard *et al.*, 2004). Greater PAH removal is seen in WWTPs utilizing activated sludge treatment, than in WWTPs with mechanical settling only (Vogelsang *et al.*, 2006). In activated sludge treatment PAHs are efficiently retained in sludge (Rogers, 1996).

Typical concentrations of PAHs in treated secondary wastewater in Europe can range from 10-100 µg/L (European Communities, 2001). However, the majority of this is associated with particulate matter in the secondary wastewater. Aqueous concentrations are low, typically 1-10 ng/L in treated wastewater (Busetti *et al.*,

2006), with the light PAHs typically at higher dissolved concentrations (Sanchez-Avila *et al.*, 2009).

In this section, secondary wastewater and post-RO water are characterized for 17 PAH compounds, as listed in Table 6.7.1. The potential human health impact of the concentrations found in secondary wastewater and in post-RO water is also evaluated using toxicity equivalency factors (TEFs) to weight the toxicity of each PAH relative to the toxicity of benzo(a)pyrene (BaP), the best studied PAH.

The underlying assumptions of the use of relative toxicity factors are that the risks from the individual PAHs are additive and the sum of the individual risks adequately reflects the PAH component of the mixture being assessed. The TEF approach is widely accepted for determining health impacts of PAHs (Bruce *et al.*, 2007), although the TEF values used have varied between authors and organizations (Nisbet & LaGoy, 1992, OEHHA, 1993, Larsen & Larsen, 1998, Muller *et al.*, 1997). The values used in this study are presented in Table 6.7.1. Multiplying the concentration of each PAH by its TEF produces a toxicity equivalence quotient (TEQ as BaP equivalent), with BaP assigned a TEF of 1. The total PAH TEQ is calculated by adding the individual TEQ as BaP equivalents in order to derive a toxicity rating for the PAH mixture present (see equation below). The RQ(median) of the mixture is then calculated by dividing the total PAH TEQ as BaP equivalent by the BaP ADWG guideline value of 0.01 µg/L (ADWG, 2004).

$$TEQ_{TOTAL\ PAHs} = \text{sum}\ \{(TEF_a \times [PAH_a]) + (TEF_b \times [PAH_b]) + (TEF_c \times [PAH_c]) + \ldots..\}$$

Table 6.7.1: Priority PAHs and their chemical characteristics, carcinogenic toxicity equivalency factors (TEFs) and cancer classification. Bolded PAHs were assessed in this study

Parameter	Priority List			Chemical Characteristics		TEFs			Cancer	
	JECFA	EU	U.S. EPA	Rings	MW (g/mol)	Nisbet 1992	OEHHA 1993	Muller 1997	IARC	IRIS
2-chloronaphthalene (β)				2	162.62				2B▪	NE
5-Methylchrysene	*			4	242.31		1		2B	
7,12-Dimethylbenz-a-anthracene				4	256.34				NE	
7H-Dibenzo-c,g-carbazole				5	267.32		1		2B	
Acenaphthene			*	3	154.2	0.001			3	NE
Acenaphthylene			*	3	152.2	0.001			NE	D
Anthracene (oil)		*	*	3	178.24	0.01		0.28	3	D
Benzo-a-anthracene	*		*	4	228.3	0.1	0.1	0.014	2A	B2
Benzo-a-pyrene	*	*	*	5	252.32	1	1	1	1	B2
Benzo-b-fluoranthene	*	*	*	5	252.32	0.1	0.1	0.11	2B	B2
Benzo-c-fluorene	*			4					3	
Benzo-*e*-pyrene				5	252.32			0	3	D
Benzo-ghi-perylene		*	*	6	276.34	0.01		0.012	3	D
Benzo-j-fluoranthene	*			5	252.32		0.1	0.045	2B	B2
Benzo-k-fluoranthene	*	*	*	5	252.32	0.1	0.1	0.037	2B	B2
Carbazole				5	167.21		0.005**		3	
Chrysene	*		*	4	228.3	0.01	0.01	0.026	2B	B2
Cyclopenta-c,d-pyrene				5				0.012	3	
Dibenzo-a,e-pyrene	*			6			1		2B	
Dibenzo-a,h-pyrene	*			6			10	1.2	2B	
Dibenzo-a,i-pyrene	*			6			10		2B	
Dibenzo-a,l-pyrene	*			6			10		2B	
Dibenzo-ah-anthracene	*		*	5	278.35	5		0.89	2A	B2
Fluoranthene		*	*	4	202.26	0.001			3	D
Fluorene			*	3	166.23	0.001			3	D
Indeno-1,2,3-d-pyrene	*	*	*	6	276.34	0.1	0.1	0.067	2B	B2
Naphthalene		*	*	2	128.18	0.001			2B	C-2^
Phenanthrene			*	3	178.24	0.001		0.0064	3	D
Pyrene			*	4	202.26	0.001		0	3	D

JECFA: Joint FAO/WHO Expert Committee on Food Additives 2005; EU: European Union; U.S EPA: U.S. Environmental protection Agency; PEFs Carcinogenic potency equivalent factors; IARC: International Agency for Cancer Research; IRIS: Integrated Risk Information System; NE: Not established

IARC: Group1: carcinogenic to humans; Group 2A: probably carcinogenic to humans; Group 2B: possibly carcinogenic to humans. Group 3 have either limited or inadequate evidence in animals and are not classifiable as to their carcinogenicity in humans due to no adequate data.

IRIS: B2, Probable human carcinogen - based on sufficient evidence of carcinogenicity in animals; C, Possible human carcinogen; D, Not classifiable as to human carcinogenicity; E, Evidence of non-carcinogenicity for humans; 2^, reasonably anticipated to be a human carcinogenic (NTP 2005)

▪ as naphthalene

*** Air Toxic Program (Oregon DEQ, 2005)*

Source: (FPTCDW, 2006, IARC, 2008, California Office of Administrative Law, 2007, European Commission, 2006, Nisbet & LaGoy, 1992, Muller et al., 1997, Oregon DEQ, 2005)

Methods

PAHs were preconcentrated by stir bar sorptive extraction (SBSE) before GC-MS analysis. A polydimethylsiloxane (PDMS) coated stir bar was placed in each sample (60 mL) and analytes were sorbed onto the PDMS phase during 20 hours of constant stirring. Stir bars were then removed from the sample, dried and introduced directly into the GC using a specially modified thermal desorption inlet. Analytes were thermally desorbed from the stir bar into the GC inlet and separated using a 60 m 5% phenyl 95% dimethylpolysiloxane capillary column. Detection was performed by MS with electron ionization (EI). Peak identification and calculation of recovery was aided by inclusion of deuterated surrogate standards.

Table 6.7.2: Limits of detection (LOD) and estimation of uncertainty for PAHs.

Analyte	Average LOD (µg/L)	Standard Relative Uncertainty (%) (0.05 µg/L)
2-chloronaphthalene (β)	0.002	22%
Acenaphthene	0.001	32%
Acenaphthylene	0.001	31%
Anthracene (oil)	0.002	42%
Benzo-a-anthracene	0.001	27%
Benzo-a-pyrene	0.001	30%
Benzo-b-fluoranthene	0.002	31%
Benzo-ghi-perylene	0.006	49%
Benzo-k-fluoranthene	0.003	30%
Carbazole	0.002	33%
Chrysene	0.001	27%
Dibenzo-ah-anthracene	0.004	85%
Fluoranthene	0.002	45%
Fluorene	0.002	43%
Indeno-1,2,3-d-pyrene	0.008	47%
Phenanthrene	0.001	27%
Pyrene	0.003	64%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R and quantifying and qualifying ions), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

PAHs were analysed from Event 3 onwards. The majority of samples (94.7% of the total) were from BPP and KWRP. Only two groundwater samples were analysed (Event 4: 23rd of January 2008) and only one sample from Subiaco WWTP was analysed (Event 5: 3rd of April 2008). A total of 923 measurements corresponding to 17 analytes were analysed, excluding replicates field and trip blanks and the distribution of sampling by event and location is presented in Table 6.7.3.

Table 6.7.3: Measurement of PAHs by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	WW	Water Reclamation Plant							
				Grab	Comp	Total			Before MF		After MF		After RO		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006													
2	May/June	6	2007													
3	September	6	2007	198	181	379	0	0	102	85	0	0	96	96	0	379
4	January	6	2008	170	34	204	34	0	34	51	0	0	34	51	0	170
5	April	5	2008	204	0	204	0	17	51	51	0	17	34	51	0	187
6	June	5	2008	187	0	187	0	0	34	51	0	17	34	51	0	187
Total		32		759	215	974	34	17	221	238	0	34	198	249	0	923

Comp, composite; GW, groundwater, SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup.

Secondary wastewater characterisation

All 17 PAHs were detected in at least 1 secondary wastewater sample (Figure 6.7.1). Fluorene was the most commonly detected PAH, with a percentage detection of 64%, followed by pyrene (50%), 2-chloronapthalene (36%) and phenanthrene (32%). All of these PAHs have four benzene rings or fewer and are therefore likely to be more soluble. For the more frequently detected PAHs median concentrations ranged

from 0.001 to 0.003 µg/L. Almost half of the detected PAHs were detected in fewer than 20% of the samples indicating an inconsistent occurrence in secondary wastewater and median concentrations were heavily influenced by the LOD (e.g. indenopyrene that was only detected in 9% of wastewater samples and the median concentration was the LOD of three of the four sampling events of 0.015 µg/L).

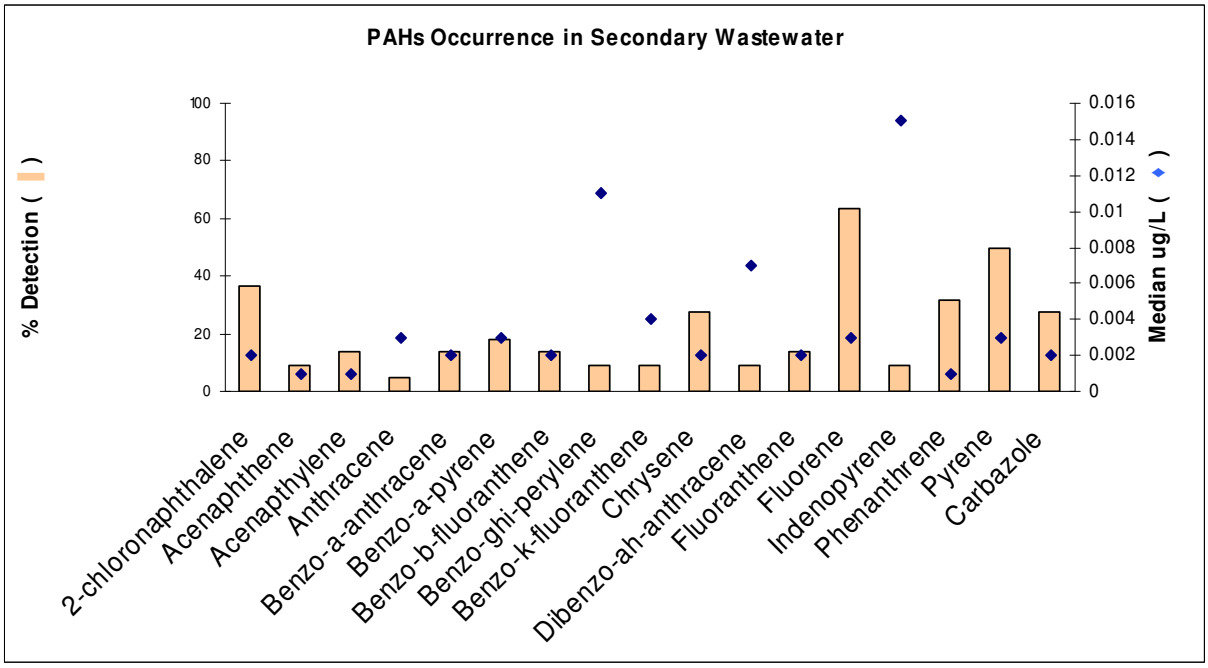

Figure 6.7.1: PAHs with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (µg/L, diamond).

Given that only one sample was analysed for Subiaco WWTP, comparison was made of median concentrations at BPP and KWRP influent only. Comparison is only made for fluorene, pyrene, phenanthrene and 2-chloronaphthalene, as these were the compounds to have percentage detections greater than 30%. For all other analytes, comparisons of median concentrations were dominated by non-detects, reported as LOD. No significant differences were observed in the median concentration of fluorene, pyrene, phenanthrene or 2-chloronaphthalene between BPP and KWRP (Figure 6.7.2). The median concentration of phenanthrene and fluorene was higher at BPP than at KWRP, while pyrene was higher at KWRP. However, none of these differences were statistically significant.

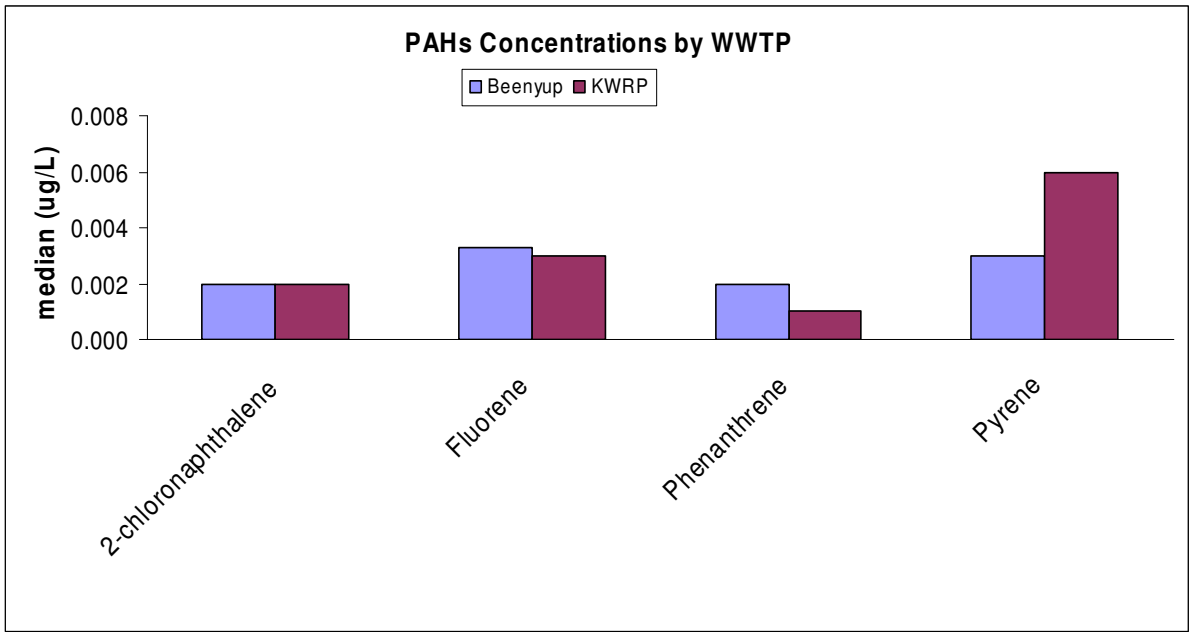

Figure 6.7.2: Median PAH concentrations by WWTP in µg/L

Comparison of seasonal trends again is only made for fluorene, pyrene, phenanthrene and 2-chloronaphthalene, as median concentrations for all other analytes were dominated by non-detects, reported as LOD. Median concentrations of fluorene and phenanthrene were highest in winter, while pyrene was highest in spring. No difference was statistically significant.

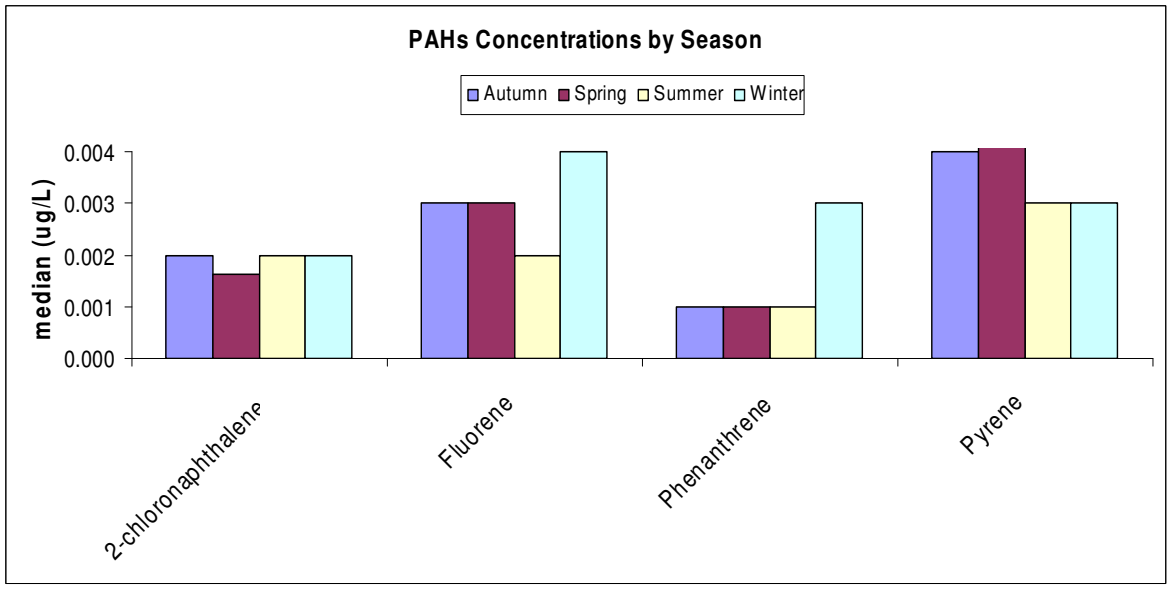

Figure 6.7.3: Median PAHs concentration by season in µg/L

RO Product water characterisation

Twelve PAHs (70%) were detected in the post-RO water. Phenanthrene was most commonly detected (24% of the RO samples) followed by fluorene (19%), carbazole (10%) and fluoranthene (10%). Benzo-a-anthracene, benzo-ghi-perylene, chrysene, dibenzo-ah-anthracene and indenopyrene were not detected in post-RO water. Median concentrations ranged from 0.0011 µg/L for acenapthylene to 0.0037 µg/L for benzo-k-fluoranthene but were heavily affected by LODs.

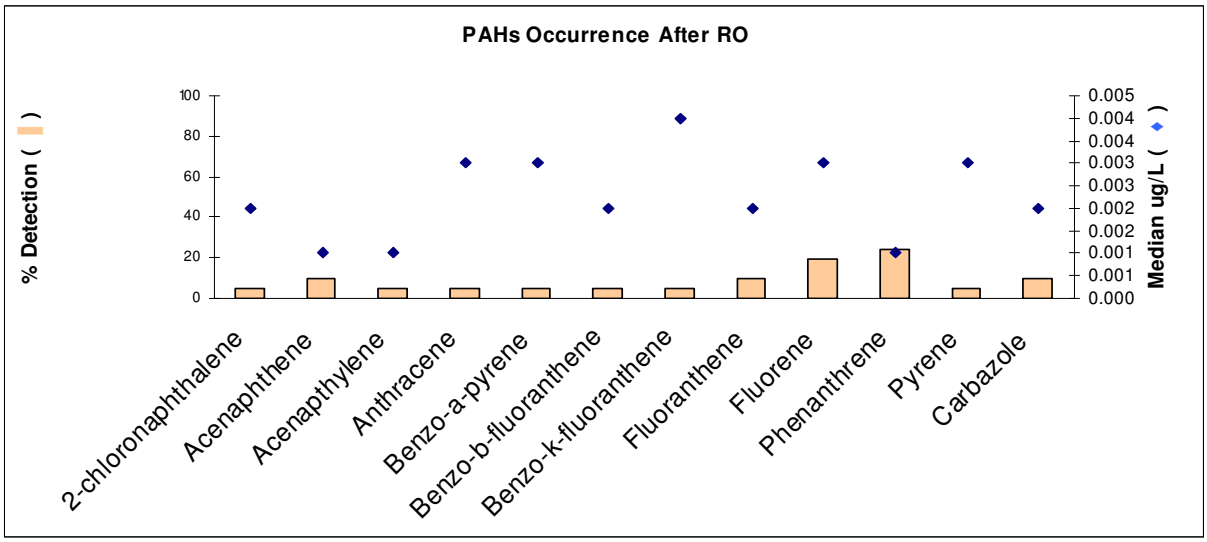

Figure 6.7.4: PAHs with percentage detections in post-RO water (vertical column) and corresponding median concentrations (µg/L, diamond).

Groundwater characterisation

Two groundwater samples were collected the 23rd of January 2008 from the Pinjar and Wanneroo bore lines. The PAHs 2-chloronapthalene, acenapthene and fluorene were all detected in the Pinjar bore line but not in the Wanneroo (Table 6.7.4). All PAHs were detected at levels close to the LOD. Field blanks for these samples also reported low concentration of these analytes, which may suggest these results are due to contamination after sampling

Table 6.7.4: Detected PAHs in Groundwater

PAHs	LOD (µg/L)	Detected concentration* (µg/L)	Field Blank
Acenaphthene	0.001	0.003	0.006
Fluorene	0.001	0.003	0.01
2-chloronaphthalene	0.002	0.004	0.003

**Actual values detected in Event 4.*

Screening health risk assessment

The median TEQ for each PAH detected in secondary wastewater and post-RO water was obtained by multiplying by the median concentrations of each PAH by the TEF as reported by Nisbet & Lagoy (1992) and Muller *et al*. (1997), as listed in Table 6.7.5. Neither Nisbet & Lagoy nor Muller *et al*. derived a TEF for carbazole and the value of 0.005 used here was reported by the Oregon Air Toxic Program (Oregon DEQ, 2005). The TEFs for acenaphthene, acenapthylene, fluoranthene and fluorene were not reported by Muller *et al*. and the values reported by Malcom and Dobson (1994) were used instead. The combined TEQ was calculated by adding the median TEQ as BaP equivalent of each detected compound and is referred to as "TEQ detected PAHs" in Table 6.7.4. PAHs that were not detected were not included in the TEF analysis.

RQs were calculated by dividing the TEQ detected PAHs by the ADWG BaP health value of 0.01 µg/L. In secondary wastewater the RQ calculated using TEF data from Nisbet & LaGoy (RQ=4.05) was higher compared with the RQ calculated using TEF data from Muller *et al*. (RQ=1.17) mainly due to the difference in the TEF for dibenzo-ah-anthracene. In post-RO water, the TEQ detected PAHs was one order of magnitude below that of the secondary wastewater. RQs in post-RO water were below 1 and have similar values using the TEFs of Nisbet & Lagoy (RQ=0.37) or Muller (RQ=0.42). Dibenzo-ah-anthracene was not detected in post-RO water and therefore the difference in TEF values determined by Nisbet and Lagoy, and Muller did not contribute to these values.

Maximum RQs in wastewater were 4.1 using Muller *et al*. TEFs and 8.9 using Nisbet & LaGoy TEFs (data not presented in table). Maximum RQs in post-RO water were below 1 using both sources of TEFs (RQmax = 0.77 and 0.79 respectively).

BaP TEQ and total TEQ detected PAHs in secondary wastewater were compared. The contribution of BaP to the total carcinogenic activity in secondary wastewater ranged from 7.5% to 25% (as per data in Table 6.7.5). This indicates that the analysis of BaP alone is insufficient to represent the potential toxicity of all PAHs in the mixture.

Table 6.7.5: Median, and maximum PAH concentrations in secondary wastewater and post-RO water samples in µg/L and TEQs calculated according to TEF values from Nisbet and LaGoy (TEQ_N) and Muller (TEQ_Mu).

			Secondary Wastewater				After Reverse Osmosis			
Parameter	**TEF Nisbet & LaGoy 1992**	**TEF Muller 1997**	**Median Conc (µg/L)**	**Max Conc (µg/L)**	**TEQ_N (median)**	**TEQ_Mu (median)**	**Median Conc (µg/L)**	**Max Conc (µg/L)**	**TEQ_N (median)**	**TEQ_Mu (median)**
2-chloronaphthalene	0	0	0.002	0.003	0	0	0.002	0.002	0	0
Acenaphthene	0.001	0.001*	0.001	0.004	0.000001	0.000001	0.001	0.006	0.000001	0.000001
Acenapthylene	0.001	0.001*	0.001	0.006	0.000001	0.000001	0.001	0.003	0.000001	0.000001
Anthracene	0.01	0.28	0.003	0.003	0.00003	0.00084	0.003	0.003	0.00003	0.00084
Benzo-a-anthracene	0.1	0.014	0.002	0.012	0.0002	0.000028			0	0
Benzo-a-pyrene	1	1	0.003	0.024	0.003	0.003	0.003	0.006	0.003	0.003
Benzo-b-fluoranthene	0.1	0.11	0.002	0.028	0.0002	0.00022	0.002	0.006	0.0002	0.00022
Benzo-ghi-perylene	0.01	0.012	0.011	0.011	0.00011	0.000132			0	0
Benzo-k-fluoranthene	0.1	0.037	0.004	0.028	0.0004	0.000148	0.004	0.010	0.0004	0.000148
Chrysene	0.01	0.026	0.002	0.011	0.00002	0.000052			0	0
Dibenzo-ah-anthracene	5	0.89	0.007	0.011	0.035	0.00623			0	0
Fluoranthene	0.001	0.001*	0.002	0.007	0.000002	0.000002	0.002	0.004	0.000002	0.000002
Fluorene	0.001	0.001*	0.003	0.009	0.000003	0.000003	0.003	0.005	0.000003	0.000003
Indenopyrene	0.1	0.067	0.015	0.015	0.0015	0.001005			0	0
Phenanthrene	0.001	0.0064	0.001	0.008	0.000001	6.4E-07	0.001	0.010	0.000001	6.4E-07
Pyrene	0.001	0	0.003	0.019	0.000003	0	0.003	0.005	0.000003	0
Carbazole**	0.005	0.005	0.002	0.010	0.00001	0.00001	0.002	0.009	0.00001	0.00001
TEQ detected PAHs					**0.040**	**0.012**			**0.004**	**0.004**
RQ (median) PAHs					**4.05**	**1.17**			**0.37**	**0.42**

TEF = Toxic Equivalency Factors with respect to Benzo-a-pyrene

*TEF from Malcolm & Dobson (1994); **TEF from Oregon Air Toxic Program (Oregon DEQ, 2005)

Treatment performance

Treatment efficiency was calculated for PAHs detected in secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were paired for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD as a worst-case scenario. The number of paired samples for each PAH was relatively low and ranged from 3 for acenapthylene and anthracene to 15 for pyrene. Median percentage removal ranged from 50% for carbazole to 99% for benzo-a-pyrene (Figure 6.7.5). Removal efficiency was generally good for all PAHs with only three PAHs having a median removal efficiency less than 75%.

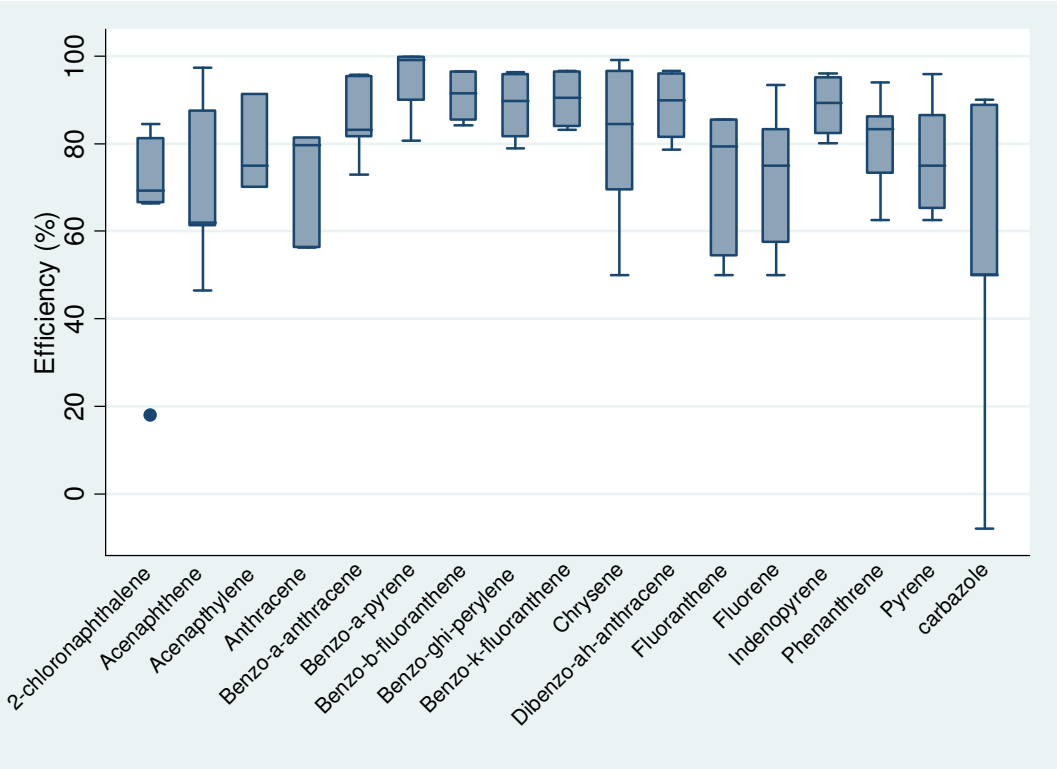

Figure 6.7.5: MF/RO removal efficiency of detected PAHs in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Discussion

All PAHs were detected on at least one occasion in secondary wastewater, which reflects their status as ubiquitous environmental contaminants (Wenzl *et al.*, 2006, WHO, 2003), although almost half of the detected PAHs were detected in fewer than

20% of the samples indicating an inconsistent occurrence. Fluorene was the most commonly detected PAH (66% of detections) followed by pyrene, phenanthrene and 2-chloronaphthalene. These four PAHs all consist of 4 benzene rings or fewer and are therefore likely to be more soluble. It should be noted that 2-chloronaphthalene, one of the most frequently detected PAHs has not undergone a complete evaluation for evidence of human carcinogenic potential and no TEF values has been assigned (USEPA, 2008). The low number of percentage detections for many compounds confounded comparison by WWTP and season and there were no statistically significant differences seen. However, median concentrations of fluorene and phenanthrene were highest in winter, while pyrene was highest in spring. This may suggest that a greater atmospheric emission of PAHs occurs during colder seasons (e.g. winter) due to a higher incidence of domestic heater use (Grynkiewicz *et al*., 2002, Mastral *et al*., 2003).

A total of 12 PAHs were also detected in the post-RO water of which phenanthrene was the most commonly detected (24% of the RO samples) followed by fluorene (19%). Treatment removal was above 75% for all except 3 PAHs. It is possible that the removal efficiencies measured in this study are lower than expected because the analytical method used only measures dissolved PAHs (i.e. in aqueous solution), and would not measure PAHs sorbed on the suspended solids present in secondary wastewater.

Rejection of PAHs in MF/RO systems is predicted to be moderate to high (Bellona *et al*., 2004). Rejection of PAHs by nanofiltration has been measured at greater than 70% (Yoon *et al*., 2006) and RO rejection is expected to be better still. Factors driving the rejection mechanism include size exclusion through steric hindrance (PAH molecular weight higher than molecular weight membrane cut off, molecular size and geometry) as well as hydrophobic-hydrophobic interactions between the membrane and solute. The PAHs selected in this study are strongly hydrophobic, ($3<\log K_{ow}<7$) and this could also lead to adsorption of the compounds onto the membranes (Yoon *et al*., 2006). Thus rejection also depends on diffusion and partitioning phenomena (Yoon *et al*., 2006, Bellona *et al*., 2004, Drewes *et al*., 2008). Rejection is expected to increase with the increase of log K_{ow} (Drewes *et al*., 2008).

Only three light PAHs (2-chloronapthalene, acenapthene, and fluorene) were detected in groundwater, all detected in one of two samples analysed for PAHs. Detected concentrations in groundwater were very close to LOD and detections in related field blanks suggest that samples could have been contaminated after sampling. PAHs are generally well absorbed to soils and particles and extensive movement of PAHs in groundwater is not expected (Mikkelsen *et al*., 1996, Murakami *et al*., 2008). Removal of light PAHs has also been observed during experimental groundwater infiltration tests (Murakami *et al*., 2008) and it is expected that more extensive testing of groundwater for PAHs would confirm that no contamination exists.

Calculated RQs using the TEQ approach indicate that levels of PAHs in secondary wastewater have potential to impact human health (RQ up to 4.05), however the advanced MF/RO treatment reduced the concentration of these compounds such that the RQ in post-RO water was always below 1.

As fluorene was the most commonly detected PAH in wastewater (64%) it might provide the best indication of PAH occurrence. Fluorene is a light PAH (with two benzene rings linked by a five member ring, molecular weight of 166 Da) and therefore may provide the best indication of treatment performance of the PAHs measured. However fluorene is much less toxic than BaP and therefore total PAH toxicity is better characterised by monitoring BaP, although a safety factor would be required in order to estimate the toxicity of all the PAHs.

Several PAHs potentially present in secondary wastewater remain untested and consequently have not been considered in risk estimates. This situation contributes to uncertainty in the screening risk assessment. Methylated-PAHs, oxy-PAHs and nitro-PAHs were not tested during this study. Nitro-PAHs have been detected in diesel particle extracts of which 2-nitrofluoranthene and 2-nitropyrene have been reported as air pollutants (Tsapakis & Stephanou, 2007). Nitro-PAHs are mutagenic (Watanabe *et al*., 1997), and may be present in wastewater (Filipic & Toman, 1996). However, studies of nitro-PAHs in road run-off suggested that the overall risk of nitro-PAHs were smaller than those of PAHs, despite higher potency factors (Murukami *et al*., 2008). Oxy-PAHs are not commonly included in monitoring programs and they are more likely to be present in water due to their higher solubility compared with other PAHs (Lundstedt *et al*., 2007). Measurement of six additional priority PAHs with TEFs greater than 1 (i.e. methylchrysene, 7H-dibenzo(c,g)carbazole and four dibenzopyrenes with TEFs up to 10) that are common air pollutants would better characterise the potential risk of PAHs in recycled water.

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6.8 Dioxins, Furans and dioxin-like PCBs

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), commonly known as dioxins and furans respectively, and polychlorinated biphenyls (PCBs) are persistent compounds with a high potential for accumulating in biological tissues. The World Heath Organisation (WHO) consider 29 of these PCDDs, PCDFs and dioxin-like PCBs to have significant toxicity (Van den Berg *et al.*, 2006) and these compounds (listed in Table 6.8.1) were analysed to determine their health significance in IPR. A screening health risk assessment was performed using toxic equivalents (TEQ) to determine whether increased human dioxin exposure occurs when secondary wastewater is treated to augment drinking water supplies

Many dioxin and dioxin-like compounds have been tested for their toxic effects using *in vivo* and *in vitro* studies. Studies of chronic exposure in mammals to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have demonstrated that the compound is associated with adverse reproduction outcomes, birth defects, hepatotoxicity, immunological suppression and carcinogenicity. The no observed adverse effect level (NOAEL) for TCDD for chronic exposure in rats (cancer and reproduction) is approximately 1 ng/kg bw/day. Based on this NOAEL and a 100-fold uncertainty factor, it is concluded that human intakes should be below 10 pg TEQ/kg bw/day averaged over a lifetime (Canadian Environmental Protection Act, 1990).

The U.S. EPA, International Agency for Research on Cancer (IARC), and the World Health Organization (WHO) list 2,3,7,8-TCDD as a Group 1 human carcinogen (California OEHHA, 2005). Most studies suggest that TCDD acts only as a promoter and not as an initiator of cancer and therefore dioxins are considered to be non-genotoxic carcinogens with a threshold in their dose-response relationships. The IARC has also determined that PCBs are probably carcinogenic to humans (Group 2A) (IARC, 2008). To date, there is inconsistent evidence that human populations exposed to dioxins have suffered excess cancer. Although some epidemiological studies found that the exposure to dioxins and dioxin-like substances result in a range of cancers, others have reported no positive association. Thus, evidence is conflicting and data are confounded by exposure to other chemicals, incomplete health records, inadequate case identification and small sample size (Australian Department of Environment and Heritage, 2005).

The NHMRC and the Therapeutic Goods Administration have concluded that a tolerable intake of 70 pg TEQ/kg bw/month from all sources (including dioxins, furans and dioxin-like PCBs) could be established on the basis that a threshold exists for all observed adverse effects, including cancer (NHMRC & TGA, 2002). This recommended tolerable maximum intake is equivalent to that set by the Joint Expert Committee on Food Additives of the United Nations Food and Agriculture Organization and the WHO. Assuming a body weight of 70 kg, 2 litres of water

consumption per day and an allocation of 20% TEQ intake to water this corresponds to 16 pg TEQ/L, which is also the guideline value in the Australian Guidelines for Water Recycling (AGWR) - Phase 2 - Augmentation of Drinking Water Supplies.

The toxicity of different dioxins, furans and dioxin-like PCBs is expressed on a common basis by comparing the toxicity of the 17 most toxic dioxins and furans and the 12 most toxic dioxin-like PCBs to that of 2,3,7,8-tetrachlorodibenzodioxin (TCDD). The toxic equivalent factors (TEQs) values for the dioxins and furans have been refined several times by different regulatory and health agencies as new data have become available. The WHO proposed the most recent version in 2006 (Van den Berg *et al.*, 2006). Two values of TEQs were calculated. The upper bound defines all congeners values reported below the limit of reporting (LOR) as equal to the LOR and it is considered the worst-case scenario. The middle bound of the TEQs defines all congeners values reported below the LOR as equal to half the LOR.

Methods

The NMI analytical methodology for the determination of PCDDs & PCDFs and PCBs are based on U.S. EPA methods 1613B and 1668A, respectively. The methods are NATA accredited and provides data on 7 dioxins, 10 furan isomers and 12 dioxin-like PCBs. The detection limits and quantification levels are usually dependent on the level of interferences rather than instrumental limitations. Samples were spiked with a range of isotopically labelled surrogate standards and clean-up was achieved by partitioning with sulphuric acid then distilled water. Further purification was performed using column chromatography on acid and base modified silica gels, neutral alumina and carbon dispersed on celite. After cleanup, the extract was concentrated to near dryness. Immediately prior to injection, internal standards were added to each extract, and an aliquot of the extract was injected into the gas chromatograph. The analytes were separated by the gas chromatography (GC) and detected by a high-resolution (≥10,000) mass spectrometer (MS). The quality of the analysis was assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems. Detailed description of the method is presented in Appendix 4.

Quality assurance/ Quality control

The quality of the analysis was assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems. Acceptable initial and ongoing calibration criteria as well as other QA/QC aspects are reported in Appendix 4

Results

The distribution of sampling days by location is presented in Table 6.8.1

Table 6.8.1: Frequency of dioxin sampling events by location

Event	Month	No days	Year	Location					No of samples
				Groundwater	Subiaco WWTP	Beenyup Pilot Plant Kwinana WRP			
						Secondary wastewater	Post-MF water	Post-RO water	
0	June	3	2005		2	-		1	3
1	November	4	2006	-	-	-	-	-	-
2	May – June	6	2007	2	4		3	3	12
3	September	5	2007			6		6	12
4	January	3	2008	2		2		2	6
Total		17		4	6	8	3	12	33

Secondary wastewater characterisation

The pooled mean TEQ (middle bound) in secondary wastewater samples was 4.50 pg TEQ/L. The dioxin-like compounds with detections in secondary wastewater included PCB 77, 81, 105, 118, 126, 156, 167, 169, OCDD, 1,2,3,4,6,7,8-Heptachlorodibenzofuran and OCDF. All detected dioxins and dioxin-like PCBs, except PCB 169, have TEFs of 0.01 or lower, which signifies very low toxicity relative to TEQ in the additive model.

RO Product water characterisation

The lowest mean TEQ were observed in the post-RO water of both KWRP and BPP and the mean TEQ were similar in the product water of both plants. Concentrations of TEQs of dioxin, furans and dioxin-like PCBs were higher before MF compared to concentrations post-RO water, indicating that the advanced treatment further reduce the concentrations of these contaminants in the product water.

Groundwater characterisation

None of the PCDDs, PCDFs and dioxin-like PCBs were detected in groundwater and the mean TEQ (middle bound) from the groundwater samples was 4.34 pg TEQ/L.

Screening health risk assessment

None of the water samples taken was above the health standard of 16 pg TEQ/L, using either the middle bound or the upper bound TEQ calculated from the 29 congeners. The TEQ (middle bound) of all dioxin-like compounds produces a combined TEQ of 3.34 pg TEQ/L before MF and a 2.45 pg TEQ/L post-RO water. Expressed as risk quotients (RQ) these values were below 1 (RQ before MF=0.21 and RQ post-RO water=0.15). Risk quotients were all below 1, even when the upper bound TEQ was used as a "worst case" scenario for the screening health risk assessment.

Table 6.8.2: Middle and upper bound TEQ (pg/L) by location

TEQ	Location	Type of sample	Mean	Median	Min	Max	RQ(mean)	RQ(max)
Middle bound	KWRP and BPP	Before MF	3.34	2.94	2.23	5.07	0.21	0.33
		Post-MF water	3.55	3.56	3.21	3.88	0.22	0.24
		Post-RO water	2.44	2.06	0.16	4.38	0.15	0.27
	Wanneroo	Groundwater	4.34	4.34	2.01	7.05	0.27	0.44
	Woodman Point, Beenyup and Subiaco WWTP	Secondary wastewater	4.5	4.4	3.17	6.03	0.28	0.38
Upper bound	KWRP and BPP	Before MF	6.61	5.72	4.31	10.13	0.41	0.63
		Post-MF water	7.10	7.12	6.41	7.76	0.44	0.49
		Post-RO water	4.49	4.15	0.30	8.77	0.28	0.55
	Wanneroo	Groundwater	8.68	8.68	4.01	14.1	0.54	0.94
	Woodman Point, Beenyup and Subiaco WWTP	Secondary wastewater	9.00	8.80	6.35	12.05	0.56	0.75

Treatment performance

For the BPP, the presumptive percentage of removal ranged from 24% to 43% while for the KWRP the presumptive percentage of removal was more variable (ranged from 4% to 47%). The average treatment efficiency expressed as TEQ was 26% between the influent and the product water in both plants.

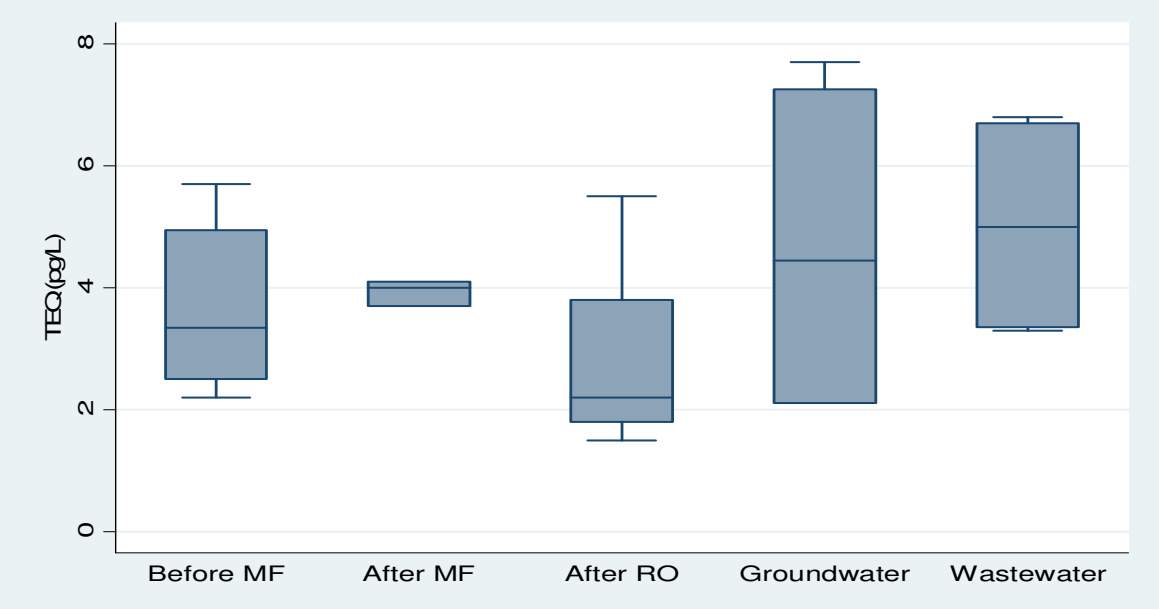

Figure 6.8.1: MF/RO removal efficiency of detected dioxins, PCBs and furans in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Middle bound dioxin concentrations (TEQ basis) from secondary wastewater, groundwater and during the advanced treatment (post-MF water and post-RO water) in the water reclamation plants. Secondary wastewater samples from Beenyup and Kwinana were composite and from Subiaco WWTP were grab samples.

