SUBSTANCES OF EMERGING CONCERN IN SOUTH AFRICAN AQUATIC ECOSYSTEMS

Volume 1:
Fate, environmental health risk characterisation and substance use epidemiology in surrounding communities

Report

to the Water Research Commission

by

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The publication of this report emanates from Water Research Commission (WRC) Project K5/2733, titled “Micropollutants and endocrine-disrupting contaminants (EDCs) in wastewater treatment systems: Approach towards effective water reclamation, new treatment technologies and development of an adverse outcome pathway network for risk assessment”.

Volume 1: Substances of emerging concern in South African aquatic ecosystems: Fate, environmental health risk characterisation and substance use epidemiology in surrounding communities (this report)

Volume 2: Substances of emerging concern in South African aquatic ecosystems: Evaluation of antibiotic resistance bacteria and chemical contaminants’ removal efficiency using various treatment technologies (**WRC Report no. 2733/2/20**)

EXECUTIVE SUMMARY

BACKGROUND

Freshwater resources are under constant threat of being polluted by a diverse range of man-made chemicals on a global scale. These contaminants are of concern because they may have detrimental health effects on both humans and wildlife. Various monitoring approaches worldwide have shown that several recalcitrant pollutants and their associated metabolites (breakdown products) are indeed not removed during water treatment systems before being discharged into recipient environmental waters or redistributed for (non)-potable reuse purposes. Toxicological data of pharmaceutical transformation products is limited due to less frequent monitoring of such compounds in surface waters, which warrants the need for the continuous surveillance of freshwater resources for “known” and “newly identified” contaminants of emerging concern (CECs), which may impact on both ecological and human health. Additionally, the vast amount of organic pollutants known to be present in surface water systems makes it difficult to evaluate each compound for its potential to cause adverse health effects. Thus, there is a need for continuous effort towards advancing water treatment technologies, the concurrent development of analytical techniques and the expansion of databases on the toxicity risks of CECs in order to protect public and ecosystem health. In terms of risk characterisation, the prioritisation of CECs through a weight-of-evidence approach is an important first step to improve risk-based management within surface water systems.

THE PRESENCE OF CONTAMINANTS OF EMERGING CONCERN IN SOUTH AFRICAN WATER SYSTEMS

The current report presents a comprehensive review of a summarised list of pharmaceuticals and personal care products (PPCPs) and other endocrine-disrupting contaminants (EDCs) detected to date within South African water systems, as well as their possible endocrine-disrupting effect *in vitro* and *in vivo*. This review further addresses other factors that should be investigated in future studies, including endocrine disruption, the stability and toxicity of PPCP metabolites in surface waters, effect-based monitoring to evaluate sub-lethal health risk outcomes, and wastewater-based epidemiology (WBE) to estimate substance use and abuse trends in human populations. The report further proposes a list of priority CECs that should receive attention based on their regular occurrence in surface waters, along with their established and/or proposed ability to act as stressors for various non-communicable health effects in wildlife and humans. It is clear from the work presented here that setting out a universal priority list of CECs may prove difficult, as each location is impacted on by varying types of wastewater and surrounding human activities. Nevertheless, from the investigation on the loads of CECs detected during wastewater treatment and in environmental surface waters, it becomes clear that more information is required in future studies on the adverse health risks of pharmaceutical breakdown products for more advanced risk assessment approaches. Targeted chemical determination during water treatment and in surface water shows strength in providing information to assess the performance of wastewater treatment works (WWTW) and the fate of recalcitrant CECs in recipient surface waters. It also reveals novel insights into the chemical use patterns of the surrounding communities through a WBE approach.

USING WASTEWATER-BASED EPIDEMIOLOGY TO MONITOR SUBSTANCE USE IN COMMUNITIES

Apart from conventional CEC monitoring approaches to evaluate the extent of CEC persistence during wastewater treatment, the investigation of target analytes at the influent of WWTW may be used to estimate the “upstream” amount of consumption or use of the target analytes in the community that is connected to the sewage system.

This approach, known as wastewater-based epidemiology, has been widely adopted to estimate the use of illicit drugs in communities, and may aid in the surveillance of substance abuse in communities, as the methods used to collate information on drug use are largely limited to substance abuse treatment centres and law enforcement reports, which may lead to inaccurate or under-estimations of drug abuse. However, the concept of WBE shows promise to be expanded to address other health-related issues apart from substance abuse.

Wastewater-based epidemiology was performed for the first time on the African continent in this study, and showed a high illicit drug usage in South African communities compared to other developed and developing countries. Although such high usage trends are indeed reported by other regulating bodies, the concept of WBE proved to supplement such databases in the country by serving as a non-intrusive tool to estimate substance use and abuse on a community level. Moreover, the concept of WBE was extended to include pharmaceutical use profiling, which is shown to be an emerging discipline in the field of environmental analytical chemistry to address public health.

The CEC monitoring results highlight the extent of previously unreported CECs in the country, whereby their potential environmental risks were evaluated using lethal and sub-lethal toxicity endpoints. The recalcitrance of various PPCPs and metabolic breakdown products during various types of wastewater treatment in the country thus raised concern that such CECs should receive higher priority to establish their human and environmental health risks. Moreover, the case studies found that, for many of the monitoring campaigns, CEC pollution also originates upstream from the discharge of the WWTWs that were investigated during the study, raising concern about the extent of alternative pollution sources in low- and middle-income countries (LMICs) where communities are not connected to municipal sanitation infrastructure.

