Wastewater Reclamation using Ozonation combined with Biological Activated Carbon Filtration

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The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South-East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South-East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Description: Advanced treatment train at Gerringong Gerroa (NSW) wastewater treatment plant.
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FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.

Chris Davis
Chair, Urban Water Security Research Alliance

CONTENTS

Acknowledgements i

Foreword ii

Executive Summary 1

1. Introduction 4

1.1. Water Reuse: a Sustainable Solution to Water Scarcity 4
1.2. Alternative Treatment Trains are needed to Promote Potable Reuse 4
1.3. Chemical Water Quality of Reclaimed Water for Potable Reuse is of Paramount Importance 4
1.4. Ozonation and Biological Activated Carbon Filtration: a Combination to Produce High Quality Reclaimed Water 5
1.5. Bioanalytical Tools: a New Way to Assess Water Quality 5
1.6. Objectives of the Enhanced Treatment Project 5

2. Chemical Water Quality across South Caboolture Water Reclamation Plant 6

2.1. South Caboolture Water Reclamation Plant 6
2.2. Fate of Organic Micropollutants 6
2.2.1. The Challenge of Organic Micropollutants in Indirect Potable Reuse 6
2.2.2. Sampling and Organic Micropollutants Quantification 8
2.2.3. Results and Discussion 8
2.3. Toxicity Assessment with Bioanalytical Tools 13
2.3.1. Bioanalytical Tools for Water Quality Assessment 14
2.3.2. Sampling and Bioanalytical Tool Methods 15
2.3.3. Results and Discussion 15
2.4. Comparison of Chemical Analysis and Bioanalytical Tools 20
2.4.1. Effect of Treatment Processes 20
2.4.2. Non-Specific Toxicity: Baseline-TEQ_{bio} and Baseline-TEQ_{chem} 21
2.4.3. Estrogenicity 21
2.4.4. Phytotoxicity 22
2.5. Fate of Disinfection By-Product Precursors 22
2.5.1. Relevance of Disinfection By-Product Precursors in Wastewater Reuse 22
2.5.2. Sampling Strategy, DBP Formation Potential Tests and Quantification 23
2.5.3. Results and Discussion 23
2.6. Final Water Quality: Indirect Potable Reuse Considerations 26
2.6.1. Organic Micropollutants 26
2.6.2. Toxicity 26
2.6.3. Disinfection By-Products 26

3. Comparison of Three Full Scale Reclamation Plants 28

3.1. Reclamation Plants Sampled 28
3.1.1. Sampling Strategy 28
3.2. Analytical Methods 29
3.2.1. Organic Micropollutants 29
3.2.2. Bioanalytical Tools 30
3.3. Water Quality before Ozonation 30
3.4. Ozonation 30
3.4.1. Dissolved Organic Carbon 31
3.4.2. Fate of Organic Micropollutants 31
3.4.3. Estrogenicity 33
3.4.4. Non-Specific Toxicity 33
3.5. Biological Activated Carbon 34

3.5.1. Dissolved Organic Carbon ... 34
3.5.2. Fate of Organic Micropollutants ... 35
3.5.3. Estrogenicity ... 37
3.5.4. Non-Specific Toxicity ... 37

4. Conclusion and Recommendations ... 38

4.1. Ozonation followed with BAC Filtration: an Effective Combination for Wastewater Reclamation ... 38
4.2. Bioanalytical Tools for Water Quality Analysis: a Complement to Chemical Analysis ... 38

Appendix 1. QHFSS Organic Micropollutants Analytical Method ... 40

Appendix 2. List of QHFSS Compounds and Properties ... 44

Appendix 3. AWMC Organic Micropollutants Analytical Method ... 47

Appendix 4. List of AWMC Compounds and their Properties ... 50

Appendix 5. Organic Micropollutant Concentration Ranges in Full Scale Reclamation Plants ... 51

Appendix 6. Reactivity of Selected Organic Micropollutants with Ozone and Hydroxyl Radicals and Removal in Treated Effluents ... 53

Glossary ... 57

Publications ... 58

References ... 59

LIST OF FIGURES

Figure 1. South Caboolture Water Reclamation plant treatment train with sampling points (S1 to S7). Contact time in BAC filtration is empty bed contact time. 7
Figure 2. Number of compounds quantified and DOC after indicated stage along the treatment train. Bars represent the number of compounds with a median concentration above the limit of quantification (four samples). Dots represent DOC on two different sampling days. 9
Figure 3. Median relative concentrations of selected compounds (median of influent concentration > 0.10 g L-1) after indicated treatment stages (error bars represent maximum and minimum values). C is the concentration after the specified treatment step and the reference concentration, C0 is the concentration in WWTP effluent. 11
Figure 4. Median removal of selected compounds (median of influent concentration > 0.10 µg L-1) by the main ozonation stage and the combination of the main ozonation and the BAC filtration stages. Error bars represent minimum and maximum removal, no error bar means that the compound was below LOQ after treatment; therefore removal was calculated as a minimum using the LOQ. CS4: concentration before main ozonation; CS5: concentration after main ozonation; CS6: concentration after BAC filtration. 12
Figure 5. Relative response of the bioassays and relative DOC after indicated stage along the treatment train compared to the WWTP effluent. Bars are the median of 4 values for bioassays and error bar represent maximum and minimum. Dots are the average of 2 values for DOC and error bars represent maximum and minimum. 16
Figure 6. Bar charts correspond to NDMA precursors measured by NDMA formation potential test (FP) across South Caboolture Water Reclamation Plant. Error bars correspond to the standard deviation (n=2). Dot points correspond to the cumulative removal percentage of NDMA precursors relative to the WWTP effluent across the plant. 24
Figure 7. THMs (left) and HAAS (right) precursors and DOC across South Caboolture Water Reclamation Plant, error bars correspond to standard deviation (n=3). 25
Figure 8. Treatment trains of the three investigated full scale reclamation plants, the dots indicate the sampling points. Ozonation: number in brackets is ozone dose relative to DOC. BAC: number in brackets is EBCT. EP=equivalent people; MF = microfiltration. 29
Figure 9. Removal of dissolved organic carbon (DOC), baseline-toxicity equivalent concentrations (baseline-TEQbio and baseline-TEQchem) and estradiol equivalent concentration (EEQ) in the reclamation plants. The hatched sections represent removal by ozonation only and the full bars represent total removal achieved after ozonation and BAC filtration (average of 3 independent samples ± standard deviation). 31
Figure 10. Removal of selected OMPs by ozonation (average of 3 independent samples ± standard deviation). 32
Figure 11. Comparison of the removal of OMPs by ozonation in reclamation plants, mgO3 mgDOC-1 indicated in brackets (average of 3 independent values ± standard deviation). 32
Figure 12. Removal of selected OMPs by BAC filtration, empty bed contact time is indicated in the legend (average of 3 independent values ± standard deviation). No bar means a removal could not be calculated because concentrations were either too low or below the LOQ. Letters in brackets indicate removal generally observed in WWTP estimated from Onesios et al. (2009): P=poor (<20%); I=intermediate (20-80%); G=good (>80%). 36
Figure 13. Comparison of the removal of organic micropollutants in BAC filters in reclamation plants (average of 3 independent values ± standard deviation). 36

LIST OF TABLES

Table 1. Selected compounds, classification, hydrophobicity expressed as logarithm of octanol-water partition coefficient (log Kow), limit of quantification (LOQ) by LC/MS-MS analysis, influent concentrations to the water reclamation plant and guideline values from the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies. 10
Table 2. DOC removal and fate of 25 selected compounds (initial concentration > 0.01 µg L-1) in each stage of the treatment train. 13
Table 3. Description of the bioassays used 15
Table 4. Maximum, median and minimum biological activity of the water entering (WWTP effluent) and leaving the reclamation treatment (final ozonation) and overall maximum, median and minimum decrease observed through the reclamation plant. 16
Table 5. Summary of reduction of DOC, selected compounds' concentrations and toxic levels observed in each treatment stage. 20
Table 6. Fraction of the observed baseline-TEQbio explained by chemical analysis. 21
Table 7. DBPs formation potential after BAC filtration (after final ozonation for NDMA). 27
Table 8. Water quality parameters before the ozonation stage in reclamation plants (N/D = not determined). 30
Table 9. Baseline-TEQbio, baseline-TEQchem and estrogenicity. 33
Table 10. Long term mean recovery of surrogate chemicals from samples and standard deviation. 40
Table 11. Recoveries, liquid chromatography retention times and MS/MS parameters. 41
Table 12. Compounds quantified, classification, hydrophobicity expressed as logarithm of octanol-water partition coefficient (log Kow), limit of quantification (LOQ) by LC/MS-MS analysis, influent concentrations to the water reclamation plant and guideline values from the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies. 44
Table 13. HPLC eluents composition and gradients (total flow rate = 1 mL min-1). 47
Table 14. QLIT-MS source parameters. 48
Table 15. Target compounds, retention times and optimized QLIT-MS parameters (Rt = retention time; DP = declustering potential; Q1 = parent ion; Q3quant = fragment ion used for quantitation; Q3conf = fragment ion used for confirmation; CE = collision energy; CXP = collision cell exit potential). 48
Table 16. Physico-chemical properties and relative potency of the compounds in the bioluminescence inhibition test with Vibrio fischeri (in relation to a reference virtual baseline toxicant); removal generally observed in full scale WWTP (P=poor, <20%; I=intermediate, 20-80%; G=good, >80%). NA = not applicable. NAv = not available. 50
Table 17. Organic micropollutant concentration ranges before ozonation (ng L-1). OoR= out of calibration range; N/Q=not quantified (due to interferences). 51
Table 18. Organic micropollutant concentration ranges after biological activated carbon (ng L-1). 52
Table 19. Reactivity of selected OMPs with ozone and hydroxyl radicals and removal in treated effluents. 53

EXECUTIVE SUMMARY

The recent millennium drought that hit Australia highlighted the need for a change in water resource management because of increasing pressure on drinking water sources due to factors such as population growth, climate change impacts and pollution. Wastewater reclamation and reuse appear to be sustainable ways to reduce water extraction from surface and underground fresh water bodies. Indirect potable reuse, in particular, allows augmenting drinking water supplies by replenishing reservoirs. This requires the production of very high quality reclaimed water with a high degree of safety. The inactivation of pathogens is of paramount importance as well as chemical contaminant removal. Treated effluents contain residual organic matter which is a complex matrix composed of natural organic matter and thousands of organic micropollutants (OMPs) such as pharmaceuticals and their human metabolites, personal care products, pesticides, and industrial chemicals, as well as their biodegradation products. OMPs are typically present at sub $\mu g\ L^{-1}$ levels but there is concern about the long term exposure effects on human health. Effluent organic matter (EfOM) can also lead to the formation of disinfection by-products (DBPs) during the final addition of chlorine or chloramine provided to prevent bacterial growth in the distribution system. DBP exposure has been associated with some forms of cancer.

Most of the indirect potable reuse schemes in the world, such as the Western Corridor Recycled Water Scheme in South East Queensland (SEQ), use a multiple barrier approach to contaminants in which reverse osmosis is a key component. Indeed, reverse osmosis is an almost universal barrier to pathogens and chemicals also allowing salt removal. However, reverse osmosis is very energy intensive and concentrates the contaminants in a waste stream representing up to 20% of the total volume of water treated. This waste stream contains high levels of salts and chemical contaminants and is therefore difficult to manage and dispose of. The current solution is to discharge it into the sea but this is not suitable for inland locations. There is therefore a need to develop alternative treatment trains with no or low production of waste stream to promote indirect potable reuse as an alternative water supply option.

Inactivation of pathogens can be achieved with disinfection processes that do not produce waste; such as ozonation, chlorination and UV and was therefore out of the scope of this project which focused on EfOM removal and particularly OMPs. Oxidation with ozone and activated carbon adsorption have been shown to effectively remove organic micropollutants, but have some limitations. Ozonation leads to the formation of transformation products which are still mostly unknown. Activated carbon has a limited adsorption capacity and needs to be regularly renewed or regenerated. Biological activated carbon (BAC) is activated carbon that is neither renewed nor regenerated, which gives a bacterial community the opportunity to establish in the filter. While less effective than activated carbon adsorption, BAC filtration relies on biodegradation and can therefore operate for years without the need to replace the media. BAC filtration has been used for many years in drinking water treatment, typically after ozonation, for the removal or natural organic matter and pesticides. Ozonation typically leads to the formation of products that are more degradable than the parent compounds, increasing the efficiency of the BAC filters. Although it seems to have great potential for the removal of organic contaminants from treated effluents, there has been little investigation of the combination of ozonation and BAC filtration in the context of water reclamation and reuse.

The aim of the enhanced treatment project was to assess the potential of ozonation and BAC filtration as a barrier to organic contaminants in wastewater reclamation and, in particular, OMPs. The project used conventional chemical analysis and innovative bioanalytical tools to determine water quality and treatment effectiveness. These tools are in vitro bioassays measuring the non-specific or specific toxicity of samples. While *in vitro* chemical analysis allows quantifying of a limited number of contaminants, bioanalytical tools take into account the whole matrix of OMPs and can therefore provide useful information on unknown compounds. The project investigated three Australian full scale water reclamation plants using ozonation and BAC filtration. First, the entire treatment train of one reclamation plant was studied to identify key treatment processes for the removal of EfOM, OMPs

and the reduction of toxicity. Then, the ozone and BAC filtration combination was compared with the other two plants. In this context, the objectives of the enhanced treatment project were to:

- Evaluate the chemical quality of treated effluent using chemical analysis and *in vitro* bioassays to quantify OMPs;
- Quantify the improvement of chemical water quality achieved by the combination of ozonation and BAC filtration as a barrier to EfOM, OMPs and DBP precursors;
- Identify additional treatment processes that have an impact on contaminant removal;
- Identify key operational parameters affecting ozonation and BAC filtration;
- Evaluate the suitability of the reclaimed water for indirect potable reuse in the Australian context;
- Determine whether chemical analysis and *in vitro* bioassays are complementary or redundant tools for the assessment of water quality and treatment processes.

The treatment train of the first reclamation plant investigated (Caboolture) consists of biological denitrification, pre-ozonation, coagulation-flocculation followed by dissolved air flotation and sand filtration (DAFF), main ozonation, BAC filtration and final ozonation for disinfection. The EfOM (as measured by dissolved organic carbon) is removed mainly by the coagulation-flocculation followed by DAFF (40-50%) and the BAC filtration (20-30%). As expected, ozonation does not lead to any significant EfOM removal confirming that it leads to the production of transformation products. The overall removal of EfOM across the plant is approximately 60%. Out of 85 targeted compounds, 54 are detected in the treated effluent entering the reclamation plant.

The main ozonation and BAC filtration are the key processes responsible for OMP removal. The main ozonation reduces the concentration of selected OMPs by 55% to more than 95% depending on their chemical structure. BAC filtration further reduces the concentration of OMPs by up to 90%. This result is surprising as some of these OMPs are known to be refractory to biodegradation. Since the activated carbon have been renewed only a few month before the sample collection, it is possible that adsorption is responsible. Combined, both processes lead to more than 90% removal of the selected OMPs leading to final concentrations orders of magnitude lower than guideline values proposed in the Australian Water Recycling Guidelines for Drinking Water Augmentation. While not playing a direct role in OMP removal, the coagulation-flocculation followed by DAFF increases the ozonation efficiency by allowing a higher ozone dose to dissolved organic carbon ratio. This ratio is a key parameter as the preliminary ozonation, with a much lower ratio, does not lead to OMP removal.

Bioanalytical tools demonstrate that the treatment train reduces the non-specific and specific toxicity of the samples by 60% to almost 100%, depending on the bioassay considered. The key treatment processes are again the coagulation-flocculation followed by DAFF, the main ozonation and the BAC filtration. Toxicity levels of the reclaimed water are equivalent or close to blank levels. The bioassays show that ozonation does not lead to any increase in toxicity, demonstrating that the mixture of transformation products is less toxic than the mixture of parent compounds. Finally, the removal of DBP precursors by ozonation and BAC filtration was assessed using formation potential tests for nitrosamines (in particular N-nitrosodimethylamine or NDMA), trihalomethanes (THMs) and haloacetic acids (HAAs). Ozonation removed 65% of NDMA precursors but had no effect on THMs and HAAs precursors. On the contrary, BAC filtration removed approximately 30% of THM and 35% of HAA precursors while further removing 40% of NDMA.

The second part of the project compared three full scale reclamation plants using ozonation and BAC filtration. The plants use various ozone doses in the ranges of 0.6-0.8; 0.2-0.3 and 0.4-0.5 mg_{O3} mg_{DOC}^{-1} for Caboolture, Landsborough and Gerringong respectively. They also use different empty bed contact times in the BAC filters: 18, 9 and 45 minutes respectively. This allowed assessment of the influence of these parameters on EfOM and OMP removal. The water quality before ozonation is very similar in the three plants in terms of dissolved organic carbon, nutrients, OMP concentrations, non-specific toxicity and estrogenicity. Out of 41 targeted OMPs, 35 are detected in all three plants,

showing the ubiquitous presence of these compounds in treated effluents. The dissolved organic carbon measurement shows no removal of EfOM in the ozonation stage. The removal in the BAC filters ranges from 20% to 50%, increasing with the empty bed contact time, which is consistent with a biological process.

The fate of OMPs in ozonation shows they can be divided in two groups. In the first group, compounds are well removed by ozonation (>90%) regardless of the ozone dose. In this group are compounds that have been shown to be very reactive with ozone. In the second group, the removal of OMPs is lower and depends on the ozone dose. Among these OMPs are compounds that have been shown to be refractory to ozone and are mainly oxidised by hydroxyl radicals. Ozone dose is therefore a key process parameter and a sufficiently high ozone dose is required to allow for the removal of refractory compounds. BAC filtration further removes the remaining OMPs by up to 99%, the removal increases when empty contact time increases from 9 to 18 minute but no significant difference is observed when increasing to 45 minutes. As the BAC filters have been in use for several years and filtered tens of thousands of bed volume at the time of sampling, it is thought that adsorption capacity is exhausted. However, some OMPs known to be refractory to biodegradation are well removed, indicating there could be some remaining adsorption capacity for traces of pollutants. The fate of OMPs in BAC filters is currently under further investigation in the laboratory to elucidate the removal mechanisms. The overall removal of OMPs is exceeds 90% for most compounds at Caboolture and Gerringong, but it varies from 40% to 99% at Landsborough, which has the lowest ozone dose and empty bed contact time. This highlights the importance of both parameters to achieve effective removal of OMPs.

Non-specific toxicity was measured across the plants and is reduced by 30 to 40%, but no clear trend related to the ozone dose can be observed. This confirms that the mixture of transformation products is less toxic than the mixture of parent compounds. The fact that toxicity reduction does not depend on the ozone dose indicates that it is partially caused by compounds that are very reactive with ozone. BAC filtration further reduced toxicity by 30 to 50%. As was found for OMPs, this increased from 9 to 18 minutes empty bed contact time but not from 18 to 45 minutes. Finally, estrogenicity is reduced by more than 90% after ozonation in all three plants. Estrogenic compounds are typically reactive with ozone and their transformation products lose their potential. The levels are so low after ozonation that the effect of BAC could not be estimated; but more reduction is observed down to levels below the limit of quantification of the bioassay.

The enhanced treatment project demonstrated that the combination of ozonation and BAC filtration is an effective barrier to EfOM, OMPs and DBP precursors in treated effluents. It also reduces non-specific and specific toxicity as measured by bioanalytical tools. The ozone dose and the empty bed contact time are key parameters controlling the process effectiveness. Therefore, this treatment option could be implemented to reduce the environmental impact of treated effluent discharge or it could be integrated in a multiple barrier treatment train to produce water suitable for indirect potable reuse. However, further consideration needs to be given to risk assessment and control. Also, this treatment does not remove dissolved solids, which may be required in some cases and would require additional treatments to be employed. The use of both chemical analysis and bioanalytical tools proved to bring complementary information and allow further insight in treatment processes efficiency and water quality. Particularly, bioanalytical tools allow assessment of the relevance and fate of oxidation transformation products. Also, when non-specific toxicity levels observed in the bioassay are compared with toxicity levels calculated from chemical analysis, it shows that the quantified compounds account for less than 1% of the toxicity measured, demonstrating the importance of non-targeted compounds in the mixture. To date, bioanalytical tools are essentially used for research purposes but they have a great potential to become conventional monitoring tools and efforts should be pursued to consolidate their use.

1. INTRODUCTION

1.1. Water Reuse: a Sustainable Solution to Water Scarcity

The ever increasing pressure on drinking water sources due to factors such as population growth, climate change impacts and pollution calls for a shift in water sources management. In Australia, the millennium drought recently highlighted the limits of surface water resources and the need for new solutions. South East Queensland (SEQ) was particularly affected, with dam levels falling to less than 20% in 2007. Water reclamation has emerged in recent decades as a sustainable solution to reduce pollution and water abstraction, while providing a climate resilient source of water (Shannon *et al.*, 2008; Rodriguez *et al.*, 2009). Water reuse can be classified in three main categories: irrigation, non-potable reuse and potable reuse. For the first two, the quality parameters are mainly based on pathogens and the required treatment consists essentially of disinfection (NRMMC *et al.*, 2006). Salt removal might also be necessary for irrigation to avoid increasing the soil salinity. The additional treatment costs are limited but there is a need for a second distribution network to bring the reclaimed water to the fields or to the taps in households (toilets, gardening…). This can add significant costs and also poses a risk of cross connection where households are connected to both drinking water and reclaimed water. On the other hand, potable reuse (either direct or indirect) requires more extensive treatment trains, following the multi-barrier concept, to provide a high quality reclaimed water at minimal risk for the consumer.

1.2. Alternative Treatment Trains are needed to Promote Potable Reuse

Most state of the art potable reuse facilities, such as the Advanced Water Treatment Plants of the Western Corridor in South East Queensland, use reverse osmosis as an almost universal barrier against contaminants. Reverse osmosis is capable of producing very high quality water; however it has two main drawbacks:

- it is a very energy intensive process compared to other treatment options;
- it concentrates contaminants in a side stream representing about 15-20% of the total treated volume.

The waste stream contains high concentrations of salts and organic contaminants which make it difficult to manage. Today, the main option is to discharge it directly into the sea or a brackish water body where salinity is not an issue and dilution mitigates the environmental impacts. This is a serious limitation for inland application of potable reuse as the concentrate management would add prohibitive costs to the treatment train (Helmy *et al.*, 2009). Therefore, alternative treatment trains not producing a waste side stream have to be investigated.

1.3. Chemical Water Quality of Reclaimed Water for Potable Reuse is of Paramount Importance

One of the main concerns regarding reclaimed water quality for potable reuse, after the presence of pathogens, is the residual organic matter. Pathogens can be effectively inactivated with conventional disinfectants (UV, ozone, chlorine, chloramine). The residual organic matter in treated effluents from a wastewater treatment plant (WWTP) contains thousands of organic micropollutants (OMPs) such as pharmaceuticals and their human metabolites, personal care products, pesticides and industrial chemicals, as well as their biodegradation products. Although they are typically present at sub $\mu g\ L^{-1}$ concentrations, there are concerns regarding the adverse effects these compounds could have on human health for a lifetime exposure to low doses. Pharmaceuticals received particular attention because they have been designed to be bioactive. While there is still no evidence of their potential impact on human health, it has been demonstrated that WWTP effluent discharge can affect a rivers' wildlife. For instance, feminisation of male fishes due to the presence of estrogenic compounds at $ng\ L^{-1}$ levels in effluents has been observed (Sumpter, 2005). Therefore, following the precautionary principle, these compounds should be removed from the reclaimed water. Another problematic aspect

of the presence of organic matter is the potential production of disinfection by-products (DBPs) due to reaction with chlorine or chloramine which are systematically used to provide a disinfectant residual in the distribution network, thus preventing undesired bacterial growth. Indeed, bladder and colorectal cancers have been associated with exposure to chlorination by-products in drinking water. DBP precursors should therefore be removed from reclaimed water to avoid or at least reduce DBP formation during disinfection.

1.4. Ozonation and Biological Activated Carbon Filtration: a Combination to Produce High Quality Reclaimed Water

Oxidation with ozone and activated carbon adsorption has been shown to be effective technologies to remove effluent organic matter and OMPs from treated wastewater. While ozonation can degrade the OMPs in situ, it leads to the formation of by-products rather than to their complete degradation. There is concern regarding the potential impact of these by-products, moreover they remain mostly unidentified to date. On the contrary, activated carbon adsorption removes the OMPs from the water but, as its adsorption capacity is limited, it needs to be regularly regenerated or renewed, which increases operating costs. Biological activated carbon (BAC) is activated carbon that is neither renewed nor regenerated, which gives a bacterial community the opportunity to establish on the media (Simpson, 2008). The efficiency of BAC is typically lower than for new activated carbon but it can maintain a significant removal of organic matter for a longer time, typically years. The combination of ozonation followed by BAC filtration is classically used in drinking water treatment to remove organic matter, pesticides and prevent DBP formation; however, it has not been extensively investigated in the context of wastewater reclamation. This combination could therefore provide a double barrier to the contaminants by first oxidising OMPs and DBP precursors then adsorbing and/or biodegrading the transformation products formed.

1.5. Bioanalytical Tools: a New Way to Assess Water Quality

As stated above, treated wastewater contains thousands of OMPs as well as human metabolites and biodegradation products. Ozonation and BAC filtration will produce even more transformation products. With the recent progress in chemical analysis, it is possible to investigate the fate of a number of known compounds down to a few ng L^{-1}. While this provides useful information, it only looks at the fate of a limited fraction of the OMPs, mostly parent compounds and only very few transformation products. Recently, new tools have been employed to assess water quality by taking into account the whole matrix of OMPs present in water (Macova *et al.*, 2010a; Poulsen *et al.*, 2011). These so called bioanalytical tools are based on *in vitro* bioassays measuring the toxic effect of samples on biological processes such as cell growth or bioluminescence. They can be non-specific (general toxicity) or specific (toxicity via a particular mode of action). Because they have the ability to take into account all the OMPs present in the matrix, they can provide useful information on the fate of compounds not quantified by chemical analysis and on the formation of by-products.

1.6. Objectives of the Enhanced Treatment Project

In this context, the enhanced treatment project investigated three full scale water reclamation plants in Australia using the combination of ozonation and BAC filtration in order to:

- evaluate the chemical quality of treated effluent using chemical analysis to quantify OMPs and *in vitro* bioassays;
- quantify the improvement of chemical water quality achieved by treatment trains using the combination of ozonation and BAC filtration as a barrier to organic matter, OMPs and DBP precursors;
- identify additional treatment processes that have an impact on contaminant removal;
- identify key operational parameters affecting ozonation and BAC filtration;
- evaluate the suitability of the reclaimed water for indirect potable reuse in the Australian context;
- determine whether chemical analysis and *in vitro* bioassays are complementary or redundant tools for the assessment of water quality and treatment processes.

2. CHEMICAL WATER QUALITY ACROSS SOUTH CABOOLTURE WATER RECLAMATION PLANT

Several sets of samples were collected at South Caboolture Water Reclamation Plant to assess the water quality of the treated effluent, to determine the effect of the reclamation treatment train on the measured parameters and to identify the key process(es). Chemical water quality was assessed on three aspects:

- organic micropollutant concentrations;
- non-specific and specific toxicity levels;
- disinfection by-product precursors.

2.1. South Caboolture Water Reclamation Plant

The South Caboolture Water Reclamation Plant was designed to reduce riverine pollution from the 40,000 population equivalent WWTP and to provide recycled water to industry and community consumers. The plant capacity is 10 ML d^{-1} but it operates usually at 8 ML d^{-1}; a balance tank placed between the wastewater treatment plant and the reclamation plant allows a steady flow of approximately 90 L s^{-1}. Whilst the plant provides water for non-potable applications, it has been designed to meet drinking water standards. The treatment process detailed in Figure 1 incorporates biological denitrification, pre-ozonation and coagulation-flocculation, followed by dissolved air flotation and sand filtration (DAFF), main ozonation, BAC filtration and final ozonation for disinfection. The activated carbon of the BAC filter was renewed in March 2008 after 9 years of operation and had filtered about 9,600 bed volumes at the time of sampling; it might therefore still have had a significant adsorption capacity at the time of sampling. Van Leeuwen *et al.* (2003) published more details on the process and its performance.

2.2. Fate of Organic Micropollutants

- What are the levels of organic micropollutant concentrations in the secondary effluent and reclaimed water?
- What is the fate of organic micropollutants along the treatment train?
- Does chemical structure influences the fate of organic micropollutants?
- What are the key processes responsible for organic micropollutant removal?
- What are the key parameters responsible for organic micropollutant removal?

2.2.1. The Challenge of Organic Micropollutants in Indirect Potable Reuse

The term "organic micropollutants" (OMPs) refers to organic contaminants present at trace levels in water (generally in the µg L^{-1} range and below). The presence of OMPs in the environment, and their potential to induce adverse biological effects, have been known for many years (Tabak and Bunch, 1970; Aherne and Briggs, 1989).During the last few decades, the drinking water industry has become increasingly concerned about the presence of these substances in water sources used for drinking water supply. Attention was first on pesticides but shifted towards other OMPs which were found in increasing concentrations in ground- and surface water. Pharmaceuticals received particular attention because they were originally designed to be bioactive. WWTPs were identified as major sources of OMPs in the environment. Indeed, while some are effectively removed by conventional biological treatments (e.g. ibuprofen, paracetamol), others (e.g. carbamazepine, diclofenac) are barely affected (Onesios *et al.*, 2009). The presence of these compounds is therefore of even higher relevance in the context of potable reuse of wastewater where human exposure can potentially be increased.