Other analysis

Bootstrap simulations were performed in Stata in order to infer the 95% confidence intervals (CI). The observations were assumed to be from an independent and identically distributed population and re-samples of equal size of the observed dataset were obtained by random sampling with 250 and 1000 replacements from the original dataset. The calculated 95% CI suggest that if secondary wastewater conditions remain unchanged, the middle bound TEQ will be between 3.24 pg TEQ/L and 5.03 pg TEQ/L in the plant influent, which is below the health value of 16 pg TEQ/L.

Discussion

The results indicate that dioxin and dioxin-like compounds, expressed as TEQ, are present only at low concentrations in secondary wastewaters and that advanced treatment is able to reduce those concentrations to levels below health significance. The low concentrations observed in the secondary wastewater may be due in part to the high affinity of these compounds to sludge solids during conventional wastewater treatment due to their low water solubility. These results are also consistent with other studies in which the release of dioxins in sewage treatment plants was small (Australian Department of Environment and Heritage, 2005) and with data from

indirect potable reuse projects around the world. Reported concentrations of PCBs and TCDD are below the guideline values after the advanced treatment in water recycling schemes in the U.S. and Singapore (OCWD, 2006, WBMWD, 2006, Singapore Government, 2002).

Australia has emission controls in place for the main potential point sources of dioxin-like compounds, and therefore the detected dioxins and dioxin-like compounds in wastewater may enter principally from diffuse atmospheric deposition and environmental cycling. Therefore, point source control offers limited scope for further reduction of inputs and concentrations of these persistent organic substances in secondary wastewater. Results also indicate that the concentrations of these compounds in the recycled water are of low health significance. As all calculated RQs were below 1 even when the upper bound TEQ was used as a worst case scenario.

Despite the fact that none of the congeners were detected in the groundwater, calculated RQs in recycled water post-RO water were lower than the RQs of the raw groundwater (0.15 and 0.27 respectively). This was a result of lower LOR achieved for post-RO recycled water than for groundwater, suggesting that the RQ for groundwater may be less accurate. Regardless, the importance of dioxins in recycled water are significantly diminished by MF/RO treatment, and there would be little practical or public health benefit gained from adopting frequent regular monitoring for dioxins and dioxin-like compounds after the advanced MF/RO treatment.

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6.9 Radionuclides

Introduction

Radionuclides may enter wastewater by natural or man-made sources. Natural sources of radiation include geologic formations and soils that contain uranium, radium, thorium, radon, and other nuclides that are radioactive. Among man-made sources, medical procedures such as thallium heart stress tests and tumour irradiation therapies are an important source of radionuclides in wastewater (U.S. EPA, 2005). Other sources of anthropogenic radiation include consumer products such as static eliminators (containing polonium-210), smoke detectors (containing americium-241), cardiac pacemakers (containing plutonium-238), fertilizers (containing isotopes from uranium and thorium decay series), and tobacco products (containing polonium-210 and lead-210).

Cancer is the principal endpoint used to evaluate the health risk from alpha and beta particle emitters, and risk assessment procedures to estimate the cancer risk from radionuclides have been comprehensively developed. Screening levels for gross alpha and gross beta particle activity have been established based on the carcinogenic potency of the radionuclides included in these categories. The recommended screening level in the ADWG for each of gross alpha and gross beta particle activities is 0.5 Becquerel per litre (Bq/L). For gross beta, the screening value of 0.5 Bq/L excludes the contribution from Potassium 40 (^{40}K), a natural beta emitter that is normally absorbed from ingested food and does not accumulate in the body. The total dose of both alpha and beta activity at the screening level corresponds to approximately 0.35 mSv per year, which is one-third of the minimum dose at which intervention is recommended (ADWG, 2004).

Methods

Gross alpha and gross beta radioactivity concentrations were determined by gas flow proportional counting (ERH_RAS_SOP_0100) by ARPANSA, a NATA accredited laboratory. Limits of reporting (LOR) for gross alpha and gross beta particles were variable as they are dependent on the concentration of dissolved solids in the sample. Typical limits of reporting were 0.01 Bq/L for both gross alpha and gross beta particle activity. To account for the variability on radiation emissions, the reported uncertainty (coverage factor K=2) was 24% for gross alpha and 12% for gross beta. Potassium-40 (^{40}K) was determined by measurement of potassium by flame AAS with a LOR of 0.05 mBq/L and an uncertainty (coverage factor K=2) of 10% (at 0.5 Bq/L). Alpha measurements were ^{241}Am equivalent and the beta measurements were ^{40}K equivalent.

Samples analysed by the Department of Health Radiation Health Branch (RHB), Western Australia were preserved in nitric acid and filtered (0.45 µm) and evaporated on a stainless steel planchet by Chemistry Centre of Western Australia according to the ISO method 9697:1992 and following the sample collection and preservation standards in AS/NZS 5667.1 (1998). The alpha and beta count rate of each sample was measured (in 2 pi geometry) using an internal gas flow proportional chamber over a 180 minute time period. The reported alpha efficiency was 33.4%±1.6% and the beta efficiency was 54.7%±2.5%. Beta contribution from ^{40}K was not subtracted from the gross beta counts because potassium was not measured. Alpha measurements were ^{241}Am equivalent and the beta measurements were ^{90}Sr equivalent. In addition to the uncertainty in the counts, the measurement results have an additional total uncertainty of 5% which is based on the sum of random counting error and the estimated upper limits of systematic error in the measurement.

Quality assurance/ Quality control

Samples were collected using consistent protocols and procedures as described in Methods. Analysis was undertaken by two different laboratories during the May/June 2007 sampling event. There were significant differences between the laboratories in the mean of the gross alpha particle activity, even though alpha measurements were ^{241}Am equivalent for both laboratories (For ARPANSA: n=13, mean=0.061 Bq/L, std dev=0.036 Bq/L; for RHB: n=16, mean=0.021 Bq/L, std dev=0.005 Bq/L; (K-Wallis X^2 p=0.02). The mean gross beta activity was higher for the RHB analysis but the differences were not statistically significant. (For ARPANSA: n=13, mean=0.35 Bq/L, std dev=0.30 Bq/L; for RHB: n=16, mean=0.85 Bq/L, std dev=0.43 Bq/L). The differences may be explained by the variability in radiation emissions and the different methods used by the laboratories. For example beta measurements were ^{90}Sr equivalent for the RHB laboratory and ^{40}K equivalent for the ARPANSA laboratory.

Results

Grab samples were collected from Beenyup WWTP, Subiaco WWTP, and KWRP in the May-June/2007, September/2007 and January/2008 monitoring events. A total of 46 analytical samples were reported, excluding field blanks, trip blanks and replicates. Of 46 samples, 22 (47%) were taken at KWRP and 30 (65%) were analysed during the May-June sampling event. Gross alpha and gross beta at BPP were tested during the September 2007 and January 2008 events and groundwater was tested during May-June 2007 and January 2008 events.

Secondary wastewater characterisation

None of the samples were above the screening level of 0.5 Bq/L for gross alpha or gross beta particle activity in secondary wastewater (with ^{40}K contribution excluded) (Table 6.9.1). Maximum gross alpha particle activity in the secondary wastewater was 0.11 Bq/L and maximum gross beta particle activity was 0.05 Bq/L (excluding the ^{40}K contribution). These activities correspond to a RQ max of 0.22 for gross alpha and 0.1 for gross beta particle activity respectively.

Table 6.9.1: Gross alpha and gross beta particle activity in secondary wastewater

Gross alpha								
Location	**Point**	**n**	**Mean**	**Std dev**	**Min**	**Max**	**RQ (mean)**	**RQ (max)**
KWRP	Before MF	4	0.053	0.039	0.017	0.09	0.106	0.18
Subiaco	secondary wastewater	2	0.062	0.051	0.025	0.098	0.124	0.19
BPP	Before MF	4	0.081	0.039	0.023	0.11	0.162	0.22
Gross beta								
KWRP	Before MF	2	-0.055	0.006	-0.059	-0.057	-	-
Subiaco	secondary wastewater	1	-0.026		-0.026	-0.026	-	-
BPP	Before MF	3	0.012	0.034	-0.014	0.05	0.024	0.1

n, number of samples; Std dev, standard deviation; RQ, risk quotient calculated using ADWG guideline of 0.5 Bq/L

RO Product water characterisation

Maximum gross alpha particle in the recycled water was 0.035 Bq/L and maximum gross beta (excluding ^{40}K) particle activity was 0.03 Bq/L, which correspond to a RQ (max) of 0.07 for gross alpha and 0.06 for gross beta particle activity respectively.

Table 6.9.2: Gross alpha and gross beta particle activity in product water

Gross alpha								
Location	**Point**	**n**	**Mean**	**Std dev**	**Min**	**Max**	**RQ (mean)**	**RQ (max)**
KWRP	Post-RO water	4	0.023	0.010	0.016	0.035	0.046	0.07
BPP	Post-RO water	2	0.023	0.015	0.012	0.033	0.046	0.07
Gross beta								
KWRP	Post-RO water	2	0.016	0.008	0.01	0.023	0.032	0.046
BPP	Post-RO water	2	0.017	0.019	0.003	0.03	0.034	0.06

n, number of samples; Std dev, standard deviation; RQ, risk quotient calculated using ADWG guideline of 0.5 Bq/L

Groundwater characterisation

Maximum gross alpha particle activity in the groundwater was almost double (0.062 Bq/L) compared with the maximum concentration in the product water (0.035 Bq/L). Similarly the maximum gross beta (excluding ^{40}K) particle activity in groundwater was almost double (0.057 Bq/L) compared with the observed in the product water (0.03 Bq/L). Nevertheless, the RQ(max) for gross alpha was 0.12 and for gross beta was 0.11 respectively.

Table 6.9.3: Gross alpha and gross beta particle activity in groundwater

Location	Particle activity	n	Mean	Std dev	Min	Max	RQ(mean)	RQ(max)
Wanneroo	Gross alpha	4	0.041	0.018	0.02	0.062	0.082	0.12
Wanneroo	Gross beta	3	0.048	0.008	0.042	0.057	0.096	0.114

n, number of samples; Sd, standard deviation; RQ, risk quotient calculated using ADWG guideline of 0.5 Bq/

Screening health risk assessment

The maximum and mean risk quotients RQ(max) and RQ(mean) respectively are presented in Tables 6.9.1, 6.9.2 and 6.9.3. RQs for gross alpha and gross beta particle activity were below 1 and after the advanced treatment RQs were one order of magnitude below 1 indicating a further reduction of the particle activity through RO.

Treatment performance

Advanced treatment of secondary wastewater by MF/RO reduces the concentration of gross alpha and gross beta particle activity to produce water of high quality with an average removal of 95% for gross beta (ranged from 80.5% to 98%) and almost 80% for gross alpha particle activity, (ranged from 67% to 89%). The removal efficiency of the advanced treatment is illustrated in Figure 6.9.1

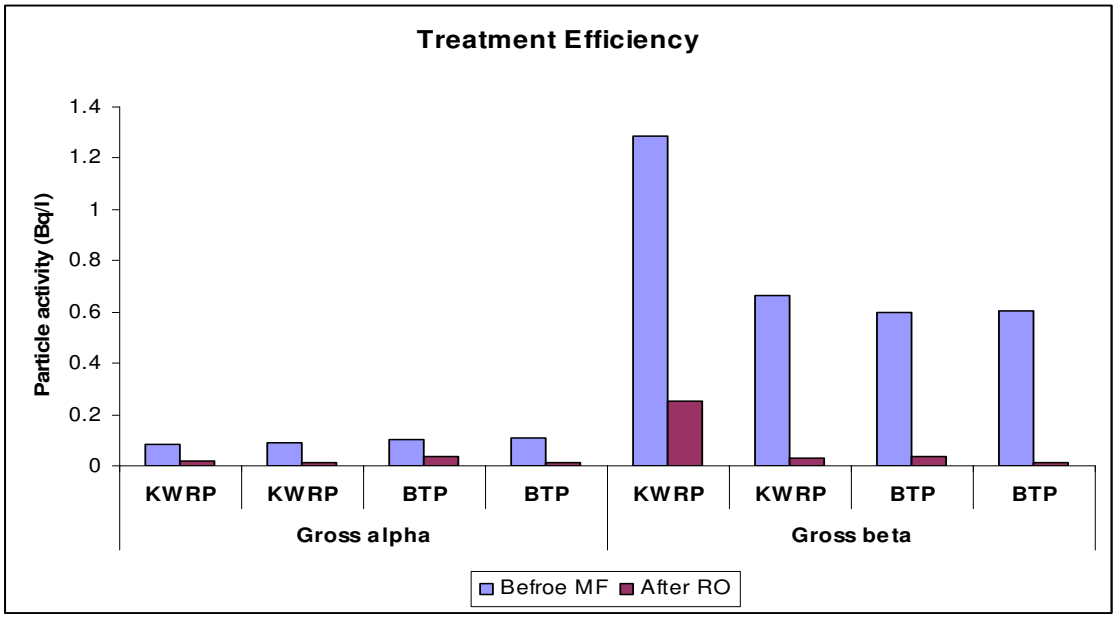

Figure 6.9.1: Removal of gross alpha and gross beta particle activity by advanced treatment

Discussion

Based on the results obtained for gross alpha and gross beta particle activity before and after the advanced treatment, no increased human health risk associated with radionuclides is anticipated if recycled water is used to augment drinking water supplies in Perth. The results are also consistent with other studies in which percentage of gross alpha and gross beta particle removal by membranes is above 90% (Asano *et al.*, 2007, Moritz *et al.*, 1995) and with the results achieved by water recycling plants using membranes for indirect potable reuse schemes (OCWD, 2006, WBMWD, 2006, Singapore Government, 2002)

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6.10 Pharmaceuticals

Introduction

Pharmaceuticals comprise a broad class of compounds developed to improve human and animal health. Pharmaceuticals are biologically active, chemically complex molecules, and they require extensive testing before registration and use. For most pharmaceuticals the ratio of the dose causing toxicity to the dose giving a beneficial effect (the Therapeutic Index) is large. Pharmaceuticals are not yet considered in drinking water quality guidelines in Australia (ADWG, 2004) or elsewhere (WHO, 2006). The wide range of chemical classes represented in the group means that generalisations on their behaviour is impossible (Daughton, 2001, Cunningham, 2004). Numerous environmental impacts may occur, including acute or chronic toxicity (Cleuvers, 2003, Nentwig *et al*., 2004, Enick and Moore, 2007, Dussault *et al*., 2008), endocrine disruption (Sumpter, 2005), interference with detoxification systems (Epel and Smital, 2001), stimulation of reproductive processes (Fong, 2001), and inhibition of primary productivity (Halling-Sorensen *et al*., 1998). Their mobility in soils and sediments can vary (Oppel *et al*., 2004), while toxicity effects may also vary depending on whether the pharmaceuticals are in aquatic or sediment phase (Nentwig *et al*., 2004). Specific classes of pharmaceuticals may share common characteristics. For example, the iodinated contrast media (ICM) are the most widely administered intravascular pharmaceuticals, administered in very high doses (60-120 g) (Christiansen, 2005). They are chemically inert, metabolically stable, and rapidly eliminated from the body via urine or faeces. While they are considered non-toxic to humans and wildlife (Christiansen, 2005, Steger-Hartmann *et al*., 1999, Putschew and Jekel, 2006), ICM persist in the aquatic environment and leach through the subsoil into groundwater aquifers (Drewes *et al*., 2001, Perez and Barcelo, 2007).

Municipal wastewater represents the main disposal pathway for pharmaceuticals consumed in household, hospital and industrial settings (Benotti *et al*., 2009). The Commonwealth government supports the safe disposal of unused medications in the community via a collection and incineration programme (Return Unwanted Medicines Project, Commonwealth Dept of Health & Ageing). Hospital wastewaters entering sewerage networks without any pre-treatment have shown pharmaceutical concentrations in WWTP primary influent in the order of µg/L (Carballa *et al*., 2004, Joss *et al*., 2005, Vieno *et al*., 2005, Al-Rifai *et al*., 2007, Santos *et al*., 2007). WWTPs are designed and regulated to remove nutrients, and any micropollutant removal is a side benefit of the existing treatment. Many pharmaceuticals have been detected in secondary wastewater at measurable concentrations (10 µg/L down to 10 ng/L) (Carballa *et al*., 2004, Carballa *et al*., 2005, Joss *et al*., 2005, Vieno *et al*., 2005, Yu *et al*., 2006, Drewes *et al*., 2008b, Kolpin *et al*., 2002, Ternes and Joss, 2006), demonstrating that classical activated sludge treatment is not capable of removing all pharmaceuticals from the influent sewage. Large variations in removal rates have been reported between different WWTP (Kanda *et al*., 2003), and even within a single WWTP, particularly with variable WWTP efficiency or seasonality

(Vieno *et al*., 2005, Ternes, 1998). Drinking water treatments such as sorption, flocculation and chloramination have also been demonstrated having variable efficiency for pharmaceutical removal, although both ozonation and sorption onto granular activated carbon (GAC) were generally more efficient (Drewes *et al*., 2008b, Ternes *et al*., 2002, Benotti *et al*., 2009).

Pharmaceuticals have also been detected at very low concentrations (ng/L) in drinking water supplies (Putschew *et al*., 2000, Loraine and Pettigrove, 2006, Rabiet *et al*., 2006, Seitz *et al*., 2006, Versteegh *et al*., 2007, Barnes *et al*., 2008, Focazio *et al*., 2008, Benotti *et al*., 2009, Ternes *et al*., 2002). While studies reported thus far indicate that no appreciable human health risk is anticipated given that measured concentrations are well below health based limits (Schulman *et al*., 2002, Webb *et al*., 2003, Blanset and Robson, 2005, Schwab *et al*., 2005, Watts *et al*., 2007). Nevertheless, the potential implications of human chronic exposure to trace levels are still unclear and pharmaceuticals have been shown to impact on aquatic organism at very low concentrations. Some pharmaceuticals such as atenolol, bezafibrate, and ciprofloxacin have significant effect on prokaryote and eukaryotic cells on *in-vitro* studies at environmental exposure levels (Pomati *et al*., 2008). For example the lowest observed effect concentration (LOEC) for fish toxicity has been reported in the range of wastewater concentrations, whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured WWTP effluent concentrations (Fent *et al*., 2006).

In this section results from the analysis of 36 pharmaceuticals are presented. The pharmaceuticals chosen represent different therapeutic drug classes of commonly prescribed pharmaceuticals in Australia, including lipid lowering agents, analgesics, antipyretics, non steroidal anti-inflammatory drugs, antidepressants, anticoagulants, tranquilizers, cytostatic agents, antiepileptics, antibiotics and x-ray contrast agents. Selection of chemicals to monitor during MF/RO treatment was based on:

- occurrence as described in published literature of pharmaceuticals in wastewater from other studies,
- prescription drug status
- volume of use
- toxicity
- pharmaceutical class, and
- laboratory capability to develop appropriate analytical methods, including availability of standards.

The description of pharmaceuticals is presented by groups. Ten antibiotics (amoxicillin, azitromycin, clarithromycin, clindamycin, erythromycin-H_2O, metronidazole, roxithromycin, sulfamethoxazole, trimethoprim and tylosin) were analysed. Eight ICM compounds (iopamidol, iotalamic acid, amidotrizoic acid, ioxaglic acid, iodipamide, iohexol, iopromide and iomeprol) and 18 pharmaceuticals

classified as “other” were analysed (clofibric acid, bezafibrate, gemfibrozil, atorvastatin, carbamazepine, phenytoin, diclofenac, ibuprofen, indomethacin, ketoprofen, naproxen, acetaminophen, cyclophosphamide, ifosfamide, warfarin, fluoxetine, diazepam and morphine).

Similar to other sections in this chapter, the characterisation of chemical contaminants in wastewater, groundwater and after the advanced treatment is presented. The efficiency of the treatment to remove these contaminants was determined and the potential human health risk was calculated by comparing measured concentrations before and after the MF/RO treatment with health values.

Methods

All pharmaceuticals were measured by liquid chromatography (LC) and tandem mass spectrometry (MS/MS). The pharmaceutical were grouped into 4 different analytical methods for ICM, antibiotics, acidic pharmaceuticals, and neutral or basic pharmaceuticals. ICM were measured using direct injection (DI) LC-MS/MS, with no sample preconcentration (Busetti *et al.*, 2008). Filtered samples (100 µL) were directly injected onto a reversed phase LC column (C18). Identification and quantification by MS/MS used positive electrospray ionization (ESI(+)) in the multi reaction mode (MRM) The antibiotic and pharmaceutical analytical methods all incorporated a solid phase extraction (SPE) preconcentration and clean-up step before analysis. For the antibiotics, filtered samples (500 mL wastewater, 1 L post-RO water) were extracted using a copolymer SPE cartridge and concentrated to ~500 µL. Extracts were separated using a reversed phase LC column (C18). Identification and quantification by MS/MS used ESI(+) and MRM (Busetti and Heitz, 2009). For neutral or basic pharmaceuticals (Busetti *et al.*, 2009), filtered samples (500 mL wastewater, 1 L post-RO water) were extracted using a surface modified styrene divinylbenzene SPE cartridge at pH=7 and concentrated to ~500 µL. Extracts were separated using a reversed phase LC column (C18). Identification and quantification by MS/MS used ESI(+) and MRM. For acidic pharmaceuticals (Busetti *et al.*, 2009), filtered samples (500 mL wastewater, 1 L post-RO water) were extracted using a surface modified styrene divinylbenzene SPE cartridge at pH=3.5 and concentrated to ~500 µL. Extracts were separated using a reversed phase LC column (C12). Identification and quantification by MS/MS used negative electrospray ionization (ESI(-)) and MRM. For all methods, analyte identification was based on chromatographic retention time (RT) and compound-specific MRM transitions. Matrix effects were overcome by inclusion of deuterated internal standards for the majority of analytes.

All methods were verified for the analytes of interest in both secondary wastewater and post-RO water. The limits of quantitation (LOQ) and estimated uncertainties for each method are listed in Table 6.10.1.

Table 6.10.1: Health values, limits of detection (LOQ) and estimation of uncertainty for pharmaceuticals. For ICM, uncertainty was calculated assuming a concentration of 10000 ng/L for both secondary wastewater and post-RO water. For all other analytes, uncertainty was calculated assuming concentration of 200 ng/L for secondary wastewater and 100 ng/L for post-RO water

Analyte	Health Value (ng/L)	MQ water LOQ[A] (ng/L) Average	Wastewater LOQ[A] (ng/L) Average	MQ Water Standard Relative Uncertainty (%)	Wastewater Standard Relative Uncertainty (%)
ICM					
Iopamidol (IOD)	360000	600	700	10.9%	18.7%
Iomeprol (IOM)	810000	700	2400	12.9%	21.8%
Iohexol (IOX)	650000	700	2700	18.6%	46.2%
*Amidotrizoic Acid (DTZ)	360000	1250	2800	11.2%	22.4%
Iothalmic Acid (ITA)	350000	1900	3200	11.8%	24.3%
Iopromide (IOP)	680000	650	670	25.0%	26.0%
Ioxaglic Acid (IXA)	350000	300	360	10.8%	15.2%
*Iodipamide (IDP)	540000	360	360	34.3%	38.9%
Antibiotics					
*Metronidazole	47000	31	53	18.1%	18.2%
Trimethoprim	63000	6.5	52	11.1%	17.7%
Sulfamethoxazole	32000	9	53	11.0%	23.3%
Azythromycin	35000	15	50	16.1%	18.2%
Clindamycin	270000	8	23	16.9%	18.9%
Tylosin	945000	7.5	27	26.0%	18.6%
Erythromycin-H_2O	15000	10	45	14.9%	21.3%
Clarithromycin	225000	7.5	30	16.4%	16.9%
Roxithromycin	135000	2.5	32	17.0%	12.5%
Amoxicillin	63000	500	1000	not determined	not determined
Pharmaceuticals extracted under neutral/basic conditions					
*Morphine	14000	25	100	17.7%	19.4%
Paracetamol	160000	10	125	12.5%	16.8%
*Ifosfamide	3000	25	100	27.5%	20.6%
Cyclophosphamide	3000	5	100	20.6%	22.0%
Fluoxetine	9000	5	25	19.6%	9.9%
*Phenytoin	135000	5	55	19.6%	36.9%
Carbamazepine	90000	1.5	40	8.5%	12.6%
Diazepam	2000	5	30	10.8%	17.6%
Ketoprofen	3000	25	275	43.9%	31.9%
*Warfarin	500	5	15	10.5%	10.4%
Bezafibrate	270	15	65	35.1%	9.4%
Atorvastatin	5000	20	150	34.3%	10.8%
Diclofenac	1600	2.5	20	13.3%	12.2%
Indometacine	22000	5	40	21.3%	13.7%
Pharmaceuticals extracted under acidic conditions					
Naproxen	200000	13	250	11.6%	20.1%
Clofibric acid	675000	1	15	11.6%	11.1%
Ibuprofen	360000	12	500	18.7%	21.9%
Gemfibrozil	540000	2.5	50	16.9%	12.3%

** Pharmaceuticals with health values calculated using lowest therapeutic dose (LTD) from Martindale, The Complete Drug Reference. All other health values from AGWR (2008)*

Quality assurance/ Quality control

Limits of detection (LODs) were calculated for every sample from the concentration equivalent to a signal to noise ratio (S/N) of 3, either using the MassLynX 4.0 software or, in some cases, manual S/N calculation using a peak of a known concentration. Limits of quantitation (LOQs) were calculated for every sample from the concentration equivalent to S/N equal to 10.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

One proficiency test for selected antibiotics was undertaken during the sampling period and the full details of these are available in Chapter 4. In summary, however, three participants (Curtin Water Quality Research Centre, National Measurement Institute, and DVGW-Technologiezentrum Wasser) analysed QA/QC samples collected during Event 2. Three antibiotics were measured by all three participants (erythromycin-H2O, roxithromycin, and sulfamethoxazole), while five other antibiotics were measured by Curtin and DVGW-Technologiezentrum Wasser (azithromycin, clarithromycin, clindamycin, metronizadole, and trimethroprim). While there was insufficient data to perform a statistical analysis, generally there was very good agreement between results from all three laboratories.

Results

A total of 2,790 measurements were analysed after excluding field blanks, trip blanks and replicates. Clofibric acid and metronidazole samples in Event 3 were contaminated (detections were seen in blanks) and therefore were excluded from the analysis. Similarly, contaminated samples occurred for ketoprofen in Event 5 and were also excluded from the analysis. Fewer pharmaceuticals were measured in Event 1 compared with other events because not all analytical methods were available at that time (Table 6.10.2). The majority of samples were taken at KWRP (50%) followed by Beenyup WWTP and BPP (37%). More than 80% of the samples were composite samples and about 6% of all samples were groundwater.

Table 6.10.2: Frequency of measurement of disinfection by products by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	WW	Water Reclamation Plant							
				Grab	Comp				Before MF		After MF		After RO		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006	141	31	172	0	0	78	0	0	0	47	0	47	172
2	May/June	6	2007	316	387	703	79	77	0	0	235	0	236	0	0	471
3	September	6	2007	0	480	480	0	0	120	120	0	0	120	120	0	480
4	January	6	2008	80	400	480	80	0	80	120	0	0	80	120	0	400
5	April	5	2008	0	480	480	0	40	80	120	0	40	80	120	0	440
6	June	5	2008	0	475	475	0	0	79	118	38	40	80	120	0	475
Total		32		537	2,253	2,790	159	117	437	478	273	80	643	480	47	2,438

Comp, composite; GW, groundwater, WW, Subiaco wastewater; MF; microfiltration, RO, reverse osmosis; K, Kwinana, B, Beenyup

Wastewater characterisation

Of 10 antibiotics tested, 9 were detected in secondary wastewater. However, the only undetected antibiotic, amoxicillin, had a significantly higher limit of quantification (LOQ=1000 ng/L in wastewater samples and LOQ=500 ng/L in post-RO water) compared to other antibiotics (see Table 6.10.1) and it seems reasonable to assume that amoxicillin would have been detected if LOQ had been comparable to that of other antibiotics.

Clarithromycin, roxithromycin, sulfamethoxazole and trimethoprim were detected in all wastewater samples, while azithromycin was detected in 89% of the samples (Figure 6.10.1). Median concentrations ranged from 20 ng/L for tylosin to 543 ng/L for sulfamethoxazole.

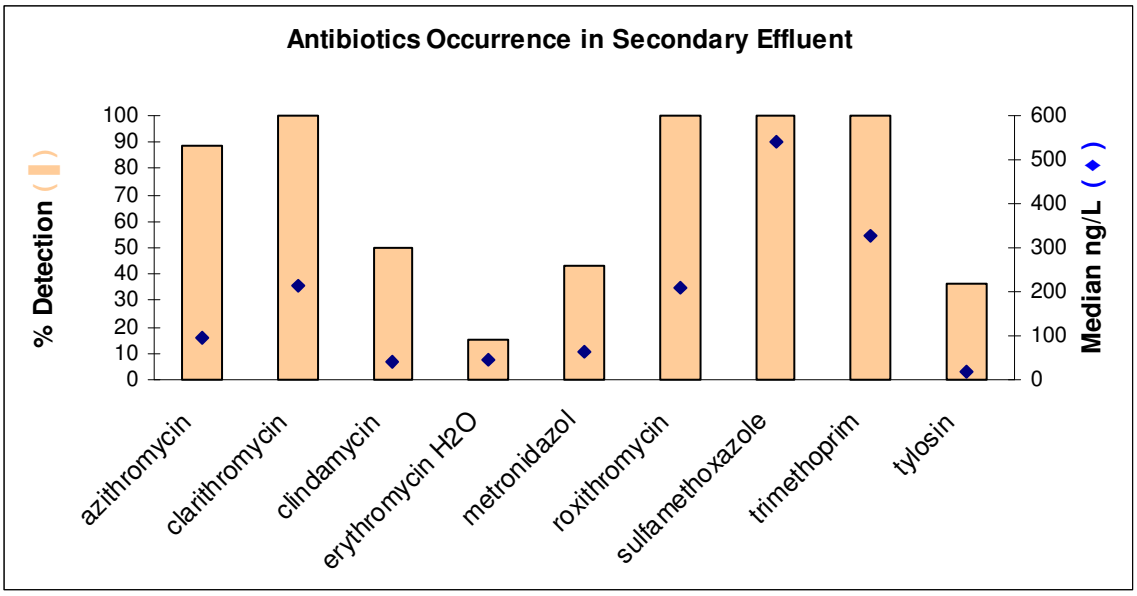

Figure 6.10.1: Antibiotics with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (ng/L, diamond).

Five of the 8 ICM were detected in secondary wastewater. Ioxaglic acid, iothalamic acid and iomeprol were not detected in any of the samples. Iopromide had the highest percentage detections (84% followed by iohexol 72%, (Figure 6.10.2). However, the median concentration of iohexol (2.3 µg/L) was higher than iopromide (1.2 µg/L). Median concentrations ranged from between 0.4 µg/L for iodipamide to 2.3 µg/L for iohexol.

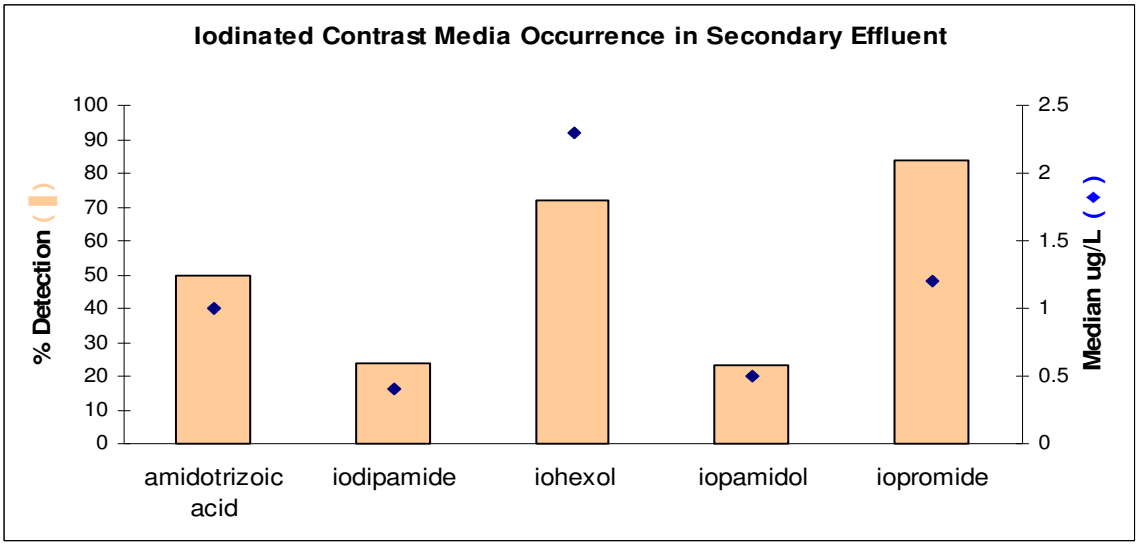

Figure 6.10.2: ICM compounds with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (ng/L, diamond).

Of the 18 pharmaceuticals classified as “other” pharmaceuticals, 15 were detected in at least one secondary wastewater sample (Figure 6.10.3). Clofibric acid and ketoprofen were the only two pharmaceuticals undetected in wastewater, although ifosfamide and cyclophosphamide were only detected once, in samples from the Subiaco WWTP (cyclophosphamide was 218 ng/L on the 12th June 2007, while ifosfamide was 199 ng/L on 3rd April 2008). Diclofenac was detected in all wastewater samples followed by carbamazepine (96.6%), indomethacin (96.6%) and gemfibrozil (96.4%). Median concentrations range from: 18 ng/L for warfarin to 940 ng/L for carbamazepine.

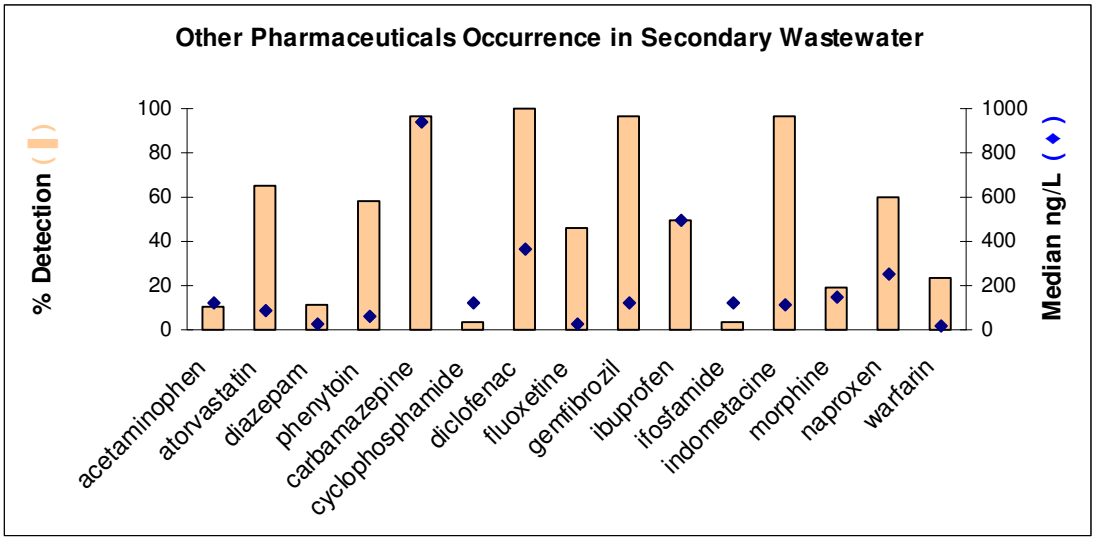

Figure 6.10.3: Other pharmaceuticals with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (ng/L, diamond).

Comparison of median concentrations of pharmaceuticals at each WWTP plant shows some differences. Median concentrations of antibiotics by WWTP are presented in Figure 6.10.4. While azithromycin, clarithromycin, clindamycin, erythromycin, metronidazole and roxithromycin showed highest median concentrations at Subiaco, the differences were not statistically significant compared to values at other plants. Erythromycin did not have statistically significant differences between plants due to the small sample size at Subiaco. Sulfamethoxazole and trimethoprim concentrations were highest at KWRP and the differences were statistically significant (K Wallis p=0.01 and p=0.0001, respectively).

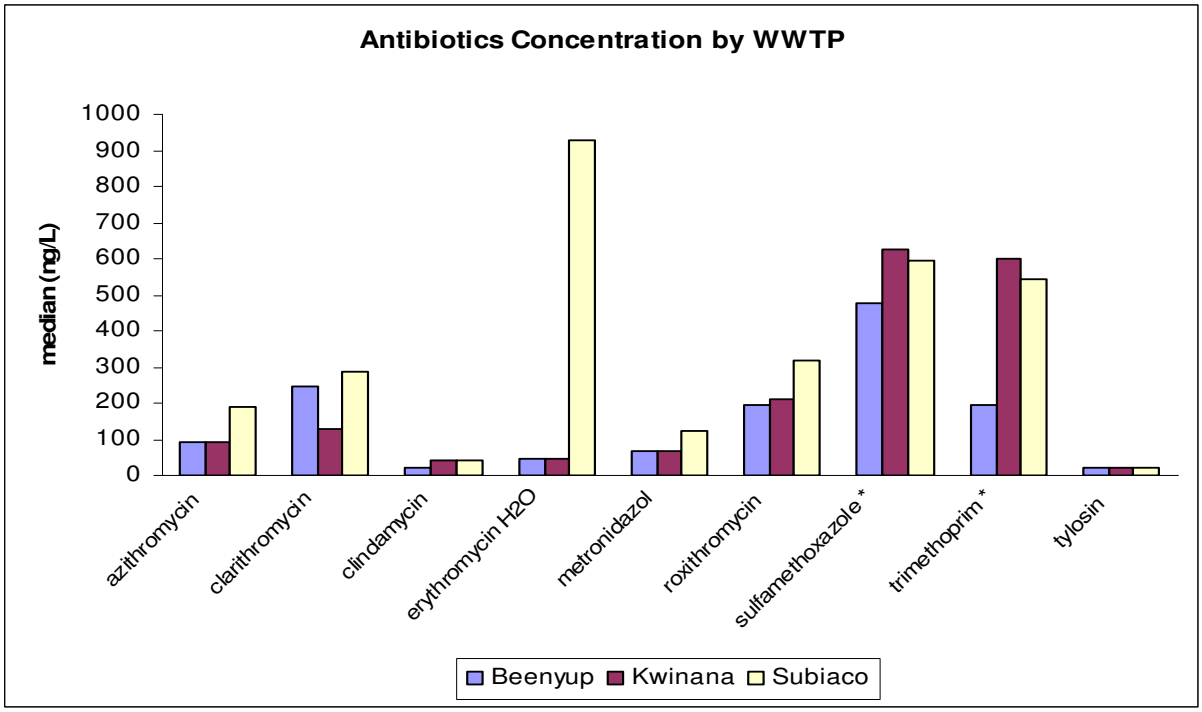

Figure 6.10.4: Median antibiotics concentration by WWTP in ng/L

**Antibiotics with statistically significant differences in concentrations among plants.*

For the ICM (Figure 6.10.5), median amidotrizoic acid, iohexol, iopamidol and iopromide concentrations were highest at Subiaco, with statistically significant differences reported for iohexol (K Wallis p=0.03) and iopamidol (K Wallis p=0.02).

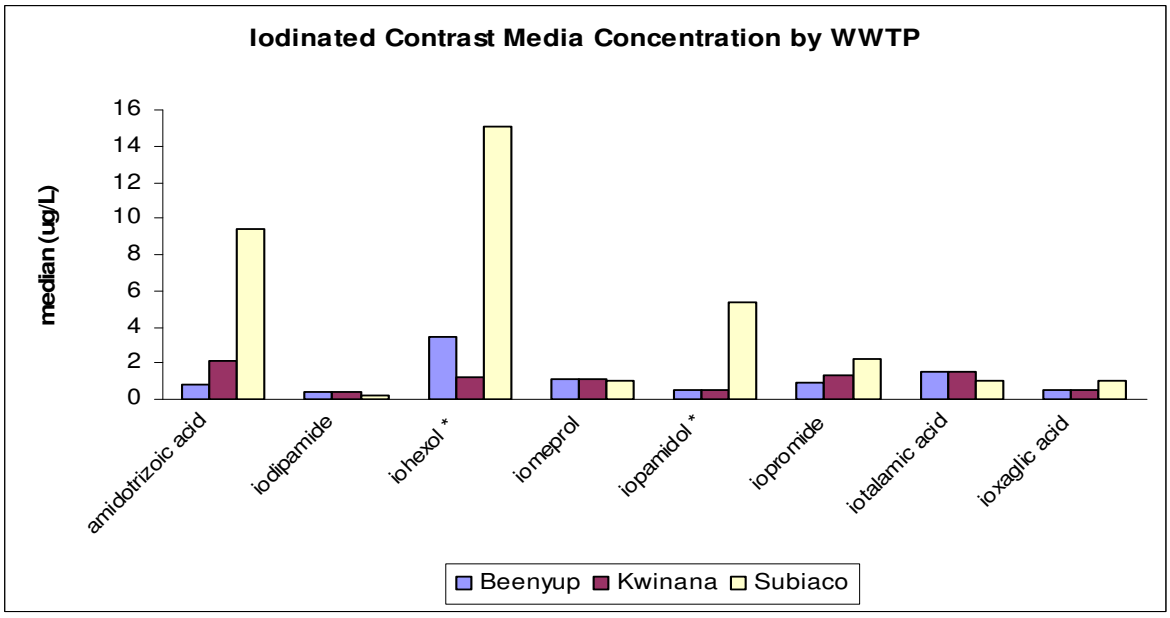

Figure 6.10.5: Median iodinated contrast media concentration by WWTP in µg/L

** ICM compounds with statistically significant differences in concentrations among plants.*

For the 'other' pharmaceuticals (Figure 6.10.6), median gemfibrozil and indomethacin concentrations were highest at Subiaco and the difference was significance (K Wallis p=0.004 and p=0.01, respectively). Median atorvastatin, diazepam, and phenytoin concentrations were also highest at Subiaco WWTP but the differences were not statistically significant. Carbamazepine, diclofenac and naproxen were statistically higher at Beenyup (K Wallis p=0.02, p=0.01 and p=0.01 respectively) while fluoxetine was slightly, but not significantly, higher. Acetaminophen was the only pharmaceutical found highest at KWRP, but the difference was not significant.