EFFECT-BASED MONITORING OF SELECTED CONTAMINANTS OF EMERGING CONCERN IN WASTEWATER

The study further investigated the removal potential of EDCs from various South African WWTWs by using an effect-based monitoring (EBM) bioassay, the Yeast Estrogen Screen (YES), in which the results showed that a large variation in wastewater treatment technologies exists to eradicate contaminants for which they were not originally designed. Furthermore, the study raised the issue of toxic masking when EBM tools are used for complex environmental monitoring such as the monitoring of waste and surface waters. Although the authors acknowledge that the YES may only serve as a first-tier screening tool and may only reveal the affinity of EDCs to bind to the human estrogen receptor (hER), the assay still proved useful as it is reported to be more robust against the toxic nature of environmental surface waters and wastewaters compared to mammalian cell lines. The results that were generated from the YES showed that estradiol-equivalent (EEQ) concentrations of WWTW discharge and environmental surface waters regularly surpassed established effect-based trigger values (EBTVs) that warrant the need for further intervention at the test sites. As with the results for CECs in the current report, the EEQ estimations in surface waters also confirmed higher levels of pollution upstream from WWTW discharge, which further raises the need for more intervention into addressing alternative pollution sources other than WWTW discharge alone.

SIGNIFICANCE OF ADVERSE OUTCOME PATHWAYS IN ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT

Although the conventional approach holds value to assess toxicity risks factors and “alarming” triggers in the environment, such conventional risk assessments have been proven to cause severe under-estimations, as they do not include sub-lethal endpoints that could lead to long-term health effects. In addition, the conventional risk characterisation of CECs and polluted water resources is based on acute and/or chronic toxicity studies, which mostly address lethal toxicity outcomes towards the most sensitive test organisms.

To add to the understanding of evolving 21st-century toxicity testing and risk assessment, this study reviews the potential application of an adverse outcome pathway (AOP) framework for risk assessment. An AOP is structured upon existing knowledge based on the relationships between physiological pathways, originating from molecular-initiating events (MIEs) and, in turn, causes a perturbation in normal biological functioning, therefore impairing a sequence of measurable key events (KEs), ranging from the cellular to the organism level. Although such an AOP framework is still in its developmental stage for decision makers providing with mitigation frameworks, there has been much advancement of this approach since its initiation in 2012, including an ever-expanding AOP knowledge base that is maintained by various international monitoring agencies. However, the African continent still seems to be under-represented.

Incorporating environmental risk assessments (ERAs) and the AOP framework into the National Toxicity Monitoring Programme (NTMP) could contribute immensely to the characterisation and interpretation of risks associated with CECs in environmental waters. For the national monitoring programme to be meaningful, more information on known predicted no-effect concentrations (PNECs) for various CECs over multiple trophic levels, along with investigating EBM methodologies to show the overall response status of the water body under investigation rather than performing unnecessary, costly, targeted chemical screenings of CECs, is needed.

SUMMARY AND RECOMMENDATIONS

The drive towards 21st-century toxicity assessment will include more interactive collaboration for EBM using high-throughput screening (HTS) to evaluate human and ecological risks from the multitude of anthropogenic stressors that are associated with rising populations and urbanisation. An evaluation of the toxicological risk of targeted CECs still holds value to understand the burden of CECs during the treatment of freshwater resources. However, using an EBM approach, along with interactive collaboration with both local and international monitoring agencies, will see the implementation of policies to safeguard the country's freshwater resources in terms of harmful contaminants that limit sustainable growth and resilience in communities.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

4-MBC	Benzedrone
β-gal	Beta Galactosidase
ADHD	Attention-deficit Hyperactivity Disorder
AMR	Antimicrobial Resistance
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
AOP-KB	Adverse Outcome Pathway Knowledge Base
ART	Antiretroviral Treatment
ARV	Antiretroviral
ATS	Amphetamine-type Stimulants
BEG	Benzoylecgonine
BEQ	Bioanalytical Equivalent Concentration
BNR	Biological Nutrient Removal
BPA	Bisphenol-A
BPS	Bisphenol-S
CA	Corrected Absorbance
CBZ-ep	Carbamazepine-10,11-epoxide
CE	Cocaethylene
CEC	Contaminant of Emerging Concern
COD	Chemical Oxygen Demand
CPRG	Chlorophenol red-b-D-galactopyranoside
CYP	Cytochrome P450 Enzyme
dh-hCBZ	10,11-dihydro-10-hydroxycarbamazepine
DHT	Dihydrotestosterone
DMDCS	Dimethyldichlorosilane
DMX	1,7-dimethylxantine
DMV	Desmethylvenlafaxine

DoA	Drug of Abuse
DTR	Drug Target Residue
DWS	Department of Water and Sanitation
DWTW	Drinking Water Treatment Works
E_2	Estradiol
EBM	Effect-based Monitoring
EBTV	Effect-based Trigger Value
EC	Emerging Contaminant
EC	European Commission
EC_{50}	Concentration which shows an effect in 50% of the experimental population
ECOSAR	Ecological Structure Activity Relationships
EDC	Endocrine-disrupting Contaminant
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Endocrine Disrupters Testing and Assessment
EE_2	Ethynyl-oestradiol
EEQ	Estradiol-equivalent
EF	Enantiomeric Fraction
EMCDDA-SCORE	European Monitoring Centre for Drugs and Drug Addiction Sewage CORe analysis Europe
EPSRC	Engineering and Physical Sciences Research Council
Erα	Estrogen Receptor
ERA	Environmental Risk Assessment
ERDC	Engineer Research and Development Centre
ERE	Estrogen-responsive Element
ERWAT	East Rand Water Care Company
ESI	Electrospray