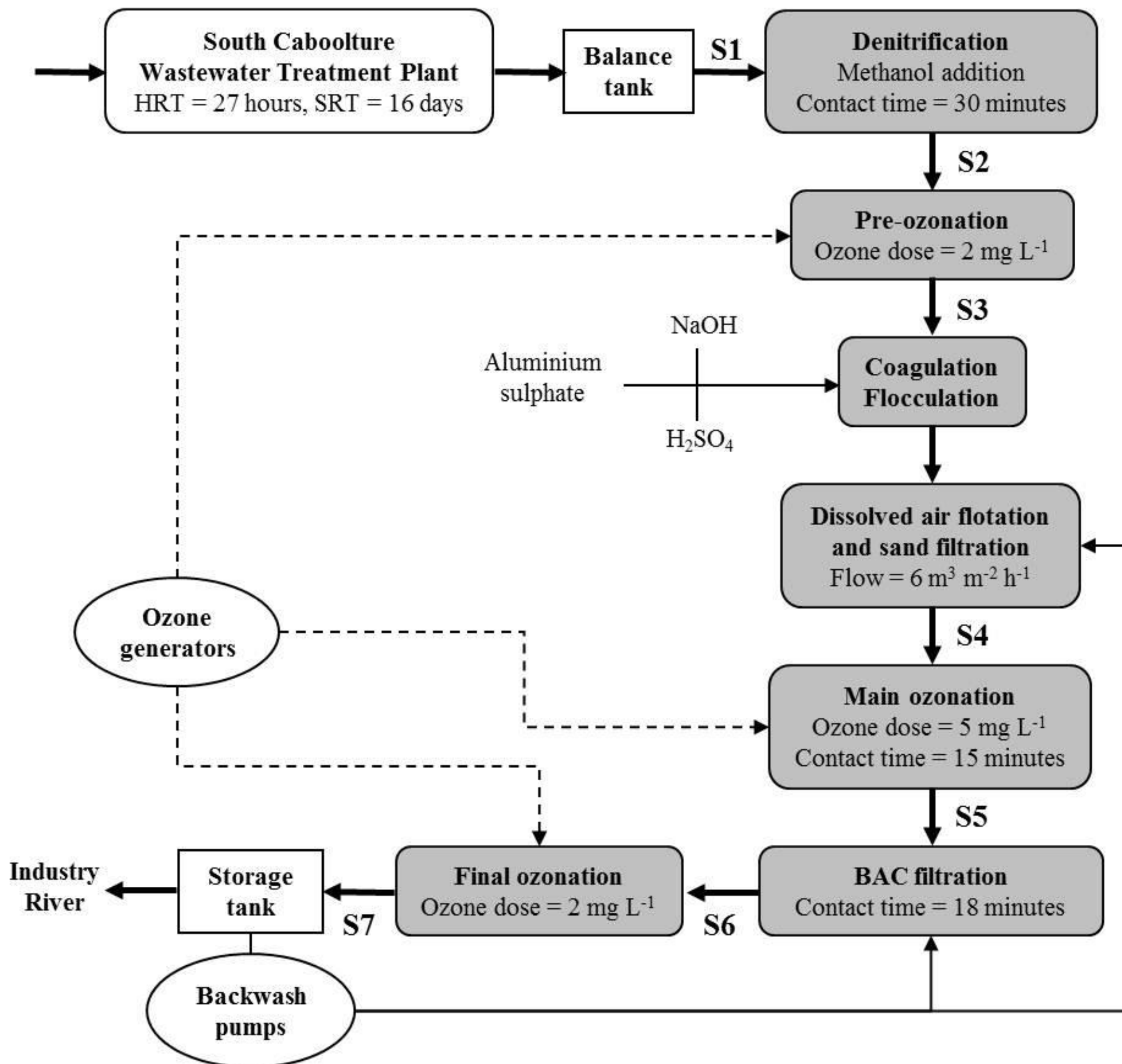

Figure 1. South Caboolture Water Reclamation plant treatment train with sampling points (S1 to S7). Contact time in BAC filtration is empty bed contact time.

Research continues to clarify the toxicological significance of these trace contaminants in the environment and drinking water. The concerns of consumers have caused increased regulatory focus on this issue, even though OMPs appear at reportedly low levels as Snyder *et al.* (2003) showed for pharmaceuticals and endocrine disruptors. Pharmaceuticals are, by design, biologically active compounds (with exception of contrast agents, which are rather diagnostic chemicals than pharmaceuticals). Their potential to affect a range of physiological processes in a large variety of non-target organisms is inherent. It has been shown that some pharmaceuticals may influence both the structure and the function of algal communities in stream ecosystems receiving treated sewage effluents (Wilson *et al.*, 2003) e.g. specific inhibition of photosynthesis in algae caused by β-blockers (Escher *et al.*, 2006). Estrogens in the environment have been implicated in adverse health effects in both animals and humans for some years (Lai *et al.*, 2002; Fent *et al.*, 2006), and there is increasing evidence that other pharmaceutical compounds may also cause harm to overall ecosystem health (Filby *et al.*, 2010). The example of the anti-inflammatory drug diclofenac, which was shown to cause for the drastic falls in vulture populations in the Indian subcontinent (Oaks *et al.*, 2004) demonstrates that pharmaceuticals can cause problems. A major concern for pharmaceuticals also includes the development of bacterial resistance (creation of "Super Bugs") from the release of antibiotics in the environment (Richardson, 2009). Others are known (or suspected) as carcinogens and ingestion of

these substances, even at very low concentrations, might be harmful in the long term. The question of mixture toxicity has recently gained more and more interest and additive effects are to be expected from mixture of EDCs (Pomati *et al.*, 2006; Kummerer, 2009). Mixtures of pharmaceuticals and endocrine disruptors at ng L^{-1} levels have the potential to induce adverse effects in human cell lines (Pomati *et al.*, 2006). Moreover, most of the studies carried out so far were limited to parent compounds and a few human metabolites and biodegradation by-products, as the chemical structures of most of these metabolites and by-products remain unknown today.

In order to reduce the discharge of OMPs into the environment and prevent human exposure in potable reuse schemes, advanced treatment processes have to be employed. Most of the OMPs are more polar than traditional contaminants and the majority have acidic or basic functional groups. These properties, coupled with occurrence at trace levels (i.e., < 1 μg L^{-1}), create unique challenges for both analytical detection and removal processes (Snyder *et al.*, 2003b). Several technologies have proven to be effective in removing OMPs from water of various qualities: activated carbon adsorption (Ternes *et al.*, 2002; Westerhoff *et al.*, 2005; Nowotny *et al.*, 2007; Snyder *et al.*, 2007; Yu *et al.*, 2008), ozonation and advanced oxidation processes (Zwiener and Frimmel, 2000; Huber *et al.*, 2003; Ternes *et al.*, 2003; Huber *et al.*, 2005; Esplugas *et al.*, 2007; Nakada *et al.*, 2007; Kim *et al.*, 2008; Hollender *et al.*, 2009; Reungoat *et al.*, 2010) and tight membrane filtration (Kimura *et al.*, 2004; Snyder *et al.*, 2007; Yoon *et al.*, 2007). However, OMPs have very diverse chemical properties, and the degree to which they are removed by these advanced treatments processes can vary from nearly complete to very little. Activated carbon adsorption and ozonation are the most cost effective options for advanced treatment of WWTP effluents (Joss *et al.*, 2008). However, ozonation is known to lead to the formation of by-products largely not identified to date, which raises concerns regarding their potential impact on the environment and human health (Benner and Ternes, 2009; Radjenovic *et al.*, 2009; Dodd *et al.*, 2010; Stalter *et al.*, 2010; Stalter *et al.*, 2011). Activated carbon adsorption following ozonation has proven to be very effective in further removing organic micropollutants and decreasing non-specific and specific toxicity, but this might not be an economically viable solution (Reungoat *et al.*, 2010). Finally, tight membrane filtration has a higher energy demand and produces a concentrated waste stream that is difficult to dispose of.

2.2.2. Sampling and Organic Micropollutants Quantification

Four sets of samples were collected over winter 2008 under dry weather conditions, including three during week days and one during a weekend (11-07-08, 22-07-08, 27-07-08 and 06-08-08). Water temperature across the plant was 22±2°C and pH was 7.0±0.5. Samples were collected at 7 sampling points along the treatment train, labelled S1 to S7 on Figure 1, in order to evaluate the performance of individual treatment steps. As the flow rate in the reclamation plant is constant representative samples were collected as time proportional 24-hour composites. At each point, samples were collected into a glass bottle pre-washed with MilliQ water and HPLC grade acetone. The samples were protected from light and refrigerated during collection and transport to the laboratory for analysis.

Organic micropollutant quantification was carried out by Queensland Health Forensic and Scientific Services (QHFSS). The method consisted of solid phase extraction (SPE), concentration and quantification by liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS). This method allowed the quantification of 85 compounds selected on the basis of quantity of usage of the particular compounds, their potential toxicity and their resistance to degradation (Appendix 1). The 85 organic micropollutants consist mainly of pharmaceuticals, a few pesticides and personal care products. Their limit of quantification (LOQ) was 0.01 μg L^{-1} in most cases. Concentrations were calculated using an internal calibration method.

2.2.3. Results and Discussion

The DOC was measured for two sets of samples (22-07-08 and 06-08-08), and varied from 14.2 to 19.7 mg L^{-1} in the influent water. In the reclamation plant's influent, 54 of the 85 targeted compounds had a median concentration above their LOQ, confirming that conventional activated sludge treatment does not completely remove these micropollutants from wastewater (Appendix 2). The concentrations

ranged from 0.01 to 2.10 µg L^{-1} with the exception of gabapentin, which was consistently found at higher concentrations ranging from 5.60 to 6.50 µg L^{-1}. The factor between the minimum and the maximum concentrations measured for each individual compound was generally close to or lower than 2, with a maximum of 3.6 observed for iopromide. No clear pattern could be distinguished between the different sampling days. The increase or decrease of single compound concentrations from one day to another appeared to be random, even when comparing the sample collected during the weekend to samples collected during weekdays. Figure 2 shows the number of compounds quantified above their LOQ and the DOC along the treatment train.

Twenty-five compounds had an influent median concentration above 0.10 µg L^{-1} (Table 1). Their removal efficiencies were determined in each treatment step except when the concentration before treatment was lower than ten times the LOQ and below LOQ after treatment. This criterion was used to allow the determination of removals up to 90% in any case and avoid underestimation. When the reported outlet concentration was below the LOQ of the compound, removal efficiency was calculated as a minimum value using the LOQ as outlet concentration. The efficiency of each treatment stage in removing these compounds is summarised in Figure 2.

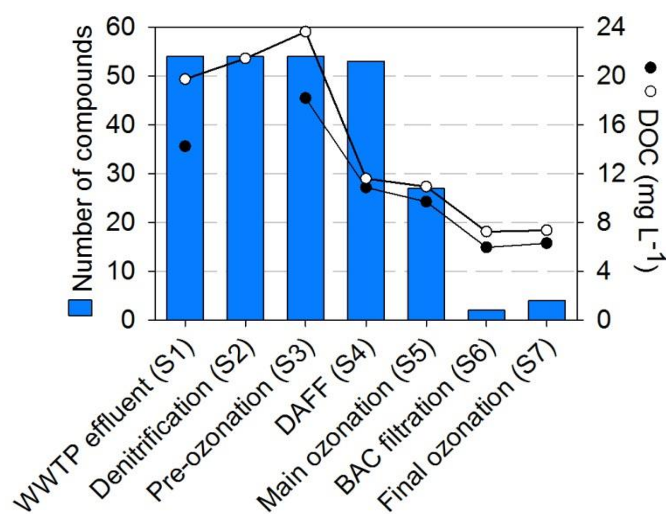

Figure 2. Number of compounds quantified and DOC after indicated stage along the treatment train. Bars represent the number of compounds with a median concentration above the limit of quantification (four samples). Dots represent DOC on two different sampling days.

The full treatment decreased the concentration of 50 of the 54 compounds quantified in the WWT effluent water to levels below LOQ (Figure 2). Concomitantly, DOC was also reduced by 55 to 60% in the treated water. Overall, among the 25 selected compounds, 22 were removed by more than 89%. The median removal of gabapentin was 86% and the removals of naproxen and iopromide were not calculated because their concentration was lower than 10 times their LOQ in the influent and below their LOQ in the effluent. The four remaining compounds were gabapentin (0.45 µg L 1), roxithromycin (0.01 µg L 1), DEET (0.03 µg L^{-1}) and caffeine (0.02 µg L^{-1}).

Table 1. Selected compounds, classification, hydrophobicity expressed as logarithm of octanol-water partition coefficient (log K_{ow}), limit of quantification (LOQ) by LC/MS-MS analysis, influent concentrations to the water reclamation plant and guideline values from the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies.

Compound name	Classification	Log K_{ow} [a]	LOQ ($\mu g\ L^{-1}$)	Influent concentrations ($\mu g\ L^{-1}$) Max	Median	Min	Guideline value ($\mu g\ L^{-1}$)
Atenolol	Beta-blocker	- 0.03	0.01	1.00	0.76	0.60	25 [v]
Caffeine		0.16	0.01	0.97	0.51	0.43	0.35 [i]
Carbamazepine	Anticonvulsant	2.25	0.01	0.95	0.70	0.39	1,000 [i]
Codeine	Analgesic	1.28	0.02	1.32	1.02	0.68	500 [i]
Diclofenac	NSAI[b]	4.02	0.01	0.27	0.20	0.14	18 [i]
Doxylamine	Sedative	2.37	0.01	0.46	0.36	0.22	12.5 [v]
Erythromycin	Antibiotic (macrolide)	2.48	0.01	0.46	0.26	0.18	175 [i]
Furosemide	Diuretic	2.32	0.01	1.30	1.07	0.89	10 [v]
Gabapentin	Anticonvulsant	- 1.37	0.10	6.50	5.45	5.10	450 [v]
Gemfibrozil	Hypolipidemic agent	4.77	0.01	0.20	0.17	0.14	600 [v]
Hydrochlorothiazide	Diuretic	- 0.10	0.01	0.90	0.79	0.50	12.5 [v]
Iopromide	Radiographic agent	-2.49	0.20	2.10	1.27	0.58	7,500 [i]
MCPA	Herbicide	2.52	0.01	0.20	0.17	0.12	2 [iii]
Metoprolol	Beta-blocker	1.69	0.01	0.48	0.39	0.35	250 [i]
Naproxen	NSAI[b]	3.10	0.10	0.51	0.29	0.24	2,200 [i]
Oxazepam	Anxiolytic	2.32	0.01	0.95	0.87	0.46	7.5 [v]
Paracetamol	Analgesic, antipyretic	0.27	0.01	0.39	0.26	0.12	1,750 [i]
Phenytoin	Anticonvulsant	2.16	0.01	0.26	0.24	0.11	140 [v]
Ranitidine	Histamine-blocker	0.29	0.01	0.36	0.31	0.22	150
Roxithromycin	Antibiotic (macrolide)	2.75	0.01	0.37	0.29	0.23	1,500 [i]
Sulfamethoxazole	Antibiotic (sulfonamide)	0.48	0.01	0.24	0.22	0.11	350 [i]
Temazepam	Sedative	2.15	0.01	0.60	0.51	0.25	50 [i]
Tramadol	Narcotic analgesic	3.01	0.01	1.42	1.22	0.88	50 [v]
Trimethoprim	Antibiotic	0.73	0.01	0.21	0.20	0.15	700 [i]
Venlafaxine	Antidepressant	3.28	0.01	1.71	1.48	1.02	37.5 [v]

[a] Calcultated with EPI SUITE 4.0
[b] NSAI: nonsteroidal anti-inflammatory agent

[i] Australian Water Recycling Guidelines for Drinking Augmentation
[ii] Australian Drinking Water Guidelines (* health value)
[iii] WHO Guidelines for Drinking Water
[iv] EU Drinking Water Guidelines
[v] Calculated following the Australian Water Recycling Guidelines for Drinking Augmentation

The first three stages of the treatment train (i.e. denitrification, pre-ozonation and coagulation/ flocculation/DAFF) did not effectively remove the OMPs. Removal of organic micropollutants in a denitrification reactor has not been reported elsewhere in the literature to our knowledge. The ozone dose used in the pre-ozonation stage is too low to induce significant removal of OMPs. Indeed, ozonation has been proved to be very effective for oxidising various micropollutants in secondary treated wastewaters but with higher ozone doses of at least 0.25 to 0.50 mg_{O3} mg_{DOC}^{-1} (Ternes *et al.*, 2003; Huber *et al.*, 2005; Snyder *et al.*, 2006; Hollender *et al.*, 2009; Wert *et al.*, 2009). The coagulation/flocculation/DAFF aims at removing colloids which are large negatively charged molecules and was therefore not expected to remove OMPs. Limited removal (50%) has also been reported in the literature except for highly hydrophobic compounds with log K_{ow}> 6 which adsorbs onto the flocs formed (Adams *et al.*, 2002; Ternes *et al.*, 2002; Westerhoff *et al.*, 2005; Vieno *et al.*, 2006; Thuy *et al.*, 2008). After these 3 stages, the concentrations of the 25 compounds that had an influent median concentration of at least 0.10 µg L^{-1} were generally still greater than 50% compared to the influent concentration (Figure 3).

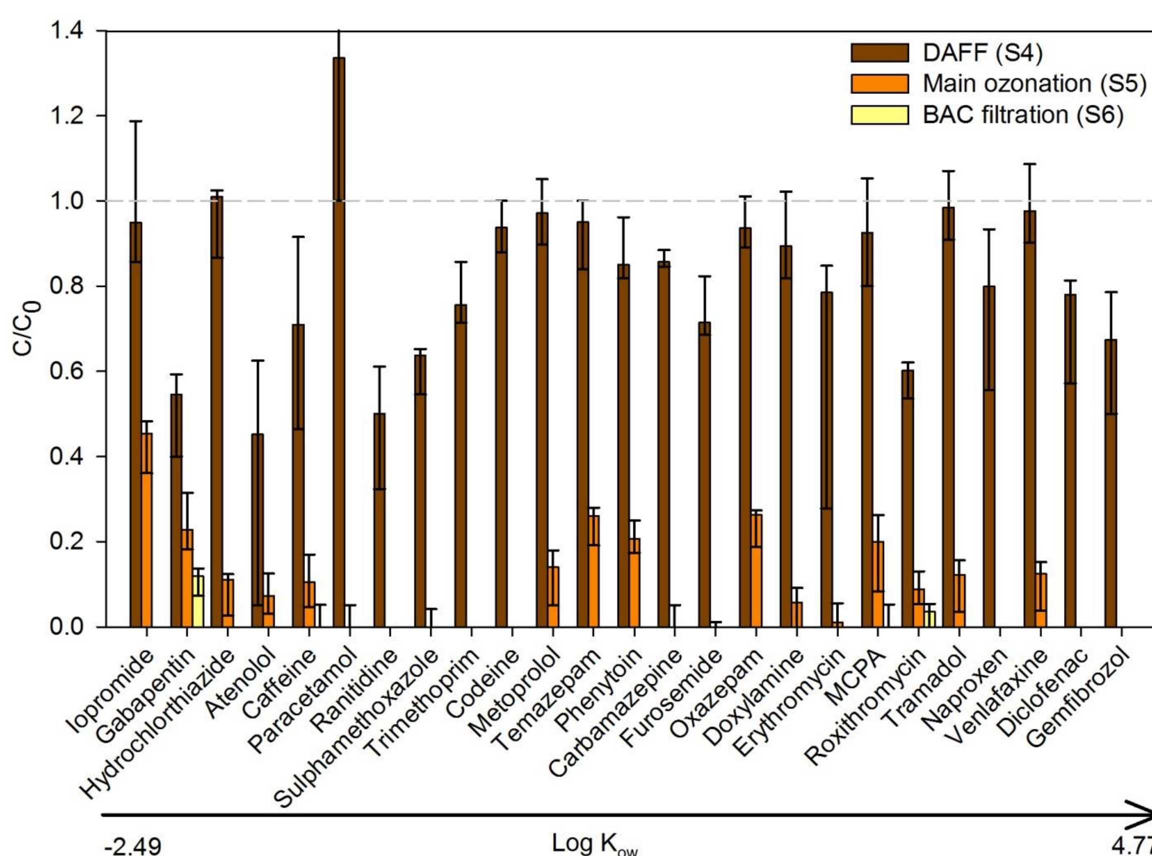

Figure 3. Median relative concentrations of selected compounds (median of influent concentration > 0.10 g L^{-1}) after indicated treatment stages (error bars represent maximum and minimum values). C is the concentration after the specified treatment step and the reference concentration, C_0 is the concentration in WWTP effluent.

The main ozonation decreased the concentration of 26 compounds below their LOQ and ozonation generally decreased the micropollutants to less than 20% of their influent concentration (Figure 3). BAC filtration further removed the compounds to levels below LOQ except for gabapentin and roxithromycin (Figure 3). Removal in the ozonation stage varied from 55% to more than 95% depending on the compound considered (Figure 4). Indeed, the reaction of organic compounds with molecular ozone is selective and only certain groups of compounds react rapidly, e.g. aliphatic molecules with double bonds, deprotonated amines and aromatics with an activating group. Other compounds are mainly oxidised by hydroxyl radicals generated during ozone decomposition.

Hydroxyl radicals are very reactive with most OMPs but are present at very low concentration during ozonation, which limits their action.

BAC filtration decreased the concentration of another 25 compounds below their LOQ and only two compounds could be quantified after: roxithromycin (0.01 µg L^{-1}) and gabapentin (0.70 µg L^{-1}).The removal efficiencies of the compounds having a median concentration of at least ten times their LOQ prior to BAC filtration were calculated: oxazepam, tramadol and venlafaxine were removed by more than 90% and gabapentin was removed by 53%. Given that the activated carbon media was renewed only 4 months before the sampling, it is likely that it still has a significant adsorption capacity and the removal observed is due to a combination of adsorption and biodegradation. Previous studies demonstrated that powdered and granular activated carbon can efficiently remove OMPs from natural water sources used for drinking water (Ternes *et al.*, 2002; Westerhoff *et al.*, 2005; Snyder *et al.*, 2007; Ormad *et al.*, 2008). Adsorption propensity of OMPs can vary greatly depending on the chemical structure and generally increases with increasing hydrophobicity (Westerhoff *et al.*, 2005). Therefore, the breakthrough for individual OMPs will occur at different filtered volumes and is not necessarily correlated to DOC breakthrough (Snyder *et al.*, 2007; Wang *et al.*, 2007).

The combined effects of the main ozonation and the BAC filtration decreased the concentration of 10 of the 25 selected micropollutants by more than 95% and by more than 89% for 12 of the 15 remaining compounds compared to their concentration prior to the main ozonation (Figure 4). Gabapentin concentration was reduced by 79%. These results show that ozonation followed by BAC filtration is a very effective combination of processes to remove micropollutants from secondary treated wastewater.

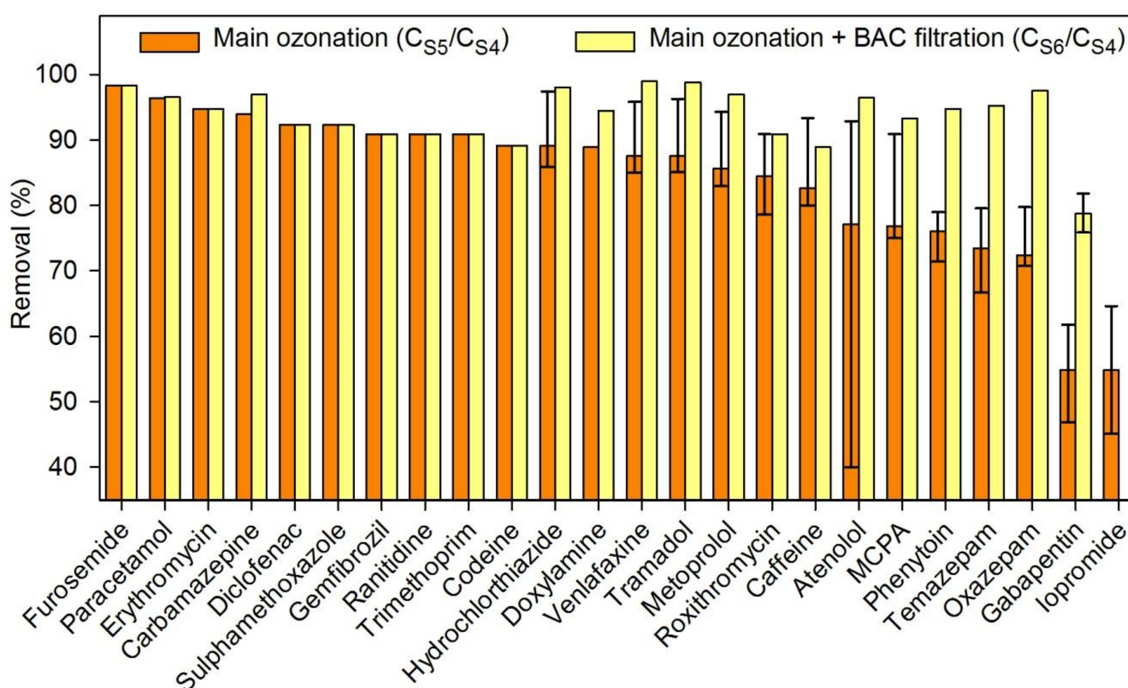

Figure 4. Median removal of selected compounds (median of influent concentration > 0.10 µg L^{-1}) by the main ozonation stage and the combination of the main ozonation and the BAC filtration stages. Error bars represent minimum and maximum removal, no error bar means that the compound was below LOQ after treatment; therefore removal was calculated as a minimum using the LOQ. C_{S4}: concentration before main ozonation; C_{S5}: concentration after main ozonation; C_{S6}: concentration after BAC filtration.

It is clear from Table 2 that the key processes responsible for OMP removal are the main ozonation and BAC filtration. However, the key steps in the removal of the DOC were the DAFF and the BAC filtration. Table 2 shows that the fate of OMPs is not correlated to the removal of DOC. This is particularly apparent for the coagulation/flocculation/ DAFF and main ozonation stages. Indeed, the

former removed 40 to 50% of the organic matter but had a limited effect on OMP concentration. On the contrary, ozonation reduced selected OMPs concentrations from 55% to more than 90% whereas DOC removal was below 10%. Ozonation is known to lead to the formation of by-products rather than to mineralisation. Nevertheless, although the coagulation/flocculation/DAFF reduced the concentration of micropollutants by less than 30%, it also played a key role in OMPs removal indirectly by reducing the DOC which enhanced the performances of the main ozonation due to a higher ozone/DOC ratio.

Table 2. DOC removal and fate of 25 selected compounds (initial concentration > 0.01 µg L^{-1}) in each stage of the treatment train.

Treatment Stage	Removal of		Comments
	DOC	Selected Compounds	
Denitrification	Nil	< 20% Exception: atenolol(38%)	Methanol addition, more biodegradable than organic micropollutants. No literature report.
Pre-ozonation	Nil	< 30%	Ozone dose too low (0.1 $mg_{O3} mg_{DOC}^{-1}$) for effective removal.
Coagulation/ flocculation/ DAFF	40-50%	< 20%. Exceptions: atenolol (42%), caffeine (29%), gabapentin (44%), gemfibrozil (32%) and roxithromycin (37%)	Literature reports removals < 50% in drinking water except for highly hydrophobic compounds (log K_{ow}> 6).
Main ozonation	< 10%	55 to > 90%	Ozone dose (0.5 $mg_{O3} mg_{DOC}^{-1}$) suitable for effective oxidation. Removal depends on individual compounds reactivity with ozone.
BAC filtration	20-30%	Oxazepam, tramadol and venlafaxine > 90%; gabapentin 53%	Only 4 compounds had a sufficiently high concentration before the filtration to calculate a removal. High influence of hydrophobicity.
Final ozonation	Nil	Gabapentin 20%	Ozone dose = 0.3 $mg_{O3} mg_{DOC}^{-1}$. Concentrations too low to assess efficiency.

- The treatment train of the South Caboolture Water Reclamation Plant can reduce the concentrations of a wide range of organic micropollutants by more than 90%; down to levels below 0.01 µg L^{-1}.
- The key treatment stages for the removal of organic micropollutants are the main ozonation and the BAC filtration.
- The ozone/DOC ratio is a key parameter in the efficiency of ozonation process.
- Oxidation efficiency of OMPs by ozonation depends on their chemical structure.
- The coagulation/flocculation/DAFF does not remove OMPs but plays a key role indirectly by reducing the DOC level before the main ozonation.
- The fate of OMPs is not correlated with DOC removal.

2.3. Toxicity Assessment with Bioanalytical Tools

- What are the toxicity levels in the treated effluent?
- What reduction of toxicity levels can be achieved by the treatment train?
- What are the key treatment stages in the reduction of toxicity levels?
- Does ozonation have the potential to form by-products increasing the toxicity levels?

2.3.1. Bioanalytical Tools for Water Quality Assessment

Chemical monitoring provides a quantitative assessment of single contaminant concentrations in a water sample but cannot account for unknown compounds including most transformation products. Effect-based monitoring complements chemical analysis. Classical ecotoxicological tests used in water quality assessment include *in vivo* fish and aquatic invertebrate assays that measure e.g. mortality, growth and feeding responses. Fish and invertebrate species are, however, not appropriate models for mammalian toxicology, which is more relevant for human exposure scenarios (e.g. indirect potable reuse). *In vitro* molecular and cell-based assays are sensitive, cost- and time-effective alternatives to whole animal testing. Implementation of human and other mammalian cell lines has facilitated evaluation of toxicological endpoints relevant for human health risk assessment.

