Chemicals in Reverse Osmosis-treated Wastewater: Occurrence, Health Risk, and Contribution to Residual Dissolved Organic Carbon

Short title: Chemicals in RO-treated Wastewater: Occurrence, Risk, and DOC Contribution

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ABSTRACT

The quality and safety of reverse osmosis (RO)-treated secondary wastewater, for indirect potable re-use, was assessed using a dataset of 375 chemicals measured in RO-treated wastewater. A screening health risk assessment indicated that four *N*-nitrosamines were of potential concern, although median concentrations of these chemicals were always below health values. The most frequently detected chemicals in RO-treated water were disinfection by-products, volatile organic compounds, metals and complexing agents, in contrast to many monitoring programs that focus on pharmaceuticals, personal care products and hormones. Frequent detections in RO-treated wastewater were most related to high concentrations in secondary wastewater, relative to limit of reporting, and the potential for chemicals to form or be added during the treatment process, rather than poor rejection by RO membranes. Between 3.7 and 10.7 µg/L of dissolved organic carbon (DOC) in RO permeate could be attributed from chemicals detected on at least one occasion, with the majority of this total attributed to chemicals detected in less than 25% of samples. While chemicals below detection may contribute a significant component of DOC, it is likely that natural organic matter and soluble microbial products still contribute the majority of DOC to RO permeate.

A high degree of safety is demonstrated for the use of RO-treated wastewater as an indirect source of potable water.

ACRONYMS AND ABBREVIATIONS

BPP: Beenyup Pilot Plant, DBP: disinfection by-product, DOC: dissolved organic carbon, DTPA: diethylenetriaminepentaacetic acid; EDTA: ethylenediaminetetraacetic acid, KWRP: Kwinana Water Recycling Plant, LOR: limit of reporting, MF: microfiltration, MW: molecular weight, NDBA: *N*-nitrosodi-n-bultyldiamine, NDMA: *N*-nitrosodimethylamine, NDPA: *N*-nitrosodi-n-propylamine, NEMA: *N*-nitrosoethylmethylamine NMOR: *N*-nitroso-morpholine, NPIP: *N*-nitrosopiperidine, NTA: nitrilotriacetic acid, PDTA: propylenediaminetetraacetic acid, RO: reverse osmosis, TEQ: toxic equivalent, TTC: threshold of toxicological concern, VOC: volatile organic compound, WW: wastewater, WWTP: wastewater treatment plant

INTRODUCTION

Drinking water augmentation is still considered a high-risk use of recycled wastewater (WW). While pathogenic microorganisms remain the greatest risk to consumers of recycled water (AGWR 2008; ADWG 2011), secondary WW also contains many chemicals with a wide range of residential, industrial, and agricultural origins and uses (Shon *et al.* 2006; Van Buynder *et al.* 2009). Reverse osmosis (RO) membrane treatment is a key barrier for chemical removal (Bellona *et al.* 2004; Snyder *et al.* 2007) and RO treatment efficiency depends on chemical concentration and properties, membrane properties, as well as specific plant design and operation. While many studies of chemical rejection by RO membranes have been published (Agenson *et al.* 2003; Bellona *et al.* 2004), many are conducted at the laboratory-scale, using 'virgin' RO membranes, and may not be

directly applicable to long-term, full-scale plant operation. Opportunities to produce a large chemical dataset for RO treated wastewater are limited, in part due to the difficulty and cost associated with the method development and analysis of large numbers of chemicals at trace and ultra-trace concentrations.

Residual dissolved organic carbon (DOC) remains in RO-treated WW, which may comprise of natural organic matter, soluble microbial products and trace concentration of anthropogenic chemicals (Shon *et al.* 2006). The bulk characteristics of DOC in RO permeate have been investigated using ion exchange (Fujita *et al.* 1996; Drewes *et al.* 2003), size exclusion chromatography with organic carbon and nitrogen detection (Henderson *et al.* 2010) and fluorescence excitation emission spectroscopy (Singh *et al.* 2009). These studies have shown that DOC in RO permeates do not normally contain organic material of molecular weight greater than 500 Da (Drewes *et al.* 2003), and that, based on Amberlite XAD™ resin fractionation, up to 50% of DOC is hydrophilic (Fujita *et al.* 1996). Molecular weight fractions <350 Da form the major components of RO DOC (Henderson *et al.* 2010), with low molecular weight acids and neutrals contribution a significant component (Drewes *et al.* 2003). In some studies no humic acids have been detected in RO permeate (Fujita *et al.* 1996), although humic acids may penetrate RO membranes when treating RO concentrates with high DOC concentrations (Henderson *et al.* 2010). Ratios of H:C and N:C are also typically higher in RO permeate than found in natural drinking waters (Fujita *et al.* 1996), also suggesting that RO DOC is non-humic. In the context of DOC characterisation, many anthropogenic chemicals are classed as low molecular weight neutral or acidic compounds. However, to date there has been no attempt to assess the contribution of anthropogenic chemicals to the DOC in RO.