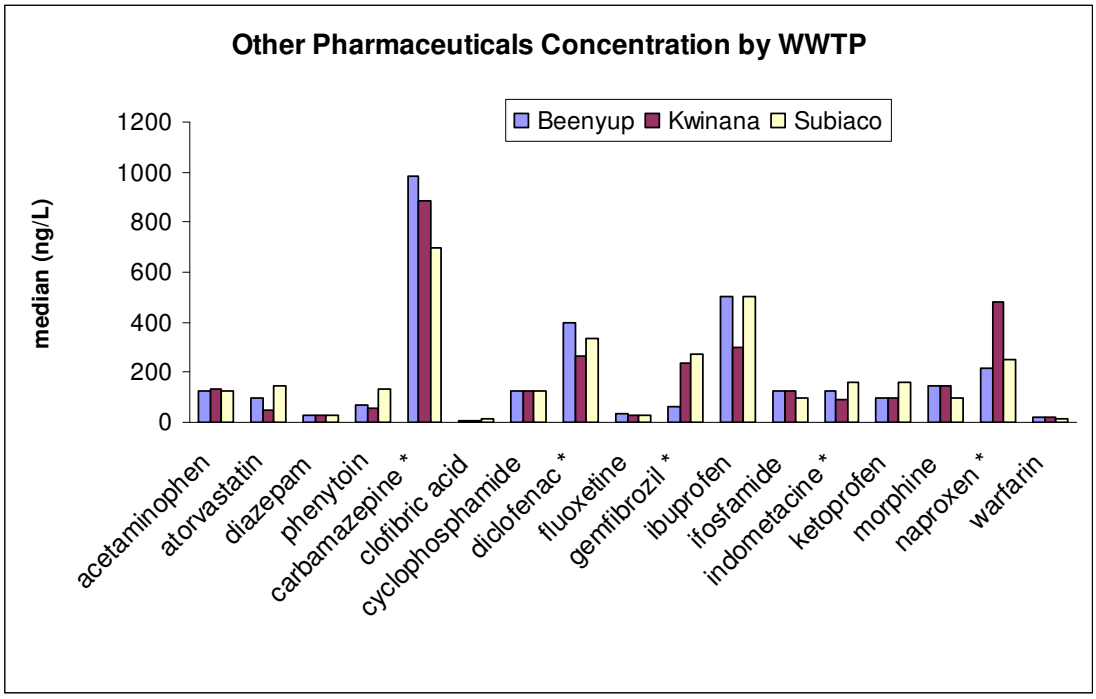

Figure 6.10.6 Median pharmaceuticals concentration by WWTP in ng/L

**Other pharmaceuticals with statistically significant differences in concentrations among plants.*

Seasonal variations in median antibiotic concentrations are shown in Figure 6.10.7. No data for metronizadole in spring was available because all concentrations were below LOQ. Differences that were not statistically significant include maximum median concentrations for azithromycin in summer, clindamycin in winter, metronidazole in autumn, and sulfamethoxazole in spring. However, the maximum median concentrations of clarithromycin, erythromycin, roxithromycin and tylosin in spring were statistically significantly higher than values in the three other seasons.

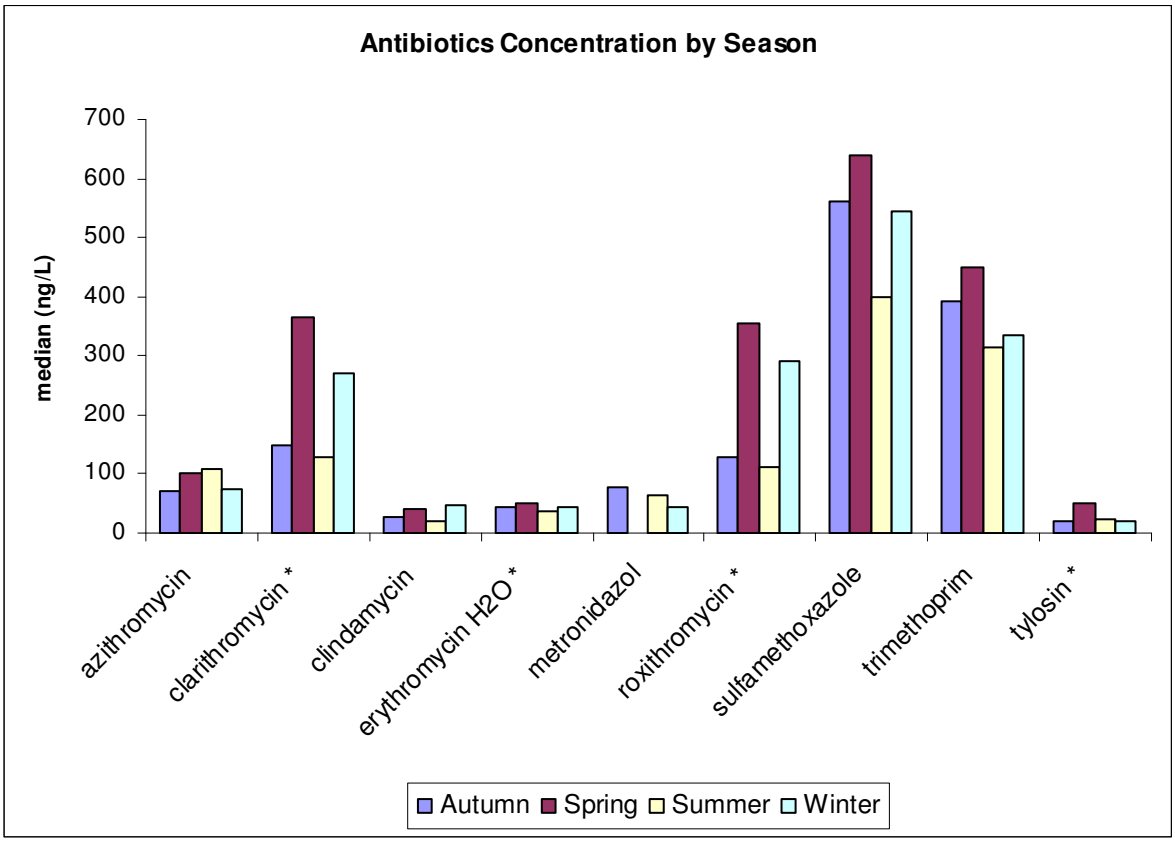

Figure 6.10.7: Median antibiotics concentration by season in ng/L

**Antibiotics with statistically significant differences in concentrations by season.*

Seasonal trends in median ICM concentrations are shown in Figure 6.10.8. All ICM except amidotrizoic acid and iohexol were detected at statistically significant higher concentrations during spring. Amidotrizoic acid was at maximal during summer while iohexol was at maximal during winter, but neither difference was statistically significant.

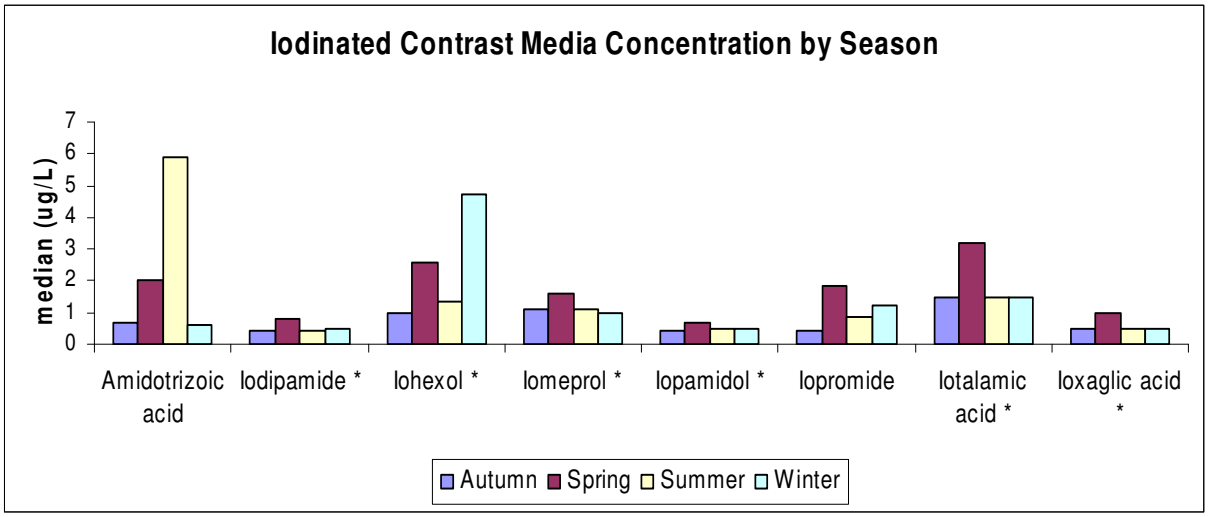

Figure 6.10.8: Median concentration (µg/L) of iodinated contrast media by season

**ICM compounds with statistically significant differences in concentrations by season.*

Seasonal variations in median 'other' pharmaceutical concentrations are shown in Figure 6.10.9. The only pharmaceutical reported to have a maximum concentration during the winter period was diazepam, but the difference was statistically significant (K Wallis p=0.003). In autumn, maximums were seen for ibuprofen and warfarin and the differences were again statistically significant (K Wallis p=0.04 and p=0.01 respectively). In summer clofibric acid, carbamazepine and indomethacin concentrations were highest but the differences were not statistically significant. All other pharmaceuticals were at maximum concentration during spring, and differences were statistically significant for phenytoin (K Wallis p=0.0003), fluoxetine (K Wallis p=0.0007), ketoprofen (K Wallis p=0.018) and morphine (K Wallis p=0.02).

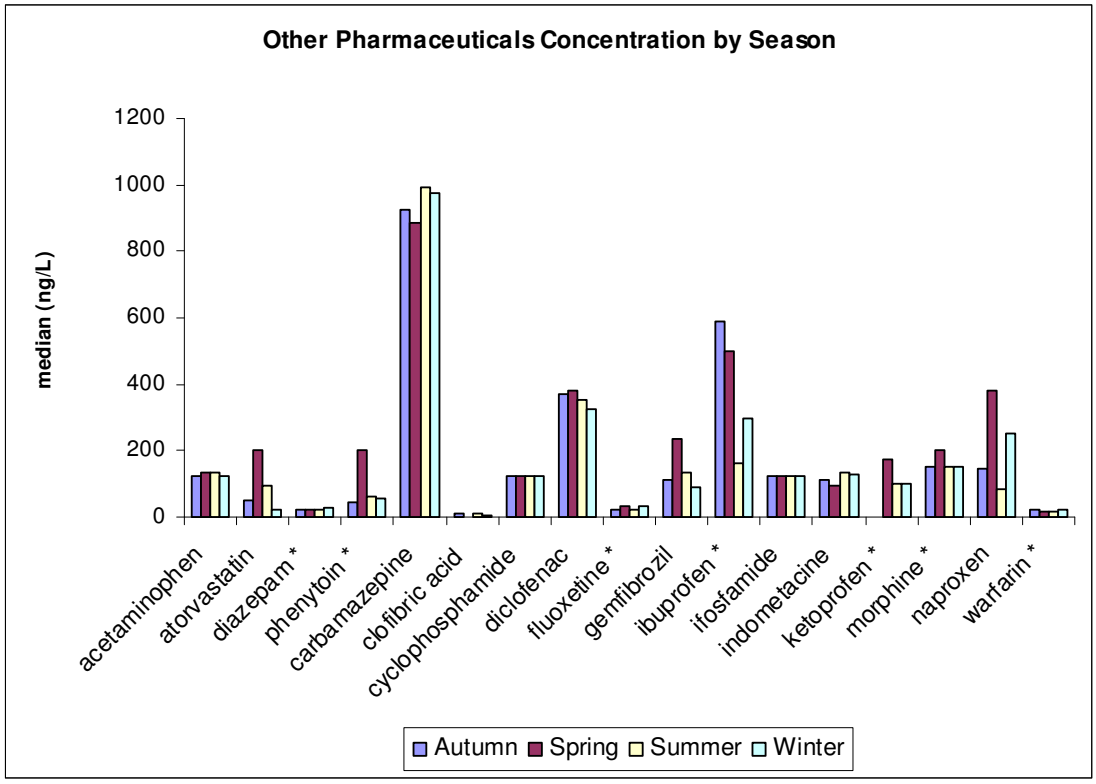

Figure 6.10.9: Median concentration (µg/L) of other pharmaceuticals by season

**Other pharmaceuticals with statistically significant differences in concentrations by season.*

RO Product water characterisation

None of the ICM or antibiotic compounds were detected in post-RO water. Of the 'other' pharmaceuticals, only diazepam, naproxen and clofibric acid were detected, and percentage detections were less than 10%. The details of detected pharmaceuticals in the post-RO water are presented in Table 6.10.3. No data on clofibric acid and naproxen concentrations before MF were available. Data presented in Table 6.10.3 corresponds to post-MF measurements.

Table 6.10.3: Pharmaceuticals detected Post-RO

Pharmaceutical	units	Date	Secondary Wastewater		Post-RO		Field blank
			LOQ	Value	LOQ	Value	
diazepam	ng/L	21/09/2007	25	26.4	7.5	18.4	<7.5
diazepam	ng/L	28/09/2007	25	<25	7.5	12	8.0
clofibric acid*	ng/L	30/05/2007	15	<15	1.0	1.6	<1.0
naproxen*	ng/L	7/06/2007	250	1210	13	15	<13

** Secondary wastewater was not sampled during these days and therefore samples from post-MF are presented as secondary wastewater data.*

Groundwater characterisation

Seven of the 36 tested pharmaceuticals analysed during the project were detected in at least one of the four groundwater samples taken during the project (Table 6.10.4). In May 2007, iodipamide, iopromide and iopamidol were detected in the Pinjar Borefield line. Fluoxetine and iodipamide were detected in the Wanneroo Borefield line in the May 2007 sampling event. Gemfibrozil, diclofenac and roxithromycin were detected in the samples collected from the Pinjar Borefield line in January 2008. Concentrations detected were close to the LOQ except for gemfibrozil and diclofenac, which were observed once each at concentrations very much below health values (>4 orders of magnitude below for gemfibrozil and at 3.5% of the health value for diclofenac).

Table 6.10.4: Pharmaceuticals detected in Groundwater

Pharmaceutical	units	LOQ	% detection	Detected Concentration
iodipamide	µg/L	0.1	50	0.18
iopromide	µg/L	0.1	25	0.53
iopamidol	µg/L	0.1	25	0.1
gemfibrozil	ng/L	3	25	22
diclofenac	ng/L	2.5	25	56
fluoxetine	ng/L	20	25	38
roxithromycin	ng/L	3.5	25	4.8

Screening health risk assessment

Health values for pharmaceuticals were calculated using the lowest therapeutic dose from the pharmacopeia (which in itself is usually significantly lower than the dose that would cause toxicity). It was assumed that 100% of pharmaceutical intake was from drinking water as per AGWR (2008). The standard safety factor was 1000 derived from: 10 for intraspecies variability; 10 for using the lowest therapeutic dose instead of the no-observed effect concentration; and 10 for protection of sensitive population subgroups including children and infants. An extra safety of factor of 10 was applied for cytotoxic/genotoxic pharmaceuticals (AGWR, 2008).

Risk Quotients for undetected pharmaceuticals are presented in Table 6.10.5 and have been calculated using the LOQ for post-RO water averaged from Event 1 to Event 6. The RQ for amoxicillin was calculated based on an ADI of 200 µg/kg/day with an extra safety factor to account for allergic reactions of 10. The guideline value in the AGWR of 1.5 µg/L was calculated using an ADI of 0.43 µg/kg/day based on the maximum permitted daily intake per day for penicillins in relation to the prevention of allergic reactions. Even when the much lower AGWR guideline value is used to calculate the RQ for amoxicillin, it was below 1 (RQ=0.33, not reported in Table 6.10.5). All RQs for other undetected pharmaceuticals were at least two orders of magnitude below 1.

Table 6.10.5: RQs for pharmaceuticals without detections in any of the samples

Parameter	Average LOQ for post-RO samples (µg/L)	n	Tier	Health value (µg/L)	Source	RQ
amoxicillin	0.5	48	2	63	ADI=200 (DHA)	0.008
bezafibrate	0.017	29	2	270	LTD = 600	0.0001
iomeprol	0.84	66	2	810	LTD =1800	0.001
iothalamic acid	1.2	66	2	350	US Food and Drug Adm	0.003
ioxaglic acid	0.5	65	2	350	US Food and Drug Adm	0.001
ketoprofen	0.024	54	2	3	ADI=1	0.008

n, number of samples; ADI, acceptable daily intake in µg/kg bw; DHA, Department of Health and Ageing – ADI list 2008; LTD, Lowest therapeutic dose.

RQs for the detected pharmaceuticals for wastewater (pre-MF) and post-RO water are presented in Table 6.10.6. RQ(max) used the maximum concentration measured for each analyte, while RQ(median) uses the median concentration measured for each analyte. Most analytes were not detected in post-RO water, and therefore RQs for post-RO water were also calculated using the LOQ averaged from Event 1 to Event 6. While diazepam, naproxen and clofibric acid were detected in post-RO water, concentrations were all below the average LOQ and therefore no RQ(max) was calculated.

Calculated RQ(median) in secondary wastewater were between one and four orders of magnitude below 1 except for diclofenac (RQ=0.2) due to its relatively high LOQ (Table 6.10.6). None of the RQ(max) was above 1 in the secondary treated wastewater. Calculated RQs of post-RO water were two to five orders of magnitude below 1. The results indicate that measured concentrations of pharmaceuticals in secondary wastewater are of low health significance and that the advanced RO treatment is able to reduce the risk by at least one order of magnitude further.

Table 6.10.6: Pharmaceuticals detected before MF and after RO corresponding RQs

		Before MF			Post-RO water		
parameter	**Health Value (ng/L)**	**LOQ (ng/L)**	**RQ(median)**	**RQ(max)**	**LOQ (ng/L)**	**RQ(median)**	**RQ(max)**
Antibiotics							
azithromycin	35000	42.5	0.003	0.006	13.8	0.0004	na
clarithromycin	225000	29.4	0.0009	0.002	7.5	0.00003	na
clindamycin	270000	27.6	0.0001	0.0004	9.1	0.00003	na
erythromycin H2O	15000	45.2	0.003	0.09	11.4	0.0008	na
metronidazol	47000	46.2	0.001	0.004	27.5	0.0006	na
roxithromycin	135000	32.2	0.002	0.003	2.5	0.00002	na
sulfamethoxazole	32000	45.8	0.02	0.03	9.3	0.0003	na
trimethoprim	63000	46.5	0.005	0.02	8.8	0.0001	na
tylosin	945000	27	0.00002	0.00006	7.6	0.000008	na
ICM							
amidotrizoic acid	360000	800	0.0003	0.03	800	0.002	na
iodipamide	540000	400	0.0001	0.002	300	0.0006	na
iohexol	650000	1000	0.0004	0.03	1000	0.002	na
iopamidol	360000	400	0.0001	0.02	300	0.0008	na
iopromide	680000	400	0.0002	0.005	300	0.0004	na
Other							
acetaminophen	160000	124	0.0008	0.005	36.3	0.0002	na
atorvastatin	5000	47	0.02	0.06	10.6	0.002	na
carbamazepine	90000	25.8	0.01	0.01	3.8	0.00004	na
clofibric acid*	675000	9.6	0.00001	na	1.3	0.000002	na
cyclophosphamide	3000	116.7	0.04	0.07	19.3	0.006	na
diazepam*	2000	27.0	0.01	0.02	7.3	0.004	na
diclofenac	1600	16.7	0.2	0.3	5.0	0.003	na
fluoxetine	9000	24.0	0.003	0.008	9.8	0.0001	na
gemfibrozil	540000	43.0	0.0002	0.002	19.4	0.00004	na
ibuprofen	360000	253	0.001	0.003	9.0	0.00003	na
ifosfamide	3000	116.7	0.04	0.07	37.5	0.01	na
indomethacin	22000	32.8	0.005	0.009	22.9	0.0010	na
morphine	14000	140	0.01	0.04	26	0.002	na
naproxen*	200000	136.6	0.001	0.007	6.2	0.00003	na
phenytoin	135000	52.3	0.0005	0.002	18.3	0.0001	na
warfarin	500	16.6	0.04	0.05	5.5	0.001	na

**Pharmaceuticals detected in post-RO water.*

Treatment performance

Treatment efficiency was calculated for analytes detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOQ after RO, the efficiency was calculated assuming a concentration equal half the LOQ as worst case scenario. For antibiotics,

the mean treatment performance ranged from 82% for clindamycin to 99% for roxithromycin (Figure 6.10.12). Most antibiotics had between 18 and 21 paired samples (before MF and after RO) to calculate the efficiency. However, only 3 samples were used to calculate the efficiency of metronidazole, which may explain the very large variability in removal efficiency compared with other antibiotics. Variability was also high for clindamycin and tylosin, with 9 and 7 paired samples respectively. Mean efficiency was above 90% for azithromycin, clarithromycin, roxithromycin, sulfamethoxazole and trimethoprim indicating a very good removal during the treatment. Antibiotics with poorer efficiency were often present in wastewater at concentrations only slightly larger than the post-RO water LOQ (e.g. tylosin, metronixadole, erythromycin-H_2O and clindamycin).

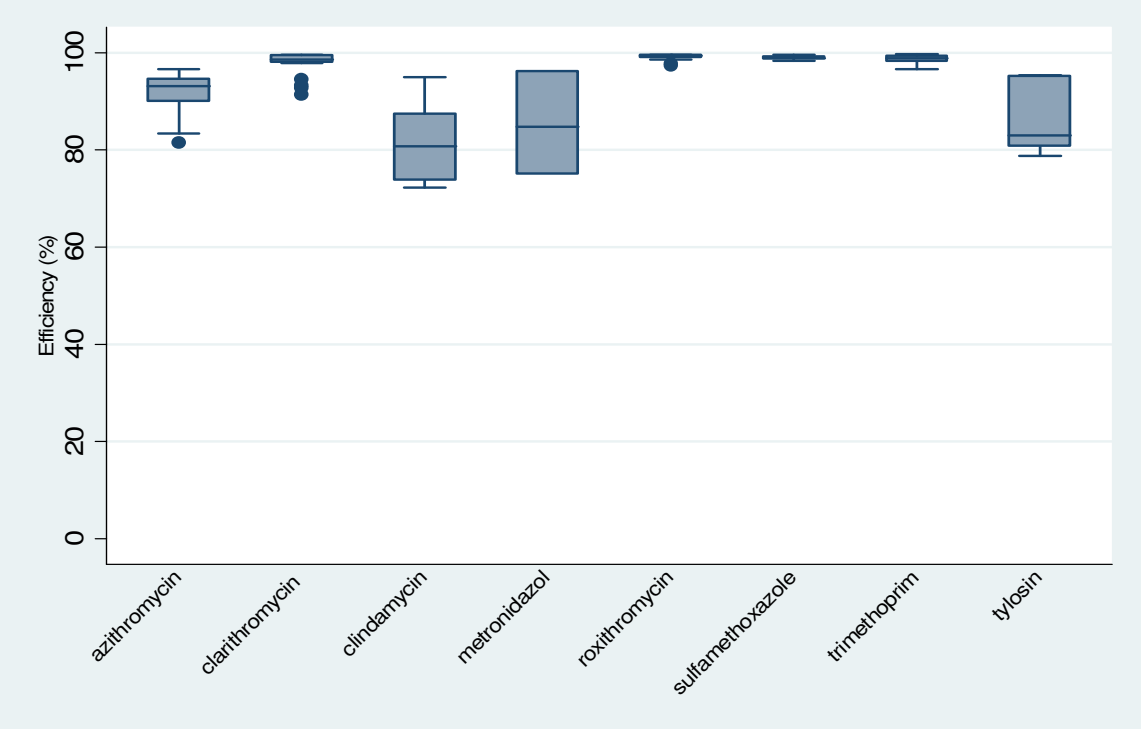

Figure 6.10.10: MF/RO removal efficiency of antibiotics detected in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

The number of measurements used in the analysis of efficiency of ICM compounds ranged between 2 (iopamidol) and 16 (iopromide). The average calculated treatment efficiency ranged from 70% for iopamidol to 85% for iopromide (Figure 6.10.13), however the RO treatment was able to consistently remove all these compounds below LOQ. Again the removal efficiency calculated was probably affected by the fact that the pre-RO concentrations were relatively close to LOQ. Variability in treatment removal was slightly higher for amidotrizoic acid compared with other ICM compounds.

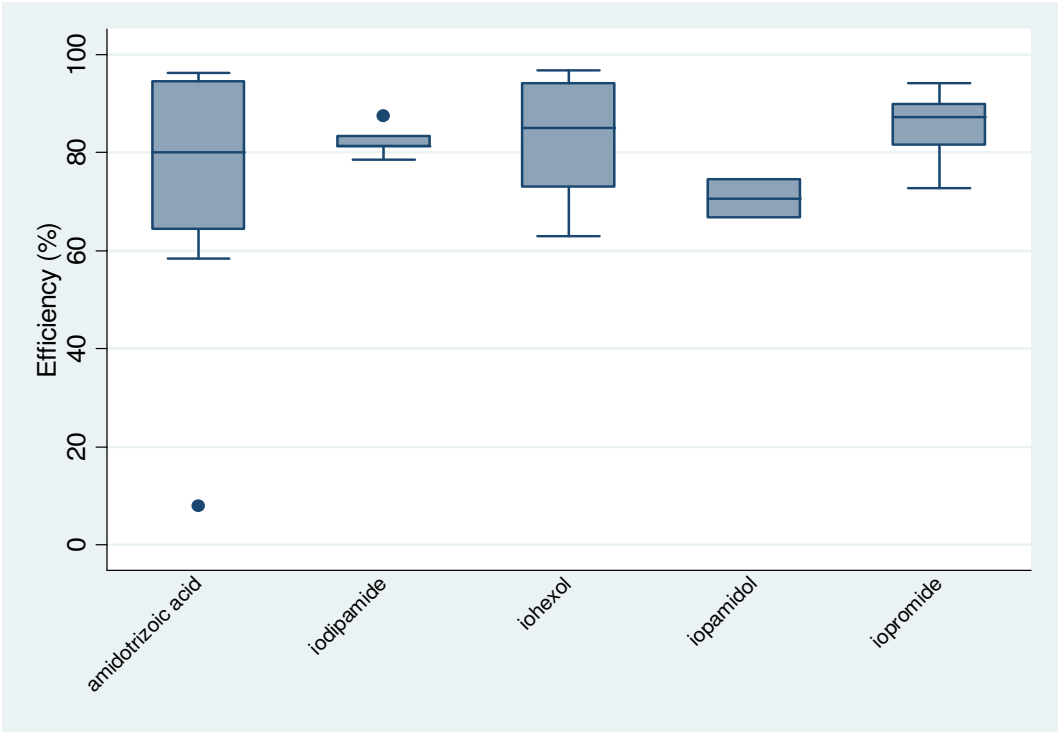

Figure 6.10.11: MF/RO removal efficiency of ICM detected in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

The number of paired samples (before MF and post-RO) to calculate removal efficiency ranged from 3 to 20. The average calculated treatment removal of all 'other' pharmaceuticals was greater than 80% except for diazepam (67%) and fluoxetine (77%), both of which were detected at low median concentrations, below 50 ng/L. The highest removal efficiencies were for carbamazepine (99.8 %) followed by naproxen (98.9%), diclofenac (98.3%) and ibuprofen (97.7%), which had the largest median concentrations in wastewater, all greater than 200 ng/L. Diazepam showed high variability of removal efficiency (range 30% to 88%), which is probably related to its low concentrations in wastewater and because only 3 paired samples before MF and after RO were available. The next most variable treatment performance was for phenytoin (n=10; std dev=11 %) followed by indomethacin (n=20; std dev=7.6 %). However, average treatment efficiency was above 90% for these pharmaceuticals (Figure 6.10.13).

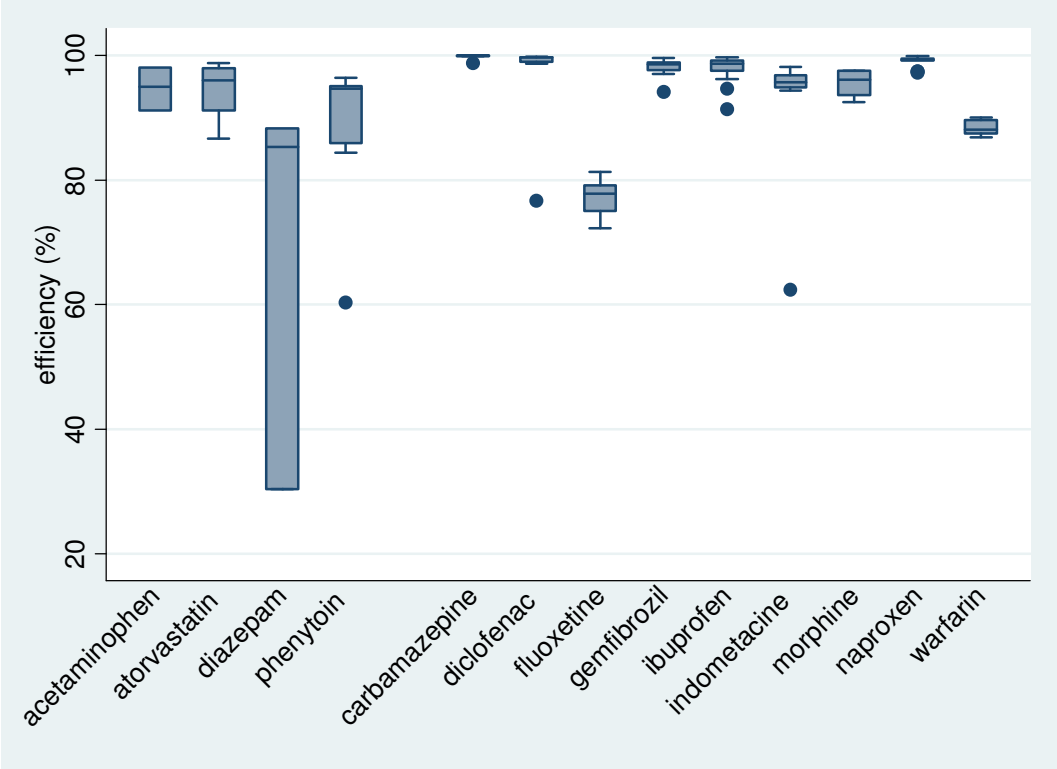

Figure 6.10.12: MF/RO removal efficiency of 'other' pharmaceuticals detected in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Grab and Composite samples

There was a good agreement between grab and composite sample results as illustrated in Figure 6.10.14. Pharmaceuticals concentrations were higher in grab samples compared with composite samples for 51% of the pairs. Grab and composite were reported with the same value for only 2% of the samples. The plot also indicates that differences tend to be higher for pharmaceuticals detected in higher concentrations and 6 outliers were observed. For samples before MF composite sample concentrations tend to be higher than grab samples while after MF and after RO grab samples tend to be higher than composite (data not shown). Two acetaminophen samples taken during the May/June 2007 sampling event reported very high concentrations (grab samples after MF=2697 ng/L and 4550 ng/L) while the composite samples concentrations were 1460 ng/L and 897 ng/L respectively. These results represent extreme outliers and were excluded from the Bland-Altman plot.

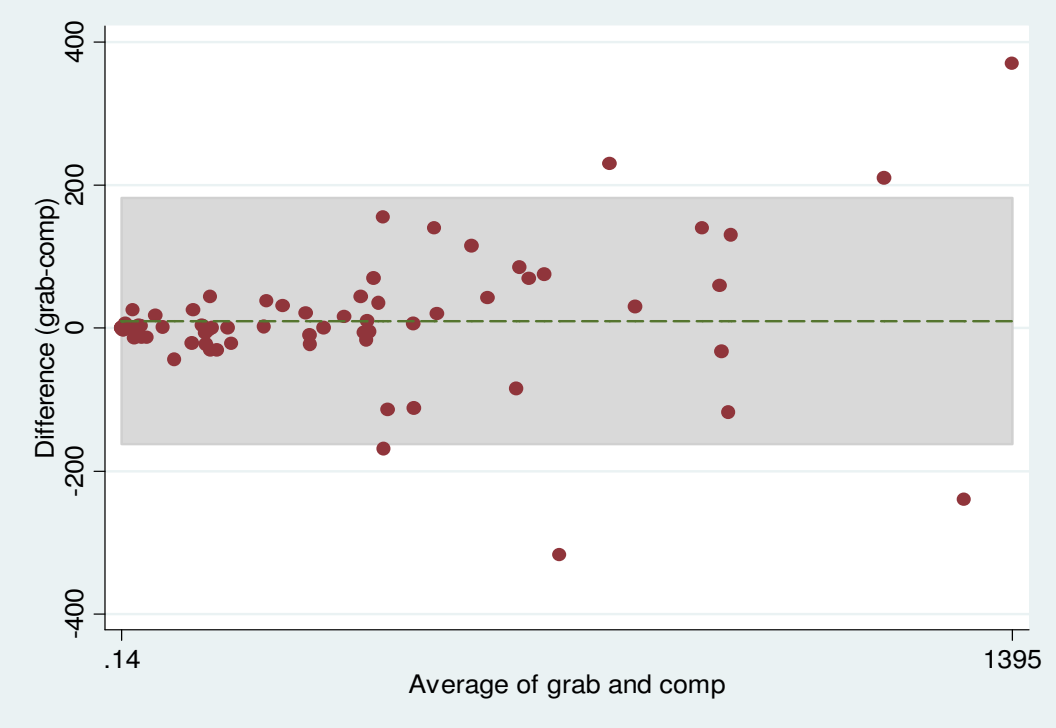

Figure 6.10.13: Bland-Altman plot comparing grab and composite samples for detected pharmaceuticals.

At KWRP, composite and grab samples were collected pre- and post-RO treatment on 3 days over a week-long period (30 May - 7 June 2007). When averaging over the 3 sampling days, there is little statistical difference in concentrations from KWRP secondary wastewater grab and composite samples except for paracetamol, gemfibrozil and naproxen. For all three however, there was significantly less variation in concentrations from composite samples than grab samples.

Discussion

A suite of 36 pharmaceuticals with diverse therapeutic properties and physico-chemical characteristics were measured in secondary treated wastewater and after MF/RO treatment. The basis of the selection of compounds, as listed in the Introduction, was similar to the criteria used by recently published studies (Benotti *et al.*, 2009). In addition to the main study, a further 20 pharmaceuticals were tested in the preliminary testing event conducted in June 2005. In this preliminary event etofibrate, fenofibrate, fenofibric acid, pentoxifylline and fenoprofen were all reported below LOR=100 ng/L, while the antibiotics oleandomycin, spiramycin, sulfadiazine, sulfadimidine, sulfamerazine, furazolidone, ronidazole, chloroamphenicol, dapsone, cloxacillin, dicloxacillin, penicillin G, penicillin V, oxacillin nafcillin, tylosin and amoxicillin were all reported below LOR=50 ng/L. Together, this work reports data for

a greater number of pharmaceuticals than have been reported for any other Australian study (Al-Rifai *et al*., 2007, Khan and Ongerth, 2004, Khan *et al*., 2004b, Watkinson *et al*., 2007).

Eighty three percent (83%) of tested pharmaceuticals (30 of 36) were detected in secondary wastewater. These results agree with other studies in which municipal wastewater represents the main source of pharmaceuticals entering the environment (Sumpter, 2005, Carballa *et al*., 2005, Al-Rifai *et al*., 2007, Focazio *et al*., 2008, Watkinson *et al*., 2007, Kim *et al*., 2007, Snyder *et al*., 2007, Heberer, 2002, Brun *et al*., 2006, Reemtsma *et al*., 2006, Suárez *et al*., 2008).

While differences were not always statistically significant, 47% of pharmaceuticals showed highest median concentration in secondary wastewater at Subiaco WWTP, compared to 17% at KWRP and only 3% at Beenyup WWTP (33% of pharmaceuticals did not have a maximum at single WWTP). The majority of hospital facilities in Perth discharge to the Subiaco WWTP. It has been estimated that the percentage inflow of hospital medical waste and non-medical waste is highest at Subiaco (0.1% and 0.2%, respectively) compared to Woodman Point WWTP, which provides KWRP influent, (0.03% and 0.04%, respectively) and Beenyup WWTP (0.01% and 0.01%, respectively). Therefore this could account for the higher concentrations of pharmaceuticals observed at Subiaco WWTP. The lack of statistical significance may be due sample size, as fewer wastewater samples were analysed from Subiaco compared to Beenyup or KWRP.

Seasonally, 64% of pharmaceuticals showed maximum median concentrations during spring, a greater percentage than all other seasons together (summer=14%, winter=8% and autumn=8%). This difference could be due to variations in pharmaceutical usage or variation in WWTP performance. Seasonal variation in antibiotic concentrations with higher concentrations in spring due to higher usage in winter-spring was expected, this was observed for four of the nine antibiotics. However, a lack of WWTP influent data and multiple years of data mean that usage variation cannot be definitively dismissed as a factor. However, it is highly unlikely that ICMs would show seasonal differences in usage and 75% of ICM showed maxima in spring. It is not expected that seasonal differences would be seen for other pharmaceuticals prescribed over the long term, such as antiepileptics, lipid lowering agents, anticoagulants, tranquilizers, or cytostatic agents. None of the samples from Subiaco WWTP were taken during the spring sampling events and therefore, the higher median concentrations measured at Subiaco are not related to seasonal differences and would have skewed the analysis of seasonal data (by recording higher concentrations in other seasons).

Six antiobiotics, azithromycin, clarithromycin, clindamycin, roxithromycin, sulfamethoxazole, and trimethroprim, were detected in 50% or more of all secondary wastewater samples, with clarithromycin, roxithromycin, sulfamethoxazole and trimethoprim detected in all wastewater samples. In contrast, the three most dispensed antibiotics in Australia in study based on 1998 data were amoxicillin, cephalexin (not studied) and erythromycin (Khan and Ongerth, 2004), with

prescription data from 2006 supporting these trends (ASM, 2006). It should be noted that amoxicillin would probably have been detected if the LOQ had been lower. Sulfamethoxazole, the next most dispensed drug (Khan and Ongerth, 2004), had the highest median concentration (543 ng/L), comparable or higher than other studies (Hirsch *et al*., 1999, Gobel *et al*., 2005). Sulfamethoxazole, an antibiotic used against the *Streptococcus* bacteria, has been reported as one of the 11 most frequently detected compounds at trace levels in drinking water (Benotti *et al*., 2009) and is one of the most abundant drugs in WWTP effluents (Hirsch *et al*., 1999, Castiglioni *et al*., 2006). Detection of macrolide antibiotics, such as azithromycin, roxithromycin, and trimethroprim, is also common in WWTP effluent (Watkinson *et al*., 2007, Hirsch *et al*., 1999, Gobel *et al*., 2005).

The possibility of chronic exposure of organisms to low levels of antibiotics through augmentation of natural water supplies with treated wastewater has led to concerns of the development of antibiotic resistance in the environment. The minimum concentration of antibiotic which will inhibit the growth of the isolated microorganism (MIC, Minimum Inhibitory Concentration) is an important factor. For example, MIC factors of single antibiotics (i.e. sulfamethoxazole, trimethoprim, erythromycin and clindamycin) for various reference bacterial strains (*S. Aureus, E. Faecalis* and *E.Coli*) often are in the range 10^1–10^3 µg/L (Wiedemann & Grimm, 1996). Thus there are several orders of magnitude of difference between the observed concentrations of antibiotics in secondary effluent (as well as post- RO treated water) and the observed MIC factors. This would imply low risk of development of antibiotic resistance in those organisms. Nevertheless, more than one compound belonging to a given class of antibiotic as well as other classes of antibiotics (e.g. fluoroquinolones, dihydrofolate reductase inhibitors, tetracyclins, beta-lactams, aminoglycosides) characterised by much lower MIC factors (i.e. MICs as low as 2 µg/L have been reported for ciprofloxacin, Wiedemann & Grimm, 1996) are likely to be present in the secondary effluents (e.g. Watkinson *et al*. 2007, Gros *et al*. 2006a, Gobel *et al*. 2004, Giger *et al*. 2003, Miao *et al*. 2004, Hirsch *et al*. 1998, Hirsch *et al*. 1999, Golet *et al*. 2001). The combination of these antimicrobial agents in secondary wastewater may well result in synergistic effects, and thus the development of antibacterial resistance should not be dismissed.

Three ICM, amidotrizoic acid, iohexol and iopromide, were detected in 50% or more of all secondary wastewater samples. The ICM showing the highest median concentrations was iohexol, confirming that the most commonly used ICM in Western Australia was probably Omnipaque 350, a commercial preparation of iohexol. Other commonly used iodinated contrast media include Ultravist 300 (iopromide) and Iopamiro 370 (iopamidol) (Blake and Halasz, 1995). Despite being detected, concentrations measured in secondary treated wastewater were still 2 to 3 orders of magnitude lower than the suggested guidelines for drinking water (AGWR, 2008) and lower than ICM concentrations previously reported in European WWTPs (Putschew *et al*., 2000, Seitz *et al*., 2006, Schittko *et al*., 2004).

Of the ‘other’ pharmaceuticals, atorvastatin, phenytoin, carbamazepine, diclofenac, gemfibrozil, indomethacin and naproxen were detected in 50% or more of all secondary wastewater samples, spanning the classes of lipid-lowering agents,

NSAIDs, and anti-epileptics. Non-prescription drugs such as NSAIDs are commonly found in secondary wastewater (Carballa *et al*., 2004, Vieno *et al*., 2005, Al-Rifai *et al*., 2007, Santos *et al*., 2007, Yu *et al*., 2006, Ternes, 1998, Khan and Ongerth, 2004, Kim *et al*., 2007, Carballa *et al*., 2005, Hilton and Thomas, 2003). While naproxen and ibuprofen have been estimated to be the highest dispensed NSAIDS in Australia (Khan and Ongerth, 2004), indomethacin and diclofenac were more frequently measured. This is probably related to the difference in LOQ, where LOQ of ibuprofen and naproxen were about an order of magnitude greater than diclofenac and indomethacin. Furthermore while diclofenac concentrations are relatively high, this is probably a reflection of its low degradability compared to ibuprofen and naproxen (Vieno *et al*., 2005, Yu *et al*., 2006). Ibuprofen degradation has recently been shown to be as high as 90% removal over six hours (Buser *et al*., 2009). The differences in secondary wastewater concentrations are most likely caused by differences in WWTP removal rather than differences in usage. In contrast, paracetamol (acetominophen) was Australia's greatest dispensed drug by mass (Khan and Ongerth, 2004), but was only measured in 11% of secondary wastewater samples in our study, confirming its high biodegradability (99% over five days, Joss *et al*., 2006). Others have measured paracetamol concentrations ranging from below detection to greater than 5 μg/L (Yu *et al*., 2006, Ternes, 1998, Hilton and Thomas, 2003, Gros *et al*., 2006a).

Atorvastatin (Lipitor) is the most commonly prescribed lipid lowering agent and, indeed, most commonly prescribed drug in Australia (ASM, 2006), but has only been found in low concentrations in our study, similar to concentrations reported in Drewes *et al*. (2008), although higher than detections by Miao and Metcalfe (2003). Low concentrations may be due to significant sorption to sludge (log K_{ow} = 6.3, Drewes *et al*., 2008). Despite the fact that Australian consumption of gemfibrozil is less than half of that of atorvastatin (ASM, 2006), gemfibrozil was detected at concentrations similar to atorvastatin but with greater frequency. Gemfibrozil is also much more commonly measured in other studies (Al-Rifai *et al*., 2007, Yu *et al*., 2006, Ternes, 1998, Kim *et al*., 2007, Gros *et al*., 2006a). Neither of these pharmaceuticals is readily biodegraded (Joss *et al*., 2006, Norwegian Scientific Committee for Food Safety, 2009), however greater detections of gemifbrozil would be associated with lower adsorption to sludge during wastewater treatment (log K_{ow} = 4.5).

The low frequency of detection of clofibric acid and non-detection of bezafibrate, despite the low LOQs achieved, and despite being poorly removed by WWTPs (Ternes, 2001, Zwiener and Frimmel, 2004), suggests that neither is commonly used in Australia and this is confirmed by data from the most recently available Australian Statistics on Medicines (ASM, 2006).