Cell-based bioassays target particular endpoints or mechanisms of toxicity and can be divided into two groups:

- bioassays with primary cells and cell lines; and
- bioassays with recombinant cell lines.

Native cells typically respond to all chemicals in a given sample and are suitable for assessment of non-specific toxicity. Non-specific toxicity is typically measured in cytotoxicity tests that quantify cell growth/viability. Cytotoxicity assays can be more specific if cells (be it primary cells or cell lines) are derived from particular tissues, e.g. pulmonary epithelial cells or liver cells. The differential toxicity between different cell types can further give an indication of the mode of action of the chemicals in the sample. Some cells react specifically to groups of chemicals with common modes of toxic action by expressing a specific physiological response, e.g. direct inhibition of photosynthesis in algae or proliferation of breast cancer cells in the presence of estrogens. Recombinant cell bioassays have emerged in the last few years to detect and amplify specific responses. Examples include hormone-mimetic activity or induction of the aryl-hydrocarbon receptor.

Most cell-based assays target a particular mode of toxic action and/or a particular recipient (e.g. human vs. fish cell line). Comprehensive risk assessment thus requires a battery of bioassays in order to cover all or many modes of toxic action and/or recipients relevant for the water sample of interest. Application of broad test batteries comprising a range of specific endpoints as well as non-specific cytotoxicity endpoints allow the assessor to account for unexpected toxicant groups that may otherwise go undetected. Two distinct approaches can be applied to design a test battery; one is driven by consideration of the protection goal, while the other is driven by detection of chemical groups of concern. In the chemical oriented design, priority is given to quantification of the risks posed by relevant groups of chemicals. Bioassays of high sensitivity towards the toxicant group of interest may hence be selected irrespective of their (lack of) direct relevance to the protection goal. For example, in order to protect our drinking water from herbicides, even though the water tested is destined for human consumption and the protection goal is to achieve good human health, it may be appropriate to include an algal assay, simply because photosynthetic organisms are particularly sensitive to herbicide exposure.

Both test battery approaches may lead to very similar and often overlapping sets of bioanalytical tools as chemicals cannot be viewed independently of their mode of action. When researchers design test batteries, they will often include considerations related to both approaches. It must also be noted that not all bioassays are fully selective and 100 % indicative of a given mode of toxic action. In all cases, a cell-based bioassay will be influenced by a combination of non-specific and specific toxicity. In a water sample, there will be thousands of chemicals, only a fraction of which will respond specifically to the endpoint featured in the applied assay. Within a range of concentrations, a window will typically exist where the specific effect sets in but is not yet compromised by overlaying cytotoxicity. The wider this window is, the more useful a given bioassay is for application in complex water matrices.

2.3.2. Sampling and Bioanalytical Tool Methods

A battery of six bioassays described in Table 3 was applied to the samples collected for OMPs quantification (2.2.2).The experimental procedure for these bioassays is available elsewhere (Macova *et al.*, 2010a). Water samples were extracted by SPE using Oasis HLB cartridges. Full dose response curves were determined for a serial dilution of the extract for each bioassay. Results were expressed as toxic equivalent concentrations (TEQ) except for the umuC assay. The TEQ represents the concentration of a given reference compound that would be required to produce the same effect as the mixture of compounds present in the sample. When the outlet TEQ was below the LOQ of the bioassay, removal efficiency was calculated as a minimum value using the LOQ as outlet TEQ. In the umuC assay, the response is determined as an induction ration (IR), an IR $\geq$ 1.5 is considered genotoxic. For genotoxicsamples, $EC_{IR1.5}$ corresponds to how many times the sample must be concentrated or diluted to elicit an IR of 1.5. Results are expressed as $1/EC_{IR1.5}$ therefore a higher number represents a greater genotoxic effect.

Table 3. Description of the bioassays used.

Toxic Mode of Action (Bioassay)	Targeted Chemicals
Baseline toxicity (*Vibrio fischeri* bioluminescence inhibition test)	Non-specific bacterial toxicity test widely recognised in the field of ecotoxicology as the standard assay for acute cytotoxicity. The assay reflects the general "energy status" of the bacteria and is sensitive to a broad spectrum of compounds with different modes of action. The toxic potential of OMPs is generally directly related to their hydrophobicity (Escher *et al.*, 2008).
Estrogenicity (E-SCREEN)	Specifically responds to natural hormones and other compounds that can mimic the activity of the female sex hormone estradiol.
AhR response (CAFLUX assay)	Dioxins and dioxin-like compounds such as polychlorinated biphenyls (PCBs) but can also respond to other chemicals such as polycyclic aromatic hydrocarbons (PAHs).
Genotoxicity (umuC assay)	Responds specifically to genotoxic compounds that cause DNA damages.
Neurotoxicity (acetylcholinesterase inhibition assay)	Organophosphate and carbamate pesticides specifically bind to this enzyme.
Phytotoxicity (PSII inhibition I-PAM assay)	Herbicides that directly inhibit photosynthesis.

2.3.3. Results and Discussion

The influent biological activity was higher than the blank (MilliQ water) in all the bioassays (Table 4). The effect of the treatment train on the toxicity levels is pictured in Figure 5. The final effluent levels and overall efficiency of the treatment train are also given in Table 4. The toxicity levels of the effluent were lower compared to the influent and close or equal to the blank levels showing that the treatment train could effectively decrease the effects observed with the bioassays; from 62% for the AhR response to more than 99% for estrogenicity. The key treatment steps responsible for the decrease of biological activity are the DAFF stage, the main ozonation and the BAC filtration. The effect of individual treatment process on each bioassay is discussed in detail below.

Table 4. **Maximum, median and minimum biological activity of the water entering (WWTP effluent) and leaving the reclamation treatment (final ozonation) and overall maximum, median and minimum decrease observed through the reclamation plant.**

			WWTP Effluent			Final Ozonation			Decrease (%)		
Bioassay	**Result Expression**	**Blank**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**
Baseline Toxicity	Baseline toxicity EqC* (TEQ, mg L^{-1})	0.21	2.9	2.1	2.0	0.72	0.52	0.31	84	78	67
Estrogenicity	EstradiolEqC (EEQ, ng L^{-1})	< 0.02	7.8	5.7	5.1	< 0.06			> 99		
AhR Response	TCDD EqC (TCDDEQ, ng L^{-1})	0.08	0.98	0.82	0.59	0.36	0.31	0.26	69	62	46
Genotoxicity	$1/EC_{IR1.5}$	< 0.01	0.32	0.19	0.13	0.04	< 0.01		> 92		83
Neurotoxicity	Parathion EqC (PTEQ, µg L^{-1})	< 0.3	3.9	3.1	2.8	1.2	<0.3		> 90		57
Phytotoxicity	DiuronEqC (DEQ, µg L^{-1})	< 0.01	0.43	0.23	0.18	0.09	0.07	0.04	85	72	66

*EqC = equivalent concentration

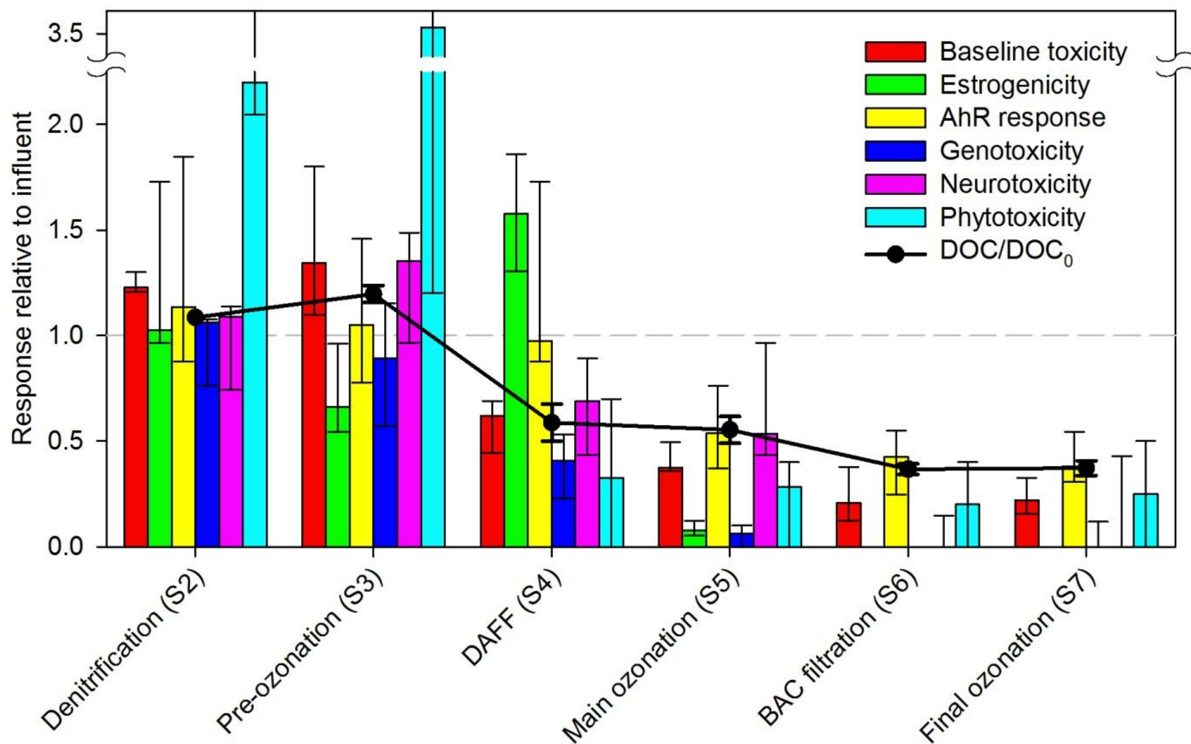

Figure 5. **Relative response of the bioassays and relative DOC after indicated stage along the treatment train compared to the WWTP effluent. Bars are the median of 4 values for bioassays and error bar represent maximum and minimum. Dots are the average of 2 values for DOC and error bars represent maximum and minimum.**

Baseline Toxicity

The *Vibrio fischeri* bioluminescence inhibition test is a non-specific bacterial toxicity test widely recognised in the field of ecotoxicology as the standard assay for acute cytotoxicity. The assay reflects the general "energy status" of the bacteria and can indicate the toxic potency of a broad spectrum of compounds with different modes of action. Denitrification and pre-ozonation did have a slight stimulatory effect, likely to be related to some non-volatile organic chemicals. The 52% decrease of TEQ in the DAFF stage is accompanied by a 40 to 50% reduction in DOC. As is discussed in more details in (Macova *et al.*, 2010b), an almost linear correlation exists between DOC level and TEQ. Although the SPE that is performed prior to toxicity testing should be able to remove a substantial fraction of the DOC, some DOC, most likely smaller breakdown products that have similar physicochemical properties and similar molecular weight, may still be present.

The main ozonation reduced the TEQ by 31% even though the DOC was not affected. It is known that some organic compounds are poorly reactive with ozone and the results of the micropollutant analysis showed that some compounds were only partially degraded in the main ozonation step (i.e. iopromide and gabapentin). Moreover, ozonation does not typically lead to complete mineralisation but to the formation of by-products. The oxidation products of ozonation are in general more polar and more hydrophilic molecules than the parent compounds but the modification is not drastic. Therefore the oxidation products of ozonation will still have a considerable effect in a non-specific assay like the bioluminescence inhibition test with *Vibrio fischeri*, where the toxicity is generally directly related to the hydrophobicity of the mixture components (Escher *et al.*, 2008).

BAC filtration reduced the baseline toxicity by 50% and the DOC by 30 to 35%. Activated carbon can effectively adsorb the more hydrophobic compounds, which is again consistent with the general trend discussed above; that the more hydrophobic compounds have a higher toxic activity than the more hydrophilic ones. Based on this fact, identification of the compounds exhibiting a high toxic activity could start with the identification of the more hydrophobic compounds.

The final ozonation did not further reduce the baseline toxicity compared to BAC filtration. The effluent TEQ was approximately 80% lower than the influent TEQ (Figure 5) and only 2.5 times higher than the blank (Table 4). This indicates that the residual toxicity is of no concern, unless the residual organic chemicals and organic matter inducing this effect were of very specific potency. This latter question was tested with a series of specific endpoints that respond to environmentally relevant modes of toxic action.

Estrogenic Activity

The E-SCREEN assay specifically responds to natural hormones and other compounds that can mimic the activity of the female sex hormone estradiol. The estrogenic activity of the samples is expressed as an estradiol equivalent concentration (EEQ). The median influent EEQ was 5.8 ng L^{-1}; higher than levels previously reported in South East Queensland. Most of the effluents from 12 activated sludge wastewater treatment plants tested by (Leusch *et al.*, 2006) had EEQs below 4 ng L^{-1} and sometimes below 1 ng L^{-1}.

Denitrification did not affect the estrogenicity (Figure 5). Pre-ozonation with an ozone dose of approximately 0.10 mg_{O3} mg_{DOC}^{-1} reduced the EEQ by 34% compared to the influent. This is higher than the removal previously observed by (Snyder *et al.*, 2006) who measured the EEQ reduction induced by various ozone doses in treated wastewater with a DOC of 6.38 mg L^{-1}. They found that an ozone dose of 2.1 mg L^{-1} (0.33 mg_{O3} mg_{DOC}^{-1}) only removed 18% of the EEQ but, with ozone doses of 3.6 mg L^{-1} (0.56 $mg_{O3}mg_{DOC}^{-1}$) and above, 90% or more removal could be achieved. In a recent study of full scale ozonation in a Swiss WWTP, the dose dependency of removal of micropollutants yielded similar results (Escher *et al.*, 2009). While most endpoints showed a clear dose-dependency of reduction of effects, the reduction of estrogenicity was already large at low ozone doses and depended more on the EEQ than on the ozone dose. When estrogenicity was already below a certain level, which was very close to the detection limit, the quantification of further reduction became difficult and prone

to large uncertainty. For the remaining samples, ozone doses of 1.6 to 5.3 mg L^{-1} in the presence of 4.2 to 6.0 mg L^{-1} DOC lead to more than 90% reduction of estrogenicity. This is consistent with laboratory experiments that demonstrated that almost all first generation transformation products of estrogenic chemicals had severely decreased estrogenic potency (Lee *et al.*, 2008). Thus ozonation can be considered as a fairly selective oxidation, where even low doses selectively target one of the most environmentally relevant modes of toxic action, namely estrogenicity.

After the coagulation/flocculation/DAFF stage the EEQ increased drastically by a median factor of 3.3 compared to the level prior to treatment. At this treatment step, the concentration of DOC is greatly reduced (by 40 to 50%), and there is a likelihood that the estrogenic chemicals that were bound to DOC were released during this treatment step. It has been previously observed with another estrogenicity assay that DOC appears to reduce the bioavailability of estrogens (Escher, unpublished results). Estrogenic chemicals are typically relatively hydrophobic and bind well to DOC (Neale *et al.*, 2008). In general DOC is not bioavailable in bioassays (the discussion on the small breakdown products above is an exception to this general paradigm) and micropollutants sorbed to DOC would not be bioavailable either. A large fraction of the matrix and also the DOC is supposed to be removed by SPE but, given the colour of the extracts, it is possible that a substantial fraction of larger DOC is co-extracted. In addition, for the E-SCREEN test, it was demonstrated that the presence of serum proteins modulates the free and bioavailable concentration of estrogenic chemicals (Heringa *et al.*, 2004). This effect was also hydrophobicity dependent and was much more pronounced for the more hydrophobic octylphenol than for the less hydrophobic estradiol. Protein binding is generally less important than binding to DOC or lipids, therefore, while the effect on bioavailability was not very large for estradiol in the study of (Heringa *et al.*, 2004); it might well be relevant under the conditions of the present study. This hypothesis needs to be evaluated in the future by exploring the correlation between size distribution of naturally occurring DOC and effect on bioavailability, estrogenicity and toxicity.

The main ozonation reduced the EEQ by a median value of 92 and 95% compared to the level of the reclamation plant's influent and to the level before treatment respectively; whereas DOC was not affected. It can be concluded that the mixture of by-products formed by the oxidation of the estrogenic compounds by ozone and hydroxyl radicals have a much lower estrogenic activity than the mixture of parent compounds, which is consistent with expectations as discussed above and in (Lee *et al.*, 2008).

BAC filtration was able to efficiently remove residual estrogenic compounds and further reduced the EEQ by another 95% to levels below the detection limit of 0.02 ng L^{-1} and the final effluent concentration was below the quantification limit of 0.06 ng L^{-1}. The overall treatment efficiency for the removal of estrogenic activity was greater than 99%. This is in good agreement with observations on full scale ozonation in a Swiss WWTP (Escher *et al.*, 2009). As discussed above the analytically determined concentrations of (xeno)estrogens were below the quantification limit, therefore for this endpoint the very sensitive bioassay poses a great advantage despite the observed limitations due to matrix effects.

Ah-Receptor Response

The CAFLUX assay targets dioxins and dioxin-like compounds such as polychlorinated biphenyls (PCBs) but can also respond to other chemicals such as polycyclic aromatic hydrocarbons (PAHs) (Macova *et al.*, 2010a). The results of the test are expressed as 2,3,7,8 tetrachlorodibenzo-p-dioxin equivalent concentration (TCDDEQ). The median TCDDEQ of the influent water was 0.82 ng L^{-1} and there was no significant variation along the first three steps of the treatment process; i.e. denitrification, pre-ozonation and coagulation/flocculation/DAFF (Figure 5). The main ozonation removed about 50% of the TCDDEQ but subsequent BAC filtration and final ozonation did not show further important removal and the median TCDDEQ of the final effluent was approximately 3.9 times higher than the blank (Table 4). Two sets of samples were submitted to a sulphuric acid silica gel clean up procedure that aims at removing organic chemicals except those that are not oxidised such as polychlorinated dibenzodioxins, furans and PCBs. The samples were then tested again with the CAFLUX assay to evaluate the contribution of these very persistent chemicals (i.e. dioxins, furans and

dioxin-like PCBs). Results showed that after clean up the TCDDEQ was not significantly different from the blank (values ranged from 0.09 to 0.11 ng L^{-1}). This shows that the effect induced by the samples without sulphuric acid silica gel clean-up is not due to the presence of dioxins, furans or dioxin-like PCBs but was caused by other chemicals. Since none of these groups of chemicals was quantified by chemical analysis in this study, no comparison between chemical and biological analysis is possible.

Genotoxicity

The umuC assay responds specifically to genotoxic compounds that cause DNA damage. To detect genotoxic effects caused by metabolites, the test is also performed in presence of a rat liver extract that can transform indirect genotoxicants to metabolites that are DNA damaging compounds. The median influent $1/EC_{IR1.5}$ were 0.19 and 0.060 in the absence and presence of the rat liver extract respectively, showing that the sample was less genotoxic after metabolisation. This is what one would commonly expect; an exception would be PAHs that are activated by metabolism. Denitrification and pre-ozonation did not have a substantial influence on genotoxicity (Figure 5). The coagulation/flocculation/DAFF stage decreased $1/EC_{IR1.5}$ by 59% compared to the influent. The main ozonation drastically reduced the genotoxicity, $1/EC_{IR1.5}$ was reduced by 80 and 93% compared to the DAFF effluent and to the influent of the plant respectively. After BAC filtration as well as in the final effluent, $1/EC_{IR1.5}$ was below the LOQ of the bioassay (Table 4). In every case, the genotoxicity of the metabolised sample was lower than the non-metabolised sample, indicating that the types of chemical inducing the genotoxic effect did not change over the treatment.

Neurotoxicity

Neurotoxicity is measured by the inhibition of the enzyme acetylcholinesterase (AChE). Organophosphate and carbamate pesticides specifically bind to this enzyme and the results are expressed as parathion equivalent concentration (PTEQ). The median PTEQ in the secondary treated wastewater was 3.1 µg L^{-1}; denitrification and pre-ozonation did not reduce the PTEQ whereas DAFF decreased it by 31% compared to influent (Figure 5). Unlike the other bioassays, the effect of the main ozonation on PTEQ was not significant but BAC filtration reduced it drastically to a level below the quantification limit of the bioassay (0.30 µg L^{-1}) which represents more than an 80% and 90% decrease compared to the main ozonation effluent and the plant influent water respectively. This observation is consistent with theoretical expectation, as it is known that compounds like diazinon and chlorpyrifos, which often constitute a large fraction of the acetylcholinesterase inhibitors, are not well oxidized by ozone. In contrast, these compounds are fairly hydrophobic ($\log K_{ow} = 3.96$ and 4.66 respectively), therefore sorption to activated carbon can be expected. A similar removal pattern has been observed for acetylcholinesterase inhibitors in the above-mentioned Swiss WWTP: none of the single removal steps (biological treatment, ozonation, sand filtration) had a high removal efficiency but all steps taken together produced a satisfactory overall removal (Escher *et al.*, 2009).

Phytotoxicity

The I-PAM assay is sensitive to herbicides that directly inhibit photosynthesis; the results are reported as a diuron equivalent concentration (DEQ). The DEQ of the influent water ranged from 0.05 to 0.22 µg L^{-1} with a median value of 0.10 µg L^{-1} (Table 4). The DEQ increased by factors of 2.2 and 3.5 after denitrification and pre-ozonation respectively but variation from one day to another was large therefore it is difficult to draw a conclusion (Figure 5). This increase was accompanied by a slight increase in baseline toxicity and could therefore be caused by baseline toxicants interfering with the measurement of the photosynthesis yield (Macova *et al.*, 2010b). The coagulation/filtration/DAFF stage reduced DEQ by 67% and 88% compared to the plant's influent water and to the pre-ozonated water respectively. The remaining treatment stages did not significantly affect the DEQ. The overall treatment achieved 75% median decrease of DEQ, the effluent median DEQ was 0.03 µg L^{-1} (Table 4).

- The treatment train of the South Caboolture Water Reclamation Plant reduced the toxicity levels observed with various bioassays down to blank levels or equivalent. This represented a total reduction from 62 to more than 90% depending on the bioassay.
- The effect of each treatment stage varied from one bioassay to another but the combination of the coagulation/flocculation/DAFF, the main ozonation and the BAC filtration was responsible for the major part of the observed reduction.
- The main ozonation leads to lower baseline and specific toxic effects showing that the mixture of degradation products formed have an overall less harmful potential than the mixture of parent compounds. This dispels concerns about the generation of highly toxic by-products during oxidation processes.

2.4. Comparison of Chemical Analysis and Bioanalytical Tools

- Are chemical analysis and bioanalytical tools complementary and/or redundant for the assessment of treatment processes?
- Do bioanalytical tools bring valuable information in addition to chemical analysis?

2.4.1. Effect of Treatment Processes

Table 5 summarises the reduction of DOC, selected compounds' concentrations and toxic levels observed in each treatment stage. It shows clearly that, taken individually, these tools lead to very different conclusions. The DOC shows that the coagulation/flocculation/DAFF and BAC filtration are the key processes in the treatment train, whereas the removal of organic micropollutants points to ozonation and BAC filtration. For the bioassays, we can also observe that the effect of each treatment stage is not the same on all toxicity levels. We can conclude that the use of these analytical tools yields complementary information that gives a more complete picture of the overall treatment train and helps in identifying the key process.

Table 5. Summary of reduction of DOC, selected compounds' concentrations and toxic levels observed in each treatment stage.

Treatment Stage	DOC	Selected Compounds	Baseline Toxicity	Estrogenicity	AhR Response	Genotoxicity	Neurotoxicity	Phytotoxicity
Denitrification	Nil	< 20%	-30 – -21%	-73 – 4%	-85 – 12%	-8 – 24%	-14 – 26%	-400 – 1.05%
Pre-ozonation	Nil	< 30%	-38 – -6%	6 – 58%	-15 – 21%	-7 – 25%	-100 – 15%	-9 – 45%
Coagulation/ flocculation/ DAFF	40–50%	< 20%	41 – 75%	-235 – -38%	-22 – 23%	32 – 63%	36 – 71%	42 – 96%
Main ozonation	< 10%	55 to > 90%	19 – 45%	-93 – 96%	41 – 61%	18 – 87%	-8 – 32%	20 – 71%
BAC filtration	20–30%	> 90%	-2 – 67%	> 88%	6 – 38%	> 9%	>81	-
Final ozonation	Nil	-	-39 – 30%	-	-30 – 20%	-	-	-

2.4.2. Non-Specific Toxicity: Baseline-TEQ_{bio} and Baseline-TEQ_{chem}

In order to assess the fraction of the baseline-TEQ measured with the bioassay (noted baseline-TEQ_{bio}) that can be explained by the quantified organic micropollutant, a quantitative structure activity relationship (QSAR) was used. The QSAR allows estimation of the relative potency (RP_i) of any organic compound from its chemical structure (Escher *et al.*, 2008). The reference compound used is the same as the one used to express the baseline-TEQ_{bio}. The concentrations of the quantified OMPs are then multiplied by their respective relative potencies and summed according to Equation 1 to derive the baseline-TEQ of the mixture of these specific OMPs then called baseline-TEQ_{chem}. The baseline-TEQ_{chem} of the mixture can be then compared to the baseline-TEQ_{bio}. As shown in Table 6, only a tiny fraction of the baseline-TEQ_{bio} could be explained by the results of the chemical analysis. This shows the limitation of chemical analysis, which targets only a limited number of compounds among the thousands present in the treated effluent. Indeed, most of the OMPs present in treated effluents are metabolites or by-products of the parent compounds and, for most of them, their structures have yet to be elucidated. For the rare ones that have been identified, the pure substance is generally not available, which does not allow quantification. The value of using bioassays is particularly apparent after ozonation, where the fraction of baseline-TEQ_{bio} explained by chemical analysis drops below 0.03%. Indeed, as discussed above, ozonation significantly reduces the OMPs' concentrations, sometimes below their LOQ, and leads to the formation of by-products that are still active in the non-specific toxicity assay.

$$\text{baseline-TEQ}_{chem} = \sum_{i=1}^{n} \text{baseline-TEQ}_i = \sum_{i=1}^{n} RP_i \cdot C_i \qquad \text{Equation 1}$$

Table 6. Fraction of the observed baseline-TEQ_{bio} explained by chemical analysis.

Treatment Stage	baseline-TEQ_{chem} / baseline-TEQ_{bio}
WWTP effluent	0.15 – 0.27%
Denitrification	0.13 – 0.17%
Pre-ozonation	0.07 – 0.22%
Coagulation/flocculation/DAFF	0.15 – 0.32%
Main ozonation	0.01 – 0.03%
BAC filtration	>0.01%
Final ozonation	>0.01%

2.4.3. Estrogenicity

Hormones and endocrine disrupting compounds (EDCs) were not quantified along the reclamation plant treatment train but an earlier sampling campaign of the influent showed that the concentrations of measured estrogenic compounds (17 β-estradiol, 17 β-ethynyl-estradiol, estrone, estriol, bisphenol A, nonylphenol) were all below the LOQ of 1 ng L^{-1}. Nevertheless, the results obtained with the bioassays show a significant estrogenic activity equivalent to 5.7 to 7.6 ng L^{-1} of estradiol. This estrogenic activity might be due to the additional effects of the mentioned compounds that can be present at concentration below their LOQ and/or to the presence of other estrogenic compounds that were not targeted by the chemical analysis. Moreover, the LOQ of the bioassays is so much lower than the chemical analysis (0.01 ng L^{-1}) that it allows assessing the efficiency of the treatment train to reduce estrogenic activity. This demonstrates the relevance of using bioassays as complementary tools to chemical analysis for the assessment of water quality and process performances.

2.4.4. Phytotoxicity

The DEQ of the influent water ranged from 0.05 to 0.22 µg L^{-1} with a median value of 0.10 µg L^{-1}. Diuron concentrations were measured by chemical analysis; it was reported in every sample of the influent water from 0.02 to 0.04 µg L^{-1}, suggesting that its contribution to the effect observed was limited. Among the other herbicides quantified, only simazine is also a photosystem II inhibitor, with a relative potency of 0.15 (Muller *et al.*, 2008). Simazine concentrations in the influent ranged from 0.05 to 0.19 µg L^{-1}. These two compounds considered together accounted for 17 to 93% of the measured DEQ. After the main ozonation the DEQ levels were below 0.08 µg L^{-1}; diuron concentrations were equal to or below the LOQ of 0.01 µg L^{-1} and simazine concentrations were between 0.02 and 0.09 µg L^{-1}, their contribution accounting for 16 to 38% of the observed DEQ. This demonstrates again the value of bioassays to take into account the effect of OMPs that are present in the mixture but not measure by chemical analysis.

- The comparison of the removal of DOC, OMPs and reduction of toxicity levels yield different information on the treatment train efficiency, showing that they are complementary tools to assess treatment performance.
- The comparison of chemicals concentrations and toxicity levels showed that a large fraction of the observed effect is due to compounds not targeted by the chemical analysis.

2.5. Fate of Disinfection By-Product Precursors

- What is the formation potential of disinfection by-products in secondary effluent and reclaimed water?
- How are the disinfection by-products precursors removed in various treatment stages?
- What are the key processes for the removal of disinfection by-product precursors?

2.5.1. Relevance of Disinfection By-Product Precursors in Wastewater Reuse

The formation of disinfection by-products (DBPs) is an unintended consequence of the necessary disinfection of drinking water and treated wastewater. They originate in the reaction of the disinfectant with the organic and inorganic compounds present in the water matrix. More than 600 DBPs have been identified so far and this is believed to be only the tip of the iceberg. Among them, the presence of trihalomethanes (THMs), haloacetic acids (HAAs) and N-nitrosamines for example N-nitrosodimethylamine (NDMA) in water is of great concern due to their adverse effects on human health. Indeed, bladder and colorectal cancers have been associated with exposure to chlorination by-products in drinking water; their presence should therefore be also avoided in potable reuse schemes. Experimental evidence suggests that exposure also occurs through inhalation and dermal absorption (Villanueva *et al.*, 2007) which are also relevant routes in the case of non-potable reuse. The U.S. Environmental Protection Agency classifies NDMA in the group B2, which includes compounds that are probably carcinogenic to humans (U.S. Environmental Protection Agency, 2012). Moreover, NDMA was recently identified as one of the DBPs with the greatest potential impact on public health (Hebert *et al.*, 2010).