An important component of achieving water re-use targets in Western Australia is augmentation of drinking water supplies through groundwater replenishment (Water Corporation 2009). In this paper

we consider data from a large study of 375 compounds in secondary WW and recycled water produced by microfiltration (MF) and RO treatment (Van Buynder *et al.* 2009), and assess the quality and safety of RO-treated WW in the context of indirect potable water re-use via groundwater replenishment. To date, this dataset represents one of the most comprehensive analyses of recycled water quality worldwide. In addition, the dataset has been used to identify key factors contributing to frequent detection of a chemical in RO permeate, and estimate the contribution of anthropogenic chemicals to DOC in RO permeate.

METHODOLOGY

Chemical selection, monitoring and analysis

A total of 375 chemicals of potential health concern, in 13 different classes, were monitored (see Table 1 for a summary, and Supporting Information Tables S1-S15 for detailed information). Chemical selection was based on volume of use in Western Australia, level of toxicological concern, environmental persistence, existence of drinking water guidelines, and prior detection in WW as reported in the scientific literature (Rodriguez *et al.* 2007a; Rodriguez *et al.* 2007b). Chemicals for which analytical methods were not available in Australia were prioritised based on health risk, and chemicals with low health toxicity or less likely to be present in concentrations of health concern were excluded. Chemicals for which analytical method development would be difficult or not feasible during the timeframe of the project were also excluded.

Chemical monitoring was undertaken over 3 years, with full details previously published (Van Buynder *et al.* 2009; Linge *et al.* 2010). Reverse osmosis permeate from two MF/RO plants was tested; the Kwinana Water Recycling Plant (KWRP), a full-scale plant (17ML/day) producing water for industrial processes only, and the Beenyup Pilot Plant (BPP), a smaller plant (~67kL/day) constructed to test MF/RO treatment at Beenyup wastewater treatment plant (WWTP). As well as operating at different scales, the plants had different loadings of industrial-sourced WW, although

both have low industrial loading by international standards (Van Buynder *et al.* 2009). Secondary treated WW was also characterised using samples from Perth's three main activated-sludge WWTPs, which treat 85% of the WW produced in the Perth metropolitan area, and this data has been reported elsewhere (Van Buynder *et al.* 2009; Rodriguez *et al.* 2010).

Sampling was carried out during seven approximately quarterly sampling events from December 2006 to October 2008 (Van Buynder *et al.* 2009; Rodriguez *et al.* 2010). Six sampling events were undertaken for all chemical classes, with an additional seventh sampling event undertaken for *N*-nitrosamines only. For most chemicals, samples were collected in every season of the year and on every day of the week over the course of the project.

The details of sampling and analytical procedures have been published previously (Van Buynder *et al.* 2009). Twenty-four-hour composite samples were collected using an automated and refrigerated ISCO 4700 sampler, except for unstable or volatile analytes, which were collected as grab samples. Sampling procedures aimed to optimise analyte preservation until analysis, and appropriate preservation agents were added to sample bottles prior to sample collection, or prior to sub-sample collection in the case of composite samples. Field and trip blanks were collected on each day of sampling, and replicate samples comprised 12% of all secondary WW samples and 8% of all RO permeate samples. A quality assurance program was implemented to ensure data quality. Analytical methods that were not accredited by the National Association of Testing Authorities, Australia (NATA) were scrutinised through appropriate peer review, and inter-laboratory testing was used in method validation where possible. In addition to concentration data, each laboratory calculated method uncertainty, limits of reporting, precision, and bias or accuracy for each method.

Data analysis

Over 20,000 analyses were conducted, not including field and trip blanks. The dataset was analysed after exclusion of trip blanks, field blanks and replicate samples, with chemical occurrence assessed by calculation of percentage detection frequency and median and maximum concentrations. Median was chosen rather than average because it is less affected by outliers and because most chemicals did not follow a normal distribution. Chemical classes were measured using a variety of analytical methods, each with different levels of sensitivity. For many analytes, the analytical limit of reporting (LOR) influenced percentage detection calculations and other statistics. Calculation of median concentrations incorporated all data points including samples reported to be below the LOR, and these were assumed to be equal to the LOR for the purposes of that calculation. While this conservative approach will overestimate the actual median concentration of chemicals reported below the LOR in more than 50% of samples, it was deemed appropriate given our primary goal of assessing the safety of RO-treated WW as potential potable water source.