Carbamazepine was detected in all wastewater samples and was the pharmaceutical with the highest median concentration in wastewater. Carbamazepine is known to be no a very persistent compound that is not adsorbed or highly biodegraded during conventional wastewater treatment (Carballa *et al*., 2004, Suárez *et al*., 2008, Daughton and Ternes, 1999, Drewes *et al*., 2002). This is reflected in the large

number of studies that measure it (Carballa *et al*., 2004, Al-Rifai *et al*., 2007, Santos *et al*., 2007, Ternes, 1998, Kim *et al*., 2007, Gros *et al*., 2006a, Khan, 2002) and the concentrations reported in this study. Phenytoin was also detected in our study, but insufficient data exists to elucidate any particular trends. Comparison is difficult because this compound has only been included in a few methods measuring a wide range of pharmaceuticals (Gros *et al*., 2006b, Snyder *et al*., 2006).

Variations observed between concentrations in grab and composite samples may indicate that pharmaceutical concentrations can vary within the plant, related to changes in usage in the community. Joss *et al*. (2005) have demonstrated diurnal variation for several pharmaceuticals in a small Swiss WWTP that was also correlated to nitrogen load, which is in line with human excretion being the major source of pharmaceuticals in wastewater. It is likely that composite sampling better represents overall plant performance compared to grab samples, where time of sampling may be a confounding factor.

In addition to detection in secondary wastewater, seven pharmaceuticals were detected in at least one of four groundwater samples taken during May 2007 or January 2008. Three ICM compounds (iodipamide, iopromide, iopamidol) one antibiotic (roxithromycin) and three 'other' pharmaceuticals (fluoxetine, gemfibrozil and diclofenac) were found. While many of these detections were at or just above detection limit, results for diclofenac and gemfibrozil in particular are about an order of magnitude higher than LOQ. Even though the blanks were below LOQ, it is possible that contamination occurred during sampling or sample preparation as these pharmaceuticals are very commonly used. Positive results are unlikely to be associated with septic tank leakage as the groundwater was a blend of multiple superficial and deep aquifer sources that have protection areas around each bore.

Analysis of post-RO water samples has confirmed that pharmaceuticals are efficiently removed by MF/RO which is in agreement with other studies investigating water recycling and RO particularly (Al-Rifai *et al*., 2007, Drewes *et al*., 2008b, Khan *et al*., 2004b, Drewes *et al*., 2001). While there were a few trace detections of diazepam, naproxen and clofibric acid, concentrations were well below drinking water guidelines or health values developed for the project. Frequent measurement of pharmaceuticals in post-MF water (data not shown) indicates the MF alone is not capable of significant pharmaceutical removal and this is in agreement with other studies that show microfiltration and nanofiltration is generally less efficient than RO for rejection (Khan *et al*., 2004b, Yoon *et al*., 2006, Drewes *et al*., 2008a).

The process of RO rejection can be influenced by many factors including compound–specific physico–chemical properties (e.g. molecular size, solubility, diffusivity, polarity, hydrophobicity, and charge), specific membrane properties (e.g., permeability, pore size, hydrophobicity, and charge), as well as membrane operating conditions (e.g., flux, transmembrane pressure, and regeneration) (Kimura *et al*., 2003, Bellona *et al*., 2004). The nominal molecular weight cut-off (MWCO) of an RO membrane is approximately 100-150 Daltons (Da). In contrast the molecular weight

of the pharmaceuticals measured here is typically greater than 200 Da, except for paracetamol (151 Da) and metronizadole (171 Da), though neither of these compounds were found at very high concentrations in secondary wastewater or post-RO. In addition, any compound where pH>pKa (e.g., acidic pharmaceuticals) will be negatively charged and RO rejection by electrostatic exclusion is also possible. Molecular width and the octanol-water partition coefficient (log K_{ow}) may also impact rejection, with rejection increasing with increase of log K_{ow} (Bellona *et al*., 2004). In general, rejection of pharmaceuticals is expected to be relatively high (>90%) in RO systems (Drewes *et al*., 2008b).

Removal efficiencies for RO can only be estimated from the data because most compounds were not detected in post-RO water. However, using half the post-RO water LOQ as upper bound produces removal efficiencies greater than 90% except for some pharmaceuticals present in wastewater at concentrations less than one order of magnitude greater than the post-RO water LOQ (e.g. clindamycin, metronizadole, phenytoin, fluoxetine, warfarin and the ICM). Even considering these lower removal efficiencies, the average removal efficiency for all pharmaceuticals is greater than 88%, which compares well to those observed in other water recycling schemes (Al-Rifai *et al*., 2007, Drewes *et al*., 2008b, Khan *et al*., 2004b, Kim *et al*., 2007, Snyder *et al*., 2007, Yoon *et al*., 2006, Khan *et al*., 2004a).

While RQ calculations indicate there is very little human health concern from pharmaceuticals in MF/RO water, regular monitoring of RO performance is recommended to ensure rejection remains high. Researchers have suggested that indicators to monitor pharmaceutical attenuation during indirect potable reuse should be present in wastewater at one to two orders of magnitude above LOQ (Sedlak *et al*., 2004). Based on this approach, sulfamethoxazole would appear to be the best choice of indicator for antibiotics, though median concentrations and percentage detections of clarithromycin, roxithormycin and trimethroprim are also high. For ICM, only iohexol fits the criteria, mostly because ICM LOQs are relatively high compared to other pharmaceuticals. Potential 'other' pharmaceutical indicators are carbamazepine and diclofenac. While ibuprofen concentrations are also relatively high in secondary wastewater, the lower number of percentage detections (50%) indicate it is less suitable as an indicator. A wide array of pharmaceuticals have been considered in this study, however it is recommended that additional compounds are studied in order to monitor other pharmaceutical classes and account for changes in pharmaceutical prescriptions with time. In particular, acetylsalicylic or salicylic acid and trichlocarban were identified as requiring investigation but were not able to be monitored during this study because of method development limitations.

References

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6.11 Estrogenic Hormones

Introduction

Environmental concern about chemicals that can alter endocrine function has been increasing in recent years due to their wide occurrence in the aquatic environment and their potential hazard to both aquatic and human life (Korner *et al.*, 2001, European Communities, 2000). Hormonally active agents, also called Endocrine Disrupting Compounds (EDCs) are natural and synthetic exogenous substances that cause adverse health effects in an intact organism, or its progeny, as a consequence of changes in endocrine function (European Communities, 2000). This class of substances is not defined by their chemical nature (i.e. chemical structure) but instead by the biological effects they can exercise on target organisms. Thousands of chemicals have been demonstrated or are suspected to modulate or mimic the action of steroidal hormones and produce biological responses qualitatively similar to those produced by endogenous hormones (Pojana *et al.*, 2004). Steroidal hormones, a particular class of EDCs, include various natural and synthetic compounds that control or regulate the endocrine function in animals and humans. In this section, three naturally occurring estrogens (17β-estradiol, estriol, and estrone) and one synthetic estrogen used for birth control (17α-ethinylestradiol) are analysed. The hormones were selected based on their estrogenic potency, their occurrence in secondary wastewater from the literature, and the high rate of prescription of ethinyl estradiol (although at low doses, ASM, 2006). The selected hormones are considered the compounds of greatest estrogenic activity in sewage effluent (Falconer, 2006). Androgenic hormones are less prescribed (compared to estrogenic hormones) and so they were not investigated during the course of this study.

Naturally occurring and anthropogenic EDCs including the reproductive hormones discussed in this chapter have been found in secondary wastewaters to occur at ng/L or sub ng/L levels around the world (Petrovic *et al.*, 2002). Conventional biological wastewater treatment can degrade estrogenic hormones such as estrone, 17β-estradiol and estrone with removal efficiencies above 95% of the estrogenic activity (Leusch *et al.*, 2005, Chapman, 2003).

Chronic, low-level (ng/L) exposure to anthropogenic sources of EDCs has been linked with a number of reproductive disorders in wildlife including abnormal sex-organ development, imposex (females developing male reproductive organs), intersex (presence of both male and female reproductive organs), uneven sex ratios (relative numbers of males and females in a population), decline in reproductive success and birth defects (Kookana *et al.*, 2007).

In humans, EDCs have been implicated in some studies in reduced sperm counts, cancers of the reproductive organs and early onset of puberty (Kookana *et al.*, 2007). However, the available information regarding the potential human health effect of EDCs is inconclusive and some consider that present data indicates that estrogenic

contamination of drinking water is very unlikely to result in physiologically detectable effects in humans (Falconer, 2006, Jugan *et al*., 2009)

Methods

Hormones were measured by liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) operated in multiple reactions monitoring mode (MRM). The method is detailed in Appendix 4 and summarized below.

The analytical method employed a solid phase extraction (SPE) preconcentration and clean-up step before analysis. For sample preparation, treated wastewater samples (250 mL) were filtered through 0.45 µm polyethersulfone membrane filters and then diluted to 500 mL with ultra pure water to reduce matrix interactions on the SPE cartridges. Post-RO water samples were already subject to microfiltration and therefore did not require further filtration. Analytes were extracted using a reverse phase C18 SPE cartridge (StrataE) and concentrated down to ~250 µL. Sample pH was adjusted to 4 by addition of formic acid. To determine analyte recoveries and to correct for matrix effects, a surrogate standard mix containing four deuterated hormones was also spiked before SPE extraction. Extracts were injected in the LC system and separated using a reversed phase LC column (C18). Identification and quantification by MS/MS used negative electrospray ionization (ESI(-)) and MRM. Analytes identification was based on chromatographic retention time (RT) and compound-specific MRM transitions. For quantitation, external calibration with deuterated surrogate standard was used. Matrix effects were overcome by inclusion of deuterated surrogate standards for all analytes.

All methods were verified for the analytes of interest in both secondary wastewater and post-RO water. Limited validation data is also present for groundwater samples. The limits of detection (LOD) and estimated uncertainties for each method are listed in Table 6.11.1.

Table 6.11.1: Health values, Limits of detection (LOD) and estimation of uncertainty for hormones

Analyte	Health value (ng/L)*	MQ water LOD (ng/L) Average (Range)	Groundwater LOD (ng/L) Average	Wastewater LOD (ng/L) Average (Range)	MQ water Standard Relative Uncertainty (5 ng/L) (%)	Wastewater Standard Relative Uncertainty (10 ng/L) (%)
Estriol	50	1.3 (0.6-3)	3.0	5.5 (1.5-15)	9.1%	10.1%
17β-Estradiol	175	1.3 (0.7-3)	1.3	5.7 (1.5-15)	9.9%	14.0%
17α-Ethynilestradiol	1.5	2.1 (0.6-6.0)	1.5	8.0 (2.3-15)	13.3%	21.4%
Estrone	30	1 (0.3-3)	1.4	4.2 (0.3-15)	13.0%	16.0%

** Health values from AGWR 2008*

Quality assurance/ Quality control

To validate the analytical procedure for hormones the following studies were undertaken: instrument linearity, instrument detection limits (IDLs), peak identification criteria (t_R and MRM ratio), accuracy, precision (SPE recoveries) method limit of quantitation (MLD), in-house reproducibility, matrix effect as well as a round-robin test for inter-laboratory comparison.

Studies concerning calibration criteria and accuracy, precision and method limit of detection and quantitation were also undertaken during the sampling campaign through spiking experiments in treated wastewater and post RO water. Laboratory blanks, trip and field blanks were also analysed and constituted about 33% of the samples analysed. Other QA/QC aspects are reported in Chapter 4.

Limits of detection were calculated for every sample from the concentration equivalent to a signal to noise ratio (S/N) of three with the MassLynX 4.0 software using a peak of a known concentration. Limits of quantitation were calculated for every sample from the concentration equivalent to S/N equal to ten (Foley & Dorsey, 1984).

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

One proficiency test for selected hormones was undertaken during the sampling period and the full details of this is available in Chapter 4. In summary, three participants (Curtin Water Quality Research Centre, National Measurement Institute, and DVGW-Technologiezentrum Wasser) analysed QA/QC samples collected during Event 2. All results were below LOD.

Results

There were a total of 308 measurements for hormones, excluding field blanks, trip blanks and replicates (Table 6.11.2). The majority of samples were composite and taken at KWRP and BPP. Measurements from Subiaco WWTP and Wanneroo groundwater correspond to 6.5% and 5.0% of the total.

Table 6.11.2: Measurement of hormones by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	SWW	Water Reclamation Plant							
				Grab	Comp				Before MF		Post-MF water		Post-RO water		dam	Total
									K	B	K	B	K	B		
1	November	4	2006	36	8	44	0	0	20	0	0	0	12	0	12	44
2	May/June	6	2007	32	40	72	8	16	0	0	24	0	24	0	0	48
3	September	6	2007	0	48	48	0	0	12	12	0	0	12	12	0	48
4	January	6	2008	8	40	48	8	0	8	12	0	0	8	12	0	40
5	April	5	2008	0	48	48	0	4	8	12	0	4	8	12	0	44
6	June	5	2008	0	48	48	0	0	8	12	4	4	8	12	0	48
Total		32		76	232	308	16	20	56	48	28	8	72	48	12	272

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

Estriol was detected in 1 of 29 secondary wastewater samples at 10.4 ng/L (Table 6.14.2). This detection occured on the 6th of June 2008 at KWRP. Ethinyl estradiol and 17β-estradiol were not detected in any of the samples. Estrone was the hormone most often detected (14 of 29 samples). Estrone was detected in 10 samples from Beenyup WWTP, 3 samples from the influent to KWRP and 1 sample from Subiaco WWTP. Hormone concentrations were higher at Subiaco WWTP but the differences were not statistically significant. No significant differences in hormone concentrations were observed by season.

Table 6.11.3: Detected Hormones in Secondary Wastewater

Hormone	% of Detection	Average LOD (ng/L)	Median (ng/L)	Detected concentration
Estriol	3.4	5.5	3.6	10.4*
Estrone	48.3	4.2	6	-

**Actual value detected for estriol in Event 6.*

RO Product water characterisation

None of the tested hormones was detected in the post-RO water

Groundwater characterisation

None of the tested hormones was detected in groundwater

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. For those hormones that were not detected, the RQ(median) was calculated using the average LOD as the observed concentration. Table 6.11.4 presents the RQs for hormones in secondary wastewater and post-RO water.

RQs were below health values in both secondary wastewater and post-RO water for estriol, 17β–estradiol and estrone (Table 6.11.4). RQ was calculated for the estriol detection value in Event 6 and the RQ was of low health significance (RQ=0.2). Ethinyl estradiol was not detected in any of the samples but the average LOD was above the health value of 1.5 ng/L and therefore calculated RQs were above 1 in both secondary wastewater and post-RO water (RQ(median)=5.3 and RQ(median)=1.4 respectively).

Table 6.11.4: Hormones before MF and post-RO water corresponding RQs

			Before MF				After RO			
Parameter	**Tier**	**Health value (ng/L)***	**LOD**	**n**	**RQ(median)**	**RQ(max)**	**LOD**	**n**	**RQ(median)**	**RQ(max)**
Hormone										
Estriol	2	50	5.5	29	0.07	0.3	1.3	30	0.03	NA
Ethinyl estradiol	2	1.5	8.0	29	5.33	NA	2.1	30	1.40	NA
17-beta estradiol	2	175	5.7	29	0.03	NA	1.3	30	0.01	NA
Estrone	2	30	4.2	29	0.20	1.83	1.0	30	0.03	NA

** Health values from AGWR 2008*

Treatment performance

MF/RO treatment was efficient in removing the detected hormones. Treatment efficiency median was 96.4% for estriol and 96.5% for estrone. Treatment efficiency was calculated using one paired sample (before and after) for estriol and 12 paired samples for estrone as shown in Figure 6.11.2. Removal efficiency for estrone ranged from 72.7% to 98.2%.

For events where post-MF water was sampled and estrone was detected, secondary wastewater and post-MF had similar concentrations although slightly higher post-MF (Beenyup 1st May 2008 6.4ng/L and 8.6 ng/L; Beenyup 25th June 2008 20.2 and 22,0

ng/L; KWRP 6th June 2008 21.8 and 23.4 ng/L). A similar result was found for estriol in the one event in which it was detected in secondary wastewater and post-MF (KWRP on the 6th June 2008 at 10.0 and 10.4 ng/L, respectively). This confirms that MF is not effective at removing hormones.

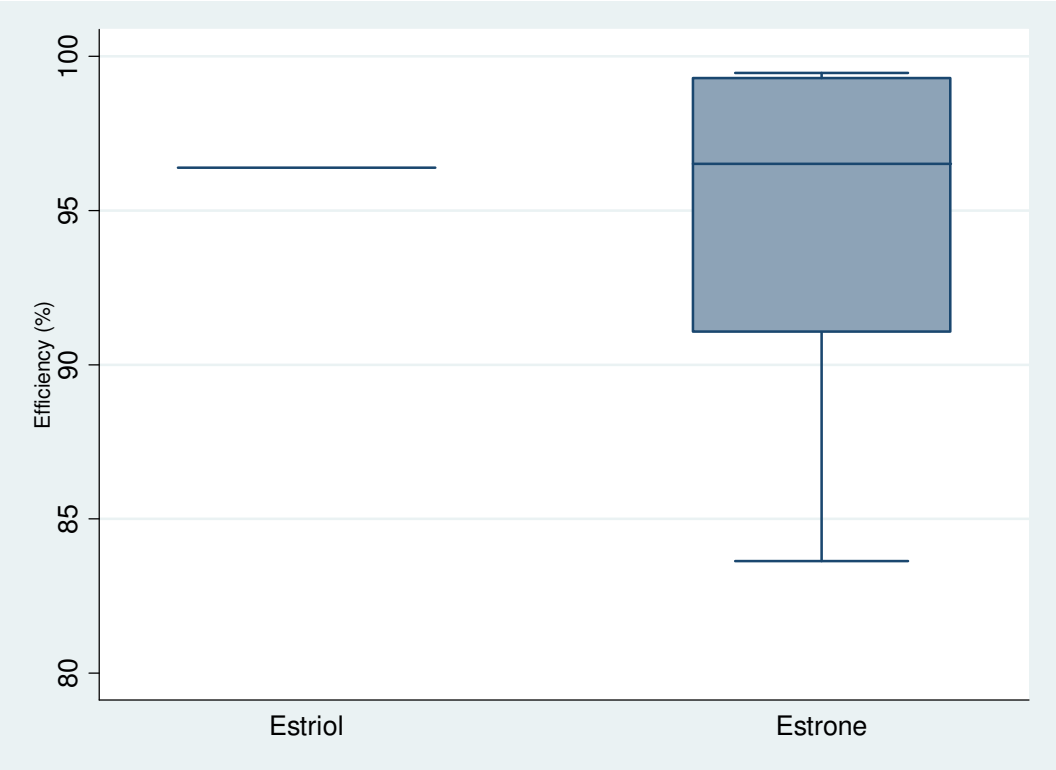

Figure 6.1.1: MF/RO removal efficiency of detected hormones in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Discussion

Four estrogenic hormones were analysed during the project. Estrone was the only hormone detected. Estrone was detected in almost 50% of the secondary wastewater samples with a median of 6 ng/L. In other studies estrone has been reported at 15 ng/L (Davis, 2000) and 23.9 ng/L (Williams *et al.*, 2007) in secondary wastewater. Estradiol and 17β-estradiol were not detected in secondary wastewater. Estradiol has been reported in secondary wastewater at a range of 1.8 ng/L to 48 ng/L with a typical value of 10 ng/L (Davis, 2000). In Australia estradiol concentrations in secondary effluent ranging from 0.05-6.3 ng/L with a median concentration of 3.8 ng/L have been reported (Williams *et al.*, 2007). Estriol was detected only in one secondary wastewater sample at 10.8 ng/L. Similar

concentration ranges (from non-detected up to tens of ng/L) have been reported previously in literature for estriol (Petrovic *et al.*, 2002).

The LOD for ethinyl estradiol was above the health value of 1.5 ng/L in secondary wastewater. The average LOD in secondary wastewater was 8.0 ng/L ranging from 2.3 to 15.0 ng/L. Therefore concentrations of ethinyl estradiol in secondary wastewater may have been present in the low ng/L range as reported in other studies. In Germany, ethinyl estradiol ranged from 9 ng/L to 15 ng/L; while in Canada concentrations up to 42 ng/L have been found (Ternes *et al.*, 1999). In Australia concentrations of ethinyl estradiol in secondary wastewater ranging from 0.01 to 1.30 ng/L with a median concentration of 0.45 ng/L has been reported (Williams *et al.*, 2007). Davis *et al*. (2000) reported ethinyl estradiol at 1 ng/L in secondary wastewater.

Ethinyl estradiol, estriol and 17β-estradiol were tested in the preliminary monitoring program and were below the LOR (50 ng/L, 50 ng/L and 250 ng/L respectively) in all secondary wastewater samples taken from KWRP influent and Beenyup WWTP. They were also all below LOR in KWRP post-RO water samlpes. An additional hormone 17α--estradiol was also below LOR (50 ng/L) in all samples taken in 2005.

Our secondary treated wastewater hormone results are consistent with other studies, but also with the evidence that suggests that secondary wastewater treatment, and in particular activated sludge treatment with sufficiently long sludge retention times, is very effective at biodegrading estrogenic hormones (Drewes *et al.*, 2006). The sludge retention time required for good estrogenic hormone removal is >2 days; as Beenyup WWTP has an average sludge retention time of between 10-15 days, this easily explains the low levels of estrogenic hormones detected in secondary treated wastewater.

None of the tested hormones was detected in the post-RO water. The results are consistent with other IPR projects in which testing of product water at the Orange County Water District, the West Basin Water Recycling Facility and the NEWater scheme in Singapore has not detected 17α ethinyl estradiol, estrone or 17β-estradiol (WBMWD, 2006, OCWD, 2006, Singapore Government, 2002).

Removal of estrogenic hormones has been demonstrated in a number of studies. The results compare well to those calculated or predicted for other water recycling plants. Drewes *et al*. (2008) predicted (for estriol and estrone), and partially verified (only for estrone) high removal (>90%) through RO rejection experiments. Similarly, Snyder *et al*. (2007) reported rejection efficiencies for estrone greater than 97% with an ultrafiltration (UF) and RO pilot system feed with treated wastewater. In the same study, using virgin RO membranes and spiking approx. 100 ng/L of ethinyl estradiol, estriol, 17β-estradiol and estrone in the feed tank (saline ground water), removal efficiencies were 85% for estrone and slightly lower for estradiol, estriol, 17β-estradiol (removal efficiencies > 80%). Removal efficiencies for 17β-estradiol and estrone were also evaluated in a full-scale (10 million gallon per day) MF/RO/AOP water

reuse facility. In that study, MF alone appeared not to be effective in removing selected hormones, while RO treatment removed target contaminants to below limits of detection. This is consistent with our results where post-MF and post-RO water was sampled. Although concentrations of estrone (and estriol) in post-MF water (data not shown) were slightly higher compared to pre-MF water, probably due to the incomplete (~95%) recovery of water through the MF membrane and a concentrating effect across the membrane. Snyder *et al*. (2007) also found higher concentrations post-MF.

Median MF/RO treatment efficiency was 96.4% for estriol and 96.5% for estrone. These results are comparable with removal efficiency for estrogenic hormones greater than 95% reported in other studies (Huang & Sedlak, 2001).

RQs were below 1 for tested hormones except for ethinyl estradiol due to the high LOD reported. Therefore additional data with a lower LOD is required to better characterise the potential health impacts of ethinyl estradiol. However, given the large molecular weight of ethinyl estradiol (296 g/mol) the treatment removal is expected to be as good if not better than observed for estrone (median 96.5%, molecular weight 270g/mol). As ethinyl estradiol was never detected in the secondary wastewater at a LOD of 8 ng/L, ethinyl estradiol can be expected to always be below the guideline of 1.5 ng/L based on a removal efficiency of RO of greater than 81% even if concentrations in secondary wastewater were close to the detection limit. No anticipated increased health risk is expected at the observed concentrations in post-RO water.

Only estrogenic hormones were measured in this study. Analysis of additional estrogenic hormones (e.g. progesterone, levonorgestrel, mestranol), androgenic hormones (e.g. testosterone, dehydroepiandrosterone, androstenedione) and thyroid hormones (e.g. thyroxine, triiodothyronine) would better characterise the hormone content. Androgenic hormones, although not often prescribed, are likely to be present in raw wastewater in higher concentrations than estrogens but are also well removed by wastewater treatment (Leusch *et al.*, 2006).

Other synthetic endocrine disrupting chemicals have been measured during this study and are addressed specifically in sections discussing phenols, pharmaceuticals, pesticides and others where relevant. Combined effects of both androgenic and estrogenic hormones and other endocrine disrupting chemicals can be assessed through a range of bioassays (whole effluent toxicity tests) however these have not been conducted for this study.

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6.12 Aminopolycarboxylate Complexing Agents

Introduction

Complexing (or chelating) agents are commonly used in industry and are commonly found in wastewater. The aminopolycarboxylate complexing agents in particular have a broad area of application because of their ability to complex a wide variety of metal ions and their high stability (Knepper, 2003). They represent group of highly polar anthropogenic organic compounds that are poorly removed by conventional WWTPs and can therefore be detected in secondary wastewater in ranges of 10-1000 µg/L (Fuerhacker *et al.*, 2003, Reemtsma *et al.*, 2006, Oviedo & Rodriguez, 2003).

The four aminopolycarboxylate complexing agents analysed in this project were ethylendiamintetraacetic acid (EDTA), nitrilotriacetic acid (NTA), propylenediamintetraacetic acid (PDTA) and diethylentriaminpentaacetic acid (DTPA), henceforward referred to as complexing agents. Worldwide, usage of EDTA predominates over other complexing agents (Knepper, 2003), however EDTA's high stability means it is one of the most prominent polar compounds found in WWTP effluent and surface waters in Europe, often with no overall removal observed in WWTPs (Reemtsma *et al.*, 2006, Oviedo & Rodriguez, 2003). As a result, other more biodegradable complexing agents have been suggested as alternatives. In particular PDTA has been considered as an alternative to EDTA (Schmidt & Brauch, 2004), while DTPA is used in pulp and paper recycling facilities that use hydrogen peroxide rather than chlorine for paper brightening (Mathur, 1993). NTA is predominantly used as a replacement for phosphate in laundry detergents because of its ability to chelate calcium and magnesium ions (Kari & Giger, 1996). The majority of research reports on EDTA only, with few measurements for NTA, PDTA, DTPA reported (Knepper, 2003).

Because of their high stability and polarity, most complexing agents are poorly removed during wastewater treatment and their high polarity also prevents efficient elimination during drinking-water treatment. Degradation varies widely with compound and also the degrading environment (Bucheli-Witschel & Egli, 2001). For example, the Fe(III)-EDTA metal complex is degraded quickly by direct photolysis, whereas other EDTA metal complexes are stable in light (Schmidt *et al.*, 2004). Biodegradation rates strongly depend on speciation, the type of microorganisms, substrate and prevailing chemical environment (Oviedo & Rodriguez, 2003, Bucheli-Witschel & Egli, 2001). Whereas NTA has been shown to be significantly removed by wastewater treatment, EDTA, DPTA, and PDTA are all generally not (Knepper, 2003, Lee *et al.*, 1996, Kari & Giger, 1996). In the environment EDTA has been found to be more persistent than DTPA, even when DTPA loadings in wastewater from pulp and paper mills were ten times higher than EDTA (Sillanpaa *et al.*, 1997).

Several European studies have found orders of magnitude greater loading of complexing agents to come from industries such as paper manufacture than from

household wastewater (Fuerhacker *et al.*, 2003, Schmidt & Brauch, 2004, Schmidt *et al.*, 2004), although some studies have shown NTA to have signficant loading from households, presumably from detergent use (Lee *et al.*, 1996). Therefore it may be expected that WWTPs which treat industrial waste may have higher effluent concentrations, though it will be very dependent on the types of industry in the wastewater catchment.

In humans, long-term toxicity of complexing agents is complicated by its ability to chelate essential and toxic metals. For example toxicological studies indicate that the cellular toxicological effects of EDTA can be attributed to the lack of metals essential for cellular function (Oviedo & Rodriguez, 2003). EDTA does not appear to be teratogenic or carcinogenic in animals and it is used in humans for the treatment of metal poisoning (WHO, 2006). IARC classified NTA as a possible human carcinogen (Group 2B) (IARC, 2008) However, the WHO guideline for NTA is based on the NOAEL for nephrotoxic effects (kidney toxicity), with a safety factor of 1000 to account for the evidence of urinary tumour induction at high doses because NTA induces tumours only after prolonged exposure to doses higher than those that produce nephrotoxicity. NTA has been proposed as therapeutic chelating agent in medicine for the treatment of manganese poisoning (Kaur *et al.*, 1980). There is no toxicity information available for DTPA or PDTA. In the environment complexing agents are unlikely to accumulate in aquatic food chains and show relatively low toxicity to aquatic organisms (Schmidt & Brauch, 2004). However, complexing agents may increase the mobility of heavy metals, causing an increase in heavy metal concentrations in secondary treated wastewater or even remobilising heavy metals adsorbed in sediments in the aquatic environment (Knepper, 2003).

Methods

All complexing agents were preconcentrated by evaporation and derivitized before being measured by gas chromatography mass spectrometry (GC-MS). Samples (250 mL) were mixed with methanol (MeOH, 2-3mL) and then concentrated at 30 °C under reduced pressure to a final volume of approximately 2 mL. After addition of formic acid, solution is evaporated to dryness under nitrogen to cleave any metal complexes. Derivatization of analytes to methyl esters using BF_3-MeOH then increased the volatility sufficiently to allow simultaneous analysis by GC-MS. Samples were injected into the GC and separated using a 5% phenyl 95% dimethylpolysiloxane capillary column. Quantification was performed by MS with Electron Ionisation (EI), with peak identification based on retention time and both quantifying and qualifying ions, where possible, and recovery aided by inclusion of deuterated internal standards.

All methods were verified for the analytes of interest in both secondary wastewater and post-RO water. The limits of detection (LOD) and estimated uncertainties for each method are listed in Table 6.12.1

Table 6.12.1: Health values, Limits of detection (LOD) and estimation of uncertainty for complexing agents

Analyte	Health value (µg/L)	Source	Average LOD (µg/L)	Standard Relative Uncertainty (10 µg/L) (%)
DTPA	0.7	TTC	2.3	57.3%
EDTA	250	ADWG, 2004	0.6	31.4%
NTA	200	ADWG, 2004	0.1	17.8%
PDTA	0.7	TTC	4.4	44.4%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (tR and quantifying and qualifying ions), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

There were a total of 264 measurements of complexing agents after excluding replicates, field and trip blanks. Complexing agents were not analysed during Event 1 and the majority of the samples were composite (87%), with some grab samples for secondary wastewater and post-RO water collected in Event 2 (Table 6.12.2). In addition, all groundwater samples were collected as grab samples.

Table 6.12.2: Measurement of complexing agents by event and location

Event	Month	No days	Year	Sample		Total	Location								
							GW	SWW	Water Reclamation Plant						
				Grab	Comp				Before MF		Post-MF water		Post-RO water		Storage dam	Total
									K	B	K	B	K	B	K	
1	November	4	2006	0	0	0	0	0	0	0	0	0	0	0	0	0
2	May/June	6	2007	32	44	76	8	20	0	0	24	0	24	0	0	48
3	September	6	2007	0	48	48	0	0	12	12	0	0	12	12	0	48
4	January	6	2008	4	40	44	4	0	8	12	0	0	8	12	0	40
5	April	5	2008	0	48	48	0	4	8	12	0	4	8	12	0	44
6	June	5	2008	0	48	48	0	0	8	12	4	4	8	12	0	48
Total		32		36	228	264	12	24	36	48	28	8	60	48	0	228

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

All complexing agents were detected in secondary wastewater (Figure 6.12.1). EDTA and NTA were detected in all wastewater samples (n=27), while DTPA and PDTA were detected in 37% and 30% of samples respectively. The highest median concentrations were measured for EDTA (145 µg/L) followed by DTPA (3.2 µg/L).

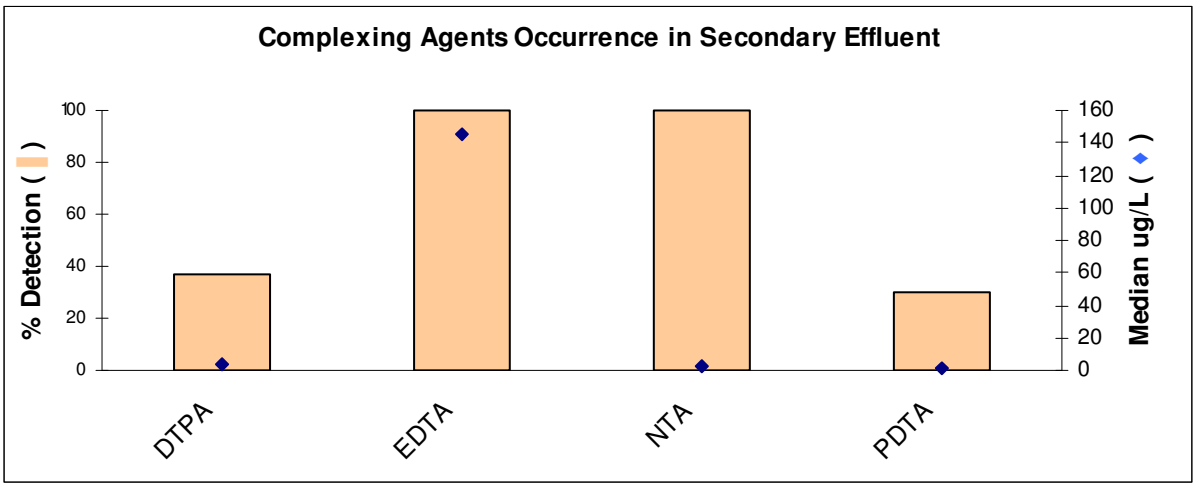

Figure 6.12.1: Complexing agents with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (µg/L, diamond).

A comparison of median concentrations at each WWTP (Figure 6.12.2) showed that EDTA was highest at Subiaco WWTP (K-Wallis $p<0.001$), while NTA was highest in KWRP influent K-Wallis $p<0.001$). PDTA was also highest at Subiaco WWTP, however the difference was not statistically significant.

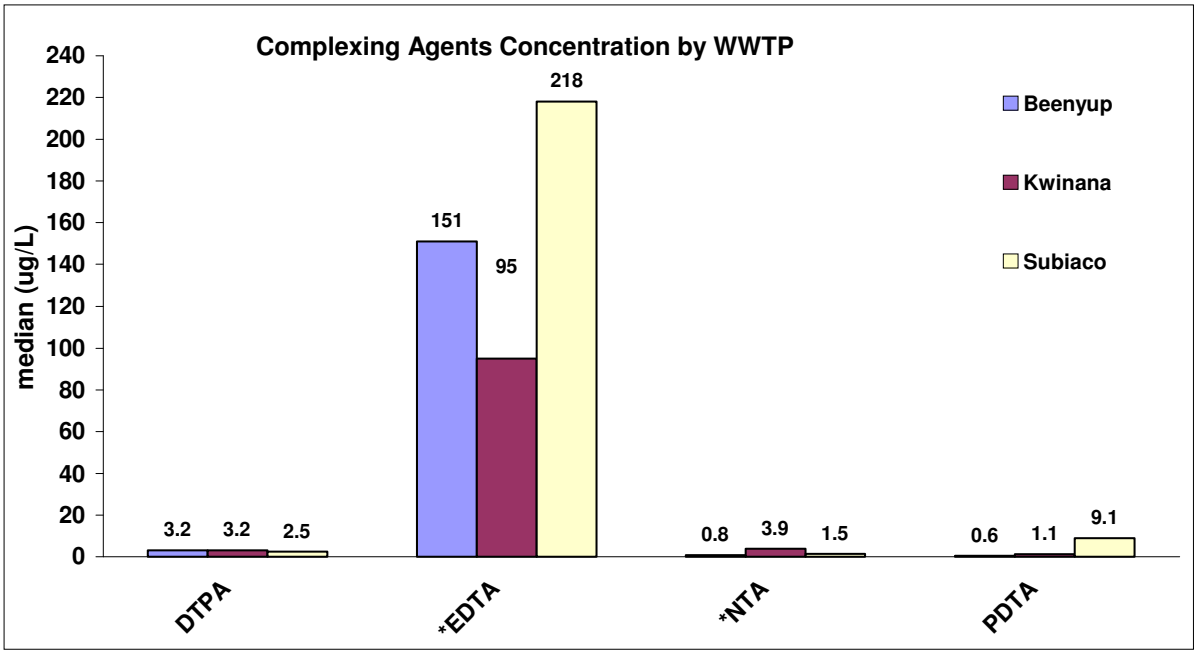

Figure 6.12.2: Complexing agents with detections in secondary wastewater and corresponding median concentrations (µg/L).

** complexing agents with statistically significant differences among plants*

Significant differences were also observed in the median concentrations of complexing agents by season, although there was no obvious relationship between the different analytes (Figure 6.12.3). EDTA concentrations were higher in winter (K-Wallis $p<0.0001$) and NTA median concentrations were higher in autumn (K-Wallis $p<0.001$). Both PDTA and DTPA had the highest median concentration in spring, but the difference was not significant.

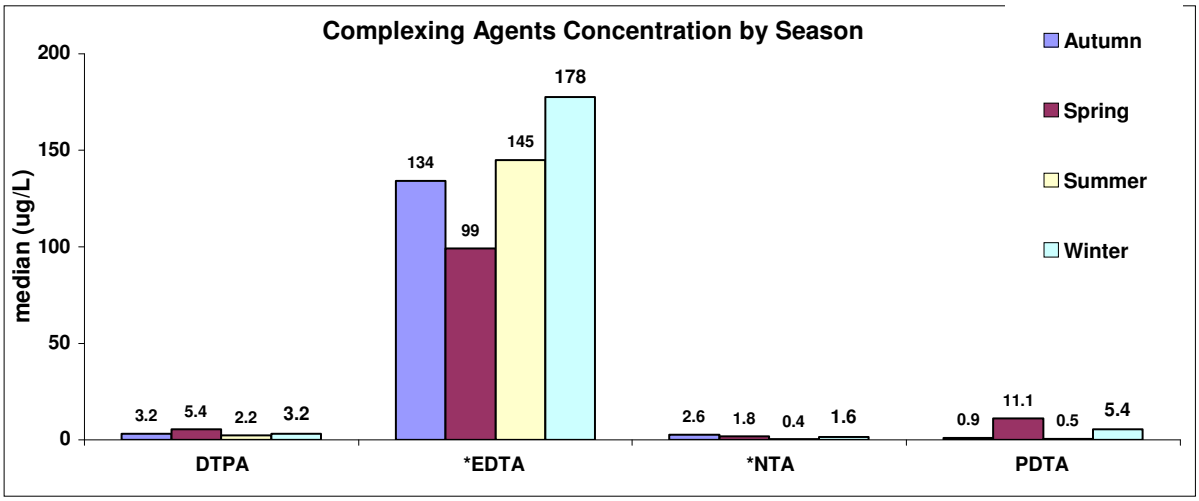

Figure 6.12.3 Median complexing agents concentration by season (µg/L).

** complexing agents with statistically significant differences among seasons*

RO Product water characterisation

All complexing agents except PDTA were detected in at least 1 post-RO water sample. The greatest percentage of detections was for EDTA (48%), followed by NTA (33%) and DTPA (4%). While median concentrations were highest for DTPA, at 2.2 µg/L, compared to a median of 0.48 µg/L for EDTA, this is mostly due to the higher limit of detection for DTPA. DTPA was only detected once in post-RO water (1.7 µg/L, KWRP 040607) and the median value reported here is actually a limit of detection.

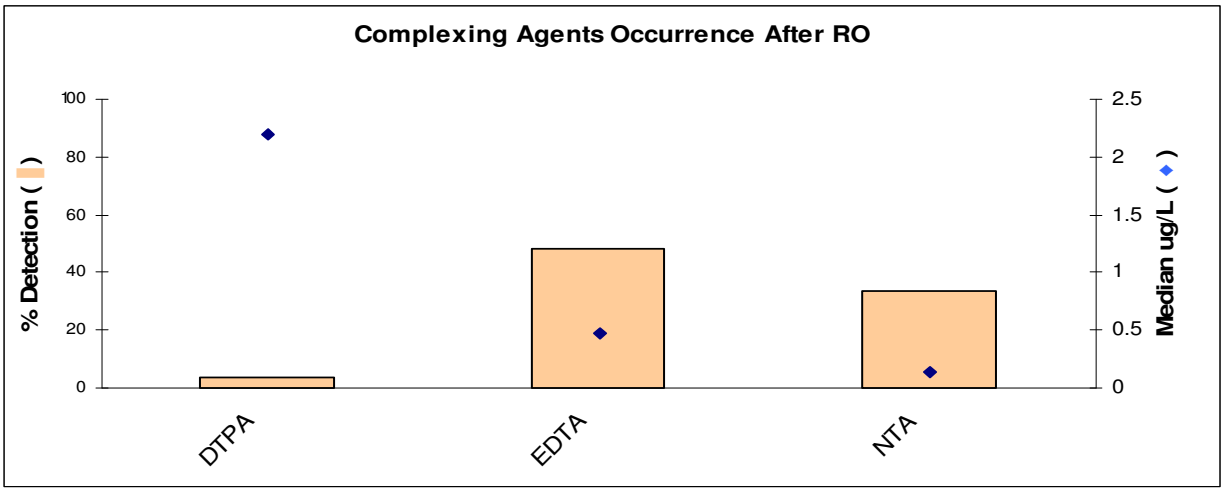

Figure 6.12.4: Complexing agents with percentage detections in post-RO water (vertical column) and corresponding median concentrations (µg/L, diamond).

Groundwater characterisation

NTA was the only complexing agent detected in groundwater and was only detected in one sample (Pinjar Boreline, May 2007), out of three groundwater samples measured for complexing agents. The concentration of NTA measured (0.2 µg/L) was very close to the LOD for that sampling event (0.19 µg/L).

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concetrations and the health values calculated in this study. There is no toxicity data available for either DTPA or PDTA and health values for these compounds were calculated using the TTC approach, as described in Chapter 3, section 3. Both DTPA and PDTA were classified in Cramer class III because they contain chemical structures that permit no strong initial impression of safety and may even suggest a significant toxicity (Kroes *et al.*, 2000, Renwick,

2004) . Comparison to health guidelines based on toxicity data (e.g. for EDTA and NTA) demonstrate that the guidelines based on TTC are significantly lower.

Table 6.12.3 presents the RQs for the complexing agents in secondary wastewater and post-RO water. In secondary wastewater RQ(max) and RQ(median) were above 1 for DTPA and PDTA, both calculated using the TTC approach. In both cases, the average LOD achieved was significantly higher than the assigned health value. In contrast, RQ(median) and RQ(max) for EDTA and NTA were always less than 1.

In post-RO water, PDTA was not detected and the RQ(median) has been calculated using the average LOD as the observed concentration. Again, RQs for DTPA and PDTA were greater than 1, indicating that the average LOD achieved was above the allocated TTC health value. RQ(median) and RQ(max) for EDTA and NTA were significantly below 1, indicating little health concern from these compounds.

Table 6.12.3: RQs for complexing agents calculated for secondary wastewater and post-RO water

						Secondary Wastewater		Post-RO water	
parameter	Health value (µg/L)	Source	Tier	LOR	n	RQ(median)	RQ(max)	RQ(median)	RQ(max)
DTPA	0.7	TTC	3	2.3	27	4.6	6.9	3.1	4.6
EDTA	250	ADWG	1	0.6	27	0.6	0.74	0.002	0.01
NTA	200	ADWG	1	0.08	27	0.01	0.02	0.001	0.005
PDTA	0.7	TTC	3	4.4	27	2.3	13	6.2	na

Treatment performance

Treatment efficiency was calculated for analytes detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD as a worst case scenario. Median treatment performance for complexing agents ranged from 99.5% for EDTA to 67.4% for PDTA (Figure 6.12.5). However the MF/RO treatment consistently removed NTA, DTPA and PDTA to below LOD, which probably indicates that the treatment efficiencies were affected by the LODs. EDTA, in contrast was often present in wastewater at concentrations two or three orders of magnitude greater than LOD and was also detected in post-RO water, meaning the measure of treatment efficiency using EDTA is more accurate and valid.