While THMs and HAAs are mainly formed when water is disinfected with chlorine (Richardson *et al.*, 2007), NDMA has been related to the presence of chloramines, specifically dichloramine generated during the disinfection process (Schreiber and Mitch, 2006). These two modes of disinfection are used for wastewater disinfection before reuse in Australia to provide a disinfectant residual in the distribution network. Studying the fate of DBP precursors in reclamation treatment trains is therefore of crucial importance. These DBPs are formed by the reaction with dissolved organic matter which, in the case of secondary treated effluent, is composed of natural organic matter and anthropogenic contaminants such as OMPs. As most DBP precursors are not characterised, a common method to measure the DBP precursors in water is by means of formation potential tests which determine the maximum quantity of DBPs that can be formed from a sample.

2.5.2. Sampling Strategy, DBP Formation Potential Tests and Quantification

Two sets of 24-hour flow proportional composite samples were collected from the sampling points indicated on Figure 1 in October 2009 to perform the NDMA formation potential test. To carry out the THMs and HAAs formation potential tests, three sets of grab samples were collected in July 2010 before the main ozonation, after the main ozonation and after the BAC filtration stages only as these processes had previously been identified as key steps. Technically, composite samples are better as they avoid having the results too much influenced by variation in water quality. However, because of the presence of the WWTP and the balance tank upstream of the reclamation plant, variations of the water quality were not expected to occur within the time of sampling.

To determine the DBP formation potential, chlorine (for THMs and HAAs) or chloramines (for NDMA) were added to a buffered sample at high concentrations and kept reacting for at least seven days to achieve the maximum formation of the specific DBPs. The THM and HAA formation potential test was performed following Standard Methods for the Examination of Water and Wastewater (Eaton *et al.*, 2005). The NDMA formation potential test closely follows the procedure described as nitrosamine precursor test by (Mitch *et al.*, 2003). The DBPs are quantified in the original sample and at the end of the formation potential test; the difference is the formation potential.

NDMA and other nitrosamines – N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosopiperidine (N-Pip), N-nirosodibuthylamine (NDBA) – were quantified using method based on U.S. Environmental Protection Agency's Method 251(Munch and Bassett, 2004). After solid phase extraction on activated carbon and elution with dichloromethane, the extracts were concentrated down by evaporation leading to a concentration factor of 1,000. Extracts were then injected in a gas chromatograph coupled with a mass spectrometer with chemical ionisation. The quantification limit for nitrosamines was 5 ng L^{-1} for NDMA, 10 ng L^{-1} for NDEA and NMOR, and 20 ng L^{-1} for N-Pip and NDBA. The THMs – chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (TBM) – were quantified using gas chromatography equipped with a purge and trap system coupled with a mass spectrometer. The limit of quantification is 1 $\mu g\ L^{-1}$ for all THMs. Five HAAs – monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA) and dibromoacetic acid (DBAA) – were extracted from aqueous samples by portioning into methyl tert-butyl ether. The analysis was carried out using gas chromatography coupled with an electron capture detector. The limit of quantification is 10 $\mu g\ L^{-1}$ for MCAA, DCAA and TCAA and 5 $\mu g\ L^{-1}$ for BCAA, MBAA and DBAA.

More details on the sampling, the formation potential tests and the quantification methods can be found in (Farre *et al.*, 2011b).

2.5.3. Results and Discussion

Fate of NDMA Precursors

NDMA, NDEA, NMOR, N-Pip and NDBA were analysed in all the samples before performing the formation potential tests. Positive results were obtained for NDMA and NMOR, but the concentrations measured along the treatment train were always lower than the limit of quantification (i.e., 5 ng L^{-1} for NDMA and 10 ng L^{-1} for NMOR) indicating that no formation occurred. This result was expected as there is no chloramination in the treatment train. Figure 6 shows the NDMA formation potential measured along the treatment train. No other N-nitrosamines, among the ones that were included in this work, were observed to be formed above their limit of quantification during the formation potential tests.

The NDMA formation potential measured at the influent of the reclamation plant was 423±55 ng L^{-1} and remained constant after denitrification confirming that this process does not affect NDMA precursors (Mitch and Sedlak, 2004). The NDMA formation potential of the secondary effluent used in South Caboolture Water Reclamation Plant was found to be similar to other domestic WWTPs in South East Queensland (Farre *et al.*, 2011a) and in other countries (Pehlivanoglu-Mantas and Sedlak,

2006) verifying that no effluents with high risk of NDMA formation potential were discharged to this specific WWTP. Pre-ozonation (0.2 mg_{O3} mg_{DOC}^{-1}) and DAFF reduced the NDMA formation potential by around 20% each, bringing the concentration down to 260±31 ng L^{-1}. The main ozonation (0.7 mg_{O3} mg_{DOC}^{-1}; 15 min contact time) was the most effective step, reducing the NDMA formation potential by another 66% to levels below 100 ng L^{-1}. This data follows the trends observed by Lee and co-authors (2007) when measuring the effect of ozone treatment on NDMA precursors in natural waters. In that study the authors reported that NDMA formation potential reduction by applying up to 40 µM (1.9 mg L^{-1}) of ozone ranged from 32 to 94%, depending on the natural water and oxidation conditions. The BAC filtration reduced the NDMA precursors further down to 58±2 ng L^{-1}. At this stage, the activated carbon had been replaced 20 months before sample collection and had filtered about 50,000 bed volumes. It is assumed that the adsorption capacity of the media is essentially exhausted and the removal observed is due to biodegradation of organic matter by the bacteria established in the filter. The final ozonation did not have a significant effect, leaving a concentration of NDMA precursors in the final effluent of 53±6 ng L^{-1}.

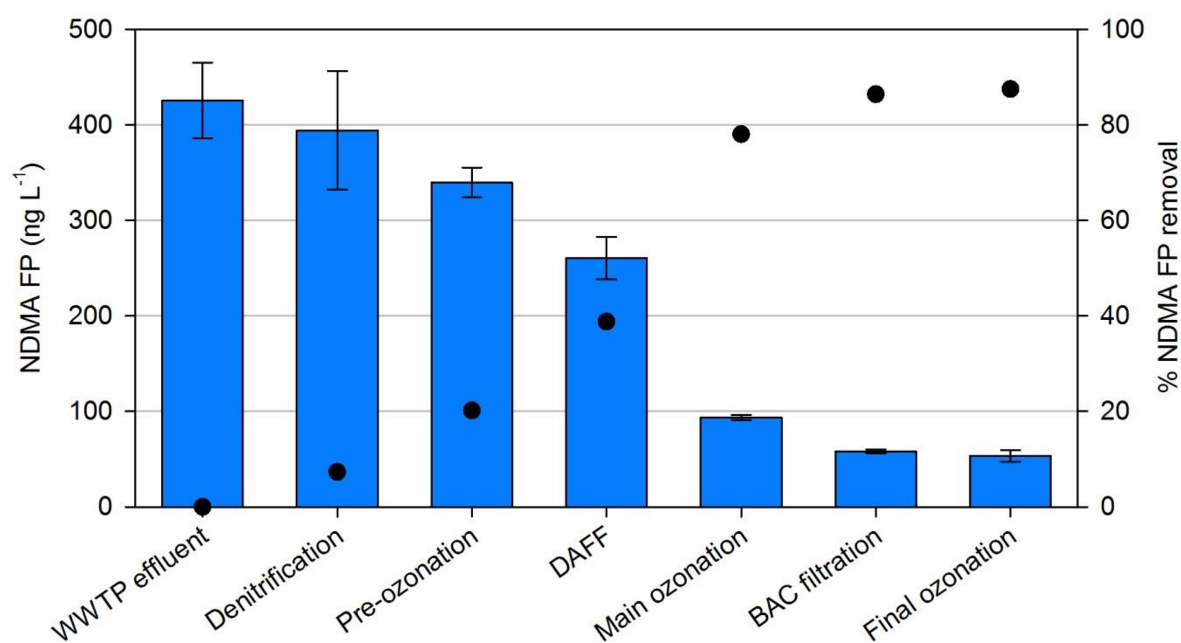

Figure 6. Bar charts correspond to NDMA precursors measured by NDMA formation potential test (FP) across South Caboolture Water Reclamation Plant. Error bars correspond to the standard deviation (n=2). Dot points correspond to the cumulative removal percentage of NDMA precursors relative to the WWTP effluent across the plant.

Fate of THM and HAA Precursors

Four THMs (TTHMs) and five HAAs (5HAAs) were quantified in the samples collected from the treatment plant before performing the formation tests. No HAAs were measured above the LOQ for any of the sampling points during the different sampling campaigns. Low concentrations of THMs were measured across the treatment train but the TTHM concentration was always below 11 µg L^{-1}. Figure 7 shows the result of HAA and THM formation potential tests of the selected samples in conjunction with 5HAAs, TTHMs and DOC data. Monohalogenated acids were not formed in the formation potential test. Among the HAAs generated during the tests, the HAAs containing only chlorine (DCAA and TCAA) had the highest concentrations, several times higher than the HAAs containing bromine. The same fact was observed for THMs.

Ozonation removes the precursors for TCAA and TCM. The increase on DBCM observed by others (Chen *et al.*, 2009) is also seen slightly in our data, since the concentration of this DBP increases from 11 µg L^{-1} to 15 µg L^{-1} when comparing the concentration of this compound after DAFF and after ozonation. Liang and Singer (2003) have suggested that bromide is more reactive with aliphatic precursors, such as hydrophilic organic material rich in aliphatic structures, than with aromatic

precursors, such as hydrophobic organic material. Ozonation is known to lead to the formation of more hydrophilic by-products and to the opening of aromatic rings. Hence, the change in the nature of the organic matter after ozonation to become more hydrophilic may explain the increase of the formation of this specific DBP.

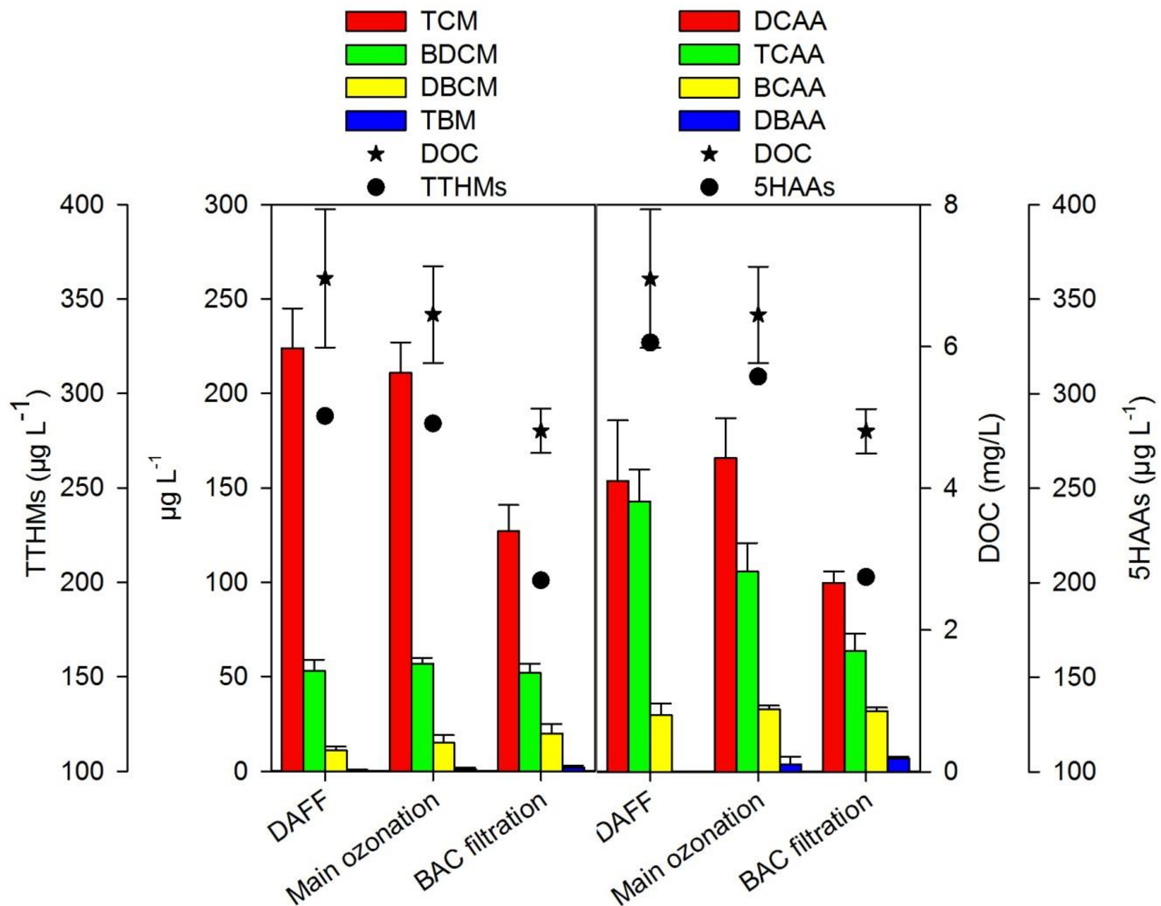

Figure 7. THMs (left) and HAAS (right) precursors and DOC across South Caboolture Water Reclamation Plant, error bars correspond to standard deviation (n=3)

The formation potential of DBPs containing only chlorine was significantly reduced by BAC filtration (39±2%, 39±2% and 40±5% for DCAA, TCAA and TCM respectively) whereas the formation of brominated DBPs was not affected. This is due to organic matter removal by the bacteria that have colonised the filtering media. The ion concentrations were not expected to be affected by the treatment, which is supported by the fact that the conductivity was stable. Since we could not measure any bromate formation above the limit of quantification (i.e. 10 µg L^{-1}), we assumed the oxidation of Br^- to BrO_3^- by ozone was minimal. Therefore all bromide (Br^-) was available to be oxidised to HOBr by HOCl during the formation potential test. The rate constant of bromide with HOCl to generate HOBr is 1.5×10^3 $M^{-1}s^{-1}$ (Kumar and Margerum, 1987) and the rate constant of THMs formation is in the range of 0.01 and 0.03 $M^{-1}s^{-1}$ (Gallard and von Gunten, 2002). It is known that once formed, bromine reacts about 10 times faster than chlorine with natural organic matter (Westerhoff *et al.*, 2004; Hua *et al.*, 2006). Hence, the formation of bromine-containing DBPs is limited by the initial Br^- concentration whereas the formation of chlorine-containing DBPs would be limited by the organic matter. Therefore, when organic matter decreases along the treatment train, the formation of chlorine-containing DBPs is reduced while the formation of bromine-containing DBPs remains constant.

The THM and HAA formation potential was not measure before the coagulation/ flocculation/DAFF stage in this campaign. However, given that these DBPs originate from the organic matter and that the formation potential and DOC follow a similar trend in the main ozonation and the BAC filtration, it can be supposed that this stage would also have a significant effect on THM and HAA formation potential as it removes about 50% of DOC of the WWTP effluent (see 2.2.3)

- The secondary effluent contains significant levels of disinfection by-product precursors. Among nitrosamines, only NDMA was formed. Among HAAs and THMs, the ones containing only chlorine were formed predominantly.
- The key process for the removal of NDMA precursors is the main ozonation although coagulation/flocculation/DAFF and BAC filtration also play a role. The coagulation/ flocculation/DAFF also plays an important indirect role by reducing the DOC concentration, therefore allowing a more efficient ozonation.
- The key process for the removal of THMs and HAAs precursor is BAC filtration. The effect of coagulation/flocculation/DAFF was not assessed but is likely to be significant as well.
- The removal of organic matter leads to a decrease in chlorinated DBP formation potential but does not impact the formation of brominated DBPs. Removal of bromide would be necessary to reduce their formation potential.

2.6. Final Water Quality: Indirect Potable Reuse Considerations

- Is the final water quality compliant with the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies?
- Would this treatment train be suitable to produce water for indirect potable reuse?

2.6.1. Organic Micropollutants

OMPs concentrations were compared to the guideline values for indirect potable reuse given in the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies (Appendix 2). The concentrations of the measured compounds were found to be below the guideline values in the WWTP effluent entering the reclamation plant before any treatment. After going through the advanced treatment train, concentrations were several orders of magnitude below the guideline values.

2.6.2. Toxicity

There is no guideline for toxicity levels observed with bioassays but, for information purposes, median equivalent concentrations obtained with the bioassays were compared to the corresponding reference compound's guideline value when available. Note however, that the effect caused by a mixture cannot be compared directly to a guideline value of a single compound. Moreover, the bioassays used here are acute tests and no conclusions can be drawn about chronic effects. Nevertheless such a comparison gives an impression of the expected hazard of the mixture but must be communicated with caution to a lay audience. For estrogenicity, neurotoxicity and phytotoxicity the reference compounds were estradiol, parathion and diuron and the guidelines values were 175 ng L^{-1}, 10 µg L^{-1} and 30 µg L^{-1} respectively. Similarly to individual compound concentrations, the bioassays equivalent concentrations were already below the guidelines values in the water entering the reclamation plant. Final effluent median equivalent concentrations were also several orders of magnitude below the corresponding guideline values, i.e. more than 2900, 33 and 428 fold for estrogenicity, neurotoxicity and phytotoxicity respectively.

2.6.3. Disinfection By-Products

Table 7 compares the formation potential after BAC filtration to the guideline values found in the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies. The final effluent values would be close to the formation potentials measured after BAC filtration as the final ozonation has little effect. For the THMs, the formation potentials are below the guideline values except for trichloromethane which is slightly above. On the contrary, for HAAs and NDMA, the formation potential is much higher than the guideline values. However, these formation potentials are obtained under conditions that are not representative of real disinfection systems. In reality, the levels formed would likely be much lower. Moreover, operational parameters during disinfection can be

optimised to limit DBP formation. Nevertheless, the treatment train significantly removes the precursors of HAAs and NDMA as well, which would also contribute to limiting their formation.

Table 7. DBPs formation potential after BAC filtration (after final ozonation for NDMA).

Disinfection By-Product	Guideline Value	Formation Potential after BAC Filtration
Trichloromethane	107 µg L^{-1}	127±14 µg L^{-1}
Bromodichloromethane	120 µg L^{-1}	52±5 µg L^{-1}
Dibromochloromethane	120 µg L^{-1}	20±5 µg L^{-1}
Monochloroacetic acid	-	< 10 µg L^{-1}
Dichloroacetic acid	0.72 µg L^{-1}	100±6 µg L^{-1}
Trichloroacetic acid	5 µg L^{-1}	64±9 µg L^{-1}
Bromochloroacetic acid	-	32±2 µg L^{-1}
Dibromoacetic acid	-	7±1 µg L^{-1}
NDMA	10 ng L^{-1}	53±6ng L^{-1}

For the parameters considered, the water quality complies with the requirements of the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies. Some DBP formation potential exceeded the guideline values but that does not mean this value would be reached under real disinfection conditions. This suggests that such a treatment train could be considered as an alternative to the combination of microfiltration and reverse osmosis for indirect potable reuse schemes. It has the advantage of not producing a waste stream and would be certainly less energy intensive. Nevertheless, before this process can be recommended for indirect potable reuse, additional consideration needs to be given to the overall risk management strategies of the treatment train. Moreover, the removal of pathogens such as viruses and bacteria has to be assessed as well. Finally, this type of treatment does not remove salts, which might be necessary in some situations.

- The concentrations of organic micropollutants were below the guideline values even before any treatment was applied; the final concentrations are several orders of magnitude lower.
- The equivalent concentrations obtained by the bioassays are below the guideline values of the corresponding compound but this is informative only as bioassay results and single compounds guideline values cannot be directly compared.
- The formation potential of THMs was below the guideline values whereas they were exceeded by the HAA and NDMA formation potential. However, these values are obtained under extreme conditions that are not representative of real disinfection systems.
- Further consideration of pathogen removal and overall risk management would be necessary.

3. COMPARISON OF THREE FULL SCALE RECLAMATION PLANTS

Following the results obtained at the South Caboolture Water Reclamation Plant, two additional full scale plants were sampled in order to:

- confirm the results obtained at Caboolture; and
- assess the influence of water quality and operating conditions.

The South Caboolture Water Reclamation Plant was also sampled again to determine the efficiency of the BAC filter after a longer period of operation and compare it to the first samples that were collected only shortly after the activated carbon had been renewed.

3.1. Reclamation Plants Sampled

Samples were collected from three full scale wastewater reclamation plants located in Australia, their treatment trains are depicted on Figure 8. All the plants receive treated effluent from WWTPs with biological nutrient removal. After various pre-treatment stages, they all use ozonation followed by BAC filtration before final disinfection using various techniques. However, the ozone dose and empty bed contact time (EBCT) in the BAC filters differ from one plant to another, providing different configurations. Relative to the DOC concentration at the time of sampling (Table 8), the ozone doses supplied were in the ranges of 0.6-0.8; 0.2-0.3 and 0.4-0.5 $mg_{O3}\ mg_{DOC}^{-1}$ for Caboolture, Landsborough and Gerringong respectively. The activated carbons used in the BAC filters were from various sources. At Caboolture, the filter media had been replaced in March 2008 and the samples were collected in July 2010, by that time approximately 68,000 bed volumes had passed through the filter. The BAC filters were commissioned in 2003 at Landsborough and the media has not been renewed since, leading to more than 350,000 bed volumes filtered at the time of sampling (March to June 2010). Finally, at Gerringong, the four BAC filters were commissioned in 2002 and the media was replaced in two of them in August 2009. Therefore, at the time of the sampling campaign in September 2010, half of the media had filtered approximately 95,000 bed volumes and the other half about 13,000 bed volumes. Given the large numbers of bed volumes filtered in each plant, it is reasonable to assume the all the filters have passed the breakthrough of organic matter and adsorption is negligible. Dissolved oxygen concentrations measured before and after filtration through the BAC showed a decrease, confirming that they were biologically active.

3.1.1. Sampling Strategy

Three sets of grab samples were collected from each plant at the sampling points indicated on Figure 8. Grab samples were collected as opposed to composite samples, since the study focuses on treatment process efficiency and not on pollutants loads. Moreover, the balance tanks allow a steady flow rate along the advanced treatment train and variations of water quality during sampling were not expected to occur in such a short timeframe.

For OMP analysis, 2 L of sample were collected into amber glass bottles pre-washed with MilliQ water and HPLC grade methanol. For the bioassays, 2 L of sample were collected in similar bottles and hydrochloric acid (36%) was added to a final concentration of 5 mM for preservation. For DOC measurements, 100 mL were collected in MilliQ washed plastic (HDPE) bottles. All bottles were rinsed a couple of times with the water to be sampled before filling. All samples were transported on ice and protected from light until they reached the laboratory where they were stored at 4°C prior to analysis (which occurred within a week).

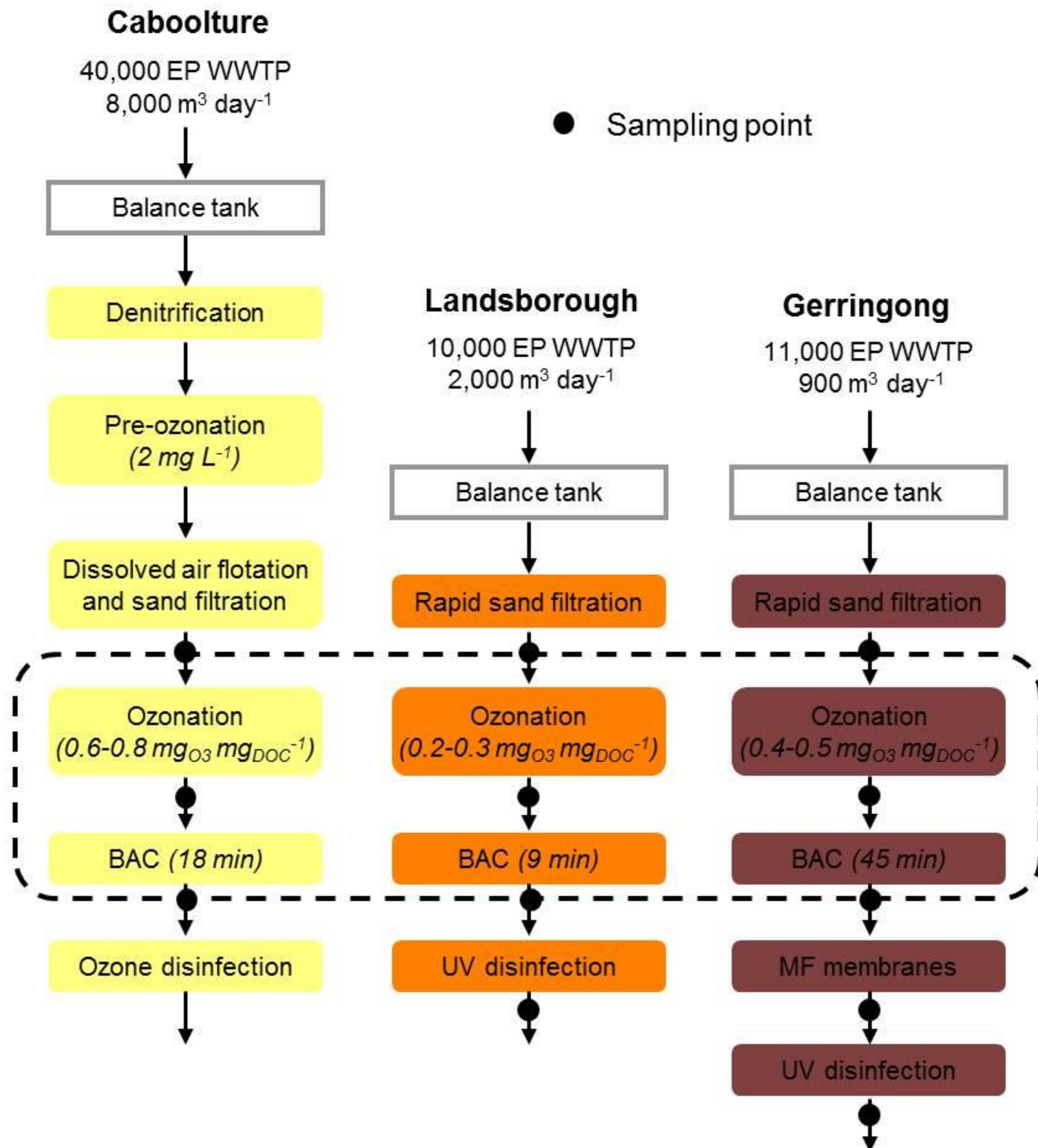

Figure 8. Treatment trains of the three investigated full scale reclamation plants, the dots indicate the sampling points. Ozonation: number in brackets is ozone dose relative to DOC. BAC: number in brackets is EBCT. EP=equivalent people; MF = microfiltration.

3.2. Analytical Methods

3.2.1. Organic Micropollutants

Forty one OMPs were quantified using the method described in detail in Appendix 3. The method consisted of SPE, elution, concentration, and analysis of the extract by liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS). The list of the quantified compounds with some of their properties is available in Appendix 4. The removal of a given OMP in a treatment stage was reported only when its concentration was above its LOQ before and after the treatment or at least ten times its LOQ when the concentration was below LOQ after the treatment. These criteria were set to allow the determination of removals up to 90% and avoid underestimating the removal of compounds that fell below their LOQ.

3.2.2. Bioanalytical Tools

We selected two bioassays from the battery presented in 2.3.2 and Table 3: the non-specific bioluminescence inhibition test with *Vibrio fischeri* and the estrogenicity specific assay E-SCREEN. The baseline-TEQ_{chem} was derived from the OMPs concentrations according to the procedure described in 1.1.1.

3.3. Water Quality before Ozonation

➢ Is the treated wastewater quality similar in different locations in Australia?

The quality of the treated effluents before the ozonation stage was similar in all the plants (Table 8). The DOC and nutrients levels were low, showing the efficacy of the WWTPs in removing these compounds. However, most of the quantified OMPs were detected before ozonation with concentrations varying from the low ng L^{-1} up to the µg L^{-1} levels, showing their incomplete removal in the WWTPs (Appendix 5). It is interesting to note that every single compound was generally quantified in a similar range of concentrations across all the plants despite the different locations and sampling times. This shows how ubiquitous these compounds are in treated effluents as well as a typical consumption pattern within Australia.

Table 8. Water quality parameters before the ozonation stage in reclamation plants (N/D = not determined).

	Caboolture	Landsborough	Gerringong
T (°C)	22.0	22.6 – 28.5	N/D
pH	6.6 – 6.7	6.7 – 7.1	6.7 – 6.9
Conductivity (µS)	879 – 910	392 – 507	520 – 563
DOC (mgC L^{-1})	6.5 – 8.1	5.8 – 6.6	4.2 – 5.8
PO_4^{3-}(mgP L^{-1})	≤ 0.02	0.22 – 2.00	< 0.02
NH_4^+ (mgN L^{-1})	< 0.03	0.22 – 0.45	0.18 – 1.36
NO_2^- (mgN L^{-1})	< 0.02	0.03 – 0.06	< 0.02 – 0.04
NO_3^- (mgN L^{-1})	<0.02 – 0.95	0.18 – 0.47	0.39 – 1.14
Baseline-TEQ_{bio}(mg L^{-1})	1.83 – 2.72	1.50 – 2.01	1.10 – 1.84
Baseline-TEQ_{chem} (µg L^{-1})	1.74 – 2.62	3.31 – 5.81	2.77 – 2.97
Baseline-TEQ_{chem}/ Baseline-TEQ_{bio}	0.10 – 0.11%	0.19 – 0.29%	0.15 – 0.26%
EEQ (ng L^{-1})	0.98 – 1.73	1.13 – 1.44	0.57 – 1.53

➢ In the three plants sampled, the secondary effluent had very similar properties, including OMP concentrations, estrogenicity and non-toxicity levels. This show the ubiquitous presence of OMPs in wastewater across Australia, and a typical consumption pattern.