All results were assessed against benchmark (non-effect) human health values, which were defined using the approach described by Rodriguez *et al.* (2007b). Chemicals were assigned to one of three categories: 1) chemicals with established regulatory guidelines, 2) unregulated chemicals with toxicity data sufficient for derivation of a health value, 3) unregulated chemicals without toxicity data. Health values for unregulated chemicals without toxicity data were derived using the concept of the threshold of toxicological concern (TTC) (Kroes *et al.* 2005). In the TTC, chemicals are allocated to a low, intermediate or high toxicity class, based on chemical structure. Health values for each toxicity class are derived from statistical analysis of a large chemical toxicology dataset, and are considered very conservative.

The screening health risk assessment of RO permeate used the concept of risk quotients (RQs) to compare health values to the concentration of each chemical, with RQ < 1 indicating that no health impacts were expected. RQ_{max} was calculated using the maximum concentration measured for each

chemical, while RQ_{med} was calculated using the median concentration. In cases when a chemical was never detected, RQ_{med} was estimated using the average LOR as a worst case scenario. The exceptions to this approach were the risk assessments of polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like poly chlorinated biphenyls. For these two classes, the concept of toxic equivalents (TEQ) was used, which expresses the toxicities of different chemicals on a common basis by comparing to a reference chemical. This is a widely accepted practice for both PAHs (Bruce *et al.* 2007) and dioxin-like compounds (Van den Berg *et al.* 2006), and the TEQ analysis for these classes are reported elsewhere (Rodriguez *et al.* 2008; Van Buynder *et al.* 2009).

The contribution to the residual DOC was estimated for each chemical with carbon incorporated in its molecular structure. Median and maximum DOC contributions were determined using previously calculated median and maximum concentrations, multiplied by the percentage carbon in each molecule. In cases where a chemical was never detected, median and maximum concentrations were estimated using half the average LOR and the average LOR, respectively. For comparison, total DOC was also determined on 15 occasions throughout the sampling program, with duplicate samples analysed on 2 occasions. Analyses were undertaken by a commercial organisation using a high-temperature combustion method based on Standard Method 5310 B (Clesceri *et al.* 1998), with a reported LOR of 0.2 mg/L.

RESULTS AND DISCUSSION

Occurrence of chemicals in RO permeate

Of the 375 chemicals analysed, 108 (29%) were detected at least once in RO permeate (Table 1), although 42 chemicals were only detected on one occasion, typically at concentrations close to the LOR. Only 29 chemicals (8%) had percentage detection in RO permeate greater than 25% (Table 2, percentage detections for all chemicals in Supporting Information Tables S1-S15). Eight of these

chemicals were classed as disinfection by-products (DBPs), including 7 halogenated DBPs, 1 *N*-nitrosamine, and 1 inorganic DBP, 8 were classed as volatile organic compounds (VOCs), 6 were metals or metalloids, and the remaining were classed as complexing agents, phenols, or miscellaneous organic chemicals. Analysis of these most frequently detected chemicals indicated several factors that contribute to higher frequency of detection in RO permeate, other than poor rejection through RO membranes. Firstly detection in RO permeate may simply indicate that the chemical's occurrence in secondary WW is high relative to the LOR achievable for RO permeate analysis, even when RO rejection is high. High frequency of detection may also be related to factors within the MF/RO treatment process that lead to formation or even addition of some chemicals during treatment, as described below.

Examples of chemicals frequently detected in RO permeate because of high detections in secondary WW include the metals and metaloids (B, Cu, Li, Si, Sr, Zn) and the complexing agents (ethylenediaminetetraacetic acid [EDTA] and nitrilotriacetic acid [NTA]), which were typically measured in secondary WW at concentrations between 8 and 1700 times the LOR. Thus, while RO removal was consistently better than 90% except for boron (Van Buynder *et al.* 2009), frequent detections at low concentrations were still recorded in RO permeate. Metals are sourced from both urban and industrial catchments, and frequent detections in secondary WW are often related to incomplete removal during WW treatment as well as very high concentrations in influent wastewater (Busetti *et al.* 2005; Choubert *et al.* 2011). Complexing agents are also used broadly, poorly removed by conventional WWTPs (Oviedo and Rodriguez 2003), and commonly detected in µg/L-concentrations in secondary WW (Reemtsma *et al.* 2006). Another chemical that was always detected in secondary WW was 1,4-dioxane, which is also widely used and not significantly removed in conventional WWTP (Zenker *et al.* 2003). However, variable RO rejection of 1,4-dioxane (Van Buynder *et al.* 2009) also contributes to the high frequency of detection in RO

permeate. Poor rejection of 1,4-dioxane is predicted because it is a small (molecular weight [MW] = 88 Da), neutral and hydrophilic molecule (Bellona *et al.* 2004).