There was lower variability in calculated treatment efficiency for EDTA than other complexing agents. While EDTA treatment efficiency was calculated from 20 paired samples (secondary wastewater and post-RO), both DTPA and PDTA had significantly fewer detections in secondary wastewater (6 and 7 paired samples respectively), which may explain the larger variability in removal efficiency. While

NTA was consistently detected in secondary wastewater and also calculated from 20 paired samples, the presence of 2 significant outliers (see Figure 6.12.5) increased variability in treatment efficiency. NTA concentrations were one or two orders of magnitude lower than EDTA concentrations, and it was often present in wastewater at concentrations much closer to LOD. Nevertheless, membrane treatment was able to achieve high and consistent removal of all complexing agents.

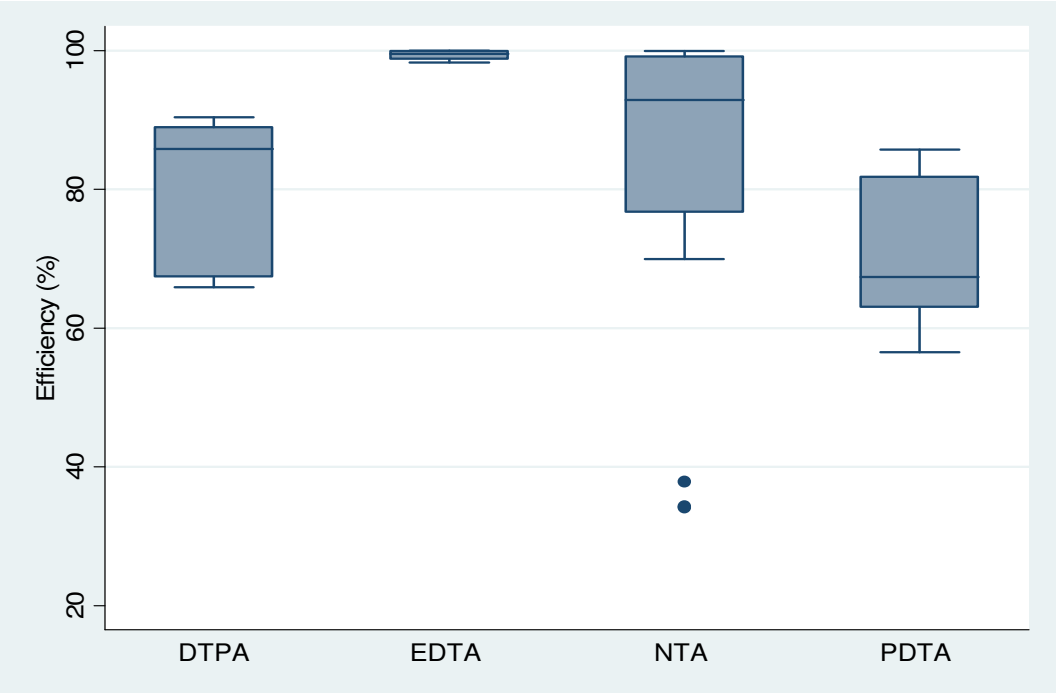

Figure 6.12.5 MF/RO removal efficiency of detected complexing agents in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Other analysis

A total of 9 paired samples after MF and 5 paired samples post-RO water were available to compare grab and composite samples. Composite samples were higher than grab in 57% of the paired samples. There was a greater difference between grab and composite samples for larger concentrations as presented in Figure 6.12.6.

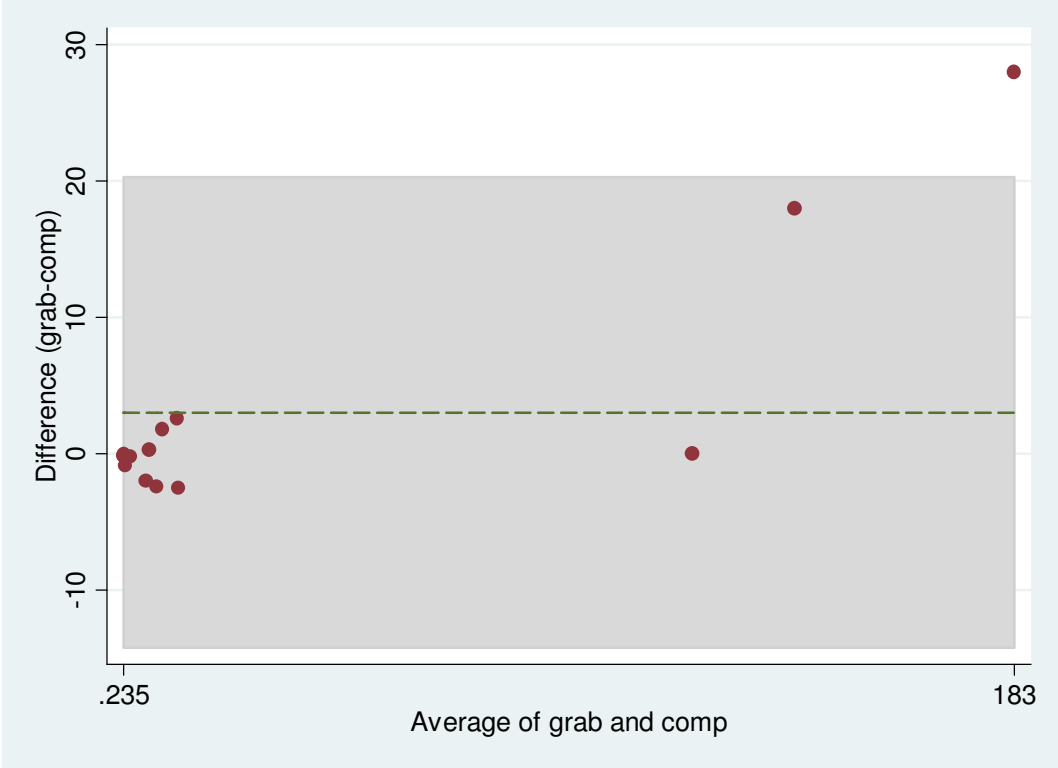

Figure 6.12.6: Bland-Altman plot comparing grab and composite (comp) samples for complexing agents.

Discussion

All 4 aminopolycarboxylate complexing agents were detected in secondary wastewater as reported in other studies (Fuerhacker *et al.*, 2003, Reemtsma *et al.*, 2006, Oviedo & Rodriguez, 2003), although no clear trend by location or season was observed. The detection of EDTA as the most abundant and most ubiquitous complexing agent also agrees with other studies that find it to be the most widely used and least biodegradable complexing agent (Knepper, 2003, Oviedo & Rodriguez, 2003, Reemtsma *et al.*, 2006)

NTA was the only complexing agent detected in groundwater, albeit at concentrations just above the LOD. NTA has previously been detected in raw drinking water samples in Canada (range <0.2–33.5 µg/L) (WHO, 2006) and NTA concentrations in groundwater samples with evidence of pollution by sewage have been 15-250 µg/L higher than at unpolluted sites (FPTCDW, 2008). However, NTA biodegradation in both oxic and anoxic environments has also been reported including rapid degradation in leachate from septic tanks (Bucheli-Witschel & Egli, 2001, Shimp *et al.*, 1994). More research would be required to determine the source and extent of NTA in groundwater.

All complexing agents were well rejected by RO. The treatment efficiency for EDTA was greater than 99%, while NTA, PDTA, and DTPA were generally removed to

below LOD, and this is consistent with findings from other studies (Drewes *et al.*, 2002, Drewes *et al.*, 2003). EDTA is an excellent chemical indicator for monitoring RO treatment efficiency because it is an excellent marker of wastewater derived micropollutants (Drewes *et al.*, 2003) and because frequent detection post-RO means accurate values can be determined for treatment efficiency.

RQ analysis indicates that the public health impact of those complexing agents with health values (EDTA and NTA) is very low. However, the conclusion for complexing agents for which the TTC was used to derive the health value (PDTA and DTPA) is less clear, as RQs were above one for these compounds. The TTC approach is a very conservative approach. It may be expected that unregulated complexing agents could have comparable human toxic effects to regulated complexing agents, based on similarity of chemical structure. In aquatic organisms there was no pronounced difference observed in impact of all 4 complexing agents when tested as the same metal complex species (Schmidt & Brauch, 2004). A less conservative approach for unregulated complexing agents could be to divide the health value of the lowest regulated complexing agents (i.e. NTA) by a safety factor of 10. More realistic health values for DTPA and PDTA are probably in the low µg/L range.

Human exposure to complexing agents through augmentation of drinking water supplies is small in comparison to that from other sources such as food additives, therapeutic uses, personal care and hygiene products (FPTCDW, 2008) Estimated NTA intake from detergent residues present on unrinsed dishes is approximately 0.175 mg/day (equal to 0.0025 mg/kg bw/day) (FPTCDW, 2008) and therapeutic doses of EDTA of up to 50 mg/kg bw/day have been recommended. Therefore, the health significance of complexing agents is considered low at the observed concentrations in post-RO water for augmentation of drinking water supplies.

While EDTA currently remains the most ubiquitous aminopolycarboxylate complexing agent, it is possible that changes in usage will result in other complexing agents becoming more abundant in secondary wastewater. In particular, methylglycinediacetic acid (MGDA) and β-alaninediacetic acid (ADA) are two other complexing agents proposed as alternatives to EDTA (Schmidt & Brauch, 2004). Both MGDA and ADA were tested in the preliminary sampling event conducted in June 2005, with all wastewater and post-RO samples reporting below the LOR of 1 µg/L. Based on European consumption data (Schmidt *et al.*, 2004), it is expected that EDTA, NTA and PDTA usage will predominate over ADA, MGDA and PDTA for some time. Future analysis of these compounds is probably only warranted if aminopolycarboxylate complexing agent usage in Western Australia is known to shift significantly from current patterns.

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6.13 Inorganic Disinfectant By-products (Anions)

Introduction

Disinfectants such as chlorine or chloramine lead to the formation of a range of disinfection by-products in drinking water and waste water, including inorganic by-products, organic oxidation by-products, and halogenated organic by-products (Clark & Boutin, 2002). This section will focus on the oxyhalide anions chlorite, chlorate and bromate, which we refer to in this section as the anions. Other disinfection by-products such as the halogenated organic disinfection by-products and *N*-nitrosamines are discussed in sections 6.3 and 6.4 respectively.

Depending on the disinfection process, different oxyhalide anions form. Chlorite and chlorate are potential by-products of disinfection by hypochlorite, chloramine or chlorine dioxide (WHO, 2000). In hypochlorite solutions, decomposition to chlorate and chlorite is pH dependent. The initial reaction to form chlorite is relatively slow, while further reaction of chlorite to form chlorate is much faster (Gordon *et al.*, 1997). In contrast, bromate forms during ozonation via oxidation (Joyce & Dhillon, 1994, WHO, 2000). The reaction of bromide with ozone initially oxidizes bromide to hypobromous acid, which then further reacts to form bromate (von Gunten & Holgne, 1994). As well as formation during disinfection itself, analysis of hypochlorite solutions used to disinfect drinking-water have shown them to contain concentrations of chlorite, chlorate and bromate between 1-100 µg/L, dependent particularly on available free chlorine concentration, and solution storage time (Asami *et al.*, 2009, Bolyard *et al.*, 1992). Bromate can also form in hypochlorite solutions contaminated with bromine. A study by Weinberg *et al* (2003) showed that bromate in hypochlorite-treated finished waters varies across the United States based on the source of the chemical feedstock, which can add as much as 3 µg/L bromate into drinking water. This study reported by Furthermore, chlorate was then measured in drinking water as a direct result of contamination in the hypochlorite solution (Bolyard *et al.*, 1992). However concentrations of chlorite and bromate were not high enough in drinking water to result in measurable concentrations.

The toxic action of both chlorate and chlorite is through oxidative damage to red blood cells (WHO, 2000). Chlorate is less effective at inducing oxidative damage and it does not appear to be teratogenic or genotoxic *in vivo*. Data on the genotoxicity of chlorite is conflicting. Bromate is also an active oxidant in biological systems and has been shown to cause cancers in animal studies, including renal tumors, although its carcinogenicity appears to be secondary to oxidative stress in the cell (WHO, 2000). It is also considered genotoxic because it generates oxygen radicals in the cell.

Methods

Ion chromatography (IC) was used for the simultaneous determination of chlorite, chlorate, and bromate using anion exchange guard and analytical columns, an anion micromembrane suppressor, and conductivity detection. A sodium carbonate buffer was used as the eluent, while sulphuric acid (1% v/v) was used as a column regenerant. Samples were not filtered before analysis, but a specially designed 20 µm filter cap placed on each sample vial removed solid materials from the sample before injection.

Table 6.13.1: Health values, Limits of detection (LOD) and estimation of uncertainty for anions

Analyte	Health value (µg/L)	Source	Average LOD (µg/L)	Standard Relative Uncertainty (%) (50 µg/L)
Bromate	20	ADWG, 2004	11	17.0%
Chlorite	300	ADWG, 2004	13	17.8%
Chlorate	700	WHO, 2006	10	19.7%

Quality assurance/ Quality control

To validate the analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty.

Results

A total of 198 measurements were analysed for anions during the project excluding replicates, field and trip blanks. All samples were grab and data was available for Events 2 to 6 (Table 6.13.2).

Table 6.13.2: Measurement of anions by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	SWW	Water Reclamation Plant							
				Grab	Comp				Before MF		Post-MF water		Post-RO water		Dam	Total
									K	B	K	B	K	B		
1	November	4	2006													
2	May/June	6	2007	48	0	48	6	9	9	6	9	0	9	0	0	33
3	September	6	2007	42	0	42	0	0	9	9	3	3	9	9	0	42
4	January	6	2008	36	0	36	6	0	6	9	0	0	6	9	0	30
5	April	5	2008	36	0	36	0	3	6	9	0	3	6	9	0	33
6	June	5	2008	36	0	36	0	0	6	9	3	3	6	9	0	36
Total		32		198	0	198	12	12	36	36	15	9	36	36	0	174

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

The occurrence of anions in secondary wastewater is presented in Figure 6.13.1. Bromate was not detected in any of the samples taken. Chlorate was detected in 37% of the secondary wastewater samples (median=12.9 µg/L, max=1600 µg/L). Chlorite was detected in 7% of secondary wastewater samples (median=7.1 µg/L, max=386 µg/L).

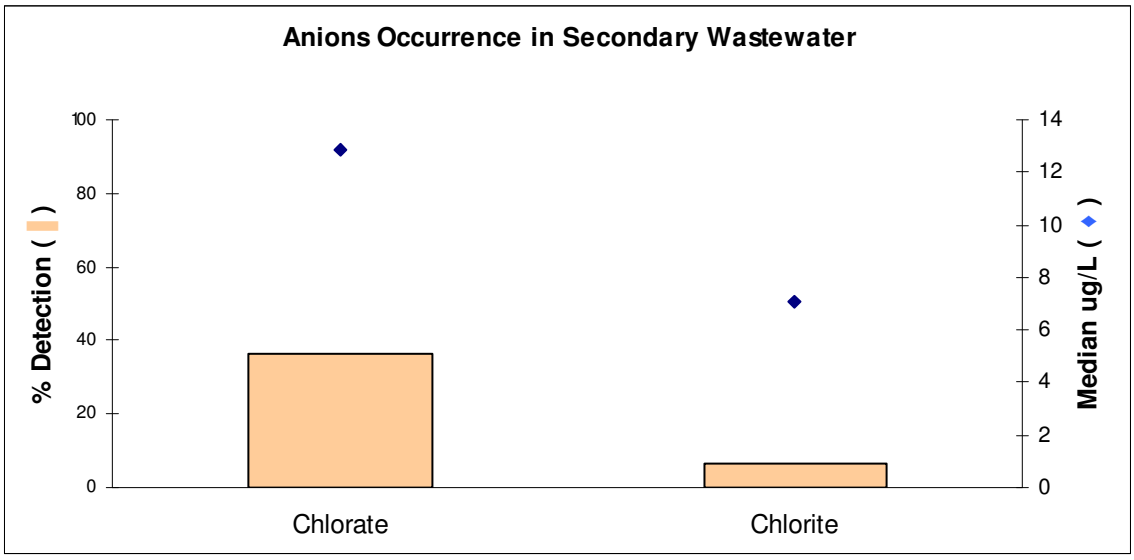

Figure 6.13.1: Anions with percentage detections in secondary wastewater (vertical column) and corresponding median concentrations (µg/L, diamond).

A comparison of median concentrations at each WWTP (Figure 6.13.2) showed that both chlorate and chlorite were detected at higher concentrations at Subiaco WWTP. However, the differences were not statistically significant and may have been affected by the small number of samples taken at Subiaco WWTP compared to KWRP or Beenyup WWTP.

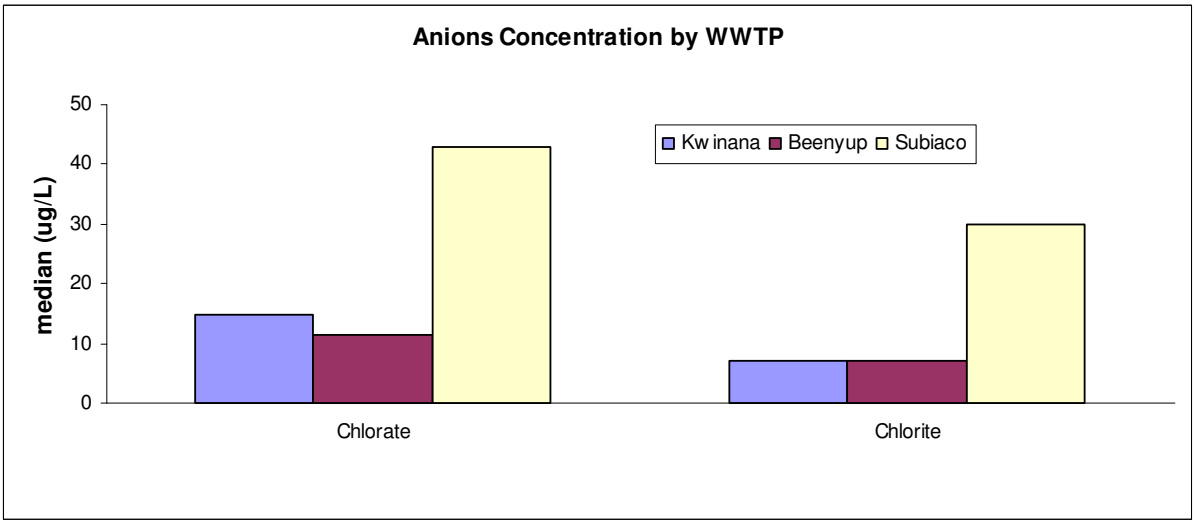

Figure 6.13.2: Anions with detections in secondary wastewater and corresponding median concentrations (µg/L).

Comparison of median concentrations by season shows that chlorate was highest in summer (Figure 6.13.3). However, the difference was not statistically significant. The

median chlorite concentration was highest in winter, although again the difference was not statistically significant. The low number of percentage detections for chlorite also means that these findings are affected by the different LODs reported in each sampling event.

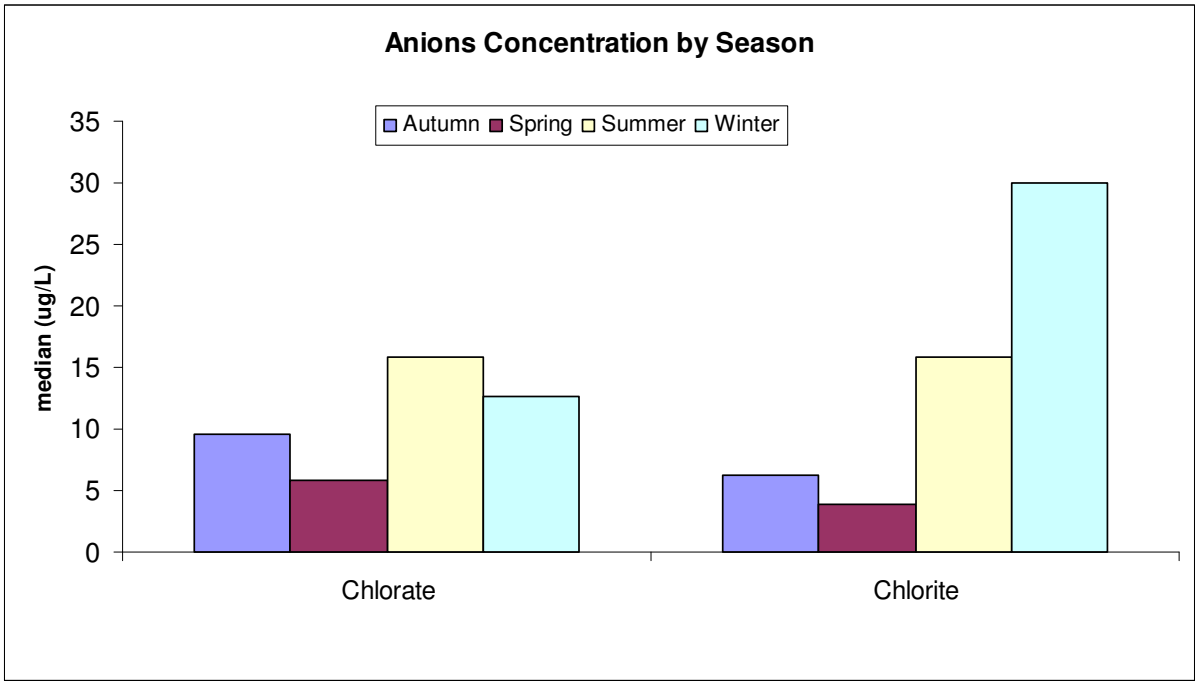

Figure 6.13.3: Anions median concentration by season (µg/L).

RO Product water characterisation

Neither chlorite nor bromate was detected in post-RO samples. Chlorate was detected in 46% of post-RO water samples, with a median concentration of 12.7 µg/L and a maximum concentration of 46 µg/L.

Groundwater characterisation

Chlorate was detected in one of four groundwater samples. The detected concentration from the Pinjar bore line on the 24th of May 2007 was 253 µg/L. Samples taken from the Wanneroo bore line and the field blank the same day were below the LOD=10 µg/L.

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. For those anions that were not detected, the RQ(median) was calculated using the average LOD as the observed concentration. Table 6.13.3 presents the RQs for anions in secondary wastewater and post-RO water.

In secondary wastewater RQ(median) was below 1 for all anions, while RQ(max) was greater than 1 for both chlorite (1.3) and chlorate (2.3). In post-RO water, RQ(median) and RQ(max) were lower than 1 for chlorate.

Table 6.13.3: Anions before MF and post-RO water corresponding RQS

					Before MF			After RO		
Parameter	**Health value (µg/L)**	**Source**	**Tier**	**LOD**	**n**	**RQ(median)**	**RQ(max)**	**n**	**RQ(median)**	**RQ(max)**
Chlorate	700	WHO	1	10.1	30	0.02	2.3	24	0.02	0.07
Chlorite	300	ADWG	1	12.6	30	0.02	1.3	24	0.04	na
Bromate	20	ADWG	1	11.2	30	0.56	na	24	0.56	na

The effect of MF/RO treatment on Anion concentrations

In both the BPP and KWRP, wastewater undergoes chloramination before MF to prevent RO membrane fouling. Over the course of the sampling period, a small number of post-MF samples were collected from within both plants in addition to the normal secondary wastewater and post-RO samples to determine the effect of chloramination during the MF/RO process. Paired wastewater, post-MF and post-RO samples were taken on 5 occasions at KWRP (Event 1: 29th November 2006, Event 2: 30th May 2007, 4th June 2007, 7th June 2007, Event 3: 21st September 2007, and Event 6: 6th June 2008) and on 3 occasions at Beenyup (Event 3: 26th September 2007, Event 4: 1st April 2008 and Event 6: 5th June 2008). There was no difference in bromate and chlorite concentrations between secondary wastewater and post-MF samples in either KWRP or BPP. However, chlorate concentrations in the BPP were significantly higher in post-MF samples than in secondary wastewater (Figure 6.13.5), with median post-MF concentration higher than health guideline. Data for chlorate is less clear at KWRP and the variation in secondary wastewater concentrations of paired samples, as measured using the standard deviation, is extremely high as a consequence of one very high value. However, post-MF samples at KWRP do not seem to show the same increased concentration as compared to secondary wastewater.

As described in section 6.3 (Halogenated DBPs), the time between the hypochlorite dosing point and post-MF sample point is 20 seconds in BPP and 25 minutes or longer in KWRP, depending on plant flow. The rate of chlorate formation is considered slow and chlorate is unlikely to form in significant concentrations in the

time available between dosing point and sample point in either KWRP or BPP Therefore this increase is attributed to chlorate, which has pre-formed in the hypochlorite solution before dosing.

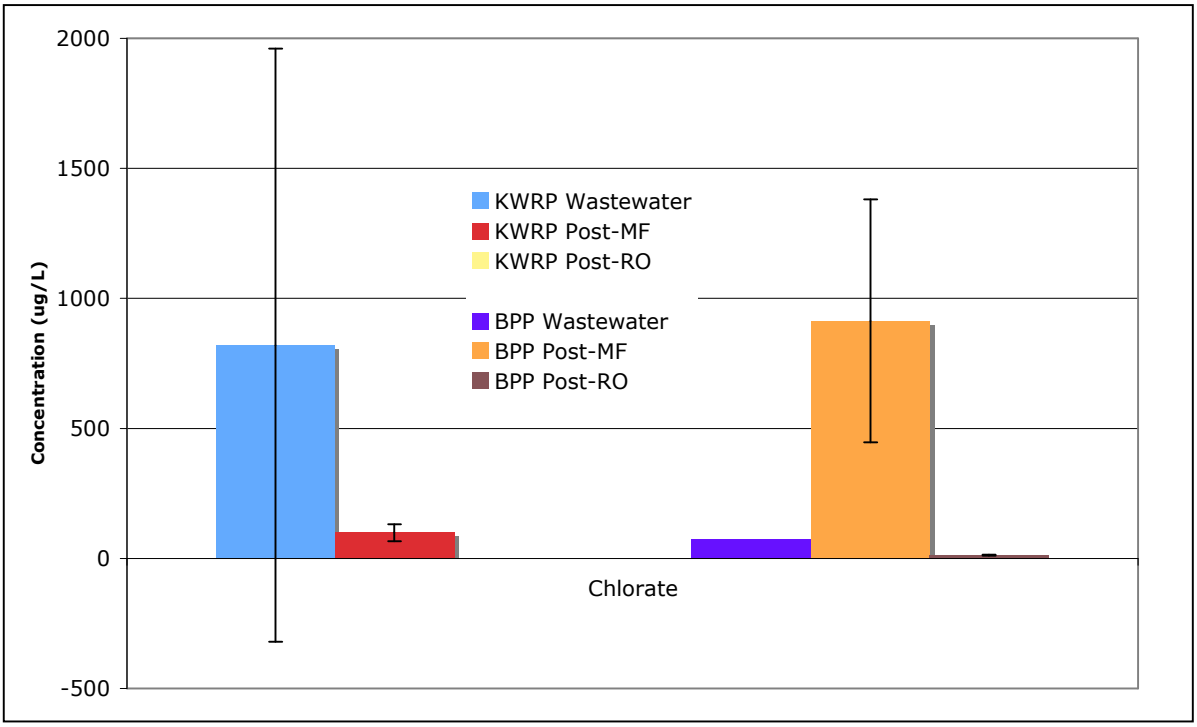

Figure 6.13.4: Median concentrations of chlorate in paired secondary wastewater, post-MF water and post-RO water samples for both KWRP (n=5) and BPP (n=3). Errors bars represent the standard deviation

Treatment performance

Treatment efficiency was calculated for anions detected in the secondary wastewater. Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were matched for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD as a worst-case scenario. Analysis of treatment efficiency was conducted with 7 paired samples for chlorate and one paired sample for chlorite. The single treatment efficiency value for chlorite was 96%, while median treatment efficiency of chlorate was 75% (Figure 6.13.5). Removal of chlorate was variable (std dev=53.3 µg/L) and for some post-RO measurements concentrations were higher than before MF.

Given the effect of chloramination on some analytes, as discussed previously, the treatment efficiency of RO alone cannot be determined using secondary wastewater as the starting concentration. As seen for other DBPs, calculation of RO treatment efficiency using the post-MF concentration as the starting concentration may be more

appropriate for chlorate. Calculations confirmed that the median RO treatment efficiency for chlorate was 96% when using paired post-MF and post-RO water samples (n=8), which was significantly higher compared to the value calculated using paired secondary wastewater and post-RO water samples (see Figures 6.13.5 and 6.13.6). The treatment efficiency for chlorite was similar using either secondary wastewater or post-MF water, although only 1 paired sample was available for each treatment efficiency calculation. Variability in efficiency calculated using post-MF and post-RO data, as represented by standard deviation, was also much lower.

Because of the differences seen in chlorate concentration in KWRP and BPP, treatment efficiency between post-MF and post-RO samples was also calculated for each plant, although this data is not plotted because of the low number of paired samples available. Treatment efficiency was very similar at both plants, with median values of 95% at KWRP and 98% at BPP.

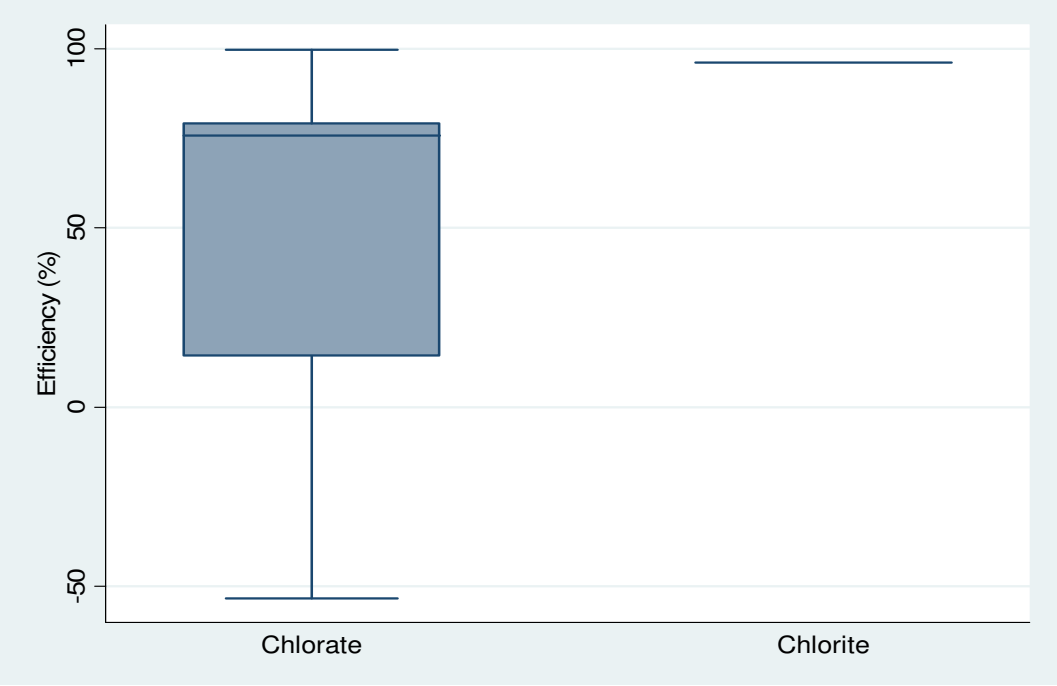

Figure 6.13.5: MF/RO removal efficiency of detected anions in secondary wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

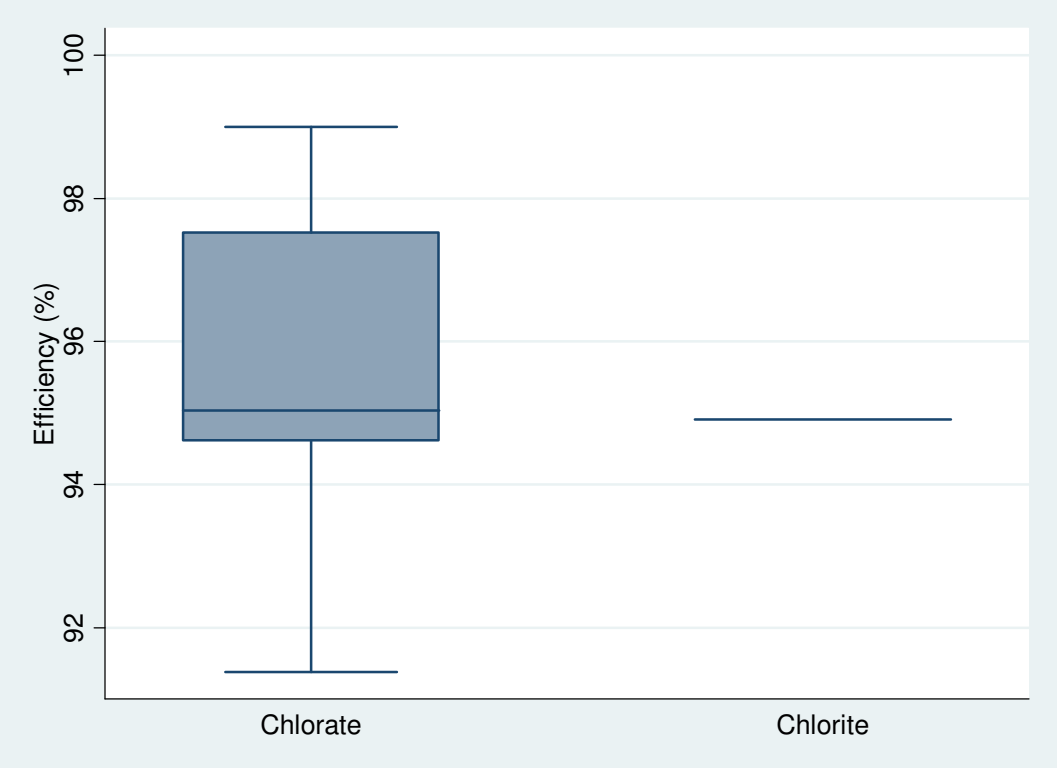

Figure 6.13.6 RO removal efficiency of detected anions in MF-treated wastewater. Bars indicate the median, 25th, 75th percentile and statistically determined minimum and maximum values, the dots represent extreme values.

Discussion

Both chlorate (37% detection) and chlorite (7% detection) were found in the secondary wastewater. Chlorine (3-5 mg/L) is added to the return activated sludge in all three WWTPs and this may be a source of these anions within each plant. The rapid rate of reaction of chlorite to chlorate including in the hypochlorite solution (Gordon *et al.*, 1997) is probably responsible for the greater percentage of chlorate detections. No bromate was measured in secondary wastewater, which is associated with there being no ozonation within any of the WWTPs.

While Subiaco WWTP tended to have the highest values of both chlorite and chlorate, the lack of statistical significance may be because fewer wastewater samples were analysed from Subiaco compared to Beenyup WWTP or KWRP. No statistically significant differences could be seen by season despite the seasonal variability in chlorine dose. Chlorate and chlorite are not expected to behave in the same way as other DBPs as they are not volatile.

Only chlorate was detected in post-RO water, and the percentage detection in post-RO samples (46%) was greater than the percentage detection in secondary wastewater (37%) and higher concentration of chlorate were measured in post-MF samples compared to secondary wastewater samples. Given that the overall rate of

chlorate formation is much slower than the residence time between hypochlorite dosing and the post-MF sampling point, it is suggested that chlorate is already present in the hypochlorite dosing solution. This is in line with other studies showing that hypochlorite solutions can contain chlorite, chlorate and bromate (Asami *et al.*, 2009, Bolyard *et al.*, 1992). While chlorite and bromate could also be expected to be present in the hypochlorite dosing solution (depending on its purity), the concentrations are still expected to be too low to be detected in post-MF water (Bolyard *et al.*, 1992, Gordon *et al.*, 1997, Weinberg *et al.*, 2003).

Only one value for chlorite treatment efficiency could be calculated using secondary wastewater, but removal was high at 96%. Calculated treatment removal for chlorate (median 76%) was variable, with some concentrations in post-RO water higher than the concentration in secondary wastewater. However, calculating treatment efficiency using post-MF and post-RO data produced higher values (95%) than using secondary wastewater and post-RO water, again supporting the hypothesis that chlorate concentrations increased during the MF/RO treatment. Treatment efficiency at KWRP was similar to Beenyup Pilot Plant, above 95%. RO removal of anions is expected to be high because they are negatively charged and repelled from the negatively changed RO membrane (Bellona *et al.*, 2004).

Maximum chlorite and chlorate concentrations were above the corresponding health values in secondary wastewater but calculated RQ(median) were below 1 for both anions. Detected anion concentrations in post-RO water were low compared to health guidelines and all RQ(median) and RQ(max) were at least one order of magnitude below 1. Bromate was never detected and risk quotients were always below 1 based on the LOD.

Detection of chlorate in one bulk groundwater sample is surprising as any chlorate that made its way into groundwater would be expected to be reduced in anoxic conditions. As the groundwater samples were actually collected at the influent to the Wanneroo drinking water treatment plant, after passing through several kilometres of pipeline, there is potential for disinfectant to have been introduced for cleaning purposes upstream (e.g. for cleaning bore screens). This single chlorate detection occurred in the same sample in which other disinfection byproducts were also detected (see section 6.3, Table 6.3.3).

Anions analysed were not detected in sufficient frequency in secondary wastewater to be selected as a chemical indicator for RO treatment. Of the three anions in this section, chlorate would be the most suitable indicator. However nitrate has similar physical and chemical properties is consistently present in secondary treated wastewater and concentrations are not affected by addition of sodium hypochlorite. Therefore nitrate is considered to be a more suitable anionic chemical indicator for RO treatment.

Perchlorate was not investigated in this study. It has been classified as an endocrine disruptor and has a low drinking water guideline (6 µg/L, Californian DHS). Given the detection of chlorate in post-RO water it might be expected that perchlorate would

also pass through RO membranes following potential introduction as an unwanted contaminant of hypochlorite during chloramination. Perchlorate can form from degradation of hypochlorite if inappropriately stored (Greiner *et al*., 2008). Therefore testing of perchlorate is recommended.

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6.14 Miscellaneous Organic Chemicals

Introduction

This section comprises a miscellaneous group of 12 chemicals that were tested during the project but that were not included in any of the other reported chemical classes. The chemicals in this chapter are: 1,4-dioxane, 4-bromophenoxybenzene, 4-chlorophenoxybenzene, acrolein, acrylonitrile, azobenzene, butylbenzylphthalate, dioctylphthalate, hexachlorobenzene, hexachlorocyclopentadiene, methyl tert-butyl ether (MTBE), and triclosan. A brief description of the uses, occurrence in wastewater and toxicity of each of these chemicals is presented below. Six of these chemicals were priority chemicals for this study (1,4-dioxane, acrolein, acrylonitrile, hexachlorobenzene, MTBE, and triclosan). The remainder were additional chemicals available through the analytical methods used.

1,4-dioxane is widely used as a solvent and as a stabilizer in the manufacture and processing of paper, cotton, textile products, automotive coolant, cosmetics and detergents (USEPA, 2008a, Zenker *et al*., 2003). It is also used in the manufacture of adhesives, sealants, pharmaceuticals, rubber chemicals and surface coatings (WHO, 2006, Zenker *et al*., 2003). In Australia, 1,4-dioxane was declared a priority chemical in 1994 due to concerns over possible human carcinogenicity, its potential for widespread public exposure, and high degree of persistence in the aquatic environment (NICNAS, 1998). IARC has classified 1,4-dioxane as possibly carcinogenic to humans (group 2B) (IARC, 2008). Exposure to the general public may occur from use of consumer products containing ethoxylated chemicals (e.g., detergents, cosmetics/toiletries, pharmaceuticals and food), via water containing 1,4-dioxane as an impurity, as well as certain foods in which it naturally occurs (NICNAS, 1998). Concentrations of around 1 μg/L have been measured in domestic WWTP effluent, attributed to the presence of 1,4-dioxane in commonly used polyethoxylated surfactants and detergents (Tanabe *et al*., 2006, Abe, 1999). 1,4-dioxane is not significantly removed during conventional wastewater treatment (Zenker *et al*., 2003), including activated sludge treatment (Tanabe *et al*., 2006, Abe, 1999), and remobilisation to groundwater can occur when sludge containing 1,4-dioxane is disposed of in landfill (Abe, 1999).

4-bromophenoxybenzene, or 4-bromophenylphenyl ether, is a brominated diphenyl ether (denoted BDE-3), 1 of 209 possible polybrominated diphenyl ether (PBDE) congeners (D'Silva *et al*., 2004). PBDEs have been used as flame-retardants in plastics since the 1970s or earlier. While 4-bromophenoxybenzene is not present in commercial PBDE mixtures (Korytar *et al*., 2005), it has been identified as a photo-degradation product of 2,2',4,4'-tetrabromodiphenylether (BDE-47) (Li *et al*., 2008), which is highly abundant in the environment because it is a major component of several commercial PBDE mixtures, and also the most thermodynamically stable PBDE conformation (D'Silva *et al*., 2004). Generally levels of 4-bromophenoxybenzene in the environment are low. It was not seen in the sludge or

wastewater effluent of a WWTP in California (North, 2004) and it was the only PBDE of 14 congeners studied that was not detected in a study of human breast milk (Sudaryanto *et al*., 2008). 4-bromophenoxybenzene was found in low concentrations (sub-ng/g dry weight) in river sediments exposed to electronic waste, including end-of-life products such as computers and printers, but was not detected in fish living in those environments (Luo *et al*., 2007).

4-chlorophenoxybenzene is a chlorinated diphenyl ether (denoted CDE-4), one of 209 possible polychlorinated diphenyl ether (PCDE) congeners (Domingo, 2006), and structurally similar to polychlorinated biphenyls (PCBs). PCDE are used as intermediates in chemical syntheses (Yang *et al*., 1997), but also enter the environment because they are present as impurities in chlorophenol preparations (Kurz and Ballschmiter, 1995). Data for environmental concentrations of PCDE is relatively scarce (Domingo, 2006), but they are significantly bioaccumulated in fish and animals (Chui *et al*., 1990, Domingo, 2006). It has also been suggested that CDE-4 mimics estrogen effects on endometriotic tissues (Yang *et al*., 1997)

Acrolein is a volatile, colourless, highly flammable liquid that is soluble in water and in organic solvents (IPCS, 1991). It is primarily used as an intermediate in chemical synthesis and as an aquatic herbicide and rodenticide (IPCS, 1991). Acrolein can enter the environment as a result of burning wood, tobacco, vehicle fuels, or through accidental releases from chemical plants and hazardous waste sites. Acrolein is very toxic for aquatic organisms and is used as an aquatic herbicide and slimicide (IPCS, 1991). However, it is rapidly degraded both in surface water and in the atmosphere, and is not expected to accumulate (Faroon *et al*., 2008). While measured in industrial waste waters, it is largely removed by wastewater treatment before wastewater discharge (USEPA, 1991). The primary effect of acrolein identified after oral exposure to acrolein in rats was gastrointestinal irritation. Altered kidney and liver weights have been also reported. The IARC classified acrolein in Group 3, not classifiable as to its carcinogenicity to humans, because the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals (IARC, 2008). The general population will normally be exposed to acrolein through the air, particularly through exposure to smoke (Faroon *et al*., 2008).