3.4. Ozonation

➢ What is the influence of the ozone dose on the reduction of DOC, OMP concentration, estrogenicity and non-specific toxicity?

➢ Is a minimum ozone dose required to observe significant removal of OMPs?

3.4.1. Dissolved Organic Carbon

In Caboolture, which uses the highest ozone dose, modest removal of DOC was observed but in the other plants DOC was not affected (Figure 9). At the doses employed, ozonation leads to limited mineralisation and oxidation by-products are generated.

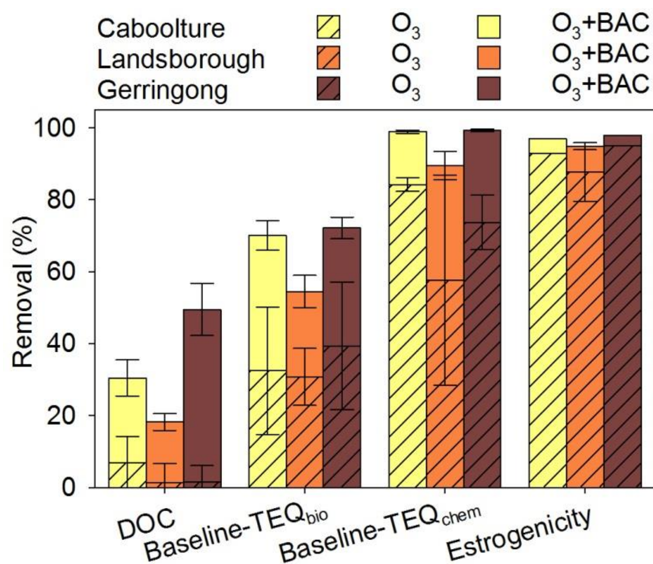

Figure 9. Removal of dissolved organic carbon (DOC), baseline-toxicity equivalent concentrations (baseline-TEQ$_{bio}$ and baseline-TEQ$_{chem}$) and estradiol equivalent concentration (EEQ) in the reclamation plants. The hatched sections represent removal by ozonation only and the full bars represent total removal achieved after ozonation and BAC filtration (average of 3 independent samples ± standard deviation).

3.4.2. Fate of Organic Micropollutants

In the three plants, ozonation achieved OMP removal to a degree depending on the compounds and the ozone dose. Some compounds were effectively removed in all plants regardless of the ozone dose, while the removal of others was lower and generally depended on the ozone dose (Figure 10). It is clear that increasing ozone to DOC ratio leads to increasing removal, particularly for compounds that show lower removal (Figure 11). In ozonation processes, organic compounds can be oxidised via two mechanisms: reaction with molecular ozone (direct pathway) and reaction with hydroxyl radical generated by ozone decomposition in water (indirect pathway). Molecular ozone reacts selectively with electron rich moieties and reaction rates vary by several orders of magnitude. On the contrary, hydroxyl radicals are not selective and reaction rates are typically >10^9 $M^{-1}s^{-1}$. However, due to $[HO^{\bullet}]/[O_3]$ ratios typically in the range of 10^{-9} to 10^{-7}(von Gunten, 2003; Buffle *et al.*, 2006b), the indirect pathway is not always the dominant one.

The compounds that were highly removed independently of the ozone dose (i.e. diclofenac, sulfamethoxazole, trimethoprim, propranolol, naproxen, carbamazepine, roxithromycin, erythromycin) have direct reaction rates with molecular ozone >10^4 $M^{-1}s^{-1}$ and/or have been previously shown to be easily removed from treated effluents, even at low ozone dosage (Appendix 6). These compounds have electron rich functional groups that are highly reactive with molecular ozone; such as aniline (diclofenac, sulfamethoxazole), pyrimidine (trimethoprim), naphthalene (propranolol, naproxen), aromatic rings and double bonds (carbamazepine) and tertiary amines (roxithromycin, erythromycin). Oxidation of these compounds occurs almost exclusively via direct reaction with molecular ozone (Buffle *et al.*, 2006b; Hollender *et al.*, 2009).

Among the compounds that showed lower removal and/or dependency on the ozone dose, metroprolol, diuron, 2,4-D, atenolol, hydrochlorothiazide and caffeine have direct reaction rates with ozone of <10^3 $M^{-1}s^{-1}$ (Appendix 6). Compounds with low direct reaction rates require exposure to higher ozone

doses to allow their effective oxidation (Hollender *et al.*, 2009; Wert *et al.*, 2009). When the direct reaction rate constant with ozone decreases, the relative importance of oxidation by hydroxyl radicals increases and, for values <10^2 $M^{-1}s^{-1}$, oxidation occurs almost exclusively via the indirect pathway (Buffle *et al.*, 2006b; Hollender *et al.*, 2009). However, during the initial phase of ozonation, ozone decomposes rapidly while reacting with the effluent organic matter and generates high amounts of hydroxyl radicals (Buffle *et al.*, 2006a; Buffle *et al.*, 2006b). Therefore, even at low ozone doses, some removal of compounds refractory to ozone can be observed (e.g. 2,4-D, diuron, caffeine).

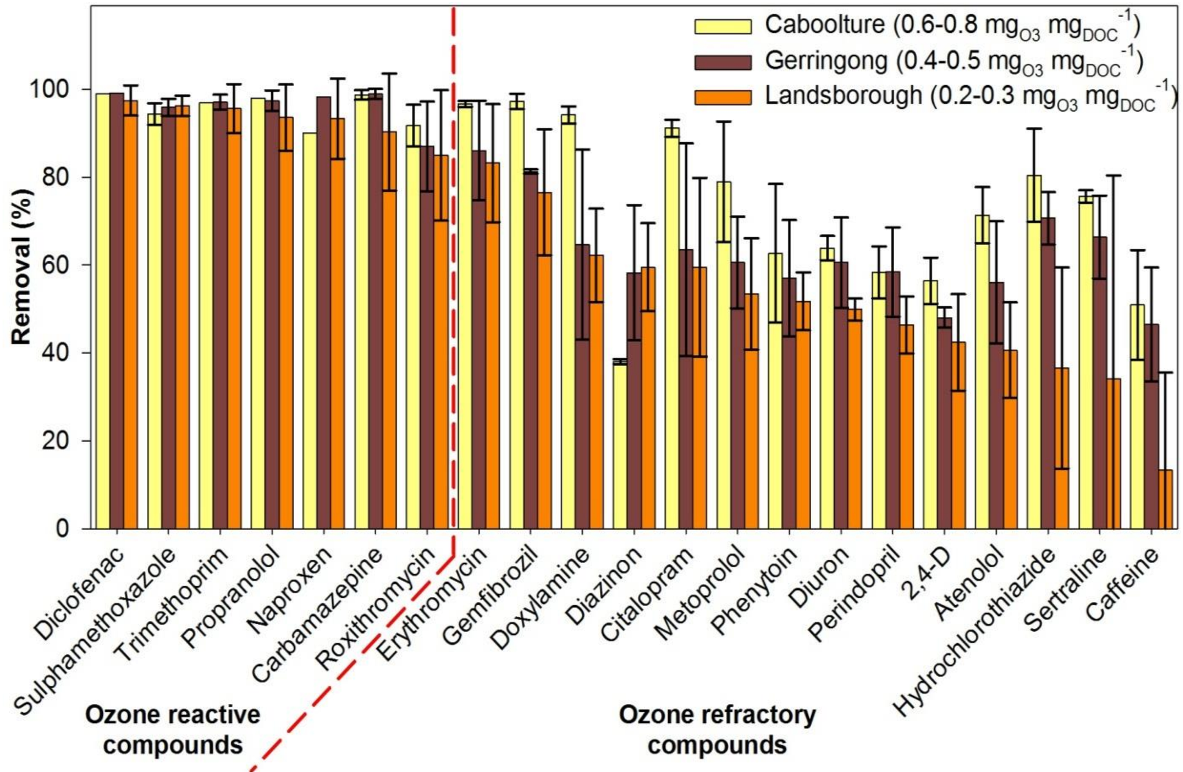

Figure 10. Removal of selected OMPs by ozonation (average of 3 independent samples ± standard deviation).

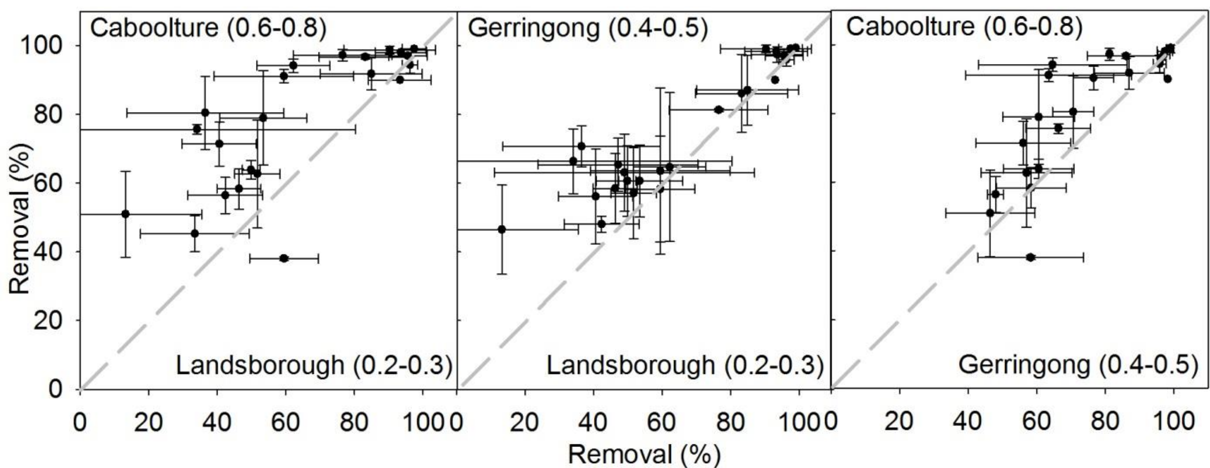

Figure 11. Comparison of the removal of OMPs by ozonation in reclamation plants, mg_{O3} mg_{DOC}^{-1} indicated in brackets (average of 3 independent values ± standard deviation).

3.4.3. Estrogenicity

Estrogenicity levels in the samples are summarised in Table 9. More than 87% reduction of estrogenicity expressed as EEQ was observed in the ozonation stage of all the reclamation plants. Even at the lowest dose of 0.2 to 0.3 mg_{O3} mg_{DOC}^{-1} high removal of estrogenicity was achieved. This is consistent with previous findings, showing that ozonation is a very effective treatment for the reduction of estrogenic activity of treated wastewater, even at relatively low ozone doses (Snyder *et al.*, 2006; Escher *et al.*, 2009; Reungoat *et al.*, 2010). Indeed, several estrogenic compounds are very reactive with molecular ozone ($k>10^4$ $M^{-1}s^{-1}$) (Deborde *et al.*, 2005) and it has been suggested that the transformation by-products lose most of their estrogenic potential (Huber *et al.*, 2004). This finding can be rationalised by the fact that receptor mediated effects require a good steric fit between the ligand (OMP or natural) and the receptor. Even mild oxidation leads to a dramatic decrease in this interaction and thus to a decrease or complete loss of estrogenic potency (Lee *et al.*, 2008).

Table 9. Baseline-TEQ_{bio}, baseline-TEQ_{chem} and estrogenicity.

	Baseline-TEQ_{bio} (mg L^{-1})			Baseline-TEQ_{chem} (µg L^{-1})			Estrogenicity ($ng_{estradiol}$ L^{-1})		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
Caboolture									
Before ozonation	1.83	2.31	2.72	1.74	2.44	2.62	0.98	1.73	1.11
After ozonation	1.55	1.60	1.33	0.27	0.34	0.47	0.07	0.08	< 0.03
After BAC filtration	0.50	0.80	0.76	0.02	0.01	0.03	0.03	< 0.01	< 0.03
Gerringong									
Before ozonation	1.56	1.10	1.84	2.97	1.10	1.84	1.53	0.57	0.64
After ozonation	0.70	0.88	1.05	0.52	0.88	1.05	< 0.03	< 0.03	< 0.03
After BAC filtration	0.42	0.28	0.57	0.02	0.28	0.57	< 0.03	< 0.01	< 0.01
Landsborough									
Before ozonation	1.97	1.50	2.01	3.83	3.31	5.81	1.44	1.13	1.15
After ozonation	1.40	0.91	1.53	2.91	0.78	1.58	0.10	0.09	0.25
After BAC filtration	0.94	0.73	0.81	0.57	0.26	0.49	0.07	0.05	0.07

3.4.4. Non-Specific Toxicity

Baseline-TEQ_{bio} and baseline-TEQ_{chem} levels in all samples are summarised in Table 9. A decrease of baseline-TEQ_{bio} between 31 and 39% was observed after the ozonation stage in all three plants (Figure 9). This indicates that the mixture of oxidation by-products has a lower non-specific toxicity potential compared to the mixture of parent compounds. Therefore, there should be no concern regarding a possible increase in non-specific toxicity due to the generation of oxidation by-products during the ozonation treatment of treated effluents. However, this assay does not take into account the formation of by-products with specific and reactive modes of toxic action that could still present a hazard to the environment and human health. Specific toxicity is usually receptor mediated and even mild oxidation leads to by-products that typically have much lower affinity to receptors as shown above for estrogenicity. In contrast, reactive intermediates can be formed and there is not enough knowledge on their effect.

The reduction of baseline-TEQ_{bio} was similar in the three plants and, contrary to what was observed for OMPs, there was no trend following the ozone dose. This observation is also not consistent with previous findings on a Swiss WWTP, where the ozone doses from 0.3 to 1 mg_{DOC}^{-1} resulted in an increased trend of reduction from 25% to approximately 70% (Escher *et al.*, 2009). It must be noted though, that the reduction of baseline-TEQ_{bio} was quite variable in that study as it would be expected that not only the ozone dose but also other determinants, for example the temperature and the type of OMPs, play a role. Nevertheless, the observed reductions were in a similar range between the Swiss study and the present study, which indicates that these case studies allow some degree of generalisation.

The baseline-TEQ_{chem} in the samples taken before the ozonation step, which were calculated from the relative potencies and concentrations of the OMP concentrations, were approximately three orders of magnitude lower than the baseline-TEQ_{bio} measured with the bioassays (Table 8). Thus, the quantified OMPs explain less than 0.3% of the non-specific toxicity and more than 99.7% of the measured non-specific toxicity is contributed by other compounds present in the water. After ozonation, the fraction of toxicity explained by chemical analysis decreases by a factor of 2 to 4, indicating that either the quantified chemicals were more degradable than the ones not on the list, or that the chemicals are just transformed and their toxicity is reduced but not fully eliminated.

Previous studies of the ozonation of effluent organic matter showed that ozone reacts preferentially with its most hydrophobic fraction, leading to the formation of more hydrophilic compounds (Gong *et al.*, 2008; Rosario-Ortiz *et al.*, 2008; Domenjoud *et al.*, 2011), which have a lower non-specific toxicity. This is also evidenced by the Quantitative Structure Activity Relationship (QSAR) used to determine the non-specific toxic potential of individual compounds, which shows that it is strongly dependent on the compounds' hydrophobicity. Indeed, a tenfold decrease in hydrophobicity, as would occur if, for example, a hydroxyl group is introduced into a molecule, would also lead to an approximately tenfold reduction of toxicity of the transformation product.

The remaining hydrophilic fraction of effluent organic matter does not react readily with ozone and/or forms by-products that conserve its toxic potential. Gong *et al.*(2008) showed that ozonation had limited effect on the more hydrophilic fractions of effluent organic matter. It is generally assumed that effluent organic matter is too large to be bioavailable but smaller breakdown products and assimilable organic carbon are likely to be and they will contribute to the baseline-TEQ_{bio}, provided they are also extracted with solid phase extraction. It can be concluded that the use of a high ozone dose does not necessarily lead to a significant toxicity reduction and may not actually lead to further toxicity reduction.

- In the range studied (0.2 to 0.8 $mg_{O3}\ mg_{DOC}^{-1}$) the ozone dose has different impacts on the reduction of DOC, OMPs, estrogenicity and non-specific toxicity.
- DOC removal is not impacted by the ozone dose: it remains low in every plant (<10%), confirming the formation of by-products.
- OMPs are impacted differently, depending on their chemical structures. OMPs that are very reactive with ozone are effectively removed (>80%) even with the lowest ozone dose. For other OMPs, the removal increases with increasing ozone dose.
- Estrogenicity is reduced by more than 87% whatever the ozone dose. This shows that estrogenic compounds are very reactive with ozone and the by-products lose their estrogenic potential.
- Non-specific toxicity reduction is significant but independent of the ozone dose (31-39%). This might indicate that ozone reacts rapidly with a fraction of the compounds and slowly with the remainder.

3.5. Biological Activated Carbon

- Does the contact time influence the reduction of DOC, OMP concentration, estrogenicity and non-specific toxicity?
- Can the fate of OMPs in BAC filters be linked to their adsorption and/or biodegradation propensity?

3.5.1. Dissolved Organic Carbon

Contrary to ozonation, BAC filtration significantly removed DOC in the three plants (Figure 9). The removal increased with increasing EBCT and reached almost 50% at Gerringong. The results obtained at Caboolture were in the same range as for the first sampling campaign (2.2.3) suggesting the adsorption capacity was already largely exhausted at the time. It shows that BAC filters can maintain

performance over a long period of time. The life of BAC filters can be divided in three phases (Simpson, 2008). During the first phase, organic matter is mainly removed by adsorption onto granular activated carbon. This phase is usually characterised by a high removal of organic matter. Rapidly, bacteria attach to the media and start growing, feeding on the organic matter and nutrients present in the water being filtered. In parallel, the adsorption efficiency starts to decrease as the activated carbon capacity becomes exhausted. During this phase, the removal of organic matter typically decreases with time. Eventually, the biomass is fully established in the filter and adsorption sites are exhausted.

In that last phase, the removal of organic matter observed is only due to biodegradation by the bacteria and typically much lower than the removal observed in the initial phase. This third phase can last for several years as the granular activated carbon does not need to be renewed. In this study, the BAC filters investigated have been in use for several years and have filtered tens of thousands of bed volumes. The bacteria therefore had ample time to establish, which was confirmed by the reduction of dissolved oxygen concentration observed across the filters in Caboolture and Landsborough. Dissolved oxygen could not be measured in Gerringong but it is reasonable to assume bacteria have developed in these filters as well.

A longer contact time allows the bacteria to degrade more organic matter as shown in previous studies on BAC filtration (Seredynska-Sobecka *et al.*, 2006) and simulated soil filtration (Rauch and Drewes, 2004; Maeng *et al.*, 2008). However, the DOC removal did not increase linearly with the contact time and a higher removal rate was observed for short EBCT (17±2%, 25±6% and 48±10% for 9, 18 and 45 minutes respectively). Indeed, the easily (rapidly) biodegradable organic matter is likely to be removed first (i.e. at short contact time) and the biodegradability of the remaining fraction decreases, leading to lower biodegradation rates. Consistently, previous simulations of soil filtration showed a faster removal of organic matter in the first stages of the filtration (Rauch and Drewes, 2004; Maeng *et al.*, 2008).

3.5.2. Fate of Organic Micropollutants

Filtration through BAC was able to further remove all the remaining compounds after ozonation, except perindopril in Landsborough (Figure 12). The removal of OMPs in Caboolture was still high and similar to what was observed during the first campaign. Removal varied from nil to more than 99% depending on the compound and the plant. The removal also depended on the EBCT: removals were higher for the filters with 18 and 45 minutes compared to 9 minutes, however there was no clear increase between 18 and 45 minutes EBCT (Figure 13). The observed removal of DOC (Figure 9) suggests that the filters are in the third phase of their life, i.e. organic matter is mainly removed by biodegradation. However, most of the compounds known to be poorly or moderately removed in the WWTP were significantly removed in the filters, even with an EBCT as short as 9 minutes, and sometimes by more than 90% for EBCT of 18 or 45 minutes.

Reungoat *et al.*(2011) observed high removal of pharmaceuticals over a long period of time in biological activated carbon filters treating non-ozonated and ozonated wastewater. This suggests that the bacterial community might adapt to the biodegradation of compounds refractory in WWTP as it has been shown in simulated aquifer recharge (Rauch-Williams *et al.*, 2010). But even though it is hypothesised that the adsorption capacity of the activated carbon in the filters is largely exhausted, the removal of specific OMPs is not correlated with the removal of bulk organic matter and OMP breakthrough can be observed much later than DOC breakthrough (Wang *et al.*, 2007). Also, OMPs with various properties can have breakthrough separated by tens of thousands of bed volumes (Snyder *et al.*, 2007).

Adsorption onto activated carbon is difficult to predict as the mechanism involves several types of interactions. Westerhoff *et al.* (2005) showed that removal efficiencies of OMPs by powdered activated carbon tend to increase with increasing octanol-water partition coefficient ($logK_{ow}$) but some protonated bases and deprotonated acids did not follow this general trend. This is partially due to the fact that charged compounds are more hydrophilic than their neutral forms. Therefore the octanol-water distribution coefficient obtained at a given pH ($logD_{ow}$) might be a better way to estimate

adsorption potential of charged compounds. The $\log D_{ow}$ (pH 7) of selected compounds were calculated from their respective $\log K_{ow}$ and pKa (Appendix 4) according to the equations proposed by Scherrer and Howard (1977). In Figure 12, compounds are presented according to increasing $\log D_{ow}$ (pH 7) from left to right but no trend of increasing removal can be seen. The removal mechanism of OMPs in biological activated carbon filters remains unclear at this stage and could be a combination of adsorption and biodegradation, depending on the compounds.

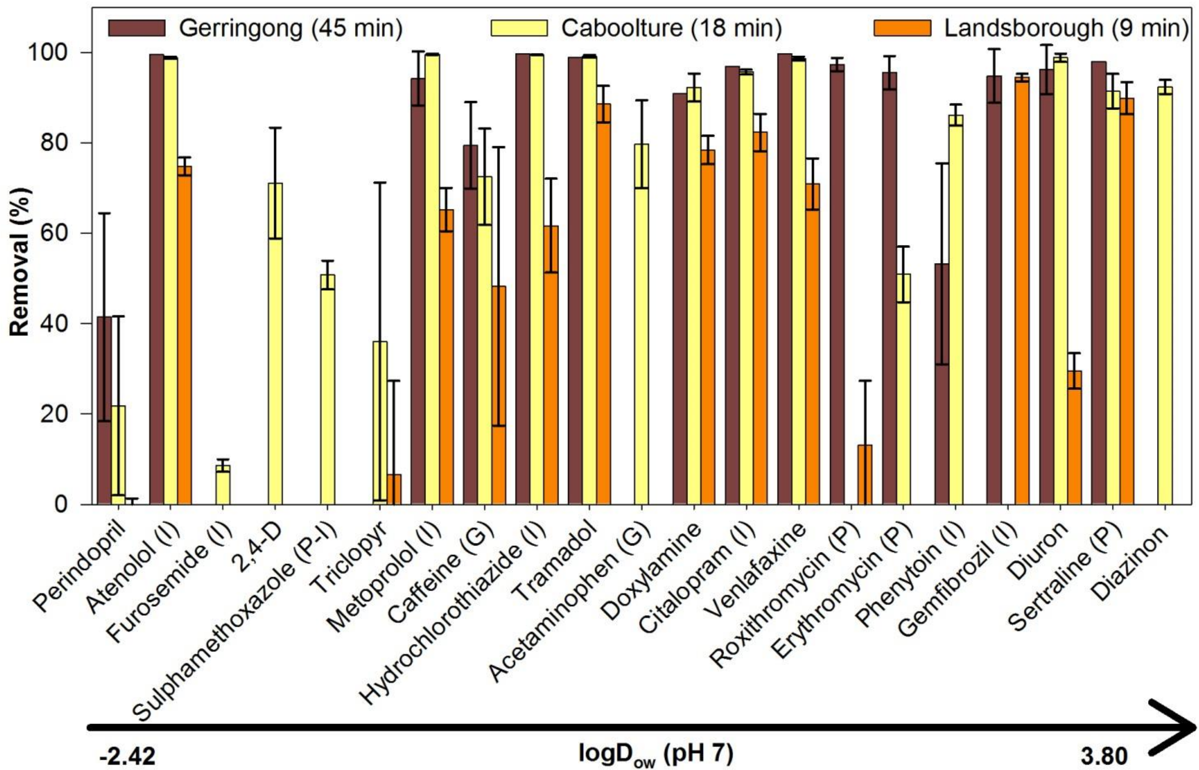

Figure 12. Removal of selected OMPs by BAC filtration, empty bed contact time is indicated in the legend (average of 3 independent values ± standard deviation). No bar means a removal could not be calculated because concentrations were either too low or below the LOQ. Letters in brackets indicate removal generally observed in WWTP estimated from Onesios *et al.* (2009): P=poor (<20%); I=intermediate (20-80%); G=good (>80%).

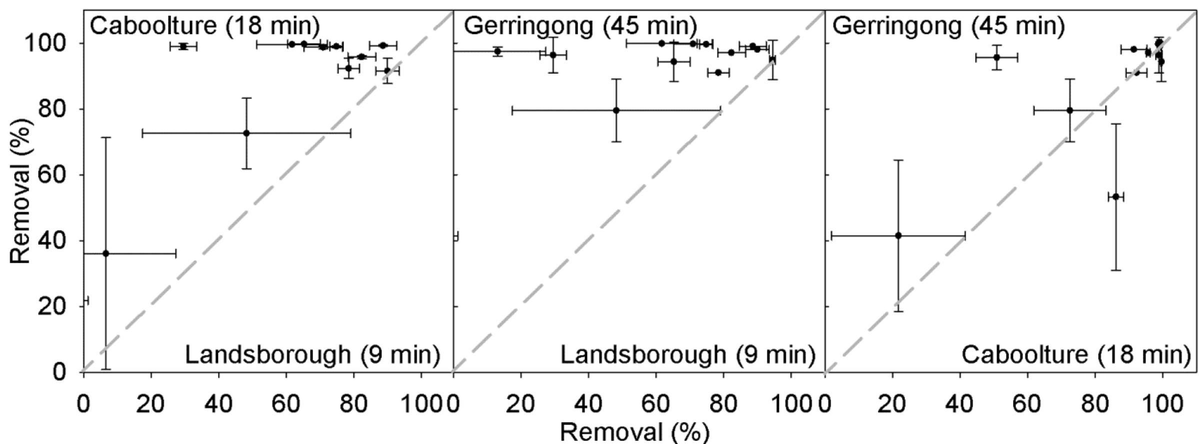

Figure 13. Comparison of the removal of organic micropollutants in BAC filters in reclamation plants (average of 3 independent values ± standard deviation).

3.5.3. Estrogenicity

BAC filtration further reduced estrogenicity in Landsborough but it is difficult to assess its efficiency as the levels were already very low after ozonation. In the other two plants, the levels were even lower before BAC filtration and close to or below the quantification limit (0.03 ng L^{-1}) after (Table 9). In the samples that were above the LOQ before and after BAC filtration the estrogenicity was only reduced by a factor of two to three, indicating that the residual estrogenic compounds that were left after ozonation are not easily biodegradable and they are likely to be xenoestrogens and/or ethinylestradiol as those are less biodegradable than the natural estrogens (Liu *et al.*, 2009).

3.5.4. Non-Specific Toxicity

BAC filtration significantly reduced the baseline-TEQ_{bio} after ozonation by 54±13, 33±13 and 51±15% in Caboolture, Landsborough and Gerringong respectively. By comparison, Caboolture had a baseline-TEQ_{bio} reduction in the range of 2 to 67% in the first campaign. The second campaign showed similar and more stable results. In parallel, the DOC was reduced by 24±6, 17±3 and 48±10% respectively, indicating that compounds contributing to the non-specific toxicity are preferentially removed or transformed to metabolites with lower toxic potential. From the point of view of specific toxicity and chemical analysis, ozonation as a single step would be sufficient for removal of OMPs. The non-specific toxicity tells us a different story because this bioassay integrates the effect of all OMPs present in the sample. Transformation products are invisible to chemical analysis and, as discussed above, will only marginally contribute to estrogenicity, but can still substantially contribute to non-specific toxicity. This is an important point and justifies the parallel application of bioassays when investigating the removal of OMPs in various wastewater treatment processes. Similarly to OMPs, the reduction of toxicity increased when EBCT increased from 9 to 18 minutes but not when it was increased to 45 minutes.

- Increasing the BAC contact time from 9 to 18 minutes leads to increased removal of DOC, OMPs, estrogenicity and non-specific toxicity. Increasing the contact time from 18 to 45 minutes leads to increased removal of DOC but does not impact other quality parameters. The various ages of the BAC filters could also have an influence the results.
- The fate of OMPs in the BAC filters could not be linked their biodegradation, nor to adsorption propensities. The removal mechanism of organic matter and OMPs is thought to be a combination of biodegradation and adsorption.

4. CONCLUSION AND RECOMMENDATIONS

4.1. Ozonation followed with BAC Filtration: an Effective Combination for Wastewater Reclamation

Ozonation followed by BAC filtration is an effective barrier for organic matter, OMPs, non-specific and specific toxicity.