In contrast to metals and complexing agents, DBPs were often more frequently detected in RO permeate than in secondary WW. As part of the MF/RO process, it is standard practice to chloraminate WW before MF to minimise RO membrane fouling (Bartels *et al.* 2005). Chlorine dosed before MF reacts with ammonia present in, or added to, the WW to form monochloramine, and this process is recognised to cause additional formation of some DBPs (Van Buynder *et al.* 2009; Farre *et al.* 2011). The phenol 2,4-dichlorophenol and the VOC chloromethane, also classed as a DBP (Krasner *et al.* 2006), also showed similar increases in concentration during MF/RO (Van Buynder *et al.* 2009), and chloramination may also play role in the frequent detection of these compounds in RO permeate. Thus for some chemicals, mitigation may be best achieved though treatment optimisation, rather than just relying on removal by RO.

In contrast to the organic DBPs, chlorate is commonly present in concentrated hypochlorite solutions (Bolyard *et al.* 1992). Thus chlorate is effectively added during MF/RO treatment during disinfection. This hypothesis is further supported by comparison of the overall treatment efficiency of the MF/RO for chlorate (76%) to the treatment efficiency for RO alone (95%) (Van Buynder *et al.* 2009). Reverse osmosis removal of anions is expected to be high because they are negatively charged and repelled from the negatively charged RO membrane (Bellona *et al.* 2004). It is noted that chlorite and bromate can also be present in hypochlorite solutions (depending on its purity), although at lower concentrations (Bolyard *et al.* 1992).

Addition during the treatment process is also hypothesised to occur for both acrylonitrile and bisphenol A. The median concentration of acrylonitrile in RO permeate (0.13 µg/L) was higher than that in secondary WW (0.04 µg/L), and percentage detections in RO permeate (83%) were also

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higher than in secondary WW (50%). Acrylonitrile can be used in the production of both RO and MF membranes (Nasef and Hegazy 2004), and therefore it is likely that the concentrations measured in RO permeate are due to acrylonitrile leaching from the treatment membranes. No measurements of acrylonitrile were made at sampling points between the MF and RO membranes and therefore it is not possible to determine whether the elevated concentrations seen in RO permeate compared are from the RO or MF membranes. 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, both membranes are made from composite polyamide and therefore either could cause elevated acrylonitrile concentrations. Further sampling and analysis is required to confirm the source of the elevated concentration, however it is noted that the maximum concentration measured in RO permeate is two orders of magnitude lower than the health value for acrylonitrile and therefore the health risk is still considered low ($RQ_{max} = 0.007$). Despite the fact that RO rejection of bisphenol A is expected to be better than 90% (Wintgens *et al.* 2004; Lee *et al.* 2008), bisphenol A was more frequently detected in RO permeate than secondary WW, and median concentrations were similar. Other studies have also reported frequent detection of bisphenol A in RO permeate at concentrations up to 0.5 μg/L (Al-Rifai *et al.* 2011), although still significantly lower than the health value of 175 μg/L. Bisphenol A is a very common water contaminant at ng/L levels, sourced from polycarbonates and epoxy resins (Tsai 2006) and thus there may be a source within the water treatment process. However, the bisphenol A dataset may also have been influenced by trace bisphenol A contamination during sample collection or analysis, with detections in several field blanks in the first sampling event, which also had the highest number of detections of bisphenol A in RO permeate. Common routes of contamination include the presence of bisphenol A in purified laboratory water and plastic components of laboratory equipment (Watabe *et al.* 2004). Further sampling is therefore required to clarify the trend.

Like the DBPs, six of the eight VOCs that were frequently detected in RO permeate (chloromethane, benzene, toluene, ethylbenzene, p-xylene and carbon disulphide) also increased in concentration during MF/RO treatment (Rodriguez *et al.* 2012). As discussed previously, chloromethane is probably formed during chloramination. In contrast, benzene, toluene, ethylbenzene and p-xylene are all aromatic compounds associated with gasoline or diesel exhaust (Watson *et al.* 2001) or with oil refinery emissions (Scheff and Wadden 1993). KWRP is located on the site of an oil refinery and the increases during MF/RO treatment have been attributed to atmospheric exposure during MF treatment (Rodriguez *et al.* 2012). It is not clear whether increases in carbon disulphide, a volatile solvent with poor rejection, can also be attributed to this exposure as carbon disulphide is not normally associated with petrol-based contamination, and was also evident at BPP, which was not influenced by oil refinery omissions (Rodriguez *et al.* 2012).