Acrylonitrile is an important monomer for in the manufacture of plastics, synthetic fibres, resins, fumigants and rubber. In Australia, acrylonitrile was declared a priority existing chemical for preliminary assessment in 1998 because of public concern about health effects (NICNAS, 2000). Acrylonitrile is not manufactured in Australia, but is imported for polymer manufacture and it has been predicted in a worst case scenario that concentrations of up to 3.1 µg/L could exist in WWTP effluent assuming no removal (NICNAS, 2000). However, in a study of five Taiwanese WWTPs, acrylonitrile was not detected in treated wastewater even when WWTP influent concentrations were over 7000 µg/L, and emissions by volatilisation were seen throughout the plant (Cheng *et al*., 2008). Acrylonitrile shows acute toxicity by all exposure avenues (NICNAS, 2000, Long *et al*., 2001). Tumours in rats have been consistently observed following both ingestion and inhalation in chronic exposure

studies (Long *et al*., 2001), while epidemiological studies provide some indications that acrylonitrile exposure is associated with cancer, particularly of the lung (IPCS, 1983). The IARC have classified acrylonitrile as possibly carcinogenic to humans (group 2B) (IARC, 2008).

Azobenzene contains two phenyl rings linked by an N=N double bond that can adsorb light. A wide class of molecules have the core azobenzene structure, with many used as industrial dyes. Azobenzene has been used as an intermediate in the manufacture of dyes and insecticides (Fishbein, 1979), and environmental residues have been seen from the biochemical synthesis from aniline-based herbicides in soils (Bordeleau and Bartha, 1970). Azobenzene is relatively insoluble in water (log K_{ow} ~4) and therefore removal in WWTPs probably occurs in the primary settling tank, along with other water insoluble azoic dyes (Hai *et al*., 2007). Anaerobic degradation of azo dyes also can occur in the activated sludge (Hai *et al*., 2007). Azobenzene is classified as a probable human carcinogen based on sufficient evidence of carcinogenicity in animals by IRIS (USEPA, 2008b) and as not classifiable as to its carcinogenicity to humans (Group 3) by IARC (IARC, 2008).

Butylbenzylphthalate is used mainly as a plasticizer for polyvinyl chloride, softening the final product and increasing its flexibility. In Canada and the USA the majority of environmental releases of butylbenzylphthalate are to the atmosphere (IARC, 1999, Canadian Environmental Protection Act, 2000), but the substance has also been detected in industrial and municipal wastewater effluents in the µg/L range and in sludge from municipal wastewater plants in µg/g levels (Canadian Environmental Protection Act, 2000). In Europe it was not detected (LOD=10 ng/L) in urban wastewater samples of Granada City in Spain, (Casajuana and Lacorte, 2003), and was detected in sub-µg/L concentrations in the treated wastewater from a WWTP in France (Dargnat *et al*., 2009). Degradation of butybenzylphthalate occurs by photo-oxidation in the atmosphere, with a half life of 6-60 hours, while biodegradation in aerobic water has a half live of 2-10 days depending on temperature (Canadian Environmental Protection Act, 2000). Butylbenzylphthalate has been detected in surface water, groundwater and drinking water (IARC, 1999), though it was rarely detected in drinking water in Canada (Canadian Environmental Protection Act, 2000). Water from drinking water distribution systems which include plastic or painted concrete reservoirs and pipes were sometimes found to contain tens of ng/L of butylbenzylphthalate (Casajuana and Lacorte, 2003). The compound has been associated with reproductive toxicity in rats, however it is considered that the estrogenic activity of phthalates identified in the in vitro studies is not relevant to human exposure (Tyl *et al*., 2004). The IARC has determined that there is inadequate evidence for the carcinogenicity of butylbenzylphthalate in humans and limited evidence in experimental animals and therefore it has been classified as Group 3 (IARC, 2008).

Dioctylphthalate (Di-n-octyl phthalate) is a hydrophobic (log K_{ow}~7) phthalic acid ester that is widely used as a plasticizer to impart flexibility to polymers, particularly polyvinyl chloride. It is found in a variety of products, including building materials,

vinyl gloves and hoses, and cements, however a paucity of concentration and leaching data means human exposure cannot be reliably calculated (Kamrin and Kamrin, 2009). Biomonitoring indicates that dioctylphthalate exposure is at or below 1 µg/kg/d in humans of all ages. Concentrations in industrial effluent have been measured up to 10 µg/L (Canadian Environmental Protection Act, 1993, Jackson and Sutton, 2008), but concentrations in municipal WWTP influent are low, and it has not been detected in secondary treated wastewater (Dargnat *et al*., 2009, Jackson and Sutton, 2008). Dioctylphthalate has been detected in municipal sludge at mg/kg concentrations, suggesting adsorption to the sludge is a likely removal mechanism (Canadian Environmental Protection Act, 1993, Dargnat *et al*., 2009). Dioctylphthalate is rarely detected in raw surface waters, usually in the range of ng/L (Canadian Environmental Protection Act, 1993) The photo-oxidation half-life for dioctylphthalate in air has been estimated to be less than 2 days, while aerobic biodegradation half-lives in soil and surface water ranged between 1 and 4 weeks (Canadian Environmental Protection Act, 1993). Scientific evidence suggests that human risks from dioctylphthalate exposure is negligible (Kamrin and Kamrin, 2009), although toxicological studies in rats indicate that dioctylphthalate produced thyroid, hepatic and hematological alterations (Poon *et al*., 1997).

Hexachlorobenzene is a fungicide formerly used as a seed and grain treatment but banned globally under the Stockholm Convention since 2001 because of its persistence in the environment (UNEP, 2005). It may also form as a waste product in the production of several chlorinated hydrocarbons, and remains an impurity in several registered pesticides (ATSDR, 2002). While it is practically insoluble in water, it is highly lipid soluble. In waters it will partition to solid particles and it is considered impervious to leaching in soils, where it has a degradation half-life of 3-6 years. Hexachlorobenzene is released to the environment almost entirely as a result of industrial activity. Concentrations measured in surface waters are generally 0.01-10 ng/L(ATSDR, 2002), though concentrations are usually higher near point sources (IPCS, 1997). Hexachlorobenzene residues are almost always detected in the adipose tissues of humans and this is attributed to trace concentrations in food, which is considered the major exposure route for humans (ATSDR, 2002). Hexachlorobenzene has been found to be clinically toxic and may cause death in incidences of high accidental exposure (IPCS, 1997). It can cause systemic, neurological, developmental, endocrine and immunological toxicity in humans (ATSDR, 2002). The IARC classified hexachlorobenzene as possibly carcinogenic to humans (IARC, 2008), while the US EPA have classed it as a probable human carcinogen (USEPA, 2008b).

Hexachlorocyclopentadiene is an organochlorine compound used as a precursor for several pesticides (e.g., aldrin, dieldrin, endrin, heptachlor, isodrin, mirex, and pentac), although few remain in use because of their persistence in the environment (ATSDR, 1999). Waste containing hexachlorocyclopentadiene is considered hazardous by the US EPA and requires disposal by incineration or burial in specially designated chemical landfills. Therefore, emissions to WWTP are expected to be low, and the main emission path to the environment via atmosphere (ATSDR, 1999).

Biomagnification through the food chain is unlikely to occur because hexachlorocyclopentadiene degrades rapidly by photolysis and by hydrolysis, and can also be biodegraded (ATSDR, 1999). It is rarely detected in ground or surface waters. While acutely toxic, hexachlorocyclopentadiene is not listed on the register of IARC carcinogens (IARC, 2008) and is considered unclassifiable by the US EPA because of insufficient information (USEPA, 2008b).

Methyl tertiary butyl ether (MTBE) is a volatile oxygenate commonly used as an additive to increase the octane number in fuels and also improve air quality by reducing the level of carbon monoxide in vehicle exhausts (Deeb *et al*., 2003). Spills and leaking petrol storage tanks can cause groundwater contamination and MTBE is a frequent contaminant of shallow groundwater (Deeb *et al*., 2003). MTBE has been detected in groundwater and drinking water at concentrations in the ng/L to µg/L range (Hartley *et al*., 1999, Wright *et al*., 2005), with areas of high population density and high recharge rates more likely to have MTBE groundwater contamination (Moran *et al*., 2005). Wastewater from municipal WWTPs discharging into the coastal waters of California have been found to vary in concentration between 1 and 100 µg/L (Brown *et al*., 2001). The principal human health concerns associated with MTBE are possible cancer effects. Limited laboratory evidence suggests that MTBE may act as a carcinogen on rodents (WHO, 2006). While the guideline value used was based on levels of health concern, this is higher than the concentration that can be detected by odour (5 µg/L, California DPH) and this may be more relevant as taste and odour at concentrations above this value make the water unpleasant to drink.

Triclosan is a polychlorophenoxyphenol, with wide spectrum antibacterial and antifungal properties. It is used in a variety of household and personal care products, including hand soaps, surgical scrubs, shower gels, deodorants, hand creams, toothpastes, and mouthwashes (Jones *et al*., 2000). Because of its widespread use, triclosan influent concentrations to WWTPs are high (µg/L) (Heidler and Halden, 2008). However, conventional wastewater treatment is very efficient in removing triclosan (>95%) and it is readily biodegradable under aerobic conditions (Heidler and Halden, 2008, Paxéus, 2004, McAvoy *et al*., 2002, Kanda *et al*., 2003, EPA South Australia, 2007). Mass balance calculations show that 30-50% of triclosan sequestered by sludge and 40-70% is lost by biotransformation or other removal mechanisms (Heidler and Halden, 2008). In a study of 16 different wastewater sources, the highest concentrations of triclosan were seen in effluent from medical facilities (Jackson and Sutton, 2008). While there are concerns that widespread use of triclosan in non-healthcare settings may promote antibiotic resistance, prolonged clinical studies with varying use patterns have not shown changes in pathogenicity, overgrowth of the normal flora by opportunistic species, or development of resistance among the skin flora (Jones *et al*., 2000). Triclosan is not acutely toxic, but is well absorbed through the skin and via oral administration (SCCP, 2009). Triclosan may have cytotoxic effects on human breast cancer cells (Darbre, 2006), and has been shown to be weakly androgenic (Foran *et al*., 2000). Triclosan has been classified as

not likely to be carcinogenic to humans by the US EPA (USEPA, 2008b), and is not classifiable as a carcinogen by the EU classification system (SCCP, 2009)

Methods

All analytes were measured by gas chromatography mass spectrometry (GC-MS), although different sample preparation methods were used for different groups.

Four compounds (1,4-dioxane, acrolein, acrylonitrile, MTBE) were extracted and pre-concentrated by headspace solid-phase microextraction (SPME) before GC-MS analysis. Samples (10 mL) were placed in 20 mL glass vials, treated with a sodium sulphate and a pH 7 buffer to enhance recovery, and a stirbar was added. Analytes were sorbed onto a polydimethylsiloxane (PDMS) SPME fibre exposed to the sample headspace for 30 minutes. Extraction was accelerated by sample agitation through stirring, and heating the vial to 60 °C. The SPME fibre was then placed in the GC inlet, where analytes were thermally desorbed from the fibre and then separated using a 30m 5% phenyl 95% dimethylpolysiloxane capillary column. Detection was performed by MS with electron ionization (EI). Peak identification and calculation of recovery was aided by inclusion of deuterated surrogate standards.

Eight compounds (4-bromophenoxybenzene, 4-chlorophenoxybenzene, azobenzene, butylbenzylphthalate, dioctylphthalate, hexachlorobenzene, hexachlorocyclopentadiene) were measured in the same analytical method as the polycyclic aromatic hydrocarbons (PAHs). Analytes were preconcentrated by stir bar sorptive extraction (SBSE) before GC-MS analysis. A PDMS coated stir bar was placed in each sample (60 mL) and analytes were sorbed onto the PDMS phase during 20 hours of constant stirring. Stir bars were then removed from the sample, dried and introduced directly into the GC using a specially modified thermal desorption inlet. Analytes were thermally desorbed from the stir bar into the GC inlet and separated using a 60m 5% phenyl 95% dimethylpolysiloxane capillary column. Detection was performed by MS with EI. Peak identification and calculation of recovery was aided by inclusion of deuterated surrogate standards.

Triclosan was measured in the same analytical method as the phenol compounds. These analytes were also preconcentrated by SBSE before GC-MS analysis. However, to increase sensitivity, each analyte was derivitized before preconcentration to its corresponding acetate by adding acetic anhydride and sodium carbonate to each sample (60 mL). After derivitisation, a PDMS coated stir bar was placed in each sample and analytes were sorbed onto the PDMS phase during 20 hours of constant stirring. Stir bars were then removed from the sample, dried and introduced directly into the GC using a specially modified thermal desorption inlet. Analytes were thermally desorbed from the stir bar into the GC and separated using a 60 m 5% phenyl 95% dimethylpolysiloxane capillary column. Detection was performed by MS with electron ionization (EI), with peak identification and calculation

of recovery was aided by inclusion of deuterated surrogate standards. The limits of detection (LOD) and estimated uncertainties for each analyte are listed in Table 6.14.1.

Table 6.14.1: Health values, limits of detection (LOD) and estimation of uncertainty for miscellaneous chemicals. Uncertainty calculated at 5 μg/L for MTBE and 1,4-dioxane, and, 0.05 μg/L for all other chemicals

Analyte	Health value (μg/L)	Source	Average LOD (μg/L)	Standard Relative Uncertainty (%)
MTBE	13	CDPH, 2009	1.48	32%
1,4-Dioxane	50	WHO, 2006	0.083	7.2%
Acrolein	3.5	TGA, 2008	0.3	Not determined
Acrylonitrile	32	(Kirman *et al.*, 2005)	0.03	Not determined
4-bromophenoxybenzene	0.7	TTC	0.002	32%
4-chlorophenoxybenzene	0.7	TTC	0.003	25%
azobenzene	0.3	IRIS, 1993	0.004	41%
butylbenzylphthalate	140	IRIS, 1993	0.032	85%
dioctylphthalate	8	WHO, 2006	0.004	62%
hexachlorobenzene	0.1	WHO, 2006	0.002	19%
hexachlorocyclopentadiene	50	OEHHA, 1999	0.007	34%
triclosan	0.35	TTC	0.0065	34%

Quality assurance/ Quality control

To validate each analytical procedure, the following studies were undertaken: instrument linearity, peak identification criteria (t_R and quantifying and qualifying ions), limit of detection, and in-house reproducibility. In addition, additional analyses during the sampling campaign were used to confirm calibration curves, limits of detection, accuracy and precision. Laboratory blanks, trip and field blanks were also analysed and constituted about one third of the samples analysed. All QA/QC and validation is reported in the descriptions of the analytical method in Appendix 4. Acrolein and Acrylonitrile were not validated to the same extent as MTBE and 1,4-dioxane because they were only analysed during one event.

Limits of detection were determined for every analytical run and were calculated using the standard deviation of replicate analyses of a standard solution of appropriate concentration. Standard deviations were then converted to 95% confidence intervals using the student's t-test.

Relative standard uncertainties were calculated using an uncertainty budget that incorporated precision, calibration standard preparation, sample volume, and linear regression of the calibration curve. Sample homogeneity was considered a negligible source of uncertainty

.

Results

A total of 538 measurements of miscellaneous chemicals were made during Events 2 to 6. Forty four percent of measurements were made in Event 3 (Table 6.14.2). Acrolein, acrylonitrile, hexachlorocyclopentadiene and dioctylphthalate were only tested in Event 3.

Table 6.14.2: Measurement of miscellaneous organic chemicals by event and location

Event	Month	No days	Year	Sample		Total	Location									
							GW	SWW	Water Reclamation Plant							
				Grab	Comp				Before MF		After MF		After RO		Storage dam	Total
									K	B	K	B	K	B		
1	November	4	2006													
2	May/June	6	2007	0	30	30	4	8	6	0	6	0	6	0	0	18
3	September	6	2007	85	137	222	0	0	57	50	1	0	57	57	0	222
4	January	6	2008	66	30	96	16	0	16	24	0	0	16	24	0	80
5	April	5	2008	71	24	95	0	8	16	24	0	8	15	24	0	87
6	June	5	2008	65	30	95	0	0	16	24	8	7	16	24	0	95
Total		32		287	255	538	20	16	111	122	15	15	110	129	0	502

Comp, composite; GW, groundwater; SWW, Subiaco secondary wastewater; MF, microfiltration; RO, reverse osmosis; K, Kwinana; B, Beenyup

Secondary wastewater characterisation

Ten of the miscellaneous chemicals were detected in the secondary wastewater (Figure 6.14.1). The solvent 1,4-dioxane had the highest percentage detections and was detected in all samples, with a median concentration of 0.53 µg/L followed by triclosan with 77.3% detections and a median concentration of 19 ng/L. Acrylonitrile was detected in 50% of samples but was only tested in 6 wastewater samples in Event 3. All other chemicals were detected in less than 35% of the samples and median concentrations were heavily influenced by LOD (e.g. MTBE)

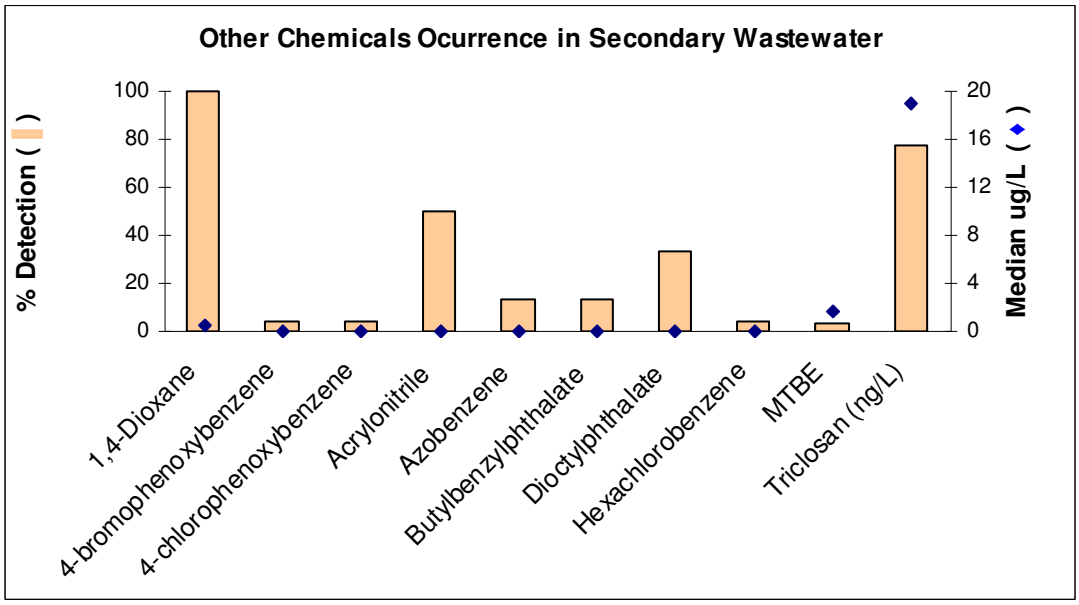

Figure 6.14.1: Miscellaneous organic chemicals with percentage detections in secondary wastewater and corresponding median concentrations (µg/L). Median concentration of triclosan is in ng/L

Given that only one sample was analysed for Subiaco WWTP for most of the miscellaneous analytes, comparison was made of median concentrations at Beenyup WWTP and KWRP influent only. Comparison was only made for 1,4-dioxane and triclosan, as these were the only compounds to have percentage detections greater than 50% and that were measured in more than one event. For all other analytes, comparisons of median concentrations were dominated by non-detects, reported as LOD. Median concentrations of 1,4-dioxane were similar at KWRP and Beenyup (0.54 µg/L and 0.53 µg/L respectively). Median concentrations of triclosan were highest at KWRP (20 ng/L vs 18 ng/L at Beenyup). However, the difference was not significant.

Comparison of seasonal trends was again only made for 1,4-dioxane and triclosan. The median concentration of 1,4-dioxane was highest in spring but the difference was not significant (0.63 µg/L, K-wallis p=0.06). Median concentrations of triclosan were highest in winter and the difference was significant (41 ng/L, K-wallis p=0.0005).

RO Product water characterisation

All miscellaneous chemicals that were detected in wastewater were also reported in at least one post-RO water sample except for triclosan and dioctylphthalate (Figure 6.14.2). Acrylonitrile was detected in 83% of the post-RO samples followed by 1,4-dioxane (29%), azobenzene (24%) and butylbenzylphthalate (14%). All other analytes were only measured in one sample; 4-chlorophenoxybenzene, 4-bromophenoxybenzene and hexachlorobenzene were all detected in the same post-

RO sample taken from KWRP on 28th September 2007. They were also detected on the same day in KWRP secondary wastewater, while the field blank was below LOD. MTBE was also detected in the KWRP post-RO sample on 28th September 2007 at 1.66 µg/L. However in this case MTBE was not detected in the corresponding wastewater sample (LOD=0.7 µg/L). The high concentration in post-RO water may indicate sample contamination, although no field blank ever showed concentrations above the LOD. Apart from MTBE, the highest median concentrations were 0.12 µg/L for 1,4-dioxane and 0.13 µg/L for acrylonitrile. The median concentration of all other chemicals was lower than 0.04 µg/L.

For acrylonitrile, the median concentration in post-RO water (0.13 µg/L) was higher than that in secondary wastewater (0.04 µg/L), and percentage detections in post-RO water (83%) were also higher than in secondary wastewater (50%).

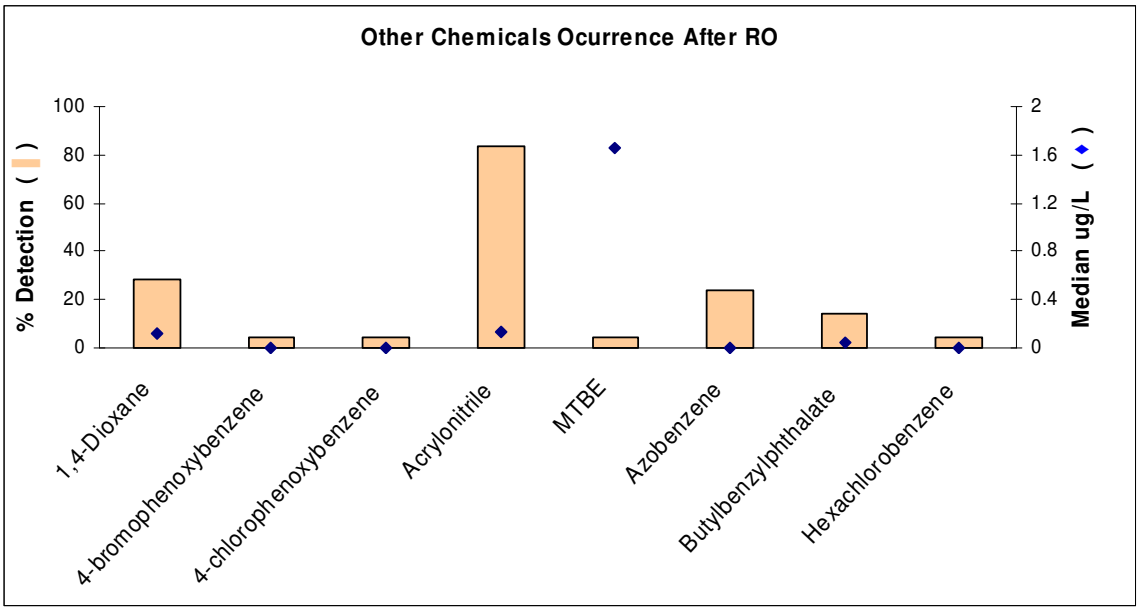

Figure 6.14.2: Miscellaneous organic chemicals with percentage detections in post-RO water and corresponding median concentrations (µg/L).

Groundwater characterisation

None of the miscellaneous chemicals analysed in this section was detected in either of the two groundwater samples tested.

Screening health risk assessment

Risk quotients (RQs) were calculated for secondary wastewater and post-RO water using maximum and median concentrations and the health values calculated in this study. For those analytes that were not detected, the RQ(median) was calculated using the average LOD as the observed concentration. Table 6.14.3 presents the RQs for miscellaneous chemicals in secondary wastewater and post-RO water. None of the RQ(median) or RQ(max) calculated for secondary wastewater or post-RO water were above 1. Furthermore, all RQs in post-RO water were 1 to 3 orders of magnitude below 1 except for MTBE (RQ(median)=0.1 and RQ(max)=0.1). The results indicate a very low health significance at the concentrations observed in wastewater and that the advanced MF/RO treatment further reduced the risks.

Table 6.14.3: Miscellaneous organic chemicals in secondary wastewater and post-RO water and corresponding RQs

					Secondary Wastewater			Post-RO water		
Parameter	**Health value (µg/L)**	**Source**	**Tier**	**LOR**	**n**	**RQ(median)**	**RQ(max)**	**n**	**RQ(median)**	**RQ(max)**
1,4-Dioxane	50	WHO 2006	1	0.083	29	0.01	0.6	21	0.002	0.01
4-bromophenoxybenzene	0.7	TTC	3	0.00175	22	0.003	0.005	21	0.003	0.01
4-chlorophenoxybenzene	0.7	TTC	3	0.00275	22	0.004	0.005	21	0.004	0.01
Acrylonitrile	32	(Kirman *et al*., 2005)	2	0.03	6	0.001	0.004	6	0.004	0.007
MTBE	13	CDPH 2009	1	1.48	29	0.1	0.5	21	0.1	0.1
azobenzene	0.3	IRIS 1993	2	0.0035	22	0.01	0.3	21	0.13	0.7
butylbenzylphthalate	140	IRIS 1993	2	0.032	22	0.0003	0.001	21	0.0003	0.001
dioctylphthalate	8	WHO 2006	1	0.004	6	0.001	0.004	6	0.0005	na
hexachlorobenzene	0.1	WHO 20006	1	0.0015	22	0.01	0.05	21	0.04	0.3
Triclosan*	0.35	TTC	2	0.0065	22	0.02	0.06	22	na	na
acrolein	3.5	TGA 2008	2	0.3	12	0.09	na	12	na	na
hexachlorocyclopentadiene	50	OEHHA 1999	2	0.007	12	0.0001	na	12	na	na

LOR, limit of reporting; n, total number of samples; CDPH, California Dept of Public Health; IRIS, Integrated Risk Information System USEPA; TGA, Therapeutic Goods Administration; TTC, threshold of toxicological concern; OEHHA, California Office of Environmental Health Hazard Assessment

Treatment performance

Treatment efficiency was calculated for all miscellaneous chemicals detected in secondary wastewater (Figure 6.14.3). Treatment efficiency was calculated as a proportion of removal, comparing wastewater and post-RO samples that were paired for plant and date. For those parameters reported below LOD after RO, the efficiency was calculated assuming a concentration equal to half the LOD as a worst-case scenario. The number of paired samples for each chemical was relatively low and ranged between 1 and 3 for all chemicals except for 1,4-dioxane, which had 22 paired samples, and triclosan, which had 14 paired samples.

Median removal efficiency was greater than 71% for all compounds except for acrylonitrile. For acrylonitrile, concentrations in post-RO water were higher than in the secondary wastewater for all paired samples except one. No post-MF samples were analysed for acrylonitrile and therefore it is not possible to determine where the increase in concentration of acrylonitrile occurred.

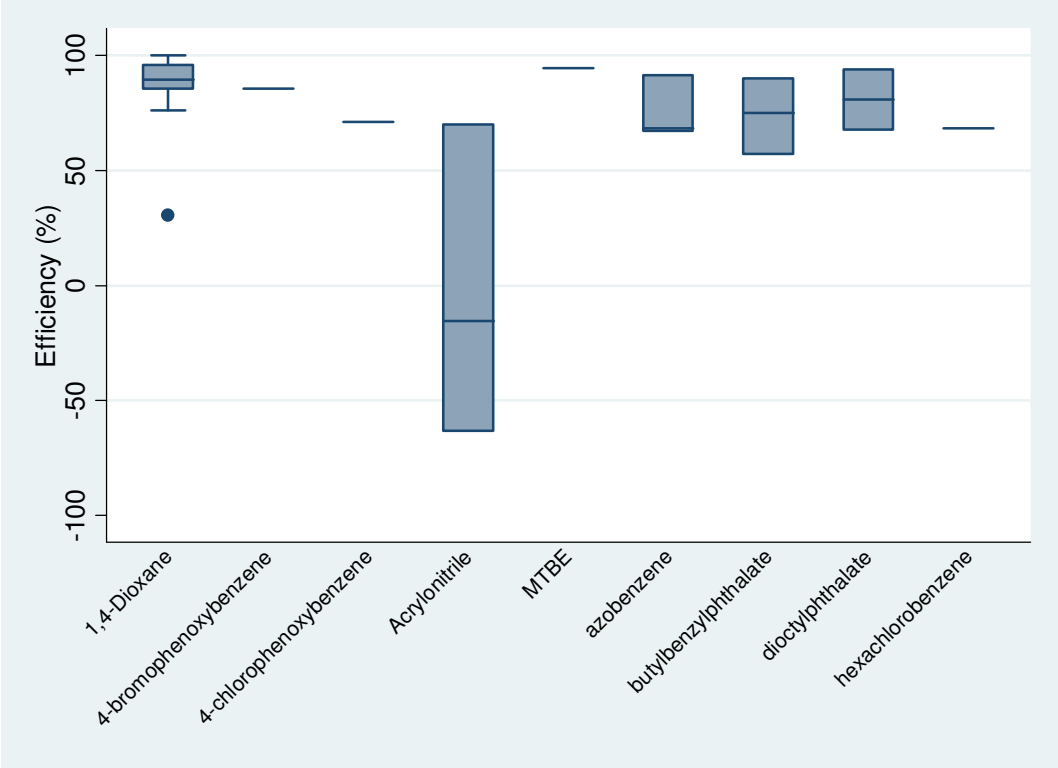

Figure 6.14.3: MF/RO removal efficiency of detected miscellaneous organic chemicals in secondary wastewater.

Discussion

Ten of the miscellaneous chemicals were detected in the secondary wastewater, with 1,4-dioxane (100% detections) and triclosan (77.3%) the most commonly detected chemicals. The low number of percentage detections for many compounds confounded comparison by WWTP and season and there were no statistically significant differences seen when comparing Beenyup WWTP and KWRP (data not shown). The frequency of detection and concentration of 1,4-dioxane is comparable to that seen in other studies (Tanabe *et al*., 2006, Abe, 1999). The main sources of 1,4-dioxane are expected to be polyethoxylated surfactants and detergents, and the lack of seasonal variation is likely due to a consistent use of these products throughout the year and the overall poor removal rates seen in conventional secondary wastewater treatment (Zenker *et al*., 2003).

Triclosan concentrations in secondary wastewater in this study were lower compared with other Australian studies (median of 19 ng/L compared to 108 ng/L Australia-wide) (Ying and Kookana, 2007) (Kookana *et al*., 2009). Again triclosan is expected to have a consistent usage throughout the year. In this case, variation in secondary wastewater may indicate a variability of removal. Summer had the lowest median triclosan concentration (8 ng/L) while the highest median concentration was in winter (41 ng/L). This may suggest that biodegradation rates are higher in the warmer months or that usage in winter is higher.

Eight of the ten miscellaneous chemicals detected in secondary wastewater were also detected in post-RO water. All were detected with a lower frequency except for acrylonitrile, which was detected in five out of six post-RO samples and at higher concentration than measured in secondary wastewater.

Acrylonitrile can be used in production of both RO and MF membranes (Ghosh and Hoek, 2009, Nasef and Hegazy, 2004), and therefore it is possible that the concentrations measured in post-RO water are due to acrylonitrile leaching from the membranes. No measurements of acrylonitrile were made in post-MF water during the study and therefore it is not possible to determine whether the elevated concentrations seen in post-RO water compared to secondary wastewater results were from the RO or MF membrane. At BPP, the MF membrane is made from polyvinylidene fluoride (PVDF), while the RO membrane is a composite polyamide, and the acrylonitrile is more likely to result from the RO membrane (Nasef and Hegazy, 2004). At KWRP, the MF and RO membranes are both made from composite polyamide and therefore either membrane could cause elevated acrylonitrile concentrations. Further analysis of acrylonitrile in post-RO and post-MF water would be required to confirm the source of the elevated concentration. However, even the maximum concentration measured in post-RO water is still two orders of magnitude lower than the health guideline value for acrylonitrile and therefore the health risk is considered low.

Overall the potential health risks associated with these miscellaneous chemicals are considered to be very low. All RQs in post-RO water were one to three orders of magnitude below 1 except for MTBE (RQ(median)=0.1 and RQ(max)=0.1). It should be noted that the guideline value used here is related to the health value (California DPH, 2009). If we were to use a taste and odour threshold both maximum and median post-RO concentrations were below the aesthetic value that is lower than the health value (WHO, 2006).

1,4-dioxane was detected in all secondary wastewater samples and 29% of post-RO samples. 1,4-dioxane is a small molecule with a molecular weight of 88 Da, which is lower than the nominal RO membrane molecular weight cut-off (MWCO) of approximately 100-150 Da (Drewes *et al.*, 2003, Xu *et al.*, 2006). Compounds that are non-ionic (neutral) and small in size like 1,4-dioxane can exhibit a partial removal (Drewes *et al.*, 2008). In our study, the removal of 1,4-Dioxane was variable ranging from intermediate (30%) to good (99%) and therefore it may be considered a good indicator compound to monitor RO treatment.

Given the limited number of measurements and the increase in acrylonitrile concentration between secondary wastewater and post-RO water, it is recommended that more analyses of acrylonitrile throughout the MF/RO process be conducted to determine the source of the acrylonitrile increase. Similarly, assessment of acrylamide is recommended because membranes could be a source of this regulated chemical (Nasef and Hegazy, 2004).

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6.15 Summary Chemical Constituents in RO-treated water and Treatment Efficiency

Chemical Constituents in post-RO Water

Of the 396 compounds investigated in the study, 25 (6%) were calculated to have percentage detections in post-RO water greater than 25% (Table 6.15.1). Eight of these compounds are disinfection by-products (seven halogenated DBPs, one *N*-nitrosamine, and one inorganic disinfection by-product), while six are metals or metalloids, four are VOCs, and the remaining compounds are from the classes of complexing agents, phenols, or miscellaneous chemicals. Only the *N*-nitrosamine NDMA poses a potential concern from a health perspective (as defined by a RQmax greater than 1).

The metals and complexing agents frequently detected in post-RO water were always present in secondary wastewater at relatively high concentrations. While they were also consistently highly removed by RO (>90%) the high wastewater concentrations caused the relatively frequent detections at very low concentrations post-RO. As has been discussed in chapter 6.1 and 6.12, the health risk associated with these groups of chemicals after RO is low. The exception was boron, which had variable removal efficiency. This makes boron a good chemical indicator.

For disinfection by-products, percentage detections in post-RO water were often higher than in secondary wastewater, although median concentrations in secondary wastewater were always lower, except for dichloracetonitrile and chlorate. Other than chlorate, the presence of these DBPs in post-RO water is probably influenced by chloramination within the MF/RO process. This highlights that for some compounds mitigation can be achieved through treatment process optimization, as well as through removal by RO. In particular chloramine contact time appears to be strongly related to DBP formation, and therefore should be monitored.

As discussed in section 6.13 (Anions), chlorate is potentially present in the hypochlorite solution dosed for chloramination which would explain its increased frequency of detections post-MF and post-RO.

Acrylonitrile is one of the most frequently detected chemicals post-RO, attributed to leaching from either the MF or RO membranes. Because of the low number of samples analysed, further analysis is required to identify the source. However, it also suggests that further attention should be paid to other compounds that might leach from the membranes, such as acrylamide, as well as anti-scalants and any other compounds used within the MF/RO process.

Table 6.15.1: Chemicals detected in post-RO water in greater than 25% of samples, corresponding detections in secondary wastewater, and treatment efficiency.

	Post-RO Water			Secondary Wastewater			Overall Efficiency Using data below LOR[a]		Recalculated Treatment Efficiency[b]		Recalculated RO Efficiency[c]	
Parameter	n	Percentage detections	Median (µg/L)	n	Percentage detections	Median (µg/L)	Total Pairs	Median	Total Pairs	Median	Total Pairs	Median
Bromochloromethane	27	100%	0.11	33	94%	0.22	26	63%	27	61%	9	71%
Dibromomethane	27	96%	0.13	33	94%	0.26	26	50%	26	43%	9	66%
NDMA	26	92%	0.0045	25	96%	0.016	18	80%	19	75%	9	58%
1,4-dichlorobenzene	29	90%	0.2	37	95%	0.81	24	84%	24	86%	8	90%
Boron	28	89%	75	31	100%	160	25	62%	23	46%	8	62%
Acrylonitrile	6	83%	0.13	6	50%	0.04	3	-15%	2	-41%		
Bromodichloromethane	27	70%	0.036	33	82%	0.06	20	33%	17	7%	3	46%
Lithium	28	68%	0.2	31	100%	7.6	25	97%	19	97%	1	97%
Chloromethane	29	62%	0.089	37	59%	0.12	16	17%	16	26%	4	45%
Silicon	21	62%	0.12	22	100%	8.8	19	99%	17	99%	2	98%
Dichloroacetonitrile	24	58%	0.095	28	11%	0.02	0		0		5	66%
Chloroform	27	56%	0.137	33	85%	0.36	21	82%	15	57%	3	68%
Strontium	28	50%	0.4	31	100%	170	25	99.8%	16	99.8%	1	99.8%
EDTA	27	48%	0.48	27	100%	145	20	99.5%	6	99.5%	7	99.8%
Carbon disulfide	17	47%	0.016	20	80%	0.0185	11	57%	6	-2%	1	70%
Chlorate	24	46%	12.7	30	37%	12.85	7	76%	4	43%	3	98%
2,4-dichlorophenol	20	45%	0.01	22	55%	0.015	11	89%	7	81%	3	55%
Copper	28	43%	0.2	31	100%	7.4	25	98%	10	97%	6	97%
NTA	27	33%	0.13	27	100%	2	20	93%	9	72%	2	92%
bisphenol A	19	32%	0.012	22	23%	0.012	4	40%	2	5%	1	40%
Benzene	29	31%	0.076	37	30%	0.076	7	0%	6	-15%	0	
Chlorodibromomethane	27	30%	0.094	33	76%	0.19	18	65%	7	-6%	1	44%
Zinc	28	29%	5	31	100%	55	25	95%	10	90%	2	92%
1,4-Dioxane	21	29%	0.12	22	100%	0.515	19	89%	8	87%	2	50%
Bromoform	27	26%	0.13	33	76%	0.15	18	75%	7	59%	1	59%

n: total number of samples analysed; a) treatment efficiency calculated using secondary wastewater and post-RO samples paired for plant and date, a concentration equal to half the LOR is assumed for those parameters reported below LOR after RO; b) treatment efficiency calculated using secondary wastewater and post-RO samples paired for plant and date, paired samples in which the analyte was reported as below LOR in post-RO samples were discarded from the calculation; c) RO efficiency calculated using paired post-MF and post-RO samples, paired samples in which the analyte was reported as below LOR in post-RO samples were discarded from the calculation

Treatment Efficiency

In this study treatment efficiency has been calculated as a proportion of removal, comparing wastewater and post-RO samples that were paired for plant and date. For those parameters reported below LOR after RO, the efficiency was calculated assuming a concentration equal to half the LOR as a worst-case scenario. However, as has been discussed throughout the report, assuming a concentration of half LOR in post-RO water may result in an artificially low assessment of treatment efficiency, particularly for samples where concentrations in secondary wastewater are close to detection limits. Table 6.15.1 includes a recalculation of treatment efficiency for these frequently detected analytes post-RO, in which all paired samples with non-detects post-RO have been discarded. The same process has also been followed to calculate RO efficiency using post-MF and post-RO samples. There is a general trend that the median treatment efficiency calculated using paired detected samples only is lower. However, considering the standard deviation, there are no significant differences between the treatment efficiency calculated by either method.

Results also confirm that, for chemicals that form or are added during treatment, treatment efficiency calculations across the whole treatment train does not reflect RO removal efficiency. Further monitoring of RO treatment performance requires monitoring immediately prior to RO rather than using secondary wastewater, particularly for all disinfection byproducts and some other chemicals.

7 Microbiological Parameters

Reverse Osmosis Plant Challenge Experiment and Microbial Analysis of Wastewater at the Beenyup Plant

Introduction

The most immediate health risks that can be present in any water are microbial pathogens. There are a range of pathogen types including viruses, bacteria, protozoa and helminths. Helminths, however, are of minimal problem in most regions of Australia and thus the majority of attention is on the other three pathogen types. Also, most of the pathogens in water are enteric in origin, that is, they are excreted in the faeces of infected individuals and then infect new hosts who ingest faecally contaminated water or food. There are, however, other non-enteric pathogens that can be present in water including species of *Mycobacterium*, *Legionella* and opportunistic pathogens such as *Pseudomonas* and *Aeromonas*.

The health risk from microbial pathogens is based on the low infectious dose of many of these pathogens. Unlike chemical contaminants which usually require long term exposures to cause illness, susceptible members of the population may only need to ingest a few of these pathogens to become immediately ill. The type and severity of the illness varies depending on the type of pathogen and the susceptibility of the infected person but the most common form of illness is gastroenteris. A comprehensive list of water-borne pathogens and the illnesses they cause can be seen in the Australian Guidelines for Water Recycling: Managing Health and Environmental Risks-Phase 1 (NRMMC EPHC & AHMC, 2006).

The presence and number of pathogens in water is usually controlled via treatment and disinfection. During wastewater treatment, pathogen numbers generally decrease, although the amount of the decrease depends on the extent and type of treatment, and on the types of pathogens present. Some pathogens are more resistant to different treatment methods than others (e.g. *Cryptosporidium* is very resistant to chlorination but sensitive to UV). In comparison, adenovirus is not very resistant to chlorination but is the most UV resistant of the enteric pathogens) (Thompson *et al*., 2003). The effectiveness of the treatment process can also be affected by external issues such as climatic conditions and the sewage load passing through the plant (influencing effective residence times). The number of pathogens per volume of sewage can also have an impact on the effectiveness of wastewater treatment to reduce the number of pathogens in treated wastewater to levels that represent minimal risk to the community.

Pathogens can still be present in treated wastewater and have even been detected in low numbers in tertiary treated wastewater. For example, Costán-Longares *et al* (2008) were able to detect up to 2 infectious virus particles in 100 L of Spanish tertiary treated wastewater produced by a pilot scale wastewater treatment plant, Kistemann *et al* (2008) were able to detect at least 2 *Giardia* cysts per 100 L of tertiary treated wastewater in Italy, and Gennaccaro *et al.* (2003) detected an average of seven infectious *Cryptosporidium parvum* oocysts per 100 L of tertiary treated wastewater in the USA (it should be noted that none of these tertiary treated wastewaters cited above were treated using RO systems). The use of dual membrane filtration is considered the gold standard for removing contaminants, including microbial pathogens, from water; however, microbial pathogens can even be present in the post-RO water produced via this technology if there is a failure in the integrity of the membranes or seals. This section reports on research undertaken to determine the occurrence of microbial pathogens in secondary treated wastewater from two of Perth's larger WWTPs, as well as the outcomes of a virus challenge test on the BPP.

Methods & Quality Assurance Procedures: see Appendix 4

Results & Discussion

Viral Challenge Test

A microbial challenge test was undertaken at the BPP to assess the capacity of the membranes to exclude viruses from the post-RO water. The aim of the experiment was to demonstrate that the reverse osmosis unit could be relied upon to prevent enteric viruses from being present in the final treated water.