This study showed that ozonation followed by BAC filtration greatly improves the chemical quality of WWTP effluents by:

- removing residual organic matter (as DOC) by to 50%;
- removing a wide range of organic micropollutants by more than 90%;
- reducing non-specific as well as specific toxicity down to blank levels; and
- removing disinfection by-product precursors by up to 80%.

This process combination has therefore the potential to be used for the advanced treatment of wastewater treatment plant effluents for the protection of surface water or as one of the barrier of an indirect potable reuse scheme.

While ozonation is a very effective barrier against OMPs, estrogenic compounds and NDMA precursors; it has a more limited effect on non-specific toxicity, THM and HAAs precursors and DOC. BAC filtration is essential to reduce non-specific toxicity, THMs and HAAs precursors and DOC removal and has a polishing effect on OMPs, estrogenicity and NDMA precursors. It is therefore recommended that ozonation is always followed by BAC filtration to offer an effective barrier to a wider range of contaminants.

The results showed that, in the range studied, the ozone dose affected the removal of OMPs but not the other aspects. The results suggest that the contact time in the BAC filters is also an important operating parameter, affecting its efficiency, but this has to be confirmed. The age of the BAC might also influence performance as well as the ozone dose itself. This shows that the results achieved by the combined treatment will depend on the operating conditions and trials have to be carried out to find out the right ones to achieve the desired objective. As the ozone dose relative to the DOC is a crucial parameter, any pre-treatment applied before ozonation to remove DOC is also likely to improve its efficiency. Although it could not be evaluated in this study, the characteristics of the wastewater itself are likely to impact the treatment performance as well. Therefore, any particular situation needs a specific evaluation and a “one-size-fits-all” solution cannot be proposed.

While the mechanisms of OMPs by ozonation have been extensively studied and are well understood, they remain unclear for BAC filters. The results suggest that it is a combination of adsorption and biodegradation but their respective role is yet to be identified and quantified. Further fundamental research is necessary on BAC filters to elucidate the mechanisms and find ways to optimise operation.

4.2. Bioanalytical Tools for Water Quality Analysis: a Complement to Chemical Analysis

The use of bioanalytical tools in combination with chemical analysis brings valuable complementary information to assess water quality and treatment processes.

Chemical analysis shows that ozonation is very effective to remove OMPs but hardly reduces DOC. This suggests that OMPs are simply transformed to by-products but, as these are unknown, it is not possible to quantify them and determine whether they are more or less harmful than the parent compounds. The bioluminescence test used in this study showed a reduction in non-specific toxicity after ozonation, suggesting that the mixture of by-products formed is less harmful than the mixture of parent compounds. Also, when comparing the reduction in toxicity observed with the bioassay to the

one calculated from the chemical analysis, it can be clearly seen that chemical analysis looks only at a very limited number of the OMPs present in the water and that the ozonation by-products still express some toxicity level. Other bioassays show that the ozonation/BAC combination is also capable of reducing specific toxicity levels.

When looking only at the OMP removal, ozonation seems to be the key process. When looking at the DOC removal, BAC filtration seems to be essential. The bioluminescence test shows that both participate in the overall reduction of non-specific toxicity.

Bioanalytical tools are still mainly used for research purposes and do not have the maturity of chemical analysis, but they have great potential to become conventional monitoring tools. Bioanalytical tools should be given more consideration and an effort should be made to combine them with classical chemical analysis for water quality and treatment processes assessment. This will help their development further and consolidate the still fragile link existing between bioassays and chemical analysis.

APPENDIX 1. QHFSS ORGANIC MICROPOLLUTANTS ANALYTICAL METHOD

Extraction Method

Solid phase extraction was performed using with Waters Oasis HLB 60 mg in 3 mL cartridges. The filled cartridges were conditioned with 1 mL dichloromethane, 5 mL methanol and 5 mL MilliQ water. The samples were extracted at neutral and acidic pH on an automated SPE equipment (Gilson Aspec); 40 mL of sample was loaded on the cartridge which was then dried for 10 minutes under a nitrogen flow. For acidic extraction, 1 mL of formic acid 98-100% purity was added to 50 mL of sample. The analytes of interest were eluted with 1 mL of acetonitrile followed by 2 mL of dichloromethane for neutral analysis; or 2% ammonium hydroxide/98% acetonitrile followed by 2 mL dichloromethane for acidic analysis. Extracts were gently blown to dryness using nitrogen. 400 µL of 15% acetonitrile/water was added to the dried extracts before transfer to LC/MS-MS vial with 400 µL glass inserts. For quality control, each batch included a blank sample and a standard mixture sample (both prepared with deionised water) which were extracted following the same method. The standard mixture sample contained 31 pharmaceuticals and personal care products. A mixture of five compounds (containing caffeine D_3, Carbamazepine D_{10}, atrazine D_5, Diclofenac D_4 and $^{13}C_6$ 2,4-dichlorophenoxy acetic acid) was added to each sample, including standard mixtures and blanks prior to extraction to monitor the extraction efficiency (Table SI 1).A 10 µL spike of internal standard containing 4compounds (acetylsulfamethoxazole D_5, fluoxetine D_5, 2,4-dichlorophenyl acetic acid and simazine D_{10}) was added to each vial prior to analysis by LC/MS-MS. Long term mean recoveries are given in Table SI 2. Given very low extraction recovery, gabapentin was quantified by direct injection of the sample (i.e. without extraction and concentration).

Quantification Method

Extracts were analysed by HPLC/tandem mass spectrometry injecting 8 µLina Shimadzu Prominence HPLC system(Shimadzu Corp., Kyoto, Japan)connected to an AB/Sciex API4000QTrap mass spectrometer equipped with an electrospray (TurboV) interface (MDS Sciex, Concord, Ont., Canada). The HPLC instrument was equipped with a 3 µm 150 ×2 mm Luna C18(2) column (Phenomenex, Torrance, CA) run at 45°C. Separation was achieved with a flow rate of 0.35 ml $min^{-1}$with a linear gradient starting at 15% B for 0.3 minutes, ramped to 100% B in 10 minutes, held for 4 minute and then to 15% B in 0.2 minutes and equilibrated for 4 minutes (A = 1% acetonitrile/99% HPLC grade water, B = 95% acetonitrile/5% HPLC grade water both containing 0.1% formic acid). Each sample extract was analysed separately in both positive and negative ion multiple reaction monitoring mode, LC/MSMS parameters are given in table SI 2.Positive samples were confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Samples were only reported as positive if the two transitions are present, retention time is within 0.15 minutes of the standard and the relative intensity of the confirmation transition was within 20% of the expected value. Analyte concentrations were determined using the internal standard method and compared to a four point calibration using standard concentrations from 5 to 100 µg L^{-1}.Limits of quantification (LOQs) were set at a signal to noise ratio of 9.

Table 10. Long term mean recovery of surrogate chemicals from samples and standard deviation.

Surrogate Chemicals	Mean Recovery (%)	Standard Deviation (%)
Diclofenac D4	82	15
Carbamazepine D10	98	15
Caffeine D3	98	20
Atrazine D5	89	16
2,4-Dichlorophenoxy acetic acid13C6	91	13

Table 11. Recoveries, liquid chromatography retention times and MS/MS parameters.

POSITIVE MODE (entrance potential = 10 volts)										
Compound Name	**Rec (%)**	**Rt (min)**	**DP (V)**	**Q1 (Da)**	**$Q3_{qant}$ (Da)**	**CE (V)**	**CXP (V)**	**$Q3_{conf}$ (Da)**	**CE (V)**	**CXP (V)**
Atenolol[1]	44	2.30	71	267.2	190.1	27	10	145.0	39	8
Ranitidine[1]	50	2.44	56	315.2	176.1	25	8	130.1	35	6
Codeine[1]	58	2.85	86	300.2	215.2	35	12	152.1	89	6
Gabapentin[1]	NA	2.90	66	172.1	154.0	19	8	137.0	23	6
Lincomycin[1]	51	3.02	60	407.3	126.1	44	8	359.3	28	20
Iopromide[1]	74	3.03	100	791.7	573.1	35	14	559.1	41	14
Oxycodone[1]	73	3.03	65	316.2	298.2	26	16	241.2	42	16
Paracetamol[1]	38	3.13	61	152.1	110.0	23	6	65.1	42	6
Cephalexin[1]	ND	3.17	45	348.3	158.1	13	8	174.1	21	10
Trimethoprim[1]	85	3.17	85	291.2	230.1	35	14	123.1	35	8
Norfloxacin[1]	22	3.21	70	320.3	276.2	26	14	233.2	35	14
Ciprofloxacin[1]	32	3.26	61	332.3	231.1	54	12	288.2	24	16
Oxytetracycline[1]	30	3.31	30	461.3	426.3	28	6	443.3	17	6
Doxylamine[1]	20	3.33	40	271.2	182.0	24	8	167.1	45	7
Enrofloxacin[1]	58	3.37	28	360.3	316.2	30	15	245.2	40	15
Tetracycline[1]	19	3.41	50	445.3	410.2	28	24	154.1	40	7
Caffeine D_3*[1]	100	3.50	60	198.1	138.0	27	8	110.0	35	8
Caffeine[1]	83	3.52	61	195.1	138.1	29	6	110.1	33	4
Metoprolol[1]	78	3.53	70	268.2	116.1	28	7	191.1	27	10
Sulfadiazine[1]	40	3.54	71	251.2	92.0	37	14	65.0	61	10
Tramadol[1]	87	3.54	45	264.2	58.0	44	8	42.0	125	3
Sulfathiazole[1]	69	3.58	51	256.2	156.1	22	10	92.1	40	8
Acetylsulfamethoxazole D_5	IS1	3.87	60	302.3	202.1	26	9	138.1	36	9
Chlortetracycline[1]	16	3.90	50	479.3	444.3	32	6	154.1	42	12
Venlafaxine[1]	79	3.98	45	278.2	58.0	50	7	121.0	40	10
Desisopropylatrazine[1]	90	4.02	70	174.0	104.0	34	10	132.0	27	10
Propranolol[1]	80	4.25	70	260.2	116.1	28	8	183.1	28	8
Desmethylcitalopram[1]	70	4.58	60	311.3	109.0	35	8	262.2	25	15
Citalopram[3]	86	4.67	70	325.3	109.0	38	4	262.2	28	4
Dapsone[3]	45	4.68	60	249.2	156.0	22	7	92.0	34	14
Erythromycin[3]	42	4.90	50	734.7	576.4	27	18	158.1	45	8
Desethylatrazine[1]	100	5.01	70	188.0	146.0	26	10	104.0	37	10
Sulfamethoxazole[3]	65	5.15	51	254.2	156.0	23	8	92.1	38	8
Tylosin[3]	12	5.36	10	916.7	174.1	58	5	101.1	72	12
Fluoxetine D_5	IS2	6.04	45	315.2	44.0	42	5	153.1	14	10
Fluoxetine[2]	44	6.09	51	310.1	44.0	37	8	148.0	13	7
Sertraline[3]	57	6.12	35	306.3	159.1	35	12	275.2	18	12
Tebuthiuron[3]	120	6.32	70	229.2	172.0	27	10	116.0	40	10
Roxithromycin[3]	21	6.39	10	837.6	679.5	32	9	158.0	52	5
Hexazinone[3]	160	6.43	70	253.2	171.0	24	10	71.0	50	10

POSITIVE MODE (entrance potential = 10 volts)										
Compound Name	**Rec (%)**	**Rt (min)**	**DP (V)**	**Q1 (Da)**	**$Q3_{qant}$ (Da)**	**CE (V)**	**CXP (V)**	**$Q3_{conf}$ (Da)**	**CE (V)**	**CXP (V)**
Ametryn[3]	110	6.60	70	228.2	186.0	28	10	116.0	38	10
Bromacil[3]	110	6.73	40	261.2	205.0	23	10	207.0	23	10
Simazine D_{10}	IS3	6.9	60	212.0	137.0	40	10	134.0	38	10
Carbamazepine D_{10}*[3]	97	7.00	65	247.2	204.1	30	8	202.1	51	8
Simazine[3]	90	7.01	70	202.1	132.0	29	10	124.0	27	10
Carbamazepine[3]	90	7.06	96	237.2	194.0	31	16	193.0	47	12
Phenytoin[3]	98	7.07	66	253.2	182.0	29	18	104.0	48	14
Oxazepam[3]	96	7.42	60	287.2	241.2	32	10	104.0	52	10
Propoxur[3]	100	7.58	25	210.1	111.0	20	8	168.1	12	8
Prometryn[3]	110	7.75	70	242.2	158.0	35	10	200.1	28	10
Terbutryn[3]	90	7.79	46	242.2	91.2	39	6	71.1	45	4
Desmethyldiazepam[3]	92	7.80	70	271.2	140.1	41	15	165.1	41	15
Carbaryl[3]	110	7.93	25	202.1	145.1	13	7	127.1	41	7
Flumeturon[3]	110	8.00	70	233.1	72.0	38	10	46.0	38	10
Sulfasalazine[3]	21	8.03	30	399.3	223.1	43	11	119.1	63	7
Atrazine D_5*[3]	88	8.13	60	221.1	179.0	27	6	101.0	36	6
Atrazine[3]	100	8.18	71	216.1	174.0	27	14	96.0	36	12
Diuron[3]	120	8.26	70	235.2	72.0	40	10	46.0	38	10
DEET[3]	79	8.27	86	192.1	119.0	26	10	91.0	44	6
Temazepam[3]	96	8.29	55	301.2	255.1	32	8	283.1	21	8
3,4-dichloroaniline[3]	70	8.41	55	162.0	127.0	30	10	74.0	70	10
Naproxen[3]	100	8.49	61	231.2	185.1	19	10	170.1	37	8
Praziquantel[3]	70	8.62	70	313.3	203.2	25	10	55.0	72	8
Diazepam[3]	84	8.89	76	285.2	154.1	36	12	193.2	42	14
Atorvastatin[3]	34	9.61	70	559.5	440.3	31	10	250.2	62	10
Diclofenac D_4*[3]	80	9.75	45	300.1	219.1	30	8	218.1	46	8
Indomethacin[3]	48	9.76	66	358.3	138.9	31	10	75.0	107	12
Diclofenac[3]	52	9.78	40	296.2	214.0	50	10	250.1	21	10
Metolachlor[3]	100	10.4	76	284.2	252.0	22	18	176.0	38	18
Diazinon[3]	130	11.2	50	305.3	169.1	35	8	249.1	27	8
Simvastatin[3]	ND	11.8	62	419.3	285.2	16	15	199.1	18	15

NEGATIVE MODE (entrance potential = -10 volts)										
Compound Name	**Rec (%)**	**Rt (min)**	**DP (V)**	**Q1 (Da)**	**$Q3_{quant}$ (Da)**	**CE (V)**	**CXP (V)**	**$Q3_{conf}$ (Da)**	**CE (V)**	**CXP (V)**
Hydrochlorothiazide[4]	91	3.98	-55	296.0	205.0	-34	-12	269.0	-28	-20
Dalapon[4]	60	4.91	-43	140.9	97.0	-11	-7	105.0	-12	-7
Picloram[4]	100	5.59	-24	239.0	195.0	-13	-7	197.0	-13	-7
Acetylsalicylic acid[4]	67	5.78	-35	178.9	136.9	-9	-11	92.9	-30	-5
Chloramphenicol[4]	89	6.17	-70	321.0	152.0	-25	-13	257.0	-16	-13
Salicylic acid[4]	54	6.47	-45	137.0	93.0	-24	-6	65.0	-40	-6
Fluroxypyr[4]	100	6.90	-35	252.9	194.9	-17	-7	196.9	-17	-7
Furosemide[4]	72	6.99	-57	329.0	285.0	-21	-13	205.0	-33	-13
Dicamba[4]	100	7.54	-25	219.0	175.0	-10	-7	177.0	-10	-10
Dichlorophenylacetic acid	IS4	7.95	-25	205.0	161.0	-10	-10	159.0	-10	-7
MCPA[4]	110	8.13	-45	199.0	141.0	-22	-7	143.0	-19	-7
2,4-D or 2,4-Dichlorophenoxyacetic acid[4]	110	8.15	-36	219.0	161.0	-21	-7	163.0	-22	-7
2,4-Dichlorophenoxy acetic acid $^{13}C_6$*[4]	90	8.15	-36	225.0	167.0	-21	-10	169.0	-21	-10
Triclopyr[4]	110	8.48	-35	254.0	196.0	-19	-7	198.0	-21	-7
Mecoprop[4]	110	8.75	-45	213.0	141.0	-21	-7	143.0	-17	-7
2,4-DP or2-(2,4-dichlorophenoxy)propionic acid[4]	110	8.78	-35	233.0	161.0	-21	-7	163.0	-21	-7
Warfarin[4]	89	8.94	-170	307.0	161.0	-28	-11	250.0	-30	-9
2,4-DB or4-(2,4-dichlorophenoxy) butyric acid[4]	100	8.98	-27	247.0	161.0	-13	-7	163.0	-13	-7
MCPB[4]	110	9.03	-30	227.0	141.0	-13	-7	143.0	-20	-7
Fluvastatin[4]	30	9.34	-10	410.3	348.1	-22	-26	210.1	-42	-11
Diclofenac D_4*[4]	80	9.58	-50	302.1	258.0	-16	-8	256.0	-16	-8
Ibuprofen[4]	101	9.85	-52	205.1	161.0	-11.5	-10	159.0	-11	-10
Gemfibrozil[4]	44	10.50	-60	249.1	121.0	-18	-8	127.0	-15	-9
Triclosan[4]	30	11.00	-50	287.0	35.0	-30	-3	35.0	-30	-3

Rec = recovery (per cent) at a concentration in the sample of 1 µg L^{-1} for pharmaceuticals and 0.1 µg L^{-1} for herbicides and pesticides (ND indicates insufficient data to determine); **Rt** = retention time; **DP** = declustering potential; **Q1** = parent ion; **$Q3_{quant}$** = fragment ion used for quantitation; **$Q3_{conf}$** = fragment ion used for confirmation; **CE** = collision energy; **CXP** = collision cell exit potential.

* surrogate compounds

[1] internal standard used for quantification = Acetylsulfamethoxazole D5 (IS1)

[2] internal standard used for quantification = Fluoxetine D5 (IS2)

[3] internal standard used for quantification = Simazine D10 (IS3)

[4] internal standard used for quantification = Dichlorophenylacetic acid (IS4)

APPENDIX 2. LIST OF QHFSS COMPOUNDS AND PROPERTIES

Table 12. Compounds quantified, classification, hydrophobicity expressed as logarithm of octanol-water partition coefficient (log K_{ow}), limit of quantification (LOQ) by LC/MS-MS analysis, influent concentrations to the water reclamation plant and guideline values from the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies.

Compound Name	Classification	Log K_{ow} [a]	LOQ (µg L^{-1})	Influent Concentrations (µg L^{-1})			Guideline Value (µg L^{-1})
				Max	Median	Min	
2,4-DB or 4-(2,4-dichlorophenoxy)butyric acid	Herbicide	3.60	0.01	- [b]	-	-	90 [iii]
2,4-D or 2,4-Dichlorophenoxyacetic acid	Herbicide	2.62	0.01	0.08	0.05	0.03	30 [i]
2-4-DP or 2-(2,4-dichlorophenoxy)propionic acid	Herbicide	3.03	0.01	-	-	-	100 [iii]
3,4-dichloroaniline	Diuron and propanil metabolite	2.37	0.01	0.02	0.02	0.01	0.1 [iv]
Acetylsalicylic acid	Analgesic, antipyretic	1.13	0.01	-	-	-	29 [i]
Ametryn	Herbicide	3.32	0.01	-	-	-	5 [ii]
Atenolol	Beta-blocker	- 0.03	0.01	1.00	0.76	0.60	25 [v]
Atorvastatin	Hypolipidemic agent	6.36	0.01	0.03	0.02	0.02	50 [i]
Atrazine	Herbicide	2.82	0.01	-	-	-	20 [i]
Bromacil	Herbicide	1.68	0.01	-	-	-	10 [ii]
Caffeine		0.16	0.01	0.97	0.51	0.43	0.35 [i]
Carbamazepine	Anticonvulsant	2.25	0.01	0.95	0.70	0.39	1,000 [i]
Carbaryl	Insecticide	2.35	0.02	0.02	-	-	5 [i]
Cephalexin	Antibiotic (cephalosporin)	0.40	0.01	0.08	0.06	-	350 [i]
Chloramphenicol	Antibiotic	0.92	0.10	-	-	-	175 [i]
Chlortetracycline	Antibiotic (tetracycline)	- 0.68	0.10	-	-	-	105 [i]
Ciprofloxacin	Antibiotic (quinolone)	0.00	0.01	0.03	0.02	-	2,500 [i]
Citalopram	Antidepressant	3.74	0.01	0.11	0.08	0.06	10 [v]
Codeine	Analgesic	1.28	0.02	1.32	1.02	0.68	500 [i]
Dalapon	Herbicide	1.68	0.01	0.08	0.07	0.04	0.1 [iv]
Dapsone	Antituberculotic and antileprotic	0.77	0.01	-	-	-	25 [v]
DEET	Insect repellent	2.26	0.01	0.07	0.06	0.04	2,500 [i]
Desethylatrazine	Atrazine metabolite	1.78	0.01	-	-	-	0.1 [iv]
Desisopropylatrazine	Atrazine metabolite	1.36	0.01	0.02	0.01	-	0.1 [iv]
Desmethylcitalopram	Citalopram metabolite		0.01	0.06	0.05	0.04	N/A
Desmethyldiazepam	Anxiolytic/diazepam metabolite	2.87	0.01	0.03	0.03	0.01	3.75 [v]
Diazepam	Anxiolytic	2.70	0.01	-	-	-	2.5 [i]

Compound Name	Classification	Log K_{ow} [a]	LOQ ($\mu g\ L^{-1}$)	Influent Concentrations ($\mu g\ L^{-1}$)			Guideline Value ($\mu g\ L^{-1}$)
				Max	**Median**	**Min**	
Compound name	Classification	Log K_{ow} [a]	LOQ ($\mu g\ L^{-1}$)	Influent concentrations ($\mu g\ L^{-1}$)			Guideline value ($\mu g\ L^{-1}$)
				Max	Median	Min	
Diazinon	Insecticide	3.86	0.01	0.04	0.03	0.02	3 [i]
Dicamba	Herbicide	2.14	0.01	0.08	0.04	-	100 [ii]
Diclofenac	NSAI[c]	4.02	0.01	0.27	0.20	0.14	18 [i]
Diuron	Herbicide	2.67	0.01	0.04	0.03	0.03	30 [i]
Doxylamine	Sedative	2.37	0.01	0.46	0.36	0.22	12.5 [v]
Enrofloxacin	Veterinary antibiotic (quinolone)	0.70	0.01	0.01	-	-	22 [i]
Erythromycin	Antibiotic (macrolide)	2.48	0.01	0.46	0.26	0.18	175 [i]
Fluometuron	Herbicide	2.35	0.01	-	-	-	0.1 [iv]
Fluoxetine	Antidepressant	4.65	0.01	0.01	-	-	100 [i]
Fluroxypyr	Herbicide	1.17	0.01	0.02	-	-	0.1 [iv]
Fluvastatin	Hypolipidemic agent	4.85	0.01	-	-	-	10 [v]
Furosemide	Diuretic	2.32	0.01	1.30	1.07	0.89	10 [v]
Gabapentin	Anticonvulsant	- 1.37	0.10	6.50	5.45	5.10	450 [v]
Gemfibrozil	Hypolipidemic agent	4.77	0.01	0.20	0.17	0.14	600 [v]
Hexazinone	Herbicide	2.15	0.01	-	-	-	2 [ii]
Hydrochlorothiazide	Diuretic	- 0.10	0.01	0.90	0.79	0.50	12.5 [v]
Ibuprofen	NSAI	3.79	0.04	0.16	0.09	0.08	4,000 [i]
Indomethacin	NSAI	4.23	0.01	0.04	0.03	0.03	250 [i]
Iopromide	Radiographic agent	-2.49	0.20	2.10	1.27	0.58	7,500 [i]
Lincomycin	Antibiotic (lincosamide)	0.29	0.01	0.06	0.03	-	35,000 [i]
MCPA	Herbicide	2.52	0.01	0.20	0.17	0.12	2 [iii]
MCPB	Herbicide	3.50	0.01	-	-	-	0.1 [iv]
Mecoprop	Herbicide	2.94	0.01	0.11	0.05	0.04	10 [iii]
Metolachlor	Herbicide	3.24	0.01	-	-	-	300 [i]
Metoprolol	Beta-blocker	1.69	0.01	0.48	0.39	0.35	250 [i]
Naproxen	NSAI	3.10	0.10	0.51	0.29	0.24	2,200 [i]
Norfloxacin	Antibiotic (quinolone)	- 0.03	0.01	0.04	0.03	-	4,000 [i]
Oxazepam	Anxiolytic	2.32	0.01	0.95	0.87	0.46	7.5 [v]
Oxycodone	Narcotic analgesic	0.66	0.01	0.04	0.04	0.03	10 [v]
Oxytetracycline	Antibiotic (tetracycline)	- 2.87	0.10	-	-	-	105 [i]
Paracetamol	Analgesic, antipyretic	0.27	0.01	0.39	0.26	0.12	1,750 [i]
Phenytoin	Anticonvulsant	2.16	0.01	0.26	0.24	0.11	140 [v]
Picloram	Herbicide	1.36	0.01	0.08	0.05	-	300 [ii] *
Praziquantel	Anthelmintic	2.42	0.01	-	-	-	2,100 [v]

Compound Name	Classification	Log K_{ow} [a]	LOQ (µg L^{-1})	Influent Concentrations (µg L^{-1})			Guideline Value (µg L^{-1})
				Max	Median	Min	
Prometryn	Herbicide	3.73	0.01	-	-	-	0.1 [iv]
Propoxur	Insecticide	1.90	0.02	0.05	0.04	0.03	0.1 [iv]
Propranolol	Beta-blocker	2.60	0.01	0.05	0.04	0.03	400 [i]
Ranitidine	Histamine-blocker	0.29	0.01	0.36	0.31	0.22	150
Roxithromycin	Antibiotic (macrolide)	2.75	0.01	0.37	0.29	0.23	1,500 [i]
Salicylic acid	Acetylsalicylic acid metabolite	2.24	0.01	0.02	0.02	0.01	290 [i]
Sertraline	Antidepressants	5.29	0.01	0.02	0.02	0.01	25 [v]
Simazine	Herbicide	2.40	0.01	0.19	0.11	0.05	20 [i]
Simvastatin	Hypolipidemic agent	5.19	0.01	-	-	-	5 [i]
Sulfadiazine	Antibiotic (sulfonamide)	- 0.34	0.01	-	-	-	1,000 [i]
Sulfasalazine	Anti-inflammatory	3.81	0.01	0.04	0.03	0.02	500 [i]
Sulfathiazole	Antibiotic (sulfonamide)	0.72	0.01	-	-	-	40 [v]
Sulfamethoxazole	Antibiotic (sulfonamide)	0.48	0.01	0.24	0.22	0.11	350 [i]
Tebuthiuron	Herbicide	1.78	0.01	-	-	-	0.1 [iv]
Temazepam	Sedative	2.15	0.01	0.60	0.51	0.25	50 [i]
Terbutryn	Herbicide	3.77	0.01	-	-	-	1 [ii]
Tetracycline	Antibiotic (tetracycline)	- 1.33	0.10	-	-	-	105 [i]
Tramadol	Narcotic analgesic	3.01	0.01	1.42	1.22	0.88	50 [v]
Triclopyr	Herbicide	2.53	0.01	0.12	0.10	0.09	10 [ii]
Triclosan	Biocide	4.66	0.01	0.02	0.02	0.02	0.35 [i]
Trimethoprim	Antibiotic	0.73	0.01	0.21	0.20	0.15	700 [i]
Tylosin	Antibiotic (macrolide)	1.05	0.01	-	-	-	1,050 [i]
Venlafaxine	Antidepressant	3.28	0.01	1.71	1.48	1.02	37.5 [v]
Warfarin	Anticoagulant	2.23	0.01	-	-	-	1 [v]

[a]Calcultated with EPI SUITE 4.0

[b] below limit of quantification

[c] NSAI: nonsteroidal anti-inflammatory agent

[i] Australian Water Recycling Guidelines for Drinking Augmentation

[ii] Australian Drinking Water Guidelines (* health value)

[iii] WHO Guidelines for Drinking Water

[iv] EU Drinking Water Guidelines

[v] Calculated following the Australian Water Recycling Guidelines for Drinking Augmentation

APPENDIX 3. AWMC ORGANIC MICROPOLLUTANTS ANALYTICAL METHOD

Extraction Method

The samples were filtered within a few hours after collection (0.45µm Nylon filters, PM separation, Australia) and stored at 4°C before extraction which occurred within 3 days. The samples were split in six 200 mL subsamples, three were extracted directly and the other three were spiked with 50 µL of a 200 µg L^{-1} mix of the targeted compounds (prepared in methanol) in order to evaluate losses during extraction and matrix interference during LC-MSMS analysis. Solid phase extraction was performed using Waters Oasis HLB 60 mg (3 mL) cartridges. The cartridges were first conditioned with twice 3 mL of methanol and twice 3 mL of HPLC water. Subsamples were then extracted without any pH adjustment, under vacuum, at a flow rate of 1 mL min^{-1}. After extraction, cartridges were dried under vacuum for at least 30 minutes. The compounds of interest were eluted with 3 times 3 mL of methanol. Extracts were gently blown to dryness using nitrogen before being reconstituted in 1 mL of a water/methanol mixture (75/25, v/v).