In contrast to other frequently detected VOCs, 1,4-dichlorobenzene and tetrachloroethene did not increase in concentration during the treatment process and their frequent detection in RO permeate is again related to high frequencies of detection and concentration (27 and 5 times LOR respectively) in secondary WW, in conjunction with poor rejection by RO membranes. The high frequency of detection of 1,4-dichlorobenzene in secondary WW is attributed to its long history of domestic use in toilet products, moth repellents, and mildew control agents (NICNAS 2000), and relatively low biodegradation rates in WW treatment (Koe and Shen 1997). Tetrachloroethene is widely used for dry cleaning of fabrics, and its presence in WW input has been linked previously to dry cleaning activities (Rule *et al.* 2006). As relatively small (MW = 147 Da 1,4-dichlorobenzene, 165 Da tetrachloroethene) and uncharged molecules, with relatively high octanol-water partitioning coefficient ($logK_{ow}$ is 3.4 for both chemicals), RO rejection is expected to be poor to moderate (Bellona *et al.* 2004).

Screening health risk assessment of RO permeate

The screening health risk assessment for RO permeate assessed RQs for the 11 chemical classes summarised in Supporting Information Tables S1-S13, plus previously reported risk assessments for dioxins-like chemicals, PAHs, and gross alpha and gross beta particle activity (Rodriguez *et al.* 2008; Van Buynder *et al.* 2009). Table 3 lists all chemicals (16) for which RQ_{med} or RQ_{max} were calculated to be greater than 1. Eight of these chemicals (1 pesticide, 4 DBPs, 1 hormone, and 1 complexing agent) were never detected but had lower health values than average LOR. In many cases, these health values were very conservative and therefore elevated RQs do not necessarily constitute a health issue. For example, health values for the haloacetic acids (HAAs) and complexing agents were developed using the conservative TTC approach, all assigned a health value of 0.7 µg/L. In comparison, health values for regulated HAAs range from 60 µg/L (dibromoacetic acid & bromoacetic acid) to 150 µg/L (chloroacetic acid), while health values for the regulated complexing agents EDTA and NTA are 200 and 250 µg/L respectively. It may be expected that unregulated chemicals would have comparable toxic effects to regulated chemicals, based on similar chemical structures. For example, EDTA, NTA, diethylenetriaminepentaacetic acid (DPTA) and propylenediaminetetraacetic acid (PDTA) had no pronounced difference in impact on aquatic organisms when tested as the same metal complex (Schmidt and Brauch 2004). A less conservative approach to determine health values for these chemicals could be to divide the health value of the lowest regulated chemical (i.e. dibromoacetic acid for the HAAs, EDTA for complexing agents) by a safety factor of 10. Using this approach, all RQs would be less than 1 except for tribromoacetic acid, which had the highest LOR of all tested chemicals (29 µg/L).

While 17α-ethinylestradiol does have a health-based guideline, the anticipated health risk from ethinyl estradiol is also considered low. 17α-ethinylestradiol is a large molecule (MW = 296 Da) that was never detected in secondary WW (LOR = 8.0 ng/L). It can be calculated that 17α-ethinylestradiol will always be below 1.5 ng/L in post-RO water when RO removal efficiency > 81%. The RO removal of 17α-ethinylestradiol is expected to be similar to that observed for estrone

(96.5%) (Van Buynder *et al.* 2009). Similarly the pesticide thiophanate-methyl was never detected in either secondary wastewater or post-RO water (LOR = 5.75 µg/L for both), and therefore the actual risk quotient for post-RO water will be below 1 assuming RO removal efficiency >13%, which is expected given MW = 342 Da.

Nine chemicals that were detected on at least one occasion in RO permeate also reported a RQ >1, although assessment of these chemicals also indicates there is a low health risk, except from a few selected *N*-Nitrosamines. Only two detected chemicals had $RQ_{med} > 1$; the complexing agent DTPA and the DBP dibromochloroacetic acid, which were both detected once only at a concentration lower than the average LOR. Additionally, health values for both chemicals were determined using the TTC approach, as previously discussed. Seven other DBPs (5 *N*-nitrosamines, and 2 haloacetaldehydes) had $RQ_{med} < 1$, but $RQ_{max} > 1$, although only *N*-nitrosodimethylamine (NDMA), *N*-nitrosodi-n-propylamine (NDPA) and *N*-nitrosodi-n-bultyldiamine (NDBA) were actually detected on more than one occasion. In the context of indirect potable re-use via groundwater replenishment, it is anticipated that maximum concentrations will be smoothed by groundwater storage over months to years. The only chemical detected in more than 25% of samples with a RQ > 1 is NDMA (Table 2). However, again, the median concentration of NDMA in RO permeate was below the conservative health value of 10 ng/L, and all detections were below 100 ng/L, which is the guideline for NDMA included in the most recent revision of the Australian Drinking Water Guidelines (ADWG 2011). Fristachi and Rice (2007) have estimated that the proportional oral intake of NDMA attributable to the consumption of drinking water relative to other exogenous sources, such as beer or food, is 2.7%, and only 0.02% when endogenous sources (created in the digestive system) are also included. Thus the majority of NDMA exposure for humans is not from drinking water consumption and the increased risk to human health from NDMA provided by drinking water consumption is still relatively minor. Our assessment of a low health risk from RO-treated WW agrees with other assessments undertaken using multiple bioassays (Van Buynder *et al.*