The challenge test was undertaken using the coliphage MS2 as a surrogate enteric virus. The male-specific coliphage (MS2) is a common member of the coliphage (bacteriophage which specifically infect the coliform group of bacteria). MS2 was used in the place of pathogenic enteric viruses as it has similar capsid morphology to the enteric viruses and is the same size or smaller than human enteric viruses. It is also easy to culture up to large numbers; is easy to culture for detection in water; and is relatively easy to detect in large volumes of water using an enrichment method. The methodology for sampling and analysis for the challenge test was developed using the methods recommended in the US EPA Membrane Filtration Guidelines (U.S.EPA, 2005). The full experimental details are given in Appendix 4.

In total, two challenge tests were undertaken. The initial challenge test was undertaken by inoculating the pre-reverse osmosis storage tank with bacteriophage to a final number of approximately 10^7 plaque forming units (pfu)/mL (7 log units/mL). Samples were then collected from the outlet of the RO unit at 5 minutes after mixing

of the storage tank, then every 5 minutes for the first 20 minutes and finally a sample collected every 10 minutes for the remaining time up to 2 hours (a total of 12 samples). In this challenge test only the enrichment method was used for detecting the potential presence of MS2 bacteriophage in the post-RO water as it was anticipated that there was minimal opportunity for the bacteriophage to pass across the RO membrane.

The results indicated that MS2 was detected in the post-RO water on 7 of the 12 sample events (10, 15 and 20 minutes, 50 and 60 minutes, and 80 and 90 minutes) (Table 7.1). This most likely indicates that at the time of the challenge test the RO membrane was not capable of excluding all viruses from the RO permeate at such a heavy virus load in the feed water to the RO membranes. This is in agreement with the US EPA membrane filtration guidelines (2005) which notes that RO membranes are not specifically designed to be used as a sterilising membrane (ie, relied upon to exclude viruses from the final permeate water). The reasons given in the guidelines are that membrane joints and seals may not have perfect integrity to prevent viruses bypassing the membranes into the permeate. The US EPA Membrane Filtration Guidelines recommends for any RO system which is to be relied upon as a barrier for small microorganisms such as viruses, that a challenge test be undertaken to determine the removal efficiency of that particular membrane system (U.S.EPA, 2005). A number of previous studies have demonstrated that log removals of MS2 by RO membranes can vary from as little as 1.4 log to more than 7 log (reviewed in Kumar *et al.*, 2007). Adham *et al.* (1998) advised that RO membranes can be relied upon for pathogen removal if quality control measures are taken to ensure that consistent removal efficiencies were achieved.

Table 7.1: Results of Challenge Test 1.

Sample	Presence/Absence
Storage Tank (following inoculation)	+
5 minutes [1]	-
10 minutes[1]	+
15 minutes[1]	+
20 minutes[1]	+
30 minutes[1]	-
40 minutes[1]	-
50 minutes[1]	+
60 minutes[1]	+
70 minutes[1]	-
80 minutes[1]	+
90 minutes[1]	+
Transport Blank[2]	-
Negative Control[3]	-
Positive Control[4]	+

[1] RO permeate

[2] Transport blank consisted of 500 mL of sterile distilled water that was taken out to the field during sampling, opened during the collection of the 20 minute sampling event and then closed and taken to the laboratory for testing for the presence of MS2 using the enrichment method.

[3] Testing of 500 mL sterilised tap water which had been treated in the same manner as the RO permeate.

[4] same as for the negative control described above except that the 500 mL of tap water was also inoculated with MS2 as well as concentrated medium, supplements and *E. coli* host.

Another potential reason for the failure of the tested RO unit to exclude the coliphage particles was that just prior to the initial challenge test a membrane clean had been undertaken on the RO membranes which had caused a problem with the ability of the RO unit to reject salt, possibly due to damage to some of the seals. Kitis *et al.* (2003) have demonstrated that small imperfections and damage to the membranes and seals that cause only minor changes in conductivity can have much more significant influence on the ability of the membranes to reject viruses (for example, if there is 1% leakage, only a theoretical 99% removal of virus would be achieved, whereas this amount of leakage would not result in a significant change in electrical conductivity). This suggests that the reason for failure to reject the high MS2 numbers may have been due to some lingering problem with the seals for the RO unit despite the operators getting the permeate conductivity down to acceptable operational levels more than 24 hours prior to the commencement of the challenge test (Figure 7.1).

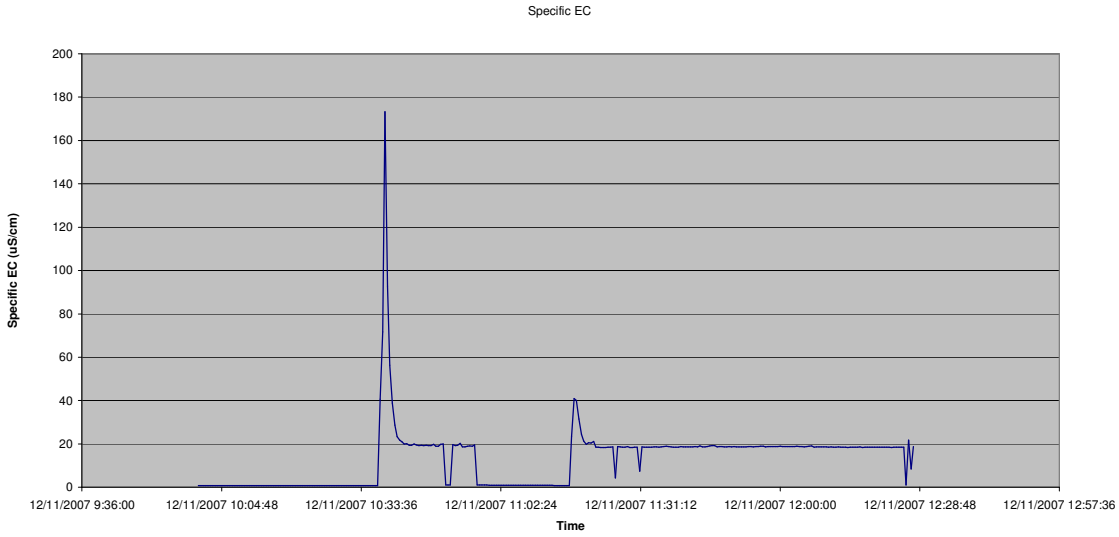

Figure 7.1: Specific Electrical Conductivity (µS/cm) in the Reverse Osmosis permeate during the first coliphage challenge test. *(The spike in electrical conductivity at 10:33 was due to a lack of water across the conductivity sensor because of the RO unit unexpectedly shutting down)*

As a response to the results of the first challenge test a second challenge test was undertaken using quantitative assessment rather than presence absence, and a lower number of bacteriophage Prior to this second challenge test samples collected from across the MF/RO treatment train were tested using the bacteriophage enrichment method to ensure that all MS2 particles from the first challenge test had been removed or inactivated. The only place where the MS2 coliphage was detected in this pre-challenge survey was the treated effluent prior to the MF (Table 7.2). This indicated that the pilot MF/RO system was operating well and that all residual MS2 coliphage particles from the first challenge test had been removed from the system.

The second challenge test was run in the same manner as the first challenge test except that:

1) the MS2 numbers added into the post MF storage tank was reduced from the amount used in the first challenge test (~7 log/mL) to achieve a final number of approximately 4 log/mL; and

2) a quantitative assessment of the number of MS2 in each sample was undertaken along with the qualitative enrichment test.

Table 7.2: Presence of Male Specific Specific (F^+) coliphage in the Beenyup pilot MF/RO train prior to the commencement of Challenge Test 2

Sample	Presence/Absence[1]
Pre-Microfiltration[2]	+
Pre-RO, Post-MF Storage Tank	-
Reverse Osmosis permeate	-
Transport Blank[3]	-
Negative Control[3]	-
Positive Control[3]	+

[1] detection undertaken using the enrichment method

[2] sample collected pre-microfiltration is the treated wastewater entering the pilot plant from the Beenyup wastewater treatment plant.

[3] Transport blank, negative control and positive control as described in Table 7.1.

The results of the second challenge test are shown as a qualitative presence or absence result in Table 7.3 and the quantitative numbers and resultant minimum log reduction in Table 7.4. The results from both the enrichment and quantitative detection of MS2 in the various water samples indicate that MS2 could always be detected in the water in the storage tank (prior to the reverse osmosis unit) but no MS2 was able to be detected in the RO permeate at any time during the challenge test. The results of the numbers detected pre- and post-reverse osmosis showed that the RO unit is capable of rejecting at least 4 log of the virus.

Table 7.3: Results of enrichment testing for Challenge Test 2

Sample	Presence/Absence
Post-RO 5 minutes [1]	-
Post-RO 10 minutes	-
Post-RO 15 minutes	-
Post-RO 20 minutes	-
Post-RO 30 minutes	-
Post-RO 40 minutes	-
Post-RO 50 minutes	-
Post-RO 60 minutes	-
Post-RO 70 minutes	-
Post-RO 80 minutes	-
Post-RO 90 minutes	-
Post-RO 100 minutes	-
Post-RO 110 minutes	-
Post-RO 120 minutes	-
Pre-Microfiltration[2]	+
Reverse Osmosis Feed Storage Tank[2]	+
Field Blank[3]	-
Negative Control[3]	-
Positive Control[3]	+

[1] The 5 minute sample was the first sample undertaken after seeding and mixing the Reverse Osmosis Feed Tank.

[2] Samples as described in Table 7.2

[3] Samples as described in Table 7.1

Table 7.4: Quantitative results of coliphage numbers in Challenge Test 2 and resulting log reductions.

Sample	Storage Tank (pfu/ml)	RO Permeate (pfu/ml)	Log Reduction
5 minutes [1,2]	15133	0	4.17
10 minutes[1]	1767	0	3.24
15 minutes[1]	967	0	2.99
20 minutes[1]	267	0	2.43
30 minutes[1]	ND	0	NA
40 minutes[1]	ND	0	NA
50 minutes[1]	ND	0	NA
60 minutes[1]	13600	0	4.13
70 minutes[1]	8000	0	3.90
80 minutes[1]	400	0	2.60
90 minutes[1]	2400	0	3.38
100 minutes[1]	2000	0	3.30
Average			3.35 (±0.63)
Pre-Microfiltration[3]	0		NA

[1] RO permeate
[2] The 5 minute sample was the first sample undertaken after seeding and mixing the Reverse Osmosis Storage Tank.
[3] Sample as described in Table 7.2

The detection of male-specific coliphage (of which MS2 is a common member) in the secondary treated wastewater prior to microfiltration using the enrichment method (Table 7.3) but the lack of detection using the quantitative method (Table 7.4) indicates that the coliphage numbers in the treated wastewater (pre-microfiltration) are less than the quantitative detection limit of 10 coliphage/mL (equivalent to 4 log of coliphage/L) but are at a number of at least 1 coliphage 500/mL (*minimum detection limit of the enrichment method). This is in agreement with reports in the literature of bacteriophage and enteric viruses numbers between 2 and 3 detectable bacteriophage or virus units per litre of treated wastewater (Gantzer *et al.*, 1998, Katayama *et al.*, 2008, Lodder & Husman, 2005). Thus, an ability of the RO membranes to reject at least 4 log of virus should be adequate to rely upon this unit as an appropriate treatment barrier for removal of at least this level of virus numbers

from treated, microfiltered wastewater. In addition, the RO units are part of a larger train including microfiltration and, potentially, natural treatment systems such as aquifers or reservoirs. Thus, the combined virus removal through the treatment across all barriers means that significant levels of virus removal should be achieved (>8 log would not be expected to be an unreasonable target) (NRMMC EPHC & AHMC, 2006). This could be further assessed by challenge testing of the MF as well as the RO membranes individually.

Detection of Specific Enteric Microorganisms in Perth Treated Wastewater

The treated wastewater from two of Perth's Wastewater Treatment Plants (WWTPs) (Beenyup and Subiaco) was tested to determine the level of detection of the enteric bacteria and viruses in the treated wastewater. The collection points at the treatment plants were in the line leading into the pilot advanced water treatment plant at the Beenyup WWTP, and from the line supplying wastewater to the Water Foundation Managed Aquifer Recharge (MAR) Floreat infiltration galleries at the Subiaco WWTP. The methods used for the detection of the different microorganisms are given in Appendix 4 and the results of the analysis are presented in Table 7.5.

Table 7.5: Detection of viruses and microbial indicators in treated wastewater from two different wastewater treatment plants. For the F+ coliphage, Adenovirus and Enterovirus qualitative data identifies the number of positive and negative results.

WWTP	Thermotolerant coliforms[a] (cfu/100mL)		Enterococci[a] (cfu/100mL)			F^+ coliphage[b]		Adenovirus[b]		Enterovirus[b]	
	Average	min	Average	Max	Min	# +'ve	# -'ve	# +'ve	# -'ve	# +'ve	# -'ve
Subiaco[c]	$1.3x10^3$ (n=45)[d]	4	$1.6x10^3$ (n=48)[d]	$1.6x10^4$	0.2	38	2	13	6	1	5
Beenyup	$>1x10^{3e}$ (n=5)[d]	NA	$>1x10^{3e}$ (n=5)[d]	NA	NA	5	0	5	0	1	4

[a] Quantitative data based on methodology for detecting number of colony forming units (cfu) per 100 mL treated wastewater. Methods used are listed in Appendix 2.

[b] Qualitative presence (presence or absence results) based on molecular methods for adenovirus and enterovirus group and enrichment method for F^+ coliphage.

[c] The results of the analysis of wastewater from the Subiaco WWTP was undertaken in conjunction with the Water Foundation's Managed Aquifer Recharge project.

[d] n = number of samples used to determine average.

[e] An accurate quantitative average could not be obtained due to excessive growth of thermotolerant coliforms and total coliforms on plates of media. An examination of the number of thermotolerant coliform colonies on each plate determined that there was in excess of 1000 cfu on each plate.

The results indicate that thermotolerant coliforms and enterococci were always detectable in the treated wastewater. As these microorganisms are common indicators of faecal contamination this is not unexpected. The numbers detected were at levels that could be considered common for treated wastewater (NRMMC EPHC & AHMC, 2006, Toze *et al*., 2004, unpublished CSIRO, 2009)

The detection of coliphage and enteric viruses, while only qualitative indicated that viruses could be regularly detected from secondary treated Perth wastewater. Male specific (F^+) coliphages were detected in 95% of the Subiaco samples and 100% of the Beenyup (although only 5 samples were tested) (Table 7.5). This indicates that testing RO permeate for the presence of coliphage could be a legitimate routine verification test for confirming the ability of operating MF/RO units to exclude enteric microorganisms, in particular enteric viruses. While not detected as frequently as the coliphage, adenovirus was detected in 68% of the Subiaco and all of the Beenyup treated wastewater samples. Adenovirus is commonly detected in wastewater and is relatively easy to detect in comparison to other enteric viruses (Toze and Sidhu 2009). The thermo- and UV-stability of adenovirus, plus its relatively high numbers in wastewater (compared to other enteric viruses) could make it another useful virus for validation and verification of treatment processes. The enterovirus group was only detected in 25% or less of the samples tested and is, thus, less suitable for use for validating wastewater treatment processes.

Conclusions

The microbiology research has shown that, without adequate integrity testing and monitoring, RO units are not able to be relied upon for complete removal on their own and that there is potential for viruses to pass to the permeate side of the membrane. However, at numbers commonly detected in treated wastewater ($<10^3$/Litre), this study has shown that the RO membranes should exclude all virus particles present in water (based on the detection level used in this study, i.e. a minimum of 1 viral unit in 500mL of RO permeate, and larger volumes of water may be needed to be tested if higher virus rejection efficiencies need to be demonstrated. Further testing of RO units using improved integrity testing and operating procedures during the GWRT is recommended to better understand virus removal which may be achieved reliably under these conditions.

The ease and frequency of detection of the male specific (F^+) coliphage in the secondary treated wastewater from both Subiaco and Beenyup wastewater treatment plants suggests that they could be a useful routine tool for ongoing monitoring and verification of the operation of RO treatment systems.

8 Toxicity Assessment

The quality of recycled water is mainly assessed based upon the measurement of water pollutant levels, and upon comparison of these levels with health threshold values. Individual chemical results indicate that traditional contaminants tested, such as metals and THMs, after the advanced treatment are below health values from the *ADWG (NHMRC and NRMMC, 2004) and the AGWR (Phase 2): Augmentation of drinking water supplies (NRMMC EPHC & NHMRC, 2008)*. However, there is a growing awareness that chemical-by-chemical approach to regulation and testing is insufficient to assess the potential human health risks of chemical mixtures.

Bioassays are useful tools to evaluate water quality and the efficiency of water treatment. Endocrine disruption (estrogenicity and androgenicity), phytotoxicity, cytotoxicity, tumour induction, mutagenicity and genotoxicity effects are commonly used endpoints used to evaluate the potential toxicity of secondary wastewater and post-RO water. For example, significant toxicity reduction has been demonstrated through secondary wastewater treatment using a suite of bioassays (Escher *et al.*, 2008). The authors reported treatment efficiency from raw wastewater to secondary wastewater typically over 90% in all bioassays tested. Moreover, removal efficiency of endocrine disrupting compounds for water recycling using the E-screen assay, reported an influent value of 1.2 ng-EEQ/L and a post-RO water value of 0.05 ng-EEQ/L (which correspond a 96% oestrogenic activity reduction) (Lee *et al.*, 2008). Similarly, quality assessment of drinking water (from source to tap) using in the ER CALUX assay, indicate that low levels of estrogenic activity could be measured after various steps of the purification process, but no health risks were identified (Oost & Heringa, 2007). Moreover, the estrogenic equivalents resulting from the E-screen assay and those calculated from the results of chemical analyses using oestradiol equivalency factors, follow a similar trend (Bicchi *et al.*, 2008, Tan *et al.*, 2007, Nelson *et al.*, 2007, Lee *et al.*, 2008).

In this section results form a cytotoxicity and genotoxicity testing of secondary wastewater and post-RO water using a human lymphoblastoid cell-line is presented. The aim was to determine any cytotoxicity and/or genotoxicity activity in secondary wastewater and post-RO water samples and to determine any changes during the treatment train. Cytotoxicity and genotoxicity bioassays were selected given the detection of some suspected or known carcinogenic substances mainly DPBs and PAHs in secondary wastewater.

Cytokinesis-block micronucleus (CBMN) assay

Cytotoxicity and genotoxicity screening were analysed by a cytokinesis-block micronucleus (CBMN) assay using a human lymphoblastoid cell-line. The test was conducted in June 2008 to evaluate potential human toxic effects of recycled water.

The study was founded by the University of Western Australia and conducted by the Australian Water Quality Centre (AWQC) in Adelaide. The CBMN assay is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity. DNA damage events are scored specifically in once-divided binucleated (BN) cells. Cytostatic effects are measured via the proportion of mono-, bi- and multinucleated cells and cytotoxicity via necrotic and/or apoptotic cell ratios (Fenech, 2007).

Methods

Grab and composite samples were collected using 200 ml glass Winchester bottles. Samples were preserved with ascorbic acid at 20 mg/L, stored at 4 $^{\circ}$C and sent to the laboratory for analysis. For each stage of treatment samples were split into 150 ml duplicates in the laboratory. Ten millilitres of each sample were filtered through an Acrodise 0.2 µm membrane. The filtrate was used for all cytotoxicity and genotoxicity tests.

The human lymphoblastoid cell line grows as a suspension culture (cell line WIL2-NS). Genotoxicity assessment was conducted using an adaptation of the CBMN assay optimised for rapid detection of micronuclei using a flow cytometer and compared with the microscopic method. The general protocol is to make up the exposure medium by adding a 10x concentrated buffered salt solution (Hanks Buffered Salts Solution) to the test sample, and then exposing the cells for 3 hours. Water samples were exposed to a minimally buffered salts medium to avoid potential reactions of active compounds in the sample with the various organic compounds in full growth medium. After this period, the test solution is removed and the cells are then cultured for 21 hrs in growth medium. At the end of this period, genotoxicity is measured as described and cytotoxicity is measured by one of two methods: Total DNA determination or metabolic (dehydrogenase) conversion of a tetrazolium salt (MTS) to a coloured product (as a measure of cell activity). The colorimetric cytotoxicity method is performed on the total DNA determination assay method.

The defined assessment criterion for genotoxicity was the lowest dilution of a sample that does not show any significant induction of micronuclei. Significant results have at least a 3-fold increase in micronuclei. Cytotoxicity was judged by determining the cell-survival index, i.e. the percentage growth rate of the cells compared with the corresponding negative controls. As supplementary qualitative criteria, the mitotic index and the proliferation index were assessed. Bromoacetic acid was used as a positive control.

Cytotoxicity screening using the total DNA quantification assay method

- 100 µl 10x HBSS + 900 µl sample prepared in an eppendorf tube.
- In a 96-well plate, 100 µl HBSS is added into each of 4 wells and a 2-fold serial dilution made in the first 3 wells by adding 100 µl of the diluted sample to the first well, mixing, and then transferring 100 µl to the next well, etc.
- Then cell suspension (100 µl of $6x10^5$ cells/ml in 1x HBSS) is added to each well. Thus a dilution series of 9/40, 9/80, 9/160, and no treatment control (NTC) were created.
- Positive control was bromoacetic acid (BA). 100 µl of a 100 µM stock solution was added to 100 µl of HBSS in the first of 3 wells, with serial dilution into the next 2 wells, and then 100 µl of cells were added. This gave final concentrations of 25, 12.5, and 6.25 µM BA.
- Mixed and incubated at 37 ^oC for 3 hrs.
- The cells are collected by centrifugation at 120 g for 5 min, the supernatant is discarded.
- Growth medium (200 µl) is added and the cells grown for 21 hrs.
- Cells were collected by centrifugation (120 g, 5 min), and the supernatant discarded.
- Lysis solution containing propidium iodide (PI) and RNAseA is added and the plates were shaken at medium speed for 1 hr.
- PI-associated fluorescence was measured using a microplate reader.

Cytotoxicity (MTS) and Genotoxicity were determined using a single minimal dilution of the samples (9/20). Bromoacetic acid was used as positive control at 12.5 µM.

Exposure and recovery

- In 24-well plate, added 100 µl 10x HBSS + 900 µl sample + 1000 µl cells ($6x10^5$ cells/ml).
- Cells were exposed for 3 hrs at 37 ºC.
- A 1.5 ml aliquot was transferred into an eppendorf tube and centrifuged at 100 g for 5 min. The supernatant was removed.

- The cell pellet was resuspended in 1 ml of growth medium and transferred into each well of a new 24-well plate containing 1 ml growth medium.
- Cells were grown for 21 hrs.

Harvest

- 1.5 ml aliquots of cells were transferred into eppendorf tubes.
- These were mixed (vortexing) and then 3 aliquots of 200 µl were added to each well of 96-well plate (containing 20 µl MTS reagent), incubated for 2 hrs at 37 ºC before the colour intensity was determined using a microplate reader.
- The remaining 900 µl in the eppendorf tube was used for flow cytometric micronucleus quantification. Lysis solution was added and the nuclei and micronuclei were collected by centrifugation before being resuspended in Isoton. Micronuclei were counted and expressed as a percentage of the number of whole nuclei in the sample. 1000 events were counted.
- A further 500 µl from the original plate was used for microscopic slide preparation. These were air-dried and then stained in DipQuik for microscopy. 1,000 cells on each of 2 replicate slides were counted for each treatment.

Results

Cytotoxicity and genotoxicity screening using the CBMN assay was conducted during the June 2008 sampling event at both KWRP and BPP. The dates and locations are presented in Table 8.1

Table 8.1: CBMN sampling dates and locations at BPP and KWRP

Beenyup pilot plant (BPP) (5th of June 2008)				
Secondary wastewater	Post-MF water	Post-RO water	Field Blanks	No of samples
Grab + Replicate	Grab + Replicate	Grab + Replicate	Field blank	7
Composite		Composite	Field blank	3
Kwinana water reclamation plant (KWRP) (6th of June 2008)				
Grab + Replicate	Grab	Grab + Replicate	Field blank	6

There was no significant cytotoxicity or genotoxicity observed when the cells were exposed for 3 hours to secondary wastewater or post-RO water samples minimally diluted in a buffered salts solution (see Figure 7.1). Significant results have at least a

3-fold increase in micronuclei. The only significant increase seen was in the positive control (bromoacetic acid) even though it was used at a concentration that induced only minimal cytotoxicity.

Cytotoxicity was initially assessed using propidium iodide (PI) to quantify the DNA of cells remaining after a treatment protocol involving a 3 hour exposure phase followed by a 21 hour recovery and growth phase. The PI assay was used to assess the potency of the samples and was run at three dilutions: 9 in 40, 9 in 80, and 9 in 160. Although some samples appeared to elicit a concentration response, none was very strong. Genotoxicity is normally assessed in the concentration range that elicits 0-30% Cytotoxicity. Given that no strong response was observed, the main genotoxicity assessment was conducted using the highest sample concentration possible (9 in 20). The same treatment and recovery protocol was used in the second experiment, and micronucleus induction was quantified by 2 methods: Flow cytometry to count the number of PI-stained particles in the expected size range for micronuclei (1–10% of the nuclei). This was expressed as a percentage of the number of whole nuclei. The induction of micronuclei was also assessed by counting micronuclei and whole nuclei under the microscope. Cytotoxicity was also assessed, using dehydrogenase-induced reduction the tetrazolium salt MTS to a coloured product as a measure of cell activity.

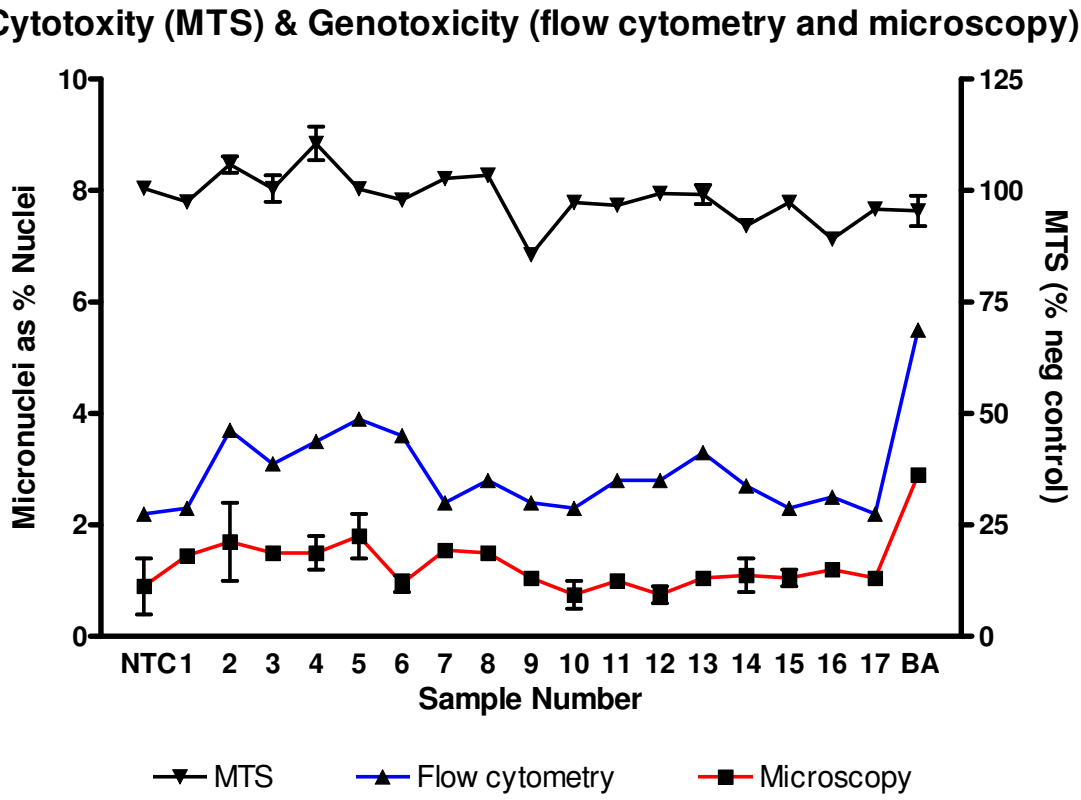

Figure 8.1 CBMN Cytotoxicity and Genotoxicity of secondary wastewater, post MF water and post-RO water at KWRP and BPP

Samples	1	2	3	4	5	6	7
Kwinana	Post-RO	Field blank	Post-RO replicate	Pre MF	Trip blank	Post MF	Pre MF replicate

Samples	8	9	10	11	12	13	14	15	16	17	BA
Beenyup	Pre MF replicate	Post-RO replicate	Post MF replicate	Post-RO composite	Field blank	Trip blank	Post-RO grab	Pre MF grab	Post-RO replicate	Pre MF composite	Positive control

Though there was some minimal reduction in MTS signal in a number of samples (sample No. 9-Post-RO replicate at BPP) this never exceeded 15% and did not correlate with the small reductions seen in the first experiment. Therefore it was concluded that these effects are not sample related. Similarly in the genotoxicity assessment, there appeared to be a small increase in micronucleus formation in samples 2, 4 & 5 (field blank, before MF at KWRP and trip blank), in the flow cytometry results (Figure 8.1) but this was not confirmed by the microscopic method. Significant results need to have at least a 3-fold increase in micronuclei seen in the microscopic method. The only significant increase seen in both methods was in the positive control, even though the bromoacetic acid was used at a concentration that induced only minimal Cytotoxicity (12.5 µM) (Figure 8.1). In conclusion, there was no

significant cytotoxicity or genotoxicity observed when WIL2-NS cells were exposed for 3 hours to the test samples minimally diluted in a buffered salts solution

Discussion

Cytotoxicity and genotoxicity screening were analysed by a cytokinesis-block micronucleus (CBMN) assay using a human lymphoblastoid cell-line. The results indicate that there was no significant cytotoxicity or genotoxicity observed in the cells exposed for 3 hours to either secondary wastewater or recycled water treated by MF/RO. Clear advantages of using in vitro human cells to evaluate potential toxic effects of chemical mixtures is the toxic significance at the relevant environmental concentrations and the elimination of the need to extrapolate from animals to humans. Nevertheless, results of *in-vitro* tests are limited to factors that may modulate toxicity in the whole organisms.

None of the secondary wastewater or post-RO water samples in the CBMN assay consistently produced a dose-related cytotoxic effect and none of them produced a significant genotoxic effect. There were some small cytotoxic effects (10-15% change from control) observed using propidium iodide but these were also observed in blank samples, and they could not be repeated using dehydrogenase-induced reduction the tetrazolium salt, suggesting random variation. Therefore, no positive results were obtained in secondary wastewater using the CBMN test.

The lack of positive results may be associated to a low sensitivity of the tests. For example genotoxins were detected with the Ames test but not with the SOS chromotest in 24 WWTP influents tested (Jolibois & Guerbet, 2005). No clear cytotoxicity or genotoxicity trend was observed between samples before and after the advanced treatment and therefore it was not possible to evaluate the efficiency of water treatment as reported in other studies (Escher *et al.*, 2008, Lee *et al.*, 2008). However, toxicity reduction was observed using RQs. Median RQs in secondary wastewater were higher than after the advanced treatment for all compounds known or suspected to be carcinogenic.

Negative results may also be due to low concentration of cytotoxic and genotoxic chemicals in both the secondary wastewater and the post-RO water samples on the day of sampling. Similarly, Jolibois & Guerbet (2005) reported that none of the tested secondary wastewaters from the two WWTPs tested was genotoxic. These results indicate that conventional wastewater treatment processes were able to remove the genotoxins detected in their influents. RQs calculated based on chemical analysis of pre-MF samples (taken on the same day as toxicity assessment) for chemicals known as carcinogens (mainly DBPs and PAHs) were all below 1. Therefore the lack of genotoxic and cytotoxic response agrees with the chemical analysis results and may indicate that the chemical analysis captured the effect of the majority of chemicals with genotoxicity or cytotoxicity effects.

A German study concluded that the *in-vitro* micronucleus test was suitable as a routine method for the wastewater testing and that the survival index was a robust measure for estimation of toxicity (Reifferscheid *et al*., 2008). It is not clear whether the use of a human lymphoblastoid cell line instead of the Chinese hamster lung fibroblast cell line V79 reported by Reifferscheid *et al*. (2008) may explain in part the differences in test sensitivities. For the CBMN analysis samples were not concentrated. Escher *et al*. (2008) recommends enrichment of the samples with solid-phase extraction given that none of the bioassays gave positive results even when raw secondary wastewaters were used. After concentration, they reported a clear response in all bioassays. Nevertheless, extrapolation of results using concentrates is also complex. The negative results may in part be explained due the lack of concentration of the water samples or the lack of metabolic activation. Previous work reported mutagenicity of WWTPs influent only in the presence of metabolic activation (Doerger *et al*., 1992). Additional CBMN analysis using concentrate samples and metabolic activation may help to clarify if there is any cytotoxicity or genotoxicity activity.

Bioassays provide responses about the possible biological effects in the receptor environment to the exposure of a mixture of pollutants. Bioassays can complement chemical analysis by evaluating potential adverse effects rather than quantifying some of the key hazards. Biological assays are becoming important in monitoring programs that aim to assess secondary wastewater treatment processes and water quality. Moreover, the large number of potentially harmful pollutants in secondary wastewater requires quick and more cost-effective analytical techniques to be used in monitoring programs. Bioassays may contribute to a reduced uncertainty in the risk assessment of both regulated and emerging chemical pollutants. Reducing uncertainty in risk assessment can support and justify the targeting of chemical monitoring activity on a regional basis to those areas validated as being at high risk based on bioassay results.

9 Conclusions

The PCRP project "Characterising Treated Wastewater for Drinking Purposes Following Reverse Osmosis Treatment" has been the first comprehensive research conducted in Western Australia to determine the feasibility to augment drinking water supplies. This research characterised chemical, physical, radiological and microbiological parameters in water after secondary treatment and after advanced treatment using microfiltration (MF) and reverse osmosis (RO). The chemical groups analysed include a broad range of analytes with different physical and chemical characteristics and toxic effects. The majority of chemicals analysed were detected in secondary wastewater and approximately 25% of the detected chemicals in wastewater were also detected at very low concentrations after RO.

The project has:

- characterised the chemical, radiological and some microbial constituents of the large Perth metropolitan Wastewater Treatment Plants (WWTP);

- assessed the performance of MF/RO treatment at the Kwinana Water Reclamation Plant (KWRP) and a pilot plant constructed at Beenyup, Beenyup Pilot Plant (BPP), and their ability to consistently produce water that is safe for human health and the environment during augmentation of drinking water supplies;

- used the research results to develop and refine health and environmental guidelines.

Methodology

A total of 34 sampling days were conducted over seven sampling events during the three year period of the project. A broad set of parameters was analysed over the first six sampling events while the seventh event consisted of two sampling days for *N*-nitrosamines at Beenyup WWTP and BPP. The KWRP was monitored for the duration of the project, while the BPP was constructed in 2007 and commissioned in time for the third sampling event in September 2007. Samples collected were secondary treated wastewater, post-MF and post-RO treated water. Raw groundwater samples were also collected from the influent to the Wanneroo Groundwater Treatment Plant, to provide a reference of raw drinking water source quality. During the project, almost 400 chemicals were tested including disinfection by-products, pesticides, metals, pharmaceuticals, industrial chemicals, endocrine disruptors, and persistent organic pollutants such as dioxins, furans and PCBs among others. Chemicals were selected for analysis based on being:

- currently or previously available for use in Western Australia;

- of toxicological concern;
- recorded elsewhere in wastewaters above guideline levels (ADWG, AGWR (Phase 2);
- small in size - that may cause low rejection efficiency by reverse osmosis treatment.

Chemical analysis was undertaken by Curtin University and Chemistry Centre WA, with specific additional analysis undertaken by National Measurement Institute, CSIRO, ARPANSA, Radiation Health WA, Water Corporation and AWQC. Some quality assurance samples were also analysed by other national and international laboratories. Analytical method development was required for many analytes. Methods were set up for all compounds except for some that were considered to be lower priority, including the brominated flame retardants and alkyltins. Analytical method validation was completed by the end of the project. Each method developed passed appropriate quality assurance requirements. Very low LODs were reported for many parameters. Laboratory proficiency tests (inter-lab comparisons) were conducted for some antibiotics, hormones, and five *N*-nitrosamines with reasonably good agreement between participating laboratories.

Screening Health Risk Assessment

A three-tiered approach was used to develop health values for chemicals as recommended in the *Australian Guidelines for Water Recycling (Phase 2) Augmentation of Drinking Water Supplies (2008).* For chemicals without existing guidelines or toxicological information, the very conservative Threshold of Toxicological Concern (TTC) approach was used to derive the health value. Health values are concentrations considered safe for lifetime consumption and are calculated assuming two litres per day of drinking water consumption as per the ADWG (2004).

A screening health risk assessment was conducted to evaluate the public health significance of the selected chemicals before and after the MF/RO treatment. The methodology for the health risk assessment of chemicals involved the application of a risk quotient (RQ) approach. The RQ is calculated as the ratio between the measured concentration of the contaminant and the health level (therefore a RQ<1 implies low health risk). Both median and maximum measured concentrations were used to assess RQ and those calculated in this study are summarised in the Chemical Analysis section below.

For most chemicals studied, this report has not discussed relative risks associated with exposure from other sources of the chemicals. It should be noted that in general the consideration of exposure in defining health-based chemical standards for drinking water results in a lower health-based guideline level when other ingestion

exposure is likely, as the overall load to the body would be increased. It should therefore be noted that when considering the relative risk of ingesting MF/RO treated wastewater that meets health values compared with other sources of exposure to the chemical, there are often much more significant sources of exposure through food or the atmosphere. As there was insignificant risk as determined by the health screening assessment, except for the *N*-nitrosamines, the specific relative risks have not been elaborated on.

Chemical Analysis

Analysis was conducted for 15 chemical groups, and a brief summary with emphasis on wastewater and post-RO water characterisation and human health significance of results is presented below. The list of all analytes tested per group, seasonal and geographical trends, groundwater characterisation and MF/RO treatment efficiency to remove the analytes and any other analysis conducted are detailed in Chapters 5 and 6.

Conclusions for each chemical group analysed are summarised below with the number of parameters analysed in each group. Concentrations after MF/RO treatment were within health values for all parameters in all groups with the exception occasionally of the *N*-nitrosamines, two disinfection byproducts, and two aminopolycarboxylate complexing agents as discussed below.

General Parameters (32)

Nutrients, alkalinity and suspended solids, as well as online data for dissolved oxygen in the aeration tanks indicated that the WWTPs were operating normally during sampling events. Turbidity, conductivity and ions indicated that KWRP and BPP, the MF/RO plants, were operating normally during sampling events. Ammonia, nitrate, turbidity, total dissolved solids and sodium exceed ADWG levels in secondary treated wastewater but were removed by MF/RO treatment to below drinking water guideline levels. Most ions of health concern (fluoride, chloride, bromide and sulphate) had close to or above 90% removal by MF/RO and met guideline levels in RO water. Nitrite was particularly poorly removed, but was always below health guideline levels.

Metals (28)

The majority of the tested metals and metalloids were detected in secondary wastewater. Arsenic, cobalt, cadmium, mercury and beryllium were never detected. Aluminium and iron were occasionally detected at levels above the health values in

secondary wastewater. However, RQs for all metals detected in the post-RO water were one to three orders of magnitude below 1 indicating low health risk. Boron was detected in secondary wastewater and has the lowest rejection during treatment (59% rejection compared with more than 90% rejection for other metals), making it a useful chemical indicator of RO treatment.

Pesticides (117)

Only 10 of the 117 tested pesticides were ever detected in the secondary wastewater. Atrazine, simazine, 2,4-D, chlorpyrifos ethyl, MCPA, metolachlor, piperonyl butoxide, propiconazole, triclopyr and trifluralin were detected in at least one secondary wastewater sample. All pesticides were below the LOR after the advanced treatment except metolachlor that was detected after RO on one occasion. However, the single detected concentration (0.08 µg/L) was well below the health value of 300 µg/L (RQ=0.0003). None of the pesticides were of health concern after RO treatment.

Disinfection by-products (DBPs) (32)

In secondary wastewater, halomethanes (HMs) were the most frequently detected DBPs (84%), followed by haloacetic acids (14%) and haloketones (6%). For some DBPs there was a greater frequency of detections of DBPs after RO than in the secondary wastewater. This is likely due to DBP formation by chloramination to reduce membrane fouling during treatment in combination with lower rejection through the RO membranes associated with the relatively small size of these DBPs. Nevertheless, median concentrations in the post-RO water were below health values for all detected DBPs. Maximum concentrations of two DBPs were above health values after RO: bromodichloroacetaldehyde (RQmax=1.4) and dibromoacetaldehyde (RQmax=1.3), both with guideline levels calculated using the threshold of toxicological concern (TTC) methodology which is very conservative.

N-nitrosamines (9):

In secondary wastewater, all *N*-nitrosamines were detected, ranging in frequency from 3.5% for NEMA to 93% for NDMA. Maximum concentrations in wastewater were above the health values for all *N*-nitrosamines except NDPhA. Median concentrations in secondary treated wastewater for NDMA, NEMA and NMOR were also above health values. Maximum concentrations of NDMA, NDBA, NDPA, NPIP and NMOR were above health values after MF/RO treatment. However, all median *N*-nitrosamine levels in post-RO water were below the health value.

The average or median concentration of NDMA is most relevant for health risk because the post-treatment groundwater storage will store water for months to years, therefore maximum values will be smoothed by retention in groundwater. In addition, concentrations may be further reduced by further treatment barriers including possible biodegradation in groundwater. It is important to acknowledge that the majority of NDMA consumption will be from food and that NDMA is also created in the digestive system. Therefore the increased risk to human health from NDMA in drinking water is relatively minor.

NDMA was selected as the chemical indicator of the group as it was the most frequently detected both before and after treatment. NDMA concentrations after RO were higher at KWRP (mean=8.5 ng/L, max=30 ng/L) than at BPP (mean=4.8 ng/L, max 9 ng/L).

Volatile Organic Compounds (57)

None of the detected VOCs was above health values in secondary treated wastewater or after RO. The highest RQmax after RO was for 1,3-dichlorobenzene (RQ max=0.17). 1,4-dichlorobenzene was detected in sufficient frequency in secondary wastewater (95%) to use this parameter as an indicator of chemical removal by RO.

Phenols (16)

Of 16 phenols measured, 11 were detected in secondary wastewater. The most commonly detected phenol in wastewater was 4 tert-butylphenol (73% of samples) while the highest median concentration was for 2 phenylphenol (69.5 ng/L). Eight phenols were occasionally detected in post-RO water but all were below levels of health concern.

Polycyclic Aromatic Hydrocarbons (PAHs)(17)

All were detected in secondary wastewater. Twelve PAHs were also detected in the post-RO water. Toxicity equivalency factors (TEFs) were used to weight each PAH's toxicity relative to the toxicity of benzo(a)pyrene (BaP), the best studied PAH. Toxic equivalency (TEQ) was calculated by adding the contribution of each PAH. This approach indicated that secondary wastewater may contain concentrations of health concern for drinking (RQ as BaP equivalents = 4.3). However, the MF/RO treatment

was able to reduce the concentration of these compounds to levels of no health concern (RQ as BaP equivalents below 1).

Dioxins, Furans and Dioxin-like PCBs (29)

None of the samples analysed was above the health standard of 16 pg TEQ/L (AGWR Phase 2). The combined toxic equivalence (TEQ) of all 29 dioxin-like compounds analysed was an average of 3.34 pg TEQ/L in secondary wastewater and 2.45 pg TEQ/L after RO. The results indicate that dioxin and dioxin-like compounds, are present only at low concentrations in secondary wastewaters and that advanced treatment further reduces these concentrations to levels well below health significance.

Pharmaceuticals (36)

Ten antibiotics, eight iodinated contrast (ICM) media and eighteen other pharmaceuticals were analysed. All antibiotics tested were detected in secondary wastewater except amoxicillin for which the LOD was relatively high (LOD = 1 µg/L). Five ICM were detected in secondary wastewater of which iopromide and iohexol were the more commonly detected. Of the other pharmaceuticals, 16 were detected in at least one secondary wastewater sample. Calculated RQ(median) in wastewater were one to five orders of magnitude below 1. After RO, calculated RQs were three to seven orders of magnitude below 1. Therefore, pharmaceuticals are of very low human health significance after MF/RO treatment.