Analytical Method

A volume of 20 µL of extract was injected in a Shimadzu UFLC connected to an AB Sciex 4000QTrap QLIT-MS equipped with a Turbo Spray source. The UFLC instrument was equipped with a SecurityGuard Gemini NX C18 4×20 mm (Phenomenex) pre-column and a 5 µm, 250×4.6 mm Altima C18 (Grace) column run at 40°C. Each sample extract was analysed separately in both positive and negative ion scheduled multiple reaction monitoring (SMRM) mode. The time window in the SMRM mode was set at 120 seconds with a target scan time of 0.5 seconds. The eluents compositions and gradients for each mode are detailed in Table SI 1. Two transitions were monitored in the SMRM mode using parameters detailed in Table SI 3. The first transition was used for quantification and the second one for confirmation only.

Table 13. HPLC eluents composition and gradients (total flow rate = 1 mL min^{-1}).

	Positive Mode		Negative Mode	
Eluent A (v/v)	95% acetonitrile 5% HPLC grade water 26.5 mM formic acid		50% acetonitrile 50% methanol	
Eluent B (v/v)	1% acetonitrile 99% HPLC grade water 26.5 mM formic acid		95% HPLC grade water 5% methanol 1mM sodium acetate	
	Analysis time(min)	**% eluent A**	**Analysis time (min)**	**% eluent A**
Gradient	0	15	0	0
	12.5	100	7	90
	15	100	10	90
	15.2	15	12	5
	21.2	15	15	5
			16	100
			21	100

Table 14. QLIT-MS source parameters.

	Positive Mode	Negative Mode
Ion spray voltage (V)	5 500.0	- 4 500.0
Source temperature (°C)	700	700
Curtain gas (arbitrary units)	30.0	30.0
Collision gas	High	High
Q1 and Q3 mass resolution	Low, Unit	Unit, Unit
Ion source gas 1 (arbitrary units)	62.0	55.0
Ion source gas 2 (arbitrary units)	62.0	55.0
Interface heater	ON	ON

Table 15. Target compounds, retention times and optimized QLIT-MS parameters (Rt = retention time; DP = declustering potential; Q1 = parent ion; $Q3_{quant}$ = fragment ion used for quantitation; $Q3_{conf}$ = fragment ion used for confirmation; CE = collision energy; CXP = collision cell exit potential).

Compound Name	Rt (min)	DP (V)	Q1 (Da)	$Q3_{quant}$ (Da)	CE (V)	CXP (V)	$Q3_{conf}$ (Da)	CE (V)	CXP (V)
Positive Mode (entrance potential = 10 volts)									
Atenolol	3.6	71	267.2	145.3	37	12	190.2	29	16
Atrazine	11.0	81	216.1	174.2	27	10	68	53	12
Caffeine	5.8	71	195.1	138.1	28	8	110.0	32	8
Carbamazepine	9.6	61	237.2	194.2	27	16	193.3	47	12
Citalopram	7.8	70	325.3	109.1	38	4	262.2	28	4
Dapsone	7.6	71	249.1	108.2	31	8	92.1	35	6
Diazinon	14.3	81	305.2	169.2	31	14	153.2	29	8
Doxylamine	5.4	40	271.2	182.2	24	8	167.2	45	7
Erythromycin	7.7	71	734.6	158.1	41	8	576.4	35	8
Indomethacin	12.4	91	358.1	139.0	27	12	111.0	71	8
Lincomycin	4.9	91	407.3	126.2	39	10	359.3	27	10
Metolachlor	13.3	61	285.1	253.2	25	16	177.3	37	16
Metoprolol	6.3	76	268.2	116.2	27	8	121.1	35	8
Perindopril	7.5	76	369.2	172.2	29	14	98.1	49	6
Phenytoin	9.4	61	253.1	182.2	27	10	104.1	51	8
Praziquantel	11.4	81	313.2	203.2	25	18	83.2	41	6
Propranolol	7.4	76	260.2	116.2	27	8	183.2	27	12
Ranitidine	3.7	61	315.3	176.1	25	14	102.2	51	16
Risperidone	6.8	96	411.2	191.2	41	12	109.9	71	6
Roxithromycin	8.8	96	837.6	679.5	31	12	158.0	49	12
Sertraline	8.9	56	306.1	159.1	39	12	275.1	19	18
Sulfamethoxazole	8.0	51	254.2	156.0	23	8	92.1	38	8
Sulfathiazole	5.9	51	256.2	156.1	22	10	92.1	40	8
Tramadol	6.4	45	264.2	58.1	44	8	42.2	125	3
Trimethoprim	5.5	86	291.2	230.3	33	4	261.2	37	6
Tylosin	7.9	151	916.6	174.2	55	14	772.5	54	10
Venlafaxine	7.1	61	278.2	58.1	41	10	260.3	19	6
Negative Mode (entrance potential = - 10 volts)									
2,4-D (2,4-Dichlorophenoxyacetic acid)	6.2	-60	218.8	160.9	-18	-5	124.9	-40	-9
Bezafibrate	6.8	-70	360.0	274.0	-26	-1	154.0	-38	-5
Chloramphenicol	7.6	-70	322.9	151.9	-26	-9	120.9	-48	-7
Diclofenac	10.1	-40	293.9	250.0	-16	-1	214.0	-30	-15
Diuron	9.7	-65	230.9	185.8	-26	-15	149.9	-36	-9
Furosemide	6.7	-70	329.0	284.8	-22	-7	204.8	-30	-11
Gemfibrozil	11.2	-85	249.0	121.0	-20	-7	127.0	-14	-5
Hydrochlorthiazide	6.4	-90	296.0	268.8	-26	-13	204.9	-30	-17
Ibuprofen	10.4	-52	205.0	161.0	-11.5	-10			
Ketoprofen	6.9	-40	253.0	209.0	-10	-11	197.0	-6	-9
Naproxen	9.4	-50	229.0	185.0	-10	-13	169.0	-38	-9
Paracetamol (Acetaminophen)	6.1	-60	150.0	106.9	-26	-7	107.8	-22	-5
Triclopyr	6.3	-55	256.0	198.0	-30	-5	196	-30	-5
Warfarin	9.5	-85	307.0	161.0	-28	-11	250.0	-32	-1

Quantification Method

The quantification of the targeted compounds in the extract was performed using 10 points external calibration curves obtained from the injection of standard solutions ranging from 0.1 to 100 µg L^{-1}. Linear or quadratic regression was used depending on the compound, which gave good fits with $r^2 > 0.99$. The concentrations measured in the three non-spiked subsamples were averaged. The spiked subsamples were used to correct the concentrations obtained for losses during the SPE and for matrix effects in the instrument (ion-enhancement or -suppression). Each spiked sample was compared to the average of non-spiked samples allowing three determination of the overall recovery efficiency of the method (by comparing the difference measured with the spiked amount). Overall recoveries were averaged and used with the average of non-spiked subsamples to calculate the actual concentration. Overall recoveries were above 20% for all compounds in all samples. The limit of quantification (LOQ) was set at a signal to noise ratio of 10 and was determined using the spiked samples. Individual recoveries and LOQs are not reported here since they were determined for each compound and sample and varied from one to another as ion-suppression and -enhancement depends largely on the matrix composition which varied with time and sample type.

QA/QC

The calibration curve was determined at the beginning of each run, typically daily, with standard solutions prepared no more than 7 days before. Blank samples and the 10 µg L^{-1} calibration curve standard were injected regularly during each run to ensure there was no contamination and that the signal intensity remained steady for each compound along the entire run.

APPENDIX 4. LIST OF AWMC COMPOUNDS AND THEIR PROPERTIES

Table 16. Physico-chemical properties and relative potency of the compounds in the bioluminescence inhibition test with *Vibrio fischeri* (in relation to a reference virtual baseline toxicant); removal generally observed in full scale WWTP (P=poor, <20%; I=intermediate, 20-80%; G=good, >80%). NA = not applicable. NAv = not available.

Compound Name	Classification	Molecular Weight (g mol^{-1})	Acidity Constant pK_a [i]	Charge pH=7 [ii]	Fraction Neutral Species at pH 7 [ii]	Octanol-Water Partition Coefficient log K_{ow} [i]	Liposome-Water Distribution Ratio at pH 7 log D_{lipw}(pH7)	Relative Potency RP	Removal in WWTP [iii]
2,4-D	Herbicide	221.04	2.73	-1	0.00	2.81	2.06	1.42E-01	
Atenolol	Beta-blocker	266.30	9.6/9.05	+1	0.01	0.23	-0.24	1.40E-03	I
Atrazine	Herbicide	215.69	1.7	0	1.00	2.61	2.88	7.08E-01	
Bezafibrate	Hypolipidemic agent	361.83	3.73/13.57	-1	0.00	4.25	3.36	1.08E+00	
Caffeine		194.19	-	0	1.00	-0.07	0.45	7.31E-03	G
Carbamazepine	Anticonvulsant	236.27	-	0	1.00	2.45	2.73	4.89E-01	P
Chloramphenicol	Antibiotic	323.13	12.66	0	1.00	1.14	1.55	3.63E-02	I
Citalopram	Antidepressant	324.39	9.63	+1	0.00	3.74	2.91	4.99E-01	I
Dapsone	Antituberculotic, antileprotic	248.30	1.28/2.09	0	1.00	0.97	1.39	3.51E-02	
Diazinon	Insecticide	304.35		0	1.00	3.80	3.95	4.01E+00	
Diclofenac	NSAI[c]	296.15	4.15/4.12	-1	0.00	4.51	3.60	2.09E+00	P
Diuron	Herbicide	233.10		0	1.00	2.68	2.94	7.40E-01	
Doxylamine	Sedative	270.37	8.73	+1	0.02	2.37	1.73	6.12E-02	
Erythromycin	Antibiotic (macrolide)	733.95	8.88/8.23	+1	0.01	3.06	2.33	7.28E-02	P
Furosemide	Diuretic	330.74	3.5	-1	0.00	2.03	1.35	2.44E-02	I
Gemfibrozil	Hypolipidemic agent	250.33	4.9	-1	0.01	4.70	3.80	3.61E+00	I
Hydrochlorothiazide	Diuretic	297.74	9.76	0	1.00	-0.07	0.45	4.78E-03	I
Ibuprofen	NSAI	206.28	4.91/4.53	-1	0.00	3.97	3.12	1.18E+00	G
Indomethacin	NSAI	357.79	4.5	-1	0.00	4.27	3.39	1.15E+00	I
Ketoprofen	NSAI	254.29	4.35	-1	0.00	3.12	2.35	2.16E-01	
Lincomycin	Antibiotic (lincosamide)	406.54	7.8	+1	0.14	0.20	0.04	1.59E-03	P
Metolachlor	Herbicide	283.8		0	1.00	3.13	3.35	1.33E+00	
Metoprolol	Beta-blocker	267.36	9.6/9.08	+1	0.01	1.88	1.25	2.46E-02	I
Naproxen	NSAI	230.27	4.15	-1	0.00	3.18	2.40	2.63E-01	I
Paracetamol	Analgesic, antipyretic	151.16	9.38/9.49	0	1.00	0.46	0.93	2.36E-02	G
Perindopril	ACE inhibitor	368.74	5.4	0	0.98	-2.42	-1.68	6.25E-05	
Phenytoin	Anticonvulsant	252.27	8.33	0	0.96	2.47	2.73	4.58E-01	I
Praziquantel	Anthelmintic	312.41	-	0	1.00	2.42	2.71	3.51E-01	
Propranolol	Beta-blocker	259.34	9.24	+1	0.01	3.48	2.69	4.11E-01	I
Ranitidine	Histamine-blocker	314.40	8.94	+1	0.01	0.27	-0.20	1.29E-03	I
Risperidone	Antipsychotic	410.50	9.59/7.99/7.06	+1	0.00	3.43	2.68	2.55E-01	
Roxithromycin	Antibiotic (macrolide)	837.10	8.3	+1	0.05	2.85	2.25	5.44E-02	P
Sertraline	Antidepressants	306.23	9.5	+1	0.00	5.97	4.93	2.62E+01	P
Sulfamethoxazole	Antibiotic (sulfonamide)	253.28	1.8/1.84	+1	0.99	0.89	1.32	2.98E-02	P-I
Sulfathiazole	Antibiotic (sulfonamide)	255.32	1.5	0	0.05	-0.09	-0.41	1.07E-03	
Tramadol	Narcotic analgesic	263.38	9.61	+1	0.00	3.01	2.25	1.72E-01	
Triclopyr	Herbicide	256.47	3.97	-1	0.00	2.53	1.81	7.58E-02	
Trimethoprim	Antibiotic	290.32	7.12	0	0.43	0.91	1.03	1.48E-02	P
Tylosin	Antibiotic (macrolide)	916.10	7.02	0	0.49	1.63	1.72	1.80E-02	
Venlafaxine	Antidepressant	277.40	9.1	+1	0.01	3.28	2.51	2.73E-01	
Warfarin	Anticoagulant	308.33	4.8	-1	0.01	2.70	1.98	8.82E-02	

i) search algorithm as described in Escher *et al.* (2011), preferentially experimental or estimated data taken from the Syracuse Research Physprop data base, http://esc.syrres.com/physprop/. If no experimental were available, SPARC (ii) was used to decide on a final value. ii) calculated with SPARC (http://ibmlc2.chem.uga.edu/sparc/), September 2009 release w4.5.1529-s4.5.1529. iii) estimated from Onesios *et al.* (2009).

APPENDIX 5. ORGANIC MICROPOLLUTANT CONCENTRATION RANGES IN FULL SCALE RECLAMATION PLANTS

Table 17. Organic micropollutant concentration ranges before ozonation (ng L^{-1}). OoR= out of calibration range; N/Q=not quantified (due to interferences).

Compound Name	Caboolture		Landsborough		Gerringong	
	Min	Max	Min	Max	Min	Max
2,4-D	43.5	101.9	4.3	7.7	5.8	8.8
Atenolol	401.6	597.6	163.4	211.7	488.0	1029.7
Atrazine	1.0	1.1	0.7	2.0	0.8	8.0
Bezafibrate	<0.5	0.9	<1.5	10.3		<0.7
Caffeine	67.7	177.8	26.9	70.7	93.1	257.8
Carbamazepine	467.7	OoR	726.7	1191.6	118.6	172.2
Chloramphenicol		<0.9	<0.5	3.0	1.7	3.1
Citalopram	167.7	207.9	160.9	313.7	124.3	155.8
Dapsone		<1.8	1.3	1.8	1.8	6.0
Diazinon	21.9	777.9	36.5	95.2	4.5	7.8
Diclofenac	193.7	239.6	161.9	315.7	139.4	205.5
Diuron	33.4	66.3	79.5	103.2	32.6	199.9
Doxylamine	233.3	721.5	104.2	192.7	84.0	106.5
Erythromycin	153.3	166.6	19.5	31.9	250.0	323.6
Furosemide	399.9	996.0	N/Q	N/Q	291.3	542.4
Gemfibrozil	84.1	154.9	82.5	190.8	35.9	59.5
Hydrochlorothiazide	719.3	2507.8	340.9	690.2	891.5	1581.4
Ibuprofen	<21.2	87.5	46.6	73.0		<24.5
Indomethacin	11.0	15.6	11.5	28.3	6.1	11.9
Ketoprofen	18.5	55.4	31.7	86.2		<21.8
Lincomycin	<0.2	3.1	0.7	1.7	0.2	0.3
Metolachlor	1.2	3.1	0.1	0.5	<0.2	0.5
Metoprolol	228.6	918.5	165.8	230.4	172.9	242.3
Naproxen	188.0	587.2	82.6	142.0	188.8	345.8
Paracetamol	117.8	153.8	<4.7	40.1	17.6	39.3
Perindopril	53.0	74.1	53.9	66.1	79.4	117.7
Phenytoin	160.8	373.3	104.2	125.0	52.8	94.0
Praziquantel	3.3	3.4	3.1	43.8	1.5	1.6
Propranolol	49.9	81.8	26.5	53.3	81.0	96.7
Ranitidine	<0.3	6.6	45.7	307.1	96.1	632.4
Risperidone	0.5	0.9	0.2	0.9	0.2	0.3
Roxithromycin	76.8	153.5	60.4	187.3	456.0	702.7
Sertraline	21.7	32.6	30.2	137.4	50.2	68.2
Sulfamethoxazole	160.0	271.9	277.8	1700.7	38.8	228.5
Sulfathiazole	<1.0	1.2		<2.6	<0.6	1.4
Tramadol	638.7	OoR	658.2	OoR	204.7	320.5
Triclopyr	27.3	114.6	35.5	184.5		<16.8
Trimethoprim	27.2	49.1	49.4	94.4	48.4	141.2
Tylosin	<0.3	0.4	0.4	3.6		<0.8
Venlafaxine	471.7	OoR	611.5	865.7	646.1	805.9
Warfarin		<0.4	8.7	11.2	4.9	5.9

Table 18. Organic micropollutant concentration ranges after biological activated carbon (ng L^{-1}).

Compound Name	Caboolture		Landsborough		Gerringong	
	Min	Max	Min	Max	Min	Max
2,4-D	7.2	10.3	2.5	4.3		<3.4
Atenolol	1.1	2.0	19.7	33.1	1.1	1.6
Atrazine	0.3	0.3	0.8	2.2		<0.5
Bezafibrate		<0.3	0.4	1.4		<0.3
Caffeine	12.0	15.3	14.7	44.6	9.9	39.4
Carbamazepine	0.3	2.3	105.5	254.5	5.6	8.5
Chloramphenicol	<0.2	0.2		<0.9		<0.3
Citalopram	0.6	0.9	10.6	15.7	<0.3	0.5
Dapsone		<0.6		<0.7		<0.7
Diazinon	1.2	5.4	16.8	32.9	0.1	0.5
Diclofenac	<0.7	1.2	0.9	6.1		<1.2
Diuron	0.1	0.4	30.3	34.2	0.3	0.9
Doxylamine		<2.0	6.9	14.4		<3.1
Erythromycin	2.5	14.7	2.3	5.1	0.9	4.5
Furosemide	9.6	20.8		<43.9		<15.7
Gemfibrozil	<0.1	0.3	0.9	2.5	0.1	0.6
Hydrochlorothiazide	0.6	1.3	90.3	178.7	0.3	0.9
Ibuprofen	1.4	4.7		<21.3		<1.4
Indomethacin	<1.2	1.9		<6.6		<2.8
Ketoprofen		<17.9		<24.3		<9.4
Lincomycin		<0.6		<0.1		<0.7
Metolachlor	6.1	8.1	0.2	0.7		<0.4
Metoprolol	0.3	0.4	28.5	35.4	0.5	8.1
Naproxen		<13.2		<6.5		<3.7
Paracetamol	1.6	3.5	<4.0	14.0		<4.4
Perindopril	15.0	29.1	31.1	37.7	15.9	27.0
Phenytoin	9.3	11.3	60.4	76.4	11.2	18.9
Praziquantel		<0.1	1.2	13.1	<0.1	0.2
Propranolol		<0.2	0.5	1.9		<0.4
Ranitidine		<1.0		<2.4		<1.1
Risperidone	0.1	0.9	0.7	2.8	<0.1	0.8
Roxithromycin	2.1	10.4	5.1	17.1	1.5	5.1
Sertraline	<0.4	0.6	<1.0	8.4	0.3	0.4
Sulfamethoxazole	5.0	6.7	17.5	74.0	0.4	1.3
Sulfathiazole		<0.5		<1.0		<0.6
Tramadol	0.9	2.3	43.6	105.6		<0.7
Triclopyr	14.4	15.2	20.1	100.3		<22.5
Trimethoprim	<0.2	0.2	1.3	6.0		<0.3
Tylosin		<0.6		<0.5		<0.9
Venlafaxine	2.0	3.3	104.1	195.4	0.5	0.9
Warfarin		<0.2		<2.9		<0.3

APPENDIX 6. REACTIVITY OF SELECTED ORGANIC MICROPOLLUTANTS WITH OZONE AND HYDROXYL RADICALS AND REMOVAL IN TREATED EFFLUENTS

Table 19. Reactivity of selected OMPs with ozone and hydroxyl radicals and removal in treated effluents.

Compound	k_{O3}($M\ s^{-1}$) (pH;T)	$k_{OH\bullet}$ (E9 $M\ s^{-1}$) (pH;T)	Degradation by ozonation in treated effluents (effluent type; DOC or TOC; pH; T; O_3 dose)	Reference
2,4-D	21.9 (2; 20)	5.1 (2; 20)		(Benitez *et al.*, 2004)
	2.4±0.1 (acid form, 21)			(Yao and Haag, 1991)
	29.1 (7.5; ?)			(Xiong and Graham, 1992) cited by (Ikehata and El-Din, 2005)
	298 (7.5; 20)			(Hu *et al.*, 2000)
Atenolol	1.7±0.4E3 (7; 20-22)	8.0±0.5 (7; 20-22)		(Benner *et al.*, 2008)
		7.05±0.27(7; room)		(Song *et al.*, 2008)
			40-93% (tertiary effluent;11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			90-99% (tertiary effluent;7.2; 7,0; 20; 0.2 mg/mgTOC)	(Dickenson *et al.*, 2009)
			55-92% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			61% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			>86% (tertiary effluent; 23.0; 7.2; ?; 0.4mg/mgDOC)	(Ternes *et al.*, 2003)
			20-60% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			40-80% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 1.0 mg/mgDOC)	(Wert *et al.*, 2009)
			>97% (tertiary effluent; 6.4±1.4; 8.5; 25; ~1.5mg/mgTOC)	(Rosal *et al.*, 2010)
Caffeine	0.82 (8; ?)			(Rosal *et al.*, 2009)
		2.6 (7; 0)		(Brezova *et al.*, 2009)
			80-93% (tertiary effluent;11.2;7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			34% (tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			>80% (tertiary effluent;7.1; 6.9; 27; 0.7 mg/mgDOC)	(Snyder *et al.*, 2006)
			50% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
Caffeine			>87% (tertiary effluent; 23.0; 7.2; ?; 0.4mg/mgDOC)	(Ternes *et al.*, 2003)
			20-60% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			80-99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 1.0 mg/mgDOC)	(Wert *et al.*, 2009)
Carbamazepine	7.81±1.31E4 (?; 25)			(Andreozzi *et al.*, 2002)
	~3E5 (7; 20)	8.8±1.2 (7; 25)		(Huber *et al.*, 2003)
		2.05±0.14 (5; ?)		(Vogna *et al.*, 2004a)

Compound	k_{O3}(M s^{-1}) (pH;T)	$k_{OH\cdot}$ (E9 M s^{-1}) (pH;T)	Degradation by ozonation in treated effluents (effluent type; DOC or TOC; pH; T; O_3 dose)	Reference
			> 94% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			>99% (tertiary effluent; 7.2; 7,0; 20; 0.2 mg/mgTOC)	(Dickenson *et al.*, 2009)
			98-100% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			50% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			>99% (tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			50-99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.9mg/mgTOC)	(Rosal *et al.*, 2010)
Citalopram			>97% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.9mg/mgTOC)	(Rosal *et al.*, 2010)
Diazinon			52-78% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
		8.4		(Real *et al.*, 2007)
		6.4-9.0		(Shemer *et al.*, 2006)
Diclofenac	6.8E5 (7; 20)			(Sein *et al.*, 2008)
	~1E6 (7; 20)	7.5±1.5 (7; 25)		(Huber *et al.*, 2003)
	1.84±0.15E4 (6; 25)			(Vogna *et al.*, 2004b)
			> 92% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			98-99% (tertiary effluent;7.2; 7,0; 20; 1 mg/mgTOC)	(Dickenson *et al.*, 2009)
			98-100% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
Diclofenac			>96% (tertiary effluent; 23.0; 7.2; -; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			>99% (tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			20-99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.3mg/mgTOC)	(Rosal *et al.*, 2010)
Diuron	14.7±0.8 (4; 20)	4.6 (>8.2; 20)		(De Laat *et al.*, 1996)
	13.3±0.95 (2.4; 22)	7.6 (8.2; 22)		(Chen *et al.*, 2008)
	16.5±0.6 (2; 20)	6.6±0.1 (9.5; 20)		(Benitez *et al.*, 2007)
		7.5-9.9		(Shemer *et al.*, 2006)
			99% (tertiary effluent; 6.4±1.4; 8.5; 25; ~2.4mg/mgTOC)	(Rosal *et al.*, 2010)
			74% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
Doxylamine			> 89% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
Erythromycin			> 95% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			>99% (tertiary effluent;7.2; 7,0; 20; 1 mg/mgTOC)	(Dickenson *et al.*, 2009)
			47-80% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			>92% (tertiary effluent; 23.0; 7.2; -; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			>99% (tertiary effluent;7.2; 7.0; 20; 0.43 mg/mgDOC)	(Snyder *et al.*, 2006)

Compound	k_{O3}(M s^{-1}) (pH;T)	$k_{OH\cdot}$ (E9 M s^{-1}) (pH;T)	Degradation by ozonation in treated effluents (effluent type; DOC or TOC; pH; T; O_3 dose)	Reference
			89% (secondary effluent; 3.2-3.5; 6.8-8.4; - ; ~1 mg/mgDOC)	(Nakada *et al.*, 2007)
			>78% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.6mg/mgTOC)	(Rosal *et al.*, 2010)
Gemfibrozil		10.0±0.6 (7;room)		(Razavi *et al.*, 2009)
			> 90% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			>93% (tertiary effluent;7.2; 7,0; 20; 0.2 mg/mgTOC)	(Dickenson *et al.*, 2009)
			>94% (tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			30-99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
Gemfibrozil			95% (tertiary effluent; 6.4±1.4; 8.5; 25; ~2.4mg/mgTOC)	(Rosal *et al.*, 2010)
Hydrochlorothiazide	5.11E3 (7; 20)	5.7±0.3 (3; 20)		(Real *et al.*, 2010)
			86-97% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			>99% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.9mg/mgTOC)	(Rosal *et al.*, 2010)
			~90% (secondary effluent; 23.3; 8.0; 20; 0.4mg/mgDOC)	(Real *et al.*, 2010)
Metoprolol	2.0±0.6E3 (7;20-22)	7.3±0.2 (7; 20-22)		(Benner *et al.*, 2008)
		8.39±0.06 (7; room)		(Song *et al.*, 2008)
	1.4E3 (7;20)			(Benitez *et al.*, 2009)
			83-94% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			75-94% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			78% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			>93% (tertiary effluent; 23.0; 7.2; ?; 0.4mg/mgDOC)	(Ternes *et al.*, 2003)
			>89% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.9mg/mgTOC)	(Rosal *et al.*, 2010)
Naproxen	~2E5 (7; 20)			(Huber *et al.*, 2005)
		9.6±0.5 (3.5; 22)		(Packer *et al.*, 2003)
	2.6E5 (7; 20)			(Benitez *et al.*, 2009)
			>96% (tertiary effluent;7.2; 7,0; 20; 1 mg/mgTOC)	(Dickenson *et al.*, 2009)
			59-98% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			>96% tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			>99% (secondary effluent; 3.2-3.5; 6.8-8.4; ? ; ~1 mg/mgDOC)	(Nakada *et al.*, 2007)
Naproxen			20-99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>89% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.35mg/mgTOC)	(Rosal *et al.*, 2010)
Phenytoin		6.28 (?;?)		(Yuan *et al.*, 2009)
			71-79% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
Propranolol	~1E5 (3-8.5; 20-22)	10±2 (7; 20-22)		(Benner *et al.*, 2008)
		10.7±0.2 (7; room)		(Song *et al.*, 2008)

Compound	k_{O3}(M s^{-1}) (pH;T)	$k_{OH\cdot}$ (E9 M s^{-1}) (pH;T)	Degradation by ozonation in treated effluents (effluent type; DOC or TOC; pH; T; O_3 dose)	Reference
			75-97% (tertiary effluent; 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			>78% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.35mg/mgTOC)	(Rosal *et al.*, 2010)
Roxithromycin	4.5±0.5E6 (7; 20)			(Huber *et al.*, 2003)
	6.3E4 (7; 20)	5.4±0.3 (7; 25)		(Dodd *et al.*, 2006)
			78-91% (tertiary effluent;7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			70-94% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			>91% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			91% (secondary effluent; 3.2-3.5; 6.8-8.4; ? ; ~1 mg/mgDOC)	(Nakada *et al.*, 2007)
Sulfamethoxazole	~2.5E6 (7; 20)	5.5±0.7 (7; 25)		(Huber *et al.*, 2003)
	5.5E5 (7; 20)			(Dodd *et al.*, 2006)
			>99% (tertiary effluent;5.3; 7,7; 20; 0.56 mg/mgDOC)	(Dodd *et al.*, 2006)
			> 93% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			>99% (tertiary effluent;7.2; 7,0; 20; 0.2 mg/mgTOC)	(Dickenson *et al.*, 2009)
			>92% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/MgDOC)	(Ternes *et al.*, 2003)
			92-98% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
			>99% (tertiary effluent;7.2; 7.0; 20; 0.43 mg/mgDOC)	(Snyder *et al.*, 2006)
			87% (secondary effluent; 3.2-3.5; 6.8-8.4; ? ; ~1 mg/mgDOC)	(Nakada *et al.*, 2007)
			20-99% (3 WWTP effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 WWTP effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>92% (tertiary effluent; 6.4±1.4; 8.5; 25; ~1.5mg/mgTOC)	(Rosal *et al.*, 2010)
Trimethoprim	2.7E5 (7; 20)	6.5±0.2 (7; 25)		(Dodd *et al.*, 2006)
			> 90% (tertiary effluent; 11.2; 7±0.5; 22±2; 0.5 mg/mgDOC)	(Reungoat *et al.*, 2010)
			93-99% (tertiary effluent;7.2; 7,0; 20; 0.2 mg/mgTOC)	(Dickenson *et al.*, 2009)
			>99% (tertiary effluent;7.2; 7,0; 20; 1 mg/mgTOC)	(Dickenson *et al.*, 2009)
			>85% (tertiary effluent; 23.0; 7.2; ?; 0.2mg/mgDOC)	(Ternes *et al.*, 2003)
			91-98% (tertiary effluent, 5.5; 7.0; 12-17; 0.62 mg/mgDOC)	(Hollender *et al.*, 2009)
Trimethoprim	2.7E5 (7; 20)	6.5±0.2 (7; 25)	>99% (WWTP tertiary effluent;7.2; 7.0; 20; 0.36 mg/mgDOC)	(Snyder *et al.*, 2006)
			96% (secondary effluent; 3.2-3.5; 6.8-8.4; - ; ~1 mg/mgDOC)	(Nakada *et al.*, 2007)
			50-99% (3 WWTP effluents; 6.6-10.3; 7.1-8.2; 18; 0.2 mg/mgDOC)	(Wert *et al.*, 2009)
			>99% (3 WWTP effluents; 6.6-10.3; 7.1-8.2; 18; 0.6 mg/mgDOC)	(Wert *et al.*, 2009)
			>97% (tertiary effluent; 6.4±1.4; 8.5; 25; ~0.6mg/mgTOC)	(Rosal *et al.*, 2010)

GLOSSARY

BAC	biological activated carbon
DAFF	dissolved air flotation and filtration
DBP	disinfection by-product
DEQ	diuron equivalent concentration
DOC	dissolved organic carbon
EBCT	empty bed contact time
EDC	endocrine disrupting compound
EEQ	estradiol equivalent concentration
EfOM	effluent organic matter
HAA	haloacetic acid
LC/MS-MS	liquid chromatography coupled with tandem mass spectrometry
LOQ	limit of quantification
OMP	organic micropollutant
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PTEQ	parathion equivalent concentration
QSAR	quantitative structure activity relationship
RP	relative potency
SPE	solid phase extraction
TCDDEQ	2,3,7,8-tetrachlorodibenzo-p-dioxin equivalent concentration
TEQ	toxicity equivalent concentration
THM	trihalomethane
WWTP	wastewater treatment plant

PUBLICATIONS

Articles published in international peer-reviewed journals issued:

Macova, M., Escher, B.I., Reungoat, J., Carswell, S., Lee, C.K., Keller, J. and Mueller, J.F. (2010) *Monitoring the Biological Activity of Micropollutants during Advanced Wastewater Treatment with Ozonation and Activated Carbon Filtration.* Water Research 44(2), 477-492.