2009; Escher *et al.* 2011; Macova *et al.* 2011). In particular, screening undertaken during the project using a cytokinesis-block micronucleus assay with a human lymphoblastoid cell-line indicated that there was no significant cytotoxicity or genotoxicity for either secondary wastewater or RO-treated wastewater (Van Buynder *et al.* 2009), and the toxicity of RO-treated WW has been shown to be indistinguishable from that of drinking water (Escher *et al.* 2011).

Anthropogenic chemicals in RO permeate DOC

The median DOC concentration measured in RO permeate was 0.2 mg/L, with a maximum detected concentration of 0.6 mg/L, and 10 out of 14 measurements (64%) above or at the LOR (0.2 mg/L). However, similar concentrations of DOC were frequently measured in both field and trip blanks, both with 53% detections. Analysis of DOC by high-temperature combustion is known to be limited by detections in blank samples, and is also typically less sensitive than methods using persulphate oxidation (Clesceri *et al.* 1998). However, the DOC concentrations measured in this study are similar to other reported values for RO treated secondary WW, which range from 0.3 to 0.8 mg/L (Fujita *et al.* 1996; Drewes *et al.* 2003; Henderson *et al.* 2010). More recently online DOC measurements at a water recycling plant utilising ultrafiltration and RO, also using secondary WW from Beenyup WWTP have been found to be less than 0.05 mg/L (Pers. Comm. Water Corporation of Western Australia 2012). Online measurements will be less impacted by DOC contamination during sampling and so it is anticipated that the calculated median of 0.2 mg/L represents an upper boundary, with the actual DOC concentration of the RO permeate likely to be closer to 0.05 mg/L.

Ninety-two per cent (344/375) of the chemicals monitored in this study incorporate one or more carbon atoms in their molecular structure and therefore could potentially contribute to DOC, the exceptions being inorganic anions and metals. Estimates of DOC contribution for all chemicals are reported in Tables S3-S15 (Supporting Information), while Table 4 summarises the overall contribution of the chemicals measured to DOC. If we consider all chemicals, regardless of whether

they were ever detected in RO permeate, the sum of median DOC contributions (DOC_{med}) was 45.8 µg/L and the sum of maximum DOC contributions (DOC_{max}) was 94.8 µg/L. However, assessment of DOC contributions of individual chemicals indicated that over 70% of this estimate (33.6 µg/L DOC_{med} or 67.2 µg/L DOC_{max}) is contributed by 16 chemicals that were never detected in RO permeate (13 pesticides, tribromoacetic acid, chloroacetic acid and PDTA), but have LORs greater than 2.5 µg/L. Recalculation of the DOC without these chemicals was much lower, 12.3 µg/L DOC_{med}, or 27.6 µg/L DOC_{max}. Furthermore, the sum of DOC is reduced even further (DOC_{med} = 3.7 µg/L and DOC_{max} = 10.4 µg/L) when only chemicals actually detected in RO permeate are included, with chemicals detected in more than 25% samples (Table 2) contributing between 19-30% of this value (DOC_{med} = 0.7 µg/L and DOC_{max} = 3.1 µg/L).

Calculation of the contribution of anthropogenic chemicals to RO permeate DOC is heavily influenced by analytical LOR. However, our calculations of DOC contribution from all detected chemicals (3.7-10.4 µg/L) is less than half than the expected concentration of DOC in RO permeate. Given that low molecular weight acids and neutrals have been found to make up to 50% of residual DOC in RO permeate (Drewes *et al.* 2003), it is likely that some DOC can be attributed to the many individual chemicals that are below detection or that were not analysed.

CONCLUSIONS

Our assessment of 375 chemicals in RO-treated WW demonstrates a high degree of safety in its use as a source of potable water. The most frequently detected chemicals in RO permeate included DBPs, VOCs and complexing agents, which is in contrast to many monitoring programs that frequently focus on pharmaceuticals, personal care products and hormones. Infrequent detection and low risk assessment of these more frequently monitored chemicals is attributed to secondary WW concentrations that are typically well below health values, and high rejection by RO membranes, often as a result of size exclusion (Bellona *et al.* 2004; Busetti *et al.* 2009).