Estrogenic hormones (4)

The four most potent estrogenic hormones were analysed. In secondary wastewater, estrone was detected in 50% of the samples with a median of 15 ng/L and estriol was detected only in one sample at 10.8 ng/L. None of the tested hormones was detected in the post-RO water. RQs were below health values in both secondary wastewater and post-RO water for estriol, 17β–estradiol and estrone. Due to the high LOD reported and low guideline level ethinyl estradiol concentrations could not be confirmed as being below guideline level. Therefore additional data with a lower LOD is required to better characterise the potential health risk associated with ethinyl estradiol. However a high percentage of removal is expected due to the large molecular size and by comparison with estrone removal efficiency this would be over 90%, therefore there is a very low risk of ethinyl estradiol exceeding the health guideline level.

Aminopolycarboxylate complexing agents (4)

All four complexing agents (EDTA, NTA, DTPA and PDTA) were detected in secondary wastewater and EDTA and NTA were detected in all wastewater samples. After RO, all complexing agents except PDTA were detected. The most commonly detected was EDTA (48% of the samples, median = 0.48 µg/L). RQs in secondary wastewater were below 1 for the complexing agents with ADWG guideline values (EDTA and NTA) and were above 1 for those without toxicological data (i.e. those with health values calculated using the TTC approach, DTPA and PDTA). Concentrations of EDTA and NTA in post-RO water were two orders of magnitude below health values. The DTPA and PDTA health values of 0.7 µg/L were calculated using the TTC approach which is very conservative. It is expected that DTPA and PDTA will have a similar toxicity to the regulated complexing agents (EDTA guideline = 250 µg/L and NTA guideline = 200 µg/L) given the similar physico-chemical properties. It is anticipated that the health values for DTPA and PDTA will increase when toxicity data becomes available.

Inorganic Disinfection Byproducts (Anions) (3)

Chlorate and chlorite were detected in secondary wastewater (37% and 7% of samples respectively) with median concentrations below guideline levels. In wastewater, maximum concentrations of chlorate and chlorite were above guideline levels. However, after RO all concentrations were below health guideline values. Bromate was not detected in any of the samples.

Miscellaneous organic chemicals (11)

Other chemicals analysed were 1,4-dioxane, methyl tertiary butyl ether (MTBE), acrylonitrile, acrolein, triclosan, phthalates and phenoxybenzenes. Of the 11 chemicals, 1,4 dioxane was detected in all wastewater samples followed by triclosan (77%) and acrylonitrile (50%). After RO acrylonitrile was commonly detected (83%) 1,4-dioxane sometimes detected (29%) while triclosan was below the LOD. None of the chemicals had concentrations of health significance after RO.

Radiation - Gross alpha and gross beta particle activity

None of the samples were above the ADWG screening level of 0.5 Bq/L for gross alpha or gross beta particle activity in secondary wastewater (with ^{40}K contribution excluded). After RO, gross alpha and gross beta particle activity levels were more than an order of magnitude below the guideline level. Therefore radiation is unlikely to be of health concern in recycled water.

Summary of chemical constituents in post-RO water and treatment efficiency

Of the 396 compounds investigated in the study, 25 were determined to have percentage detections in post-RO water greater than 25%. Eight of these compounds were disinfection by-products (seven halogenated DBPs, one *N*-nitrosamine, and one inorganic disinfection by-product), while six were metals or metalloids, four were VOCs, and the remaining compounds were from the classes of complexing agents, phenols, or miscellaneous chemicals. Only the *N*-nitrosamines pose a potential concern from a health point of view. Eight compounds had higher percentage detection in post-RO than in secondary wastewater, and this was attributed to contamination (e.g. toluene), formation during chloramination (e.g. halomethanes), or unintentional addition during the MF/RO process (e.g. acrylonitrile, chlorate). These constituents demonstrate that chloramination procedure, membrane materials and anti-scalant chemical usage need to also be considered as potential sources of chemicals in post-RO water.

For parameters detected in secondary wastewater there were no significant differences between the treatment efficiency calculated using all data pairs compared to the treatment efficiency calculated only from data pairs with detections in post-RO water. Results also confirmed that, for chemicals that form or are added during treatment, treatment efficiency calculations across the whole treatment train do not reflect RO removal efficiency. Further monitoring of RO treatment performance requires monitoring immediately prior to RO rather than using secondary wastewater, particularly for all DBPs.

Microbiological Analysis

Microbiological characterisation of secondary wastewater indicated that thermotolerant coliforms and enterococci were always detectable. Male specific (F^+) coliphages were detected in 95% of Subiaco and all Beenyup samples. Adenovirus were detected in 68% of the Subiaco and all of the Beenyup samples, while enterovirus were only detected in less than 25% of samples.

Virus challenge tests using the coliphage MS2 as an indicator of enteric virus demonstrate that RO alone was able to achieve at least 4 log removal of virus particles.

The significant natural occurrence of male specific (F^+) coliphages in the secondary treated wastewater, suggests that they could be a useful routine tool for ongoing monitoring and verification of the operation of RO treatment systems. For the MF/RO process it is considered a valid indicator for removal of viruses and other organisms (including bacteria and protozoa).

Toxicity Analysis

No significant cytotoxicity or genotoxicity was observed when human cells were exposed for 3 hours to secondary treated wastewater or post-RO water samples. These tests indicate that there are no toxic effects on cells or genes.

Although mixtures of chemicals have not been specifically addressed in the health risk assessment of chemicals, the toxicity tests indicate low cytotoxicity and genotoxicity associated with the chemical mixture.

Treatment Performance Indicators and Recycled Water Quality Indicators for future monitoring

A key outcome of this research was the identification of chemical indicators of RO treatment performance and recycled water quality indicators relevant for Western Australia.

The AGWR guidelines (2008) define an indicator as *a chemical or microbial parameter that can be used to measure the effectiveness of a process*. Indicator chemicals may be selected either to indicate specific performance of a treatment process or safety of the treated water.

Suitable Treatment Performance Indicator chemicals must:

- have characteristics that can be linked to a predominant removal mechanism (e.g. filtration, adsorption or oxidation), because different treatment processes target different properties

- be present in concentrations that are representative of the broader class of compounds and that are sufficiently high to determine a meaningful degree of reduction through a unit process or a sequence of processes

- be quantifiable using an established, and preferably accredited, analytical method.

The most sensitive indicator chemicals for assessing the performance of a specific treatment process will be those that are partially removed under normal operating conditions. If the level of removal of the indicator compound is significantly diminished, it will indicate reduced system performance. An indicator compound that is easily removed by the treatment process would be less sensitive to partial failure, and an indicator compound that is poorly removed under normal operating conditions would provide little insight into system performance under any conditions.

MF is a pre-treatment step to remove particulates and allow RO to operate efficiently. Chemical indicators of treatment performance are selected on the basis of physico-chemical properties that essentially characterise removal by RO. The key physico-chemical properties that determine chemical rejection by MF/RO are size (molecular weight, width and length), hydrophobicity (log K_{ow}, log D) and acidic/basic character (pK_a). Log K_{ow} also provides information on polarity (dipole moment) and solubility in water (associated with chemical charge). The selected indicators of treatment performance, listed in Table 9.1, cover chemical groups with different:

- molecular weights (ranged from 10.8 to 296 g/mol),
- hydrophobicity properties (log K_{ow} ranged from -0.64 to 3.4) and
- acidic/basic characteristics (pK_a ranged from 2.13 to 10.4).

The details of each chemical parameter selected as an indicator are presented in Appendix 5, Table A.5.1

Rejection of chemical contaminants by MF/RO is related to interactions between RO membrane characteristics, filtration operating conditions and compound properties. While chemicals of low molecular weight and high polarity are expected to be poorly rejected by the membranes, the presence of any of the chemical indicators with large molecular weight in the post-RO water will indicate a failure of the treatment system.

The results from this project were analysed considering characteristics of a good indicator chemical of treatment performance, to derive a group of indicators appropriate for monitoring chemical removal for different chemical groups by the MF/RO process. Selected chemical indicators of treatment performance were normally detected in secondary wastewater (most more than 90% of the time). They were usually detected at higher concentrations than other chemicals of the same group. If more than one compound was commonly detected in secondary wastewater at similar concentrations, the one with the lower percentage of rejection was selected as it is considered more sensitive to assess the performance of the treatment.

Recycled water quality indicators have the purpose of demonstrating safety of recycled water with respect to specific chemical groups, and hence provide additional confidence beyond treatment performance indicator monitoring that all chemical hazards are being mitigated. This can be particularly important for some chemical groups such as hormones and pesticides for which no chemical was selected as a treatment performance indicator.

For each of the chemical groups, suitable recycled water quality indicator chemicals have been identified (Table 9.2). Many of these parameters were also identified as suitable indicators of RO treatment performance (Table 9.1).

Table 9.1: Treatment Performance Indicator chemicals

Chemicals represented	RO Treatment Performance Indicator (Chemical Group)	Secondary Wastewater % Detection Median (Med) No samples	Post-RO % Detection Median No samples	Median (%) Removal efficiency (min – max)	Reason for selection/ comments
Small size, charged (+ or -) inorganic, very hydrophilic	Boron (Metalloid)	100 % Med=160 µg/L n=31	89% Med=75 ug/L n= 28	Intermediate 62 % (31 to 90%)	Metal with the lowest % of rejection. Only metal with median in post-RO water higher than in groundwater.
Small size, negatively charged, very hydrophilic, inorganic	Nitrate (Inorganic anion)	100% Med=3.45mg/L n=16	100% Med=0.12 mg/L n=16	Intermediate 88% (85 to 99%)	Commonly detected anionic chemical with intermediate removal by RO
Small size uncharged, very hydrophilic, highly polar organic	NDMA (*N*-nitrosamines)	96% Med=16 ng/L n=25	92% Med=4.5ng/L n=26	Intermediate 79% (30 - 95%)	Highest median concentration and high % of detection in wastewater and post-RO water. Toxicological concern
Small size, uncharged, slightly hydrophilic, non-polar, non-ionic, organic	Chloroform* (DBP)	85 % Med=0.4 µg/L n=33	56 % Med=0.14 µg/L n=27	Intermediate 82% (-412 - 98%)	Halomethanes were the most commonly detected DBP in wastewater. Selected in other IPR schemes. Represents hydrophobic compounds. Potentially adsorbing to the membrane and partitioning into the permeate
Small size, uncharged, hydrophilic, non-polar organic	Bromochloromethane * (DBP)	94 % Med=0.22 µg/L n=33	100% Med=0.11 µg/L n=27	Intermediate 63% (-50 to 99%)	May be a better indicator than chloroform based on PCRP data (Higher % detection, lower rejection by MF/RO, but lower concentrations).
Intermediate size, uncharged, hydrophobic, non-polar volatile organic	1,4-dichlorobenzene (VOC)	95 % Med=0.81 µg/L n=37	90 % Med=0.2 ug/L n=29	Intermediate 84 % (-20 to 95%)	Of VOCs, the highest median concentration and high % of detection in wastewater and post-RO water
Moderately large size, uncharged, slightly hydrophobic, non-polar organic	Carbamazepine (Non-polar pharmaceutical)	97 % Med=938 ng/L n=29	0 % Med= NA n=29	Good 99.8 % (98.8 to 99.9%)	Very persistent. Detected in all wastewater samples with the highest median concentration of pharmaceuticals. Represents very well rejected compounds by RO membranes
Large size, negatively charged, polar organic	EDTA (Complexing Agent)	100 % Med=2 µg/L n=27	48 % Med= 0.5 ug/L n=27	Good 99.5 % (98 to 99.9%)	EDTA and NTA were detected in all wastewater samples but higher concentrations were observed for EDTA.
Large size, negatively charged, slightly hydrophobic, polar organic	Diclofenac (Acidic pharmaceutical)	100 % Med=362 ng/L n=26	0 % Med= NA n=26	Good 99.6% (76.7 to 99.8%)	Detected in all wastewater samples. Represents acidic pharmaceuticals

**Only one of the halomethanes would be required as an Indicator, which is most appropriate depends on the analytical method LOD; NA: not applicable*

Table 9.2: Recycled Water Quality Indicators

Chemical groups represented	Recycled water quality Indicator	Secondary Wastewater % Detection Median (Med) No samples (n)	Post-RO water % Detection Median (Med) No samples (n)	Health Value
Metals, small	Boron	100 % Med=160 µg/L n=31	89% Med=75 ug/L n= 28	4 mg/L
Inorganic anions, small	Nitrate	100% Med=3.45mg/L n=16	100% Med=0.12 mg/L n=16	50 mg/L
N-nitrosamines, Small, uncharged	NDMA	96% Med=16 ng/L n=25	92% Med=4.5ng/L n=26	10 ng/L
Anions	Chlorate	37% Med=12.8 n=30	46% Med=12.7 µg/L n=24	700 µg/L
Miscellaneous Neutral organic compounds	1,4 Dioxane*	100% Med=0.52 µg/L n=22	28.5% Med=0.12 µg/L n=21	50 µg/L
Disinfection Byproducts	Chloroform	85 % Med=0.4 µg/L n=33	56 % Med=0.14 µg/L n=27	200 µg/L
Volatile organic compounds	1,4-dichlorobenzene	95 % Med=0.81 µg/L n=37	90 % Med=0.2 ug/L n=29	1.5 mg/L
Polycyclic aromatic hydrocarbons	Fluorene	64% Med= 0.003 µg/L n=22	19% Med=0.003 µg/L n=21	140 µg/L
Phenols	2,4,6-trichlorophenol	64% Med=44.5 ng/L n=22	0%	20000 ng/L
Non-polar pharmaceuticals	Carbamazepine	97 % Med=938 ng/L n=29	0 % Med= NA n=29	100 µg/L
Hormones	Estrone	48% Med=15 ng/L n=29	0%	30 ng/L
Large polar charged organics, Complexing Agents	EDTA	100 % Med=2 µg/L n=27	48 % Med= 0.5 ug/L n=27	250 µg/L
Acidic, polar pharmaceuticals	Diclofenac	100 % Med=362 ng/L n=26	0 % Med= NA n=26	1.8 µg/L
Pesticides	Trifluralin	91% Med=0.48 µg/L n=32	0% NA n=32	50 µg/L
Dioxins, furans & dioxin-like PCBs	Octadioxin	67% Med=16 pg/L n=14	18% Med=5 pg/L n=11	100 ng/L

** With the LOD used in this project 1,4-dioxane would also be a good RO performance indicator.*

Concluding Remarks

This research confirms that the MF/RO treatment process is effective in controlling water quality hazards and reliably producing recycled water suitable for augmenting public drinking water supplies. Chemical contaminants were removed to levels below health significance. The water quality achieved after the MF/RO treatment complies with *Australian Drinking Water Guidelines (2004)* and with the *Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies* values, except occasionally for *N*-nitrosamines.

N-nitrosamines were the only chemical group identified as a potential health concern. The selected indicator *N*-nitrosodimethylamine (NDMA) was routinely detected after the MF/RO treatment, occasionally above the *AGWR (Phase 2,* 2008) value of 10 ng/L. However, the guideline value is very stringent, being a tenth of the 100 ng/L limit in the WHO *Guidelines for Drinking-Water Quality* (2008) and proposed for the ADWG (released for public comment in 2009), which was never exceeded. The average and median NDMA concentrations complied with the AGWR guideline value during this sampling regime, of particular relevance as post-treatment maximum concentrations will be smoothed by groundwater storage for months to years. Nevertheless, *N*-nitrosamines including NDMA need to be carefully monitored, including their formation and degradation, to better understand the potential health risks.

This report provides the information necessary for government to develop regulation for conducting indirect potable reuse using MF/RO treatment. Health and environmental recommendations for the Groundwater Replenishment Trial (GWRT) are given in Appendix 5 and 6 respectively.

This research indicates that there will be a high degree of safety associated with further investigation of indirect potable reuse in Western Australia that uses MF/RO treatment in the treatment train. Identification of key chemicals (indicators of treatment performance and recycled water quality) for monitoring, along with the implementation of a risk management framework, provides confidence to proceed with the Groundwater Replenishment Trial.

Abbreviations

AAS	Atomic Absorption Spectroscopy
ADI	Acceptable Daily Intake
ADWG	Australian Drinking Water Guidelines
AGWR	Australian Guidelines for Water Recycling (Phase 2)
AOP	Advanced Oxidation Process
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Australian Water Quality Centre
AWT	Advanced water treatment
BN	Binucleated
BPP	Beenyup Pilot Plant
CAS	Chemical Abstracts Service
CBMN	cytokinesis-block micronucleus
CCP	critical control point
CCWA	Chemistry Centre of Western Australia
CDC	Centers for Disease Control and Prevention
CI(s)	confidence interval(s)
CIP	clean in place
COC	chemicals of concern
CoC	Chain of Custody
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CWQRC	Curtin Water Quality Research Centre
DBP(s)	disinfection by-product(s)
DoH	Department of Health
DoW	Department of Water
DEC	Department of Environment and Conservation
DI	de-ionised
DNA	deoxyribonucleic acid
DWGV	drinking water guideline value
EC	European Commission
EDC(s)	Endocrine Disrupting Compounds/Chemicals

EI	electron ionisation
EMEA	European Medicines Agency
EOM	effluent organic matter
EPA	Environmental Protection Agency/Authority
ESI	electrospray ionization
EU	European Union
EVs	environmental values
FDA	Food and Drug Administration (USA)
GC	gas chromatography
GV	guideline value (WHO, Australia)
GWR	groundwater replenishment
HACCP	hazard analysis and critical control points
HQ(s)	hazard quotient(s)
HQ(wcs)	hazard quotients worst case scenario
HRA	health risk assessment
HV	health value
IARC	International Agency for Research on Cancer
IC	Ion chromatography
IDL(s)	instrument detection limits
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
IPR	indirect potable reuse
IRIS	Integrated Risk Information System
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_{ow}	octanol-water partitioned coefficient
KWRP	Kwinana Water Reclamation Plant
LC	liquid chromatography
LDTD	lowest daily therapeutic dose
LLE	liquid-liquid extraction
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification

LOR	limit of reporting
MAR	Managed Aquifer Recharge
MBR	membrane bioreactor
MCL	maximum contaminant level
MLD	method limit of quantitation
MF	microfiltration
MRM	multiple reactions monitoring mode
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NMI	National Measurement Institute
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTP	National Toxicology Program
NTU	nephelometric turbidity unit
OCWD	Orange County Water District
OECD	Organization for Economic Cooperation and Development
OEHHA	Office of Environmental Health Hazard Assessment (California)
ORP	oxidation reduction potential
P	proportion from water
PCRP	Premiers Collaborative Research Project
POP(s)	persistent organic pollutant(s)
PRW	purified recycled water
PTA	Proficiency Testing Australia
QA	quality assurance
QC	quality control
QSAR	quantitative structure activity relationship
RfD	reference dose
RHB	Radiation Health Branch
RO	reverse osmosis
RQ(s)	risk quotient(s)
RIVM	Research for Man and the Environment (The Neatherlands)
s	seconds

S-ADI	surrogate acceptable daily intake
SBSE	stir bar sorptive extraction
SCHER	Scientific Committee on Health and Environment Risk
SDOO	Sepia Depression Ocean Outfall
std dev	standard deviation
SF(S)	safety factor(s)
SGS	Societe Generale de Surveilance
SHRA	screening health risk assessment
S/N	signal to noise ratio
SOM	Standard Operation Method
SOP	standard operating procedure
SP	sampling point
SPE	solid phase extraction
sPF	slope factor in (mg/kg/day)
SSTs	Secondary sedimentation tanks (or clarifiers)
TDI	tolerable daily intake
TEF(s)	toxic equivalency factor(s)
TEQ(s)	toxic equivalent(s)
TEQ_{DFP}	WHO 2005-TEQ for all analytes (dioxins, furans and PCBs)
TGA	Therapeutic Goods Administration
TMI	tolerable monthly intake
TTC	thresholds of toxicological concern
UF	ultrafiltration
U.S. EPA	United States Environmental Protection Agency
UV	ultraviolet light
UWA	University of Western Australia
WA	Western Australia
WET	whole of effluent toxicity
WHO	World Health Organisation
WRP	water reclamation plant
WWTP	wastewater treatment plant
~	approximately
>	greater than
<	less than

Chemical Abbreviations

ADA	B-alaninediacetic acid
BaP	benzo(a)pyrene
BOD	biochemical oxygen demand
COD	chemical oxygen demand
DBP(s)	disinfection by-product(s)
DEET	N,N-Diethyl-meta-toluamide
DO	dissolved oxygen
DOC	dissolved organic carbon
DMA	dimethylamine
DTPA	diethylentriaminpentaacetic acid
EDC(s)	endocrine disrupting compound(s)
EDTA	ethylenediaminetetraacetic acid
FRP	filterable reactive phosphorus
HAA(s)	haloacetic acid(s)
HAK(s)	haloketones
HAL(s)	haloaldehydes
HAN(s)	halocetonitriles
^{40}K	Potassium 40
LPG	liquefied petroleum gas
MeOH	methanol
MGDA	methylglycinediacetic acid
MtBE	methyl *tert*-butyl ether
NDBA	*N*-nitrosodi-n-bultyldiamine
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NDPA	*N*-nitrosodi-n-propylamine
NDPhA	*N*-Nitrosodiphenylamine
NEMA	*N*-nitrosoethylmethylamine
$NHCl_2$	dichloramine
NMOR	*N*-nitroso-morpholine

NPEOs	nonylphenol ethoxylates
NPIP	*N*-nitrosopiperidine
NTA	nitrilotriacetic acid
NPYR	*N*-nitroso-pyrrolidine
OCDD	octachlorodibenzodioxin
OCDF	octachlorodibenzofuran
-OH	hydroxyl
PAH(s)	polycyclic aromatic hydrocarbon(s)
PBB(s)	polybrominated biphenyl(s)
PCB(s)	polychlorinated biphenyl(s)
PCDD(s)	polychlorinated dibenzodioxin(s)
PCDF(s)	polychlorinated dibenzofuran(s)
PBDE	polybrominated diphenyl ethers
PDMS	polydimethylsiloxane
PDTA	propylenediamintetraacetic acid
PhAC(s)	pharmaceutically active compounds
PI	propidium iodide
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCE	trichloroethylene
TCEP	tris(2-chloroethyl)phosphate
TDS	total dissolved solids
THM(s)	trihalomethanes(s)
TOC	total organic carbon
TSS	total suspended solids
VOC(s)	volatile organic compound(s)

Units

Bq	Becquerel
bw	Body weight
cfu	colony forming units
d	day
EEQ	17β-estradiol equivalent
g	gram, one thousandth of a kilogram, $1x10^{-3}$ kg
kg	kilogram
L	litre
log	logarithm to base 10
ML	mega litre, 1000 litres
micron	One millionth of a metre
mg	milligram, one-millionth of a kilogram, $1x10^{-6}$ kg
mg/L	milligrams per litre
ng	nanogram (10^{-9} g)
ng/L	nanograms per litre
/	per
pfu	plaque forming units
pg	picogram (10^{-12} g)
ppb	parts per billion (10^{-9})
ppm	parts per million (10^{-6})
ppt	parts per trillion (10^{-12})
µg	microgram (10^{-6} g)
µg/L	micrograms per litre
µM	micromoles per litre or micromolar
µS	micro Siemens
TEQ	Toxic Equivalents
t_R	chromatographic retention time

Glossary

10^{-6} cancer risk	The concentration of a chemical in drinking water that corresponds to an excess estimated lifetime cancer risk (in addition to cancer risk from other causes) of 1 in 1,000,000
Acceptable daily intake (ADI)	An estimate of the amount of a substance in food and/or drinking water, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation. It is usually expressed in milligrams of the chemical per kilogram of body weight per day (mg/kg/day). ADIs are established for substances that have a reason to be found in food or water such us additives, pesticide residues and veterinary drugs in foods. For calculation of ADI based on ADWG, a standard body mass of 70 kg is used. (See also 'Tolerable Daily Intake' and 'Reference Dose')
Advanced water treatment (AWT)	Any physical, chemical or biological treatment process used to accomplish a degree of treatment greater than that achieved by secondary treatment. In this report it refers to secondary wastewater treated using microfiltration and reverse osmosis treatment at BPP and KWRP
Adverse effect	The change in morphology, physiology, growth, development or life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. Some adaptive changes are not generally considered to be adverse e.g. some changes in enzyme levels
Ames test	An in vitro mutagenicity screening test utilising histidine dependent *Salmonella typhimurium* bacteria. The test is used for screening substances for possible carcinogenicity, though the relationship between mutagenicity and carcinogenicity is not exact.
Analyte	A chemical (compound, element, ion etc.) subject to analytical determination
Anthropogenic compound	A compound that occurs in the environment primarily as a result of human activity
Aquifer	Soil, sand or rock below the land surface that contains water in recoverable quantities. A confined aquifer has layers of impermeable material above and below it and is under pressure. In an unconfined

	aquifer, the water table is at atmospheric pressure and thus is able to rise and fall
Assay	A test for a particular chemical or biological agent to determine its properties or effect
Beenyup Pilot Plant (BPP)	The pilot water reclamation plant that treats secondary treated wastewater from the Beenyup WWTP by microfiltration and reverse osmosis to produce water for water quality testing
Best available technology (BAT)	The best technology treatment techniques or other means that are available to remove a contaminant(s) to below the guideline value. BATs are designated after examination for efficacy under laboratory and field conditions (taking cost into consideration)
Bioassay	The quantitative measurement, under standardized conditions, of the biological effects of a substance on an organism or part of an organism
Bioavailability	The degree and rate at which a substance is absorbed into a living system or is made available at the site of physiological activity
Biochemical oxygen demand (BOD)	The amount of oxygen (measured in mg/L) that is required for the decomposition of organic matter by single-cell organisms, under test conditions. It is used to measure the amount of organic pollution in wastewater
Cancer slope factor	An upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent
Carcinogen	Any substance that can produce cancer in an organism
Chemicals of concern	Chemicals suspected or identified as having the potential to pose an adverse effect to human health and the environment and the subject of study
Chelating agents	Organic compounds that have the ability to bind metal ions in water solutions into soluble chemical complexes
Chemical oxygen demand (COD)	The amount of oxygen (measured in mg/L) that is consumed in the oxidation of organic and oxidisable inorganic matter, under test conditions. It is used to measure the total amount of organic and inorganic pollution in wastewater. Contrary to BOD, with COD practically all compounds are fully oxidized
Chronic exposure	Occurring over a long period of time, either continuously or intermittently; used to describe long- term low-level exposure to a toxic chemical

Chronic toxicity	Used to describe ongoing effects that develop only after a long-term low-level exposure to a toxic chemical
Common mechanism of toxicity	Pertains to two or more chemicals that cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events (i.e. interpreted as mode of action)
Composite (proportional) samples	A composite sample is a collection of individual samples obtained over a time period to determine the average conditions during the sampling period. In this report the time period was 24 hours. Composite samples may be time-weighted (i.e. samples taken every hour) or flow-weighted (samples taken at a frequency proportional to the rate of flow). Each individual sample is combined with the others and the resulting mixture (composite sample) forms a representative sample that is analysed to determine the average conditions during the sampling period. Flow-weighted composite samples were used for standard operational samples of secondary treated wastewater. In the MF/RO plants (including influent) all composite samples were time-weighted.
Concentrate (also known as retentate)	The portion of the process stream that contains salts and other organic and inorganic constituents rejected from the membrane process
Conductivity	a measure of the ability of an aqueous solution to conduct an electric charge; related to the amount of total dissolved solids (TDS)
Contaminant	Biological or chemical substance or entity that is either present in an environment where it does not belong or is present at concentrations that are capable of producing an adverse effect to humans or the environment
Contaminants of potential health concern	As used in this report, contaminants in the secondary wastewater with maximum detected concentrations within a factor of 10 of applicable human health benchmark values (i.e. RQmax greater than or equal to 0.1)
Control measure	Any action or activity that can be used to prevent or eliminate a hazard or reduce it to an acceptable level (also see HACCP)
Critical control point (CCP)	A point, step or procedure in a recycled water process at which control can be applied, and a safety hazard can as a result be prevented, eliminated or reduced to acceptable levels (also see HACCP)

Critical effect(s)	The adverse effect judged to be the most important for setting an acceptable human intake or exposure. It is usually the most sensitive adverse effect, i.e. that with the lowest effect level, or sometimes a more severe effect, not necessarily having the lowest effect level
Critical limit	The maximum or minimum value to which a physical, biological or chemical parameter must be controlled at a critical control point to prevent, eliminate or reduce to an acceptable level the occurrence of the identified safety hazard (also see HACCP)
Disinfection by product (DBP)	A range of organic and inorganic products resulting from the reaction of disinfecting oxidants with natural aquatic organic material reductants in water systems. The number and nature of all products are not precisely known at present, and vary with type of disinfectant employed. Some of the chlorination by-products are mutagenic and some are suspected animal carcinogens
Dose	A stated quantity or concentration of a substance to which an organism is exposed over a continuous or intermittent duration of exposure. It is most commonly expressed as the amount of test substance per unit weight of test animal (e.g. mg/kg body weight). Dosage **often** involves the dimension of time (e.g. mg/kg/day)
Dose additive	The assumption that each chemical behaves as a concentration or dilution of every other chemical in the mixture, when evaluating the joint risk of chemicals that are toxicologically similar and act at the same target site. The response of the combination is the response expected from the equivalent dose of an index chemical. The equivalent dose is the sum of the component doses, scaled by each chemical's toxic potency relative to the index chemical
Dose-response curves	The quantitative relationship between the dose of an agent and an effect caused by the agent
Drinking water guideline value (DWGV)	A threshold value for a maximum acceptable concentration or measure of a water quality characteristic that, based on present knowledge, does not result in any significant risk to the health of the consumer (health-related guideline value), or is associated with good quality water (aesthetic guideline value). Guidelines are issued for advisory purposes
Duplicates	Two separate samples with separate containers taken at the same time and at the same place
Emerging contaminant(s)	The natural or synthetic organic compounds where growing evidence

	suggests that adverse effects at environmentally relevant concentrations could occur but whose environmental releases are not regulated, and which are not routinely screened for their presence in water. Emerging contaminants may be candidate for future legislation due to its adverse effects and/or persistency
Emerging substance	A substance that has been detected in the water, but which is currently not included in routine monitoring programmes and whose fate, behaviour and toxicological effects are not well understood
Endocrine disrupting compound(s) (EDC(s))	A substance or mixture of substances exogenous to an organism that can interfere with normal hormone function by influencing metabolism, growth and reproduction and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations
Endpoint	An observable or measurable biological event used as an indicator of the effect of a chemical on a biological system (cell, organism, etc)
Environmental buffer	A dam, river or aquifer, where purified recycled water is allowed to mix with the natural water in the catchment, providing an important separation between the recycling process and the normal drinking water treatment process
Exposure assessment	An identification and evaluation of the human population exposed to a toxic agent, describing its composition and size, as well as the type, magnitude, frequency, route and duration of exposure to one or more contaminated media. The estimation could be qualitative or quantitative
External dose	Amount of chemical that is inhaled, ingested, or comes in dermal contact and is available for systemic absorption. External dose is usually expressed in units of mg of chemical per kg body weight per day (mg/kg/day)
Flux	The rate at which a reverse osmosis membrane allows water to pass through it
Fouling	The deposition of organic matter on the membrane surface, which causes inefficiencies
Frequently detected compound	As used in this report, a compound that is detected in at least 90 percent of the samples from any given water quality
Genotoxic	Agents for which a direct activity is the alteration of the information encoded in genetic material
Genotoxic carcinogen	A chemical which induces tumours via a mechanism involving direct damage to DNA

Groundwater	The term as used in this report means the underground water source that has the potential to be used as a drinking water supply. It is considered the background quality water for comparison with the product water quality
Groundwater recharge	The natural or intentional infiltration of surface water into the zone of saturation
Half-life	The time required for the concentration of a compound in a given environmental medium or the time required for an organ, tissue, or the whole body to reduce or eliminate (excrete) one-half of the original concentration of a chemical or its metabolite
Halogenated compound	A compound in which one or more hydrogen atoms have been replaced by a halogen atom, such as fluorine, chlorine, or bromine.
Hazard	A biological, chemical, physical or radiological agent that has the potential to produce a particular type of adverse health effect
Hazard analysis and critical control point (HACCP) system	A systematic methodology to control hazards in a process by applying a two-part technique: first, an analysis that identifies hazards and their severity and likelihood of occurrence; and second, identification of critical points where the hazards may be controlled, and the monitoring criteria to ensure that controls are working effectively. This system was introduced in the food industry and is now being applied in the Australian water industry to drinking water
Hazard identification	The identification, from animal and human studies, *in vitro* studies and structure activity relationships, of adverse health effects associated with exposure to a hazardous agent.
Health risk assessment (HRA)	The evaluation of scientific information on the hazardous properties of environmental agents (hazard characterization), the dose-response relationship (dose-response assessment), and the extent of human exposure to those agents (exposure assessment)
Human health value (or Benchmark value)	A threshold value above which the concentration of a chemical in water may have adverse effects on humans if the water is used as drinking water without treatment or other measures to lower the concentration. As used in this report, it is a standard point or reference from a variety of drinking water standards, guidelines, and threshold concentrations. These include ADWG, WHO, USEPA, Title 22 and other sources of toxicological information such as TGA, IRIS, EMEA and OEHHA
***in vitro* studies**	Studies of chemical effects conducted in tissues, cells, or subcellular extracts from an organism (i.e., not in the living organism)

***in vivo* studies**	Studies of chemical effects conducted in intact living organisms
Indicator	An individual chemical occurring at quantifiable level, which represents certain physicochemical and biodegradable characteristics of a family of trace constituents that are relevant to fate and transport during treatment, providing a conservative assessment of removal
Indirect potable reuse (IPR)	The planned reuse of highly treated recycled water for discharge into a receiving body such us a river, reservoir or aquifer before re-treatment and subsequent supply as drinking water
Influent water	Inflowing water that feeds treatment operations
Log octanol-water partitioning coefficient (Kow)	The ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. It is inversely related to the solubility of a compound in water
Log reduction	The reduction in concentration by a factor of 10. A 1 log reduction represents a 10-fold reduction in numbers or 90% removal, a 2 log reduction represents a 100-fold reduction or 99% reduction, a 3 log reduction represents a 1,000-fold reduction or 99.9% reduction, and so forth
Kwinana Water Reclamation Plant (KWRP)	The water reclamation plant that treats secondary wastewater from the Woodman Point WWTP by microfiltration and reverse osmosis to produce water for industrial use
Lethal concentration 50 (LC_{50})	In a toxicity test is the concentration of a chemical at which 50 percent of test organisms die within a specified period of time (typically 48 or 96 hours)
Lifetime Health Advisory (L-HA)	The concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects in humans over a lifetime exposure. The L-HA is based on exposure of a 70-kilogram adult consuming 2 liters of water per day, and assumes that only a portion (generally 20 percent) of the total exposure to a contaminant is from drinking water. This parameter is a guideline value issued by U.S. EPA and is not a legally enforceable federal standard
Limit of Detection (LOD)	The constituent concentration that, when processed through a complete method, produces a signal with a 99.74 percent probability that it is different from a blank. In this study LOD is the concentration producing a signal to noise ratio of three or the concentration equivalent to three times the standard deviation of an appropriate calibration standard.

Limit of quantitation (LOQ)	The lowest concentration of analyte in a sample that can be detected and quantified by the laboratory with stated level of confidence under the stated conditions of the test. In this study LOQ is the concentration producing a signal to noise ratio of ten.
Limit of reporting (LOR)	Represents the lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test. Where the instrumental signal for an analyte in a sample is lower than the signal determined for the lowest calibration standard, the sample will be reported as “less than concentration value” (<LOR). Typically, LOR is 3 to 5 times the method limit of detection for a given target analyte. (Refer to: NATA Technical Note #17 and Eurachem Guides)
Lowest-observed adverse effect level (LOAEL)	The lowest exposure level at which there are statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group
Maximum contaminant level (MCL)	The U.S. EPA drinking-water standard that is legally enforceable and that sets the highest permissible concentration of a specific contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur and which allows an adequate margin of safety
Maximum contaminant level goal (MCLG)	The U.S. EPA non-enforceable maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur and which allows an adequate margin of safety.
Metabolite	A modified chemical, produced by biological processes, of the original ‘parent’ compound
Microfiltration (MF)	Treatment process passing water through microfiltration membranes with a molecular weight cut-off of 10^5 Daltons. MF has a pore size range of approximately 0.1 – 0.2 µm (nominally 0.1 µm)
Micropollutants	Trace organic contaminants found in microgram per litre concentrations or lower
Multiple barriers	The application of individual water treatment barriers in series as a barrier against hazards
Mutagen	An agent that causes a permanent genetic change in a cell, that is change to the DNA, other than that which occurs during normal genetic recombination (e.g., mutagen MX)

Mutagenicity	The capacity of a chemical or physical agent to cause permanent alteration in the amount or structure of the genetic material within living cells or organisms
Nephelometric turbidity unit (NTU)	The unit of measure for turbidity
No observed adverse effect level (NOAEL)	The highest exposure level at which there are no statistically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or to be precursors to adverse effects
No observed effect level (NOEL)	An exposure level at which there are no statistically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control
Non-genotoxic carcinogen	A chemical which induces tumours via a mechanism which does not involve direct damage to DNA
Nonpoint source	Pollution sources which are diffuse and do not have a single point of origin or are not introduced into a receiving stream or the environment from a specific outlet. The pollutants are generally carried off the land by stormwater runoff. The commonly used categories for non-point sources are agriculture, forestry, urban, mining, construction, land disposal, and saltwater intrusion
Oxidation reduction potential	The electric potential required to transfer electrons from the oxidant to the reductant, used as a qualitative measure of the state of oxidation in water treatment systems
Parameter	A variable, measurable property whose value is a determinant of the characteristics of a system such as water. Temperature, pressure, density and chemical concentrations are examples of parameters
Permeate	The product water that passes through a reverse osmosis membrane
Persistent organic pollutants (POP's)	Chemical substances that persist in the environment, bioaccumulate through the food web and pose a risk of causing adverse effects to human health and the environment
Point source	A stationary location or fixed facility from which pollutants are discharged or emitted; also, any single identifiable source of pollution
Pollutant	A contaminant at a concentration high enough to endanger the life of organisms

Premiers Collaborative Research Project (PCRP)	A multidisciplinary project on chemicals of concern in recycled water titled “Characterising treated wastewater for drinking purposes following microfiltration and reverse osmosis treatment”
Purified recycled water (PRW)	Refers to recycled water produced following MF and RO treatment. In this report refers to the post-RO water at the Kwinana Water Reclamation Plant (KWRP) or the Beenyup pilot plant (BPP)
q1	The 95% upper confidence limit of the slope estimate used for the linearised multi-stage model
Quality assurance (QA)	All the planned and systematic activities implemented to ensure that the quality control activities are being properly implemented to provide adequate confidence that an entity will fulfill requirements for quality (AS/NZS ISO 8402:1994)
Quality control (QC)	A planned system of operational techniques and activities designed to provide a quality product (AS/NZS ISO 8402:1994)
Quantitative structure–activity relationship (QSAR)	Computer-based mathematical models, which relate the biological activity of compounds to theoretically calculated or experimental descriptors of their chemical structure
Raw water	Water that forms the source supply for potable water, before it has been treated
Raw wastewater	Untreated wastewater and its contents
Recycled water	Water generated from sewage, greywater or stormwater systems and treated to a standard that is appropriate for its intended use. In this report the term ‘recycled water” refers to secondary wastewater, treated using MF and RO to generate water intended for augmentation of drinking water supplies
Reference dose (RfD)	An estimate (with an uncertainty of perhaps an order of magnitude) of the daily exposure that is likely to be without appreciable risk of deleterious health effects in the human population (including sensitive subgroups) over an individual's lifetime. It can be derived from a NOAEL, a LOAEL, or a benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used
Reverse osmosis (RO)	Treatment process passing water through reverse osmosis membranes with a molecular weight cut-off of about 10^2 Daltons. The process involves application of pressure to a concentrated solution which causes the passage of close to pure water from the concentrated

	solution across a semi-permeable membrane
Risk	In the context of human health refers to the probability of injury, disease, or death from exposure to a chemical hazard or a mixture of chemicals. In quantitative terms, risk is expressed in values ranging from zero (representing the certainty that harm will not occur) to one (representing the certainty that harm will occur)
Risk assessment	The process of defining risk level using available information to predict how often hazards or specified events may occur (likelihood) and the magnitude of their consequences
Risk characterization	A process leading to an estimation of the probability that harm will result from exposure to a substance within a defined set of circumstances
Risk management	The process of evaluating alternative options and implementing them in response to risk assessment. The decision making could incorporate scientific, technological, social, economic and political information
Risk quotient (RQ)	Ratio of the measured concentration of a detected contaminant to its guideline value (for a regulated compound), its health value (for unregulated chemicals with toxicity information) or its threshold of toxicological concern (TTC) value (for unregulated chemicals without toxicity information)
RQmax	Ratio of the maximum detected concentration of a contaminant in a given location to its human-health value
RQmedian	Ratio of the median of detected concentrations of a contaminant in a given location to its human-health value
Safety factor	A single factor or product of several single factors used to derive an acceptable intake. These factors account for adequacy of the study, interspecies extrapolation, inter-individual variability in humans, adequacy of the overall data base, nature and extent of toxicity, public health regulatory concern and scientific uncertainty (also called uncertainty factor)
Secondary wastewater	The liquid portion of wastewater leaving a secondary wastewater treatment plant. In this report corresponds to secondary treated wastewater from Woodman Point, Subiaco or Beenyup WWTPs and the influent to KWRP and BPP
Secondary treatment	Biological treatment of wastewater using microorganisms to convert dissolved or suspended materials into a form more readily separated from the water being treated. In this report the secondary treatment processes are activated sludge treatment.

Sewage	The used water of community and/or industry, conveyed through sewers to be treated at a wastewater treatment plant
Sewerage	The sewerage system comprises the pipes and plant needed to transport and treat sewage
Slope factor	An upper bound, generally approximating or exceeding a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent. This estimate, usually expressed in units of proportion (of a population) affected per mg/kg/day, is generally reserved for use in the low-dose region of the dose-response relationship, that is, the slope of the dose-response (carcinogenicity) data extrapolated to zero using an appropriate mathematical model
Structure activity relationship (SAR)	The relationship between the biological activity of a chemical or series of chemicals and their structure. The relationships can be described qualitatively and quantitatively
Surrogate	A quantifiable bulk parameter in which a quantifiable change can serve as a performance measure of individual unit processes or operations regarding their removal of trace compounds. Generally measured in line.
Threshold	The lowest dose or exposure level below which no toxicity is observed
Threshold of toxicological concern (TTC)	The establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health. The concept proposes that a low level of exposure with a negligible risk can be identified for many chemicals, including those of unknown toxicity, based on knowledge of their chemical structures
Tolerable daily intake (TDI)	An estimate of the amount of a substance in air, food or drinking water that can be taken in daily over a lifetime without appreciable health risk. The term “tolerable” is used, as contaminants do not serve an intended function and as intake is unavoidably associated with the basic consumption of food and water. TDI is the analogous term of ADI used for contaminants
Wastewater treatment plant (WWTP)	A biological treatment plant (activated sludge plant) that treats raw sewage into secondary treated wastewater that provides the influent water to the KWRP and the BPP
Water quality	The chemical, physical, and biological condition of water related to a beneficial use

References

NB: This Reference list applies to all chapters except Chapter 6, which has reference lists within each section, but may also reference general references from the list below.

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