Reungoat, J., Macova, M., Escher, B.I., Carswell, S., Mueller, J.F. and Keller, J. (2010) *Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration.* Water Research 44 (2), 625-637.

Reungoat, J., Escher, B.I., Macova, M. and Keller, J. (2011) *Biofiltration of wastewater treatment plant effluent: Effective removal of pharmaceuticals and personal care products and reduction of toxicity.* Water Research 45(9), 2751-2762.

REFERENCES

Adams, C., Wang, Y., Loftin, K. and Meyer, M. (2002) *Removal of antibiotics from surface and distilled water in conventional water treatment processes.* Journal of Environmental Engineering-Asce 128(3), 253-260.

Aherne, G.W. and Briggs, R. (1989) *The relevance of the presence of certain synthetic steroids in the aquatic environment.* Journal of Pharmacy and Pharmacology 41(10), 735-736.

Andreozzi, R., Marotta, R., Pinto, G. and Pollio, A. (2002) *Carbamazepine in water: persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity.* Water Research 36(11), 2869-2877.

Benitez, F.J., Acero, J.L., Real, F.J. and Roldan, G. (2009) *Ozonation of pharmaceutical compounds: Rate constants and elimination in various water matrices.* Chemosphere 77(1), 53-59.

Benitez, F.J., Acero, J.L., Real, F.J. and Roman, S. (2004) *Oxidation of MCPA and 2,4-D by UV radiation, ozone, and the combinations UV/H2O2 and O-3/H2O2.* Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 39(3), 393-409.

Benitez, F.J., Real, F.J., Acero, J.L. and Garcia, C. (2007) *Kinetics of the transformation of phenyl-urea herbicides during ozonation of natural waters: Rate constants and model predictions.* Water Research 41(18), 4073-4084.

Benner, J., Salhi, E., Ternes, T. and von Gunten, U. (2008) *Ozonation of reverse osmosis concentrate: Kinetics and efficiency of beta blocker oxidation.* Water Research 42(12), 3003-3012.

Benner, J. and Ternes, T.A. (2009) *Ozonation of Metoprolol: Elucidation of Oxidation Pathways and Major Oxidation Products.* Environmental Science & Technology 43(14), 5472-5480.

Brezova, V., Slebodova, A. and Stasko, A. (2009) *Coffee as a source of antioxidants: An EPR study.* Food Chemistry 114(3), 859-868.

Buffle, M.O., Schumacher, J., Meylan, S., Jekel, M. and von Gunten, U. (2006a) *Ozonation and advanced oxidation of wastewater: Effect of O-3 dose, pH, DOM and HO center dot-scavengers on ozone decomposition and HO center dot generation.* Ozone-Science & Engineering 28(4), 247-259.

Buffle, M.O., Schumacher, J., Salhi, E., Jekel, M. and von Gunten, U. (2006b) *Measurement of the initial phase of ozone decomposition in water and wastewater by means of a continuous quench-flow system: Application to disinfection and pharmaceutical oxidation.* Water Research 40(9), 1884-1894.

Chen, K.C., Wang, Y.H. and Chang, Y.H. (2009) *Using catalytic ozonation and biofiltration to decrease the formation of disinfection by-products.* Desalination 249(3), 929-935.

Chen, W.R., Wu, C., Elovitz, M.S., Linden, K.G. and Suffet, I.H.M. (2008) *Reactions of thiocarbamate, triazine and urea herbicides, RDX and benzenes on EPA Contaminant Candidate List with ozone and with hydroxyl radicals.* Water Research 42(1-2), 137-144.

De Laat, J., Maouala-Makata, P. and Dore, M. (1996) *Constantes Cinetiques de Reaction de L'Ozone Moleculaire et des Radicaux Hydroxyles Sur Quelques Phenyl-Urees et Acetamides Rate Constants for Reactions of Ozone and Hydroxyl Radicals with Several Phenyl-Ureas and Acetamides.* Environmental Technology 17(7), 707 - 716.

Deborde, M., Rabouan, S., Duguet, J.P. and Legube, B. (2005) *Kinetics of aqueous ozone-induced oxidation of some endocrine disruptors.* Environmental Science & Technology 39(16), 6086-6092.

Dickenson, E.R.V., Drewes, J.E., Sedlak, D.L., Wert, E.C. and Snyder, S.A. (2009) *Applying Surrogates and Indicators to Assess Removal Efficiency of Trace Organic Chemicals during Chemical Oxidation of Wastewaters.* Environmental Science & Technology 43(16), 6242-6247.

Dodd, M.C., Buffle, M.O. and Von Gunten, U. (2006) *Oxidation of antibacterial molecules by aqueous ozone: Moiety-specific reaction kinetics and application to ozone-based wastewater treatment.* Environmental Science & Technology 40(6), 1969-1977.

Dodd, M.C., Rentsch, D., Singer, H.P., Kohler, H.P.E. and von Gunten, U. (2010) *Transformation of beta-Lactam Antibacterial Agents during Aqueous Ozonation: Reaction Pathways and Quantitative Bioassay of Biologically-Active Oxidation Products.* Environmental Science & Technology 44(15), 5940-5948.

Domenjoud, B., Cortes-Francisco, N., Guastalli, A.R., Caixach, J., Esplugas, S. and Baig, S. (2011) *Ozonation of Municipal Secondary Effluent; Removal of Hazardous Micropollutants and Related Changes of Organic Matter Composition.* Journal of Advanced Oxidation Technologies 14(1), 138-146.

Eaton, A.D., Clesceri, L.S., Rice, E.W. and Greenberg, A.E. (2005) Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC.

Escher, B.I., Baumgartner, R., Koller, M., Treyer, K., Lienert, J. and McArdell, C.S. (2011) *Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater.* Water Research 45(1), 75-92.

Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S. and Vermeirssen, E.L.M. (2008) *Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples.* Journal of Environmental Monitoring 10(5), 612-621.

Escher, B.I., Bramaz, N. and Ort, C. (2009) *Monitoring the treatment efficiency of a full scale ozonation on a sewage treatment plant with a mode-of-action based test battery.* Journal of Environmental Monitoring 11(10), 1836–1846.

Escher, B.I., Bramaz, N., Richter, M. and Lienert, J. (2006) *Comparative Ecotoxicological Hazard Assessment of Beta-Blockers and Their Human Metabolites Using a Mode-of-Action-Based Test Battery and a QSAR Approachâ€* Environmental Science & Technology 40(23), 7402-7408.

Esplugas, S., Bila, D.M., Krause, L.G.T. and Dezotti, M. (2007) *Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents.* Journal of Hazardous Materials 149(3), 631-642.

Farre, M.J., Keller, J., Holling, N., Poussade, Y. and Gernjak, W. (2011a) *Occurrence of N-nitrosodimethylamine precursors in wastewater treatment plant effluent and their fate during ultrafiltration-reverse osmosis membrane treatment.* Water Science and Technology 63(4), 605-612.

Farre, M.J., Reungoat, J., Argaud, F.X., Rattier, M., Keller, J. and Gernjak, W. (2011b) *Fate of N-nitrosodimethylamine, trihalomethane and haloacetic acid precursors in tertiary treatment including biofiltration.* Water Research 45(17), 5695-5704.

Fent, K., Weston, A.A. and Caminada, D. (2006) *Ecotoxicology of human pharmaceuticals.* Aquatic Toxicology 76(2), 122-159.

Filby, A.L., Shears, J.A., Drage, B.E., Churchley, J.H. and Tyler, C.R. (2010) *Effects of advanced treatments of wastewater effluents on estrogenic and reproductive health impacts in fish.* Environmental Science and Technology 44(11), 4348-4354.

Gallard, H. and von Gunten, U. (2002) *Chlorination of natural organic matter: kinetics of chlorination and of THM formation.* Water Research 36(1), 65-74.

Gong, J., Liu, Y. and Sun, X. (2008) *O3 and UV/O3 oxidation of organic constituents of biotreated municipal wastewater.* Water Research 42(4-5), 1238-1244.

Hebert, A., Forestier, D., Lenes, D., Benanou, D., Jacob, S., Arfi, C., Lambolez, L. and Levi, Y. (2010) *Innovative method for prioritizing emerging disinfection by-products (DBPs) in drinking water on the basis of their potential impact on public health.* Water Research 44(10), 3147-3165.

Helmy, M., Schimmoller, L., Bellamy, B. and Curl, J. (2009) Alternative and more sustainable treatment processes for indirect potable reuse. 7th IWA World Congress on Water Reclamtion and Reuse, Brisbane (Autsralia).

Heringa, M.B., Schreurs, R., Busser, F., Van Der Saag, P.T., Van Der Burg, B. and Hermens, J.L.M. (2004) *Toward more useful in vitro toxicity data with measured free concentrations.* Environmental Science & Technology 38(23), 6263-6270.

Hollender, J., Zimmermann, S.G., Koepke, S., Krauss, M., McArdell, C.S., Ort, C., Singer, H., von Gunten, U. and Siegrist, H. (2009) *Elimination of Organic Micropollutants in a Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed by Sand Filtration.* Environmental Science & Technology 43(20), 7862-7869.

Hu, J.Y., Morita, T., Magara, Y. and Aizawa, T. (2000) *Evaluation of reactivity of pesticides with ozone in water using the energies of frontier molecular orbitals.* Water Research 34(8), 2215-2222.

Hua, G.H., Reckhow, D.A. and Kim, J. (2006) *Effect of bromide and iodide ions on the formation and speciation of disinfection byproducts during chlorination.* Environmental Science & Technology 40(9), 3050-3056.

Huber, M.M., Canonica, S., Park, G.Y. and von Gunten, U. (2003) *Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes.* Environmental Science & Technology 37(5), 1016-1024.

Huber, M.M., Gobel, A., Joss, A., Hermann, N., Loffler, D., McArdell, C.S., Ried, A., Siegrist, H., Ternes, T.A. and von Gunten, U. (2005) *Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: A pilot study.* Environmental Science & Technology 39(11), 4290-4299.

Huber, M.M., Ternes, T.A. and vonGunten, U. (2004) *Removal of Estrogenic Activity and Formation of Oxidation Products during Ozonation of 17-Ethinylestradiol.* Environmental Science & Technology 38(19), 5177-5186.

Ikehata, K. and El-Din, M.G. (2005) *Aqueous Pesticide Degradation by Ozonation and Ozone-Based Advanced Oxidation Processes: A Review (Part I).* Ozone: Science & Engineering: The Journal of the International Ozone Association 27(2), 83 - 114.

Joss, A., Siegrist, H. and Ternes, T.A. (2008) *Are we about to upgrade wastewater treatment for removing organic micropollutants?* Water Science & Technology 57(2), 251-255.

Kim, I.H., Tanaka, H., Iwasaki, T., Takubo, T., Morioka, T. and Kato, Y. (2008) *Classification of the degradability of 30 pharmaceuticals in water with ozone, UV and H2O2.* Water Science & Technology 57(2), 195-200.

Kimura, K., Toshima, S., Amy, G. and Watanabe, Y. (2004) *Rejection of neutral endocrine disrupting compounds (EDCs) and pharmaceutical active compounds (PhACs) by RO membranes.* Journal of Membrane Science 245(1-2), 71-78.

Kumar, K. and Margerum, D.W. (1987) *KINETICS AND MECHANISM OF GENERAL-ACID-ASSISTED OXIDATION OF BROMIDE BY HYPOCHLORITE AND HYPOCHLOROUS ACID.* Inorganic Chemistry 26(16), 2706-2711.

Kummerer, K. (2009) *The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges.* Journal of Environmental Management 90(8), 2354-2366.

Lai, K.M., Scrimshaw, M.D. and Lester, J.N. (2002) *The effects of natural and synthetic steroid estrogens in relation to their environmental occurrence.* Critical Reviews in Toxicology 32(2), 113-132.

Lee, C., Schmidt, C., Yoon, J. and von Gunten, U. (2007) *Oxidation of N-nitrosodimethylamine (NDMA) precursors with ozone and chlorine dioxide: Kinetics and effect on NDMA formation potential.* Environmental Science & Technology 41(6), 2056-2063.

Lee, Y., Escher, B.I. and Von Gunten, U. (2008) *Efficient removal of estrogenic activity during oxidative treatment of waters containing steroid estrogens.* Environmental Science & Technology 42(17), 6333-6339.

Leusch, F.D.L., Chapman, H.F., van den Heuvel, M.R., Tan, B.L.L., Gooneratne, S.R. and Tremblay, L.A. (2006) *Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand.* Ecotoxicology and Environmental Safety 65(3), 403-411.

Liang, L. and Singer, P.C. (2003) *Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water.* Environmental Science & Technology 37(13), 2920-2928.

Liu, Z.H., Kanjo, Y. and Mizutani, S. (2009) *Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment - physical means, biodegradation, and chemical advanced oxidation: A review.* Science of The Total Environment 407(2), 731-748.

Macova, M., Escher, B., Mueller, J. and Toze, S. (2010a) Bioanalytical Tools to Evaluate Micropollutants across the Seven Barriers of the Indirect Potable Reuse Scheme. Urban Water Security Research Alliance, Brisbame (Australia).

Macova, M., Escher, B.I., Reungoat, J., Carswell, S., Lee, C.K., Keller, J. and Mueller, J.F. (2010b) *Monitoring the Biological Activity of Micropollutants during Advanced Wastewater Treatment with Ozonation and Activated Carbon Filtration.* Water Research 44(2), 477-492.

Maeng, S.K., Sharma, S.K., Magic-Knezev, A. and Amy, G. (2008) *Fate of effluent organic matter (EfOM) and natural organic matter (NOM) through riverbank filtration.* Water Science and Technology 57(12), 1999-2007.

Mitch, W.A., Gerecke, A.C. and Sedlak, D.L. (2003) *A N-nitrosodimethylamine (NDMA) precursor analysis for chlorination of water and wastewater.* Water Research 37(15), 3733-3741.

Mitch, W.A. and Sedlak, D.L. (2004) *Characterization and fate of N-nitrosodimethylamine precursors in municipal wastewater treatment plants.* Environmental Science & Technology 38(5), 1445-1454.

Muller, R., Schreiber, U., Escher, B.I., Quayle, P., Nash, S.M.B. and Mueller, J.F. (2008) *Rapid exposure assessment of PSII herbicides in surface water using a novel chlorophyll a fluorescence imaging assay.* Science of The Total Environment 401(1-3), 51-59.

Munch, J.W. and Bassett, M.V. (2004) Determination of nitrosamines in drinking water by solid phase extraction and capillary column gas chromatography with large volume injection and chemical ionization tandem masss spectrometry (MS/MS). U.S. Environmental Protection Agency.

Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N. and Takada, H. (2007) *Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant.* Water Research 41(19), 4373-4382.

Neale, P.A., Escher, B.I. and Schafer, A.I. (2008) *Quantification of solute-solute interactions using negligible-depletion solid-phase microextraction: Measuring the affinity of estradiol to bulk organic matter.* Environmental Science & Technology 42(8), 2886-2892.

Nowotny, N., Epp, B., vonSonntag, C. and Fahlenkamp, H. (2007) *Quantification and Modeling of the Elimination Behavior of Ecologically Problematic Wastewater Micropollutants by Adsorption on Powdered and Granulated Activated Carbon.* Environmental Science & Technology 41(6), 2050-2055.

NRMMC, EPHC and AHMC (2006) Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1), Natural Resource Management Ministerial Council, Environment Protection and Heritage Council and Australian Health Ministers Conference, Canberra (Australia).

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A. and Khan, A.A. (2004) *Diclofenac residues as the cause of vulture population decline in Pakistan.* Nature 427(6975), 630-633.

Onesios, K.M., Yu, J.T. and Bouwer, E.J. (2009) *Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review.* Biodegradation 20(4), 441-466.

Ormad, M.P., Miguel, N., Claver, A., Matesanz, J.M. and Ovelleiro, J.L. (2008) *Pesticides removal in the process of drinking water production.* Chemosphere 71(1), 97-106.

Packer, J.L., Werner, J.J., Latch, D.E., McNeill, K. and Arnold, W.A. (2003) *Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen.* Aquatic Sciences 65(4), 342-351.

Pehlivanoglu-Mantas, E. and Sedlak, D.L. (2006) *The fate of wastewater-derived NDMA precursors in the aquatic environment.* Water Research 40(6), 1287-1293.

Pomati, F., Castiglioni, S., Zuccato, E., Fanelli, R., Vigetti, D., Rossetti, C. and Calamari, D. (2006) *Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells.* Environmental Science and Technology 40(7), 2442-2447.

Poulsen, A., Chapman, H., Leusch, F. and Escher, B. (2011) Application of Bioanalytical Tools for Water Quality Assessment. Urban Water Security Research Alliance, Brisbane (Australia).

Radjenovic, J., Godehardt, M., Petrovic, M., Hein, A., Farre, M., Jekel, M. and Barcelo, D. (2009) *Evidencing Generation of Persistent Ozonation Products of Antibiotics Roxithromycin and Trimethoprim.* Environmental Science & Technology 43(17), 6808-6815.

Rauch-Williams, T., Hoppe-Jones, C. and Drewes, J.E. (2010) *The role of organic matter in the removal of emerging trace organic chemicals during managed aquifer recharge.* Water Research 44(2), 449-460.

Rauch, T. and Drewes, L. (2004) *Assessing the removal potential of soil-aquifer treatment systems for bulk organic matter.* Water Science and Technology 50(2), 245-253.

Razavi, B., Song, W.H., Cooper, W.J., Greaves, J. and Jeong, J. (2009) *Free-Radical-Induced Oxidative and Reductive Degradation of Fibrate Pharmaceuticals: Kinetic Studies and Degradation Mechanisms.* Journal of Physical Chemistry A 113(7), 1287-1294.

Real, F.J., Acero, J.L., Benitez, F.J., Roldan, G. and Fernandez, L.C. (2010) *Oxidation of hydrochlorothiazide by UV radiation, hydroxyl radicals and ozone: Kinetics and elimination from water systems.* Chemical Engineering Journal 160(1), 72-78.

Real, F.J., Benitez, F.J., Acero, J.L. and Gonzalez, M. (2007) *Removal of diazinon by various advanced oxidation processes.* Journal of Chemical Technology and Biotechnology 82(6), 566-574.

Reungoat, J., Escher, B.I., Macova, M. and Keller, J. (2011) *Biofiltration of wastewater treatment plant effluent: Effective removal of pharmaceuticals and personal care products and reduction of toxicity.* Water Research 45(9), 2751-2762.

Reungoat, J., Macova, M., Escher, B.I., Carswell, S., Mueller, J.F. and Keller, J. (2010) *Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration.* Water Research 44 (2), 625-637.

Richardson, S.D. (2009) *Water Analysis: Emerging Contaminants and Current Issues.* Analytical Chemistry 81(12), 4645-4677.

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R. and DeMarini, D.M. (2007) *Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research.* Mutation Research-Reviews in Mutation Research 636(1-3), 178-242.

Rodriguez, C., Van Buynder, P., Lugg, R., Blair, P., Devine, B., Cook, A. and Weinstein, P. (2009) *Indirect Potable Reuse: A Sustainable Water Supply Alternative.* International Journal of Environmental Research and Public Health 6(3), 1174-1203.

Rosal, R., Rodriguez, A., Perdigon-Melon, J.A., Petre, A., Garcia-Calvo, E., Gomez, M.J., Aguera, A. and Fernandez-Alba, A.R. (2009) *Degradation of caffeine and identification of the transformation products generated by ozonation.* Chemosphere 74(6), 825-831.

Rosal, R., Rodríguez, A., Perdigón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A. and Fernández-Alba, A.R. (2010) *Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation.* Water Research 44(2), 578-588.

Rosario-Ortiz, F.L., Mezyk, S.P., Wert, E.C., Doud, D.F.R., Singh, M.K., Xin, M., Baik, S. and Snyder, S.A. (2008) *Effect of ozone oxidation on the molecular and kinetic properties of effluent organic matter.* Journal of Advanced Oxidation Technologies 11(3), 529-535.

Scherrer, R.A. and Howard, S.M. (1977) *Use of distribution coefficients in quantitative structure-activity relations.* Journal of Medicinal Chemistry 20(1), 53-58.

Schreiber, I.M. and Mitch, W.A. (2006) *Nitrosamine formation pathway revisited: The importance of chloramine speciation and dissolved oxygen.* Environmental Science & Technology 40(19), 6007-6014.

Sein, M.M., Zedda, M., Tuerk, J., Schmidt, T.C., Golloch, A. and von Sonntao, C. (2008) *Oxidation of diclofenac with ozone in aqueous solution.* Environmental Science & Technology 42(17), 6656-6662.

Seredynska-Sobecka, B., Tomaszewska, M., Janus, M. and Morawski, A.W. (2006) *Biological activation of carbon filters.* Water Research 40(2), 355-363.

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marinas, B.J. and Mayes, A.M. (2008) *Science and technology for water purification in the coming decades.* Nature 452(7185), 301-310.

Shemer, H., Sharpless, C.M., Elovitz, M.S. and Linden, K.G. (2006) *Relative rate constants of contaminant candidate list pesticides with hydroxyl radicals.* Environmental Science & Technology 40(14), 4460-4466.

Simpson, D.R. (2008) *Biofilm processes in biologically active carbon water purification.* Water Research 42(12), 2839-2848.

Snyder, S., Vanderford, B., Pearson, R., Quinones, O. and Yoon, Y. (2003b) *Analytical methods used to measure endocrine disrupting compounds in water.* Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management 7(4), 224-234.

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Wert, E.C. and Yoon, Y. (2007) *Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals.* Desalination 202(1-3), 156-181.

Snyder, S.A., Wert, E.C., Rexing, D.J., Zegers, R.E. and Drury, D.D. (2006) *Ozone Oxidation of Endocrine Disruptors and Pharmaceuticals in Surface Water and Wastewater.* Ozone: Science & Engineering 28(6), 445 - 460.

Snyder, S.A., Westerhoff, P., Yoon, Y. and Sedlak, D.L. (2003) *Pharmaceuticals, Personal Care Products, and Endocrine Disruptors in Water: Implications for the Water Industry.* Environmental Engineering Science 20(5), 449-469.

Song, W.H., Cooper, W.J., Mezyk, S.P., Greaves, J. and Peake, B.M. (2008) *Free radical destruction of beta-blockers in aqueous solution.* Environmental Science & Technology 42(4), 1256-1261.

Stalter, D., Magdeburg, A. and Oehlmann, J. (2010) *Comparative toxicity assessment of ozone and activated carbon treated sewage effluents using an in vivo test battery.* Water Research 44(8), 2610-2620.

Stalter, D., Magdeburg, A., Wagner, M. and Oehlmann, J. (2011) *Ozonation and activated carbon treatment of sewage effluents: Removal of endocrine activity and cytotoxicity.* Water Research 45(3), 1015-1024.

Sumpter, J.P. (2005) *Endocrine disrupters in the aquatic environment: An overview.* Acta Hydrochimica Et Hydrobiologica 33(1), 9-16.

Tabak, H.H. and Bunch, R.L. (1970) *Steroid hormones as water pollutants I.* Dev Ind Microbiol 11, 367-376.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.J., Gulde, B.H., Preuss, G., Wilme, U. and Seibert, N.Z. (2002) *Removal of pharmaceuticals during drinking water treatment.* Environmental Science & Technology 36(17), 3855-3863.

Ternes, T.A., Stuber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M. and Teiser, B. (2003) *Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater?* Water Research 37(8), 1976-1982.

Thuy, P.T., Moons, K., van Dijk, J.C., Anh, N.V. and Van der Bruggen, B. (2008) *To what extent are pesticides removed from surface water during coagulation-flocculation?* Water and Environment Journal 22(3), 217-223.

U.S. Environmental Protection Agency (2012) Technical Fact Sheet – N-Nitroso-dimethylamine (NDMA).

van Leeuwen, J., Pipe-Martin, C. and Lehmann, R.M. (2003) *Water Reclamation At South Caboolture, Queensland, Australia.* Ozone: Science & Engineering 25(2), 107 - 120.

Vieno, N., Tuhkanen, T. and Kronberg, L. (2006) *Removal of pharmaceuticals in drinking water treatment: Effect of chemical coagulation.* Environmental Technology 27(2), 183-192.

Villanueva, C.M., Cantor, K.P., Grimalt, J.O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G., Marcos, R., Rothman, N., Real, F.X., Dosemeci, M. and Kogevinas, M. (2007) *Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools.* American Journal of Epidemiology 165(2), 148-156.

Vogna, D., Marotta, R., Andreozzi, R., Napolitano, A. and d'Ischia, M. (2004a) *Kinetic and chemical assessment of the UV/H2O2 treatment of antiepileptic drug carbamazepine.* Chemosphere 54(4), 497-505.

Vogna, D., Marotta, R., Napolitano, A., Andreozzi, R. and d'Ischia, M. (2004b) *Advanced oxidation of the pharmaceutical drug diclofenac with UV/H2O2 and ozone.* Water Research 38(2), 414-422.

von Gunten, U. (2003) *Ozonation of drinking water: Part I. Oxidation kinetics and product formation.* Water Research 37(7), 1443-1467.

Wang, H.X., Ho, L., Lewis, D.M., Brookes, J.D. and Newcombe, G. (2007) *Discriminating and assessing adsorption and biodegradation removal mechanisms during granular activated carbon filtration of microcystin toxins.* Water Research 41(18), 4262-4270.

Wert, E.C., Rosario-Ortiz, F.L. and Snyder, S.A. (2009) *Effect of ozone exposure on the oxidation of trace organic contaminants in wastewater.* Water Research 43(4), 1005-1014.

Westerhoff, P., Chao, P. and Mash, H. (2004) *Reactivity of natural organic matter with aqueous chlorine and bromine.* Water Research 38(6), 1502-1513.

Westerhoff, P., Yoon, Y., Snyder, S. and Wert, E. (2005) *Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes.* Environmental Science & Technology 39(17), 6649-6663.

Wilson, B.A., Smith, V.H., Denoyelles Jr, F. and Larive, C.K. (2003) *Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages.* Environmental Science and Technology 37(9), 1713-1719.

Xiong, F. and Graham, N.J.D. (1992) *Rate Constants for Herbicide Degradation by Ozone.* Ozone: Science & Engineering: The Journal of the International Ozone Association 14(4), 283 - 301.

Yao, C.C.D. and Haag, W.R. (1991) *Rate constants for direct reactions of ozone with several drinking water contaminants.* Water Research 25(7), 761-773.

Yoon, Y., Westerhoff, P., Snyder, S.A., Wert, E.C. and Yoon, J. (2007) *Removal of endocrine disrupting compounds and pharmaceuticals by nanofiltration and ultrafiltration membranes.* Desalination 202(1-3), 16-23.

Yu, Z.R., Peldszus, S. and Huck, P.M. (2008) *Adsorption characteristics of selected pharmaceuticals and an endocrine disrupting compound - Naproxen, carbamazepine and nonylphenol - on activated carbon.* Water Research 42(12), 2873-2882.

Yuan, F., Hu, C., Hu, X.X., Qu, J.H. and Yang, M. (2009) *Degradation of selected pharmaceuticals in aqueous solution with UV and UV/H2O2.* Water Research 43(6), 1766-1774.

Zwiener, C. and Frimmel, F.H. (2000) *Oxidative treatment of pharmaceuticals in water.* Water Research 34(6), 1881-1885.

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