While RO membranes are the key chemical removal barrier, our results suggest that poor rejection by RO membranes is not always the main reason for frequent detection in RO permeate. Instead, a high volume of use, such that concentrations are high in secondary WW relative to LOR (e.g., complexing agents), and the potential for chemicals to form (e.g. DBPs) or be added (e.g. acrylamide or bisphenol A) during the treatment process were more influential. These findings provide guidance to water industry practitioners to identify the most appropriate chemical suite for monitoring, as well as provide avenues to ensure further reduction of chemical detections in RO-treated WW. In particular, source protection via regulation of chemicals in primary wastewater could help to reduce frequent detections of some chemicals.

It is noted that frequent detection in RO permeate does not equate to a health risk, with $RQ_{max} < 0.1$ for all chemicals detected in $> 25\%$ of RO permeate samples except for NDMA ($RQ_{max} = 3$), dibromomethane (0.7), and benzene (0.14). Only *N*-nitrosamines, including NDMA, are considered of potential concern in RO permeate, with a few infrequent detections of NDMA, NDBA, NDPA and NMOR above health guidelines. While median concentrations were always below health values, further research and monitoring of *N*-nitrosamines including NDMA is recommended to understand their formation and degradation and further assess health risks. It is anticipated that appropriate WW treatment, optimisation of chloramine disinfection practices, or addition of ultraviolet irradiation or advanced oxidation treatment could all provide additional barriers to ensure *N*-nitrosamine concentrations are always below health values. There remain ongoing limitations in the health risk assessment of recycled water, particularly when the LOR of currently available analytical methods are not able to reach health guidelines. More fundamentally, a lack of toxicological data for some chemicals means that health guidelines must be estimated by conservative processes such as the TTC concept. In this case, toxicological assessment via

bioanalytical tools may provide additional confidence that there is low risk from use of RO-treated WW as a potable water source.

Assessment of the contribution of anthropogenic chemicals to residual DOC in RO permeate suggests that it is a minor component of DOC in RO permeate. Only between 3.7 and 10.7 μg/L could be attributed from chemicals detected on at least one occasion, with the majority of this total attributed to chemicals detected in less than 25% of samples. The chemicals monitored in this study were chosen based on health risk, rather than abundance in secondary WW, and it is possible that some high concentration but low toxicity anthropogenic chemicals further contribute. While chemicals below detection may contribute a significant component of DOC, it is likely that natural organic matter and soluble microbial products still contribute the majority of DOC to RO permeate. A more accurate assessment of the contribution of anthropogenic chemicals to RO permeate DOC would require additional fractionation of RO DOC before analysis, and this would be technically challenging and time consuming. While natural organic matter and soluble microbial products are unlikely to have inherent health risks, they can provide a source for DBP formation. The frequent detection of DBPs in RO permeate is likely to have occurred because of the reaction of chloramine with this fraction of WW organic matter.

Table 1: The 13 chemical classes tested, and the number of chemicals detected at least once in RO-treated wastewater. Information about individual chemicals is provided in the Supplementary Information, Tables S1-S15, available online at: .

Chemical Class	Number of chemicals	Detected in RO-treated wastewater
Metals and metalloids	28	12
Pesticides	129	1
Halogenated disinfection by-products	32	24
N-nitrosamines	9	8
Volatile organic compounds	57	25
Polyaromatic hydrocarbons	17	11
Polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like poly chlorinated biphenyls	29	4
Phenols	16	8
Pharmaceuticals	36	3
Anions	3	1
Estrogenic hormones	4	0
Complexing agents	4	3
Miscellaneous	11	8
Total	375	108

Table 2: Chemicals detected in more than 25% of all RO-treated wastewater samples, corresponding data for secondary wastewater, and a screening health risk assessment for RO-treated wastewater using median and maximum risk quotients (RQ_{med} and RQ_{max}). $n° =$ total number of samples analysed; nd = not detected.

Chemical	n°	RO-treated WW % Detects	RO-treated WW Median (µg/L)	n°	Secondary WW % Detects	Secondary WW Median (µg/L)	RQ_{med}	RQ_{max}
Bromochloromethane	27	100	0.11	33	94	0.22	0.003	0.009
Dibromomethane	27	96	0.13	33	94	0.26	0.2	0.7
NDMA	27	93	0.004	25	96	0.016	0.4	3
Boron	28	89	75	31	100	160	0.02	0.04
1,4-Dichlorobenzene	27	89	0.19	33	94	0.81	0.005	0.02
Acrylonitrile	6	83	0.13	6	50	0.04	0.004	0.007
Silicon	21	76	120	22	100	8800	0.0003	0.0007
Lithium	28	75	0.2	31	100	7.6	0.003	0.07
Bromodichloromethane	27	70	0.04	33	82	0.06	0.001	0.007
Chloromethane	27	63	0.09	33	58	0.12	0.006	0.03
Chloroform	27	56	0.14	33	85	0.36	0.001	0.004
Strontium	28	54	0.4	31	100	170	0.0001	0.0005
EDTA	27	48	0.5	27	100	145	0.002	0.01
2,4-Dichlorophenol	21	48	0.01	23	48	0.013	0.00005	0.0003
Copper	28	46	0.2	31	100	7.4	0.0001	0.0008
Chlorate	24	46	12.7	30	37	12.8	0.02	0.07
Dichloroacetonitrile	24	46	0.05	28	11	0.02	0.03	0.25
Carbon disulfide	15	40	0.02	16	81	0.02	0.00002	0.0002
Toluene	27	37	0.13	33	15	0.13	0.0002	0.002
Zinc	28	36	5.0	31	100	55	0.002	0.004
NTA	27	33	0.13	27	100	2	0.001	0.005
p-Xylene	27	33	0.13	33	0	nd	0.0002	0.0004
Bisphenol A	20	30	0.01	19	17	0.012	0.00007	0.0002
Benzene	27	30	0.08	33	24	0.076	0.08	0.14
Tetrachloroethene	27	30	0.17	33	88	0.49	0.003	0.006
Dibromochloromethane	27	30	0.09	33	76	0.19	0.001	0.005
1,4-Dioxane	21	29	0.12	22	100	0.51	0.002	0.01
Ethyl benzene	27	26	0.07	33	0	nd	0.0002	0.0005
Bromoform	27	26	0.13	33	76	0.15	0.001	0.003

Table 3: Chemicals with a calculated risk quotient (RQ) greater than 1 in RO-treated wastewater, ordered by percentage detection. Chemicals with health guidelines estimated by the threshold of toxicological concern are denoted by TTC. n° = total number of samples analysed; LOR = limit of reporting; n/a = not available.

Chemical	Health value (µg/L)	Average LOR (µg/L)	n	% Detects	RQ_{med}	RQ_{max}
NDMA	0.01 (AGWR 2008)	0.001	27	93	0.4	3.0
NDPA	0.005 (Cal DPH 2007)	0.002	27	22	0.3	1.4
NDBA	0.006 (IRIS 2011)	0.004	27	11	0.2	2.1
Bromodichloroacetaldehyde	0.7 (TTC)	0.28	24	4	0.29	1.43
Dibromoacetaldehyde	0.7 (TTC)	0.24	24	4	0.26	1.26
Dibromochloroacetic acid	0.7 (TTC)	2.3	27	4	2.6	10
NPIP	0.004 (OEHAA 2009)	0.002	27	4	0.3	1.5
NMOR	0.005 (OEHAA 2009)	0.004	27	4	0.4	2.2
DTPA	0.7 (TTC)	2.3	27	4	3.1	4.6
Thiophanate-methyl	5 (ADWG 2011)	5.75	17	0	1.15	n/a
Bromochloroacetic acid	0.7 (TTC)	1.0	27	0	1.5	n/a
Dichlorobromoacetic acid	0.7 (TTC)	1.9	27	0	2.7	n/a
Tribromoacetic acid	0.7 (TTC)	29.0	27	0	41	n/a
NEMA	0.002 (IRIS 2011)	0.002	27	0	1.0	n/a
17α-Ethinylestradiol	0.0015 (AGWR 2008)	0.002	29	0	1.40	n/a
PDTA	0.7 (TTC)	4.4	27	0	6.2	n/a

Table 4: Estimates of DOC contribution from anthropogenic chemicals measured in RO-treated wastewater. Over 70% of total DOC from all chemicals (**A**) was contributed by 16 chemicals that were never detected but with LOR greater than 2.5 µg/L (**B**). Recalculation of total DOC without these chemicals was much lower (**C**). Total DOC was also calculated for all chemicals detected in RO permeate (**D**) and for the most frequently detected chemicals (**E**), as listed in Table 2.

	Number of chemicals	DOC_{med} (µg/L)	DOC_{max} (µg/L)
A. All chemicals	344	45.8	94.8
B. Undetected chemicals with LOR > 2.5 µg/L	16	33.6	67.2
C. All chemicals except undetected chemicals with LOR > 2.5 µg/L	328	12.3	27.6
D. All detected chemicals	95	3.7	10.4
E. Chemicals detected in >25% samples	22	0.7	3.1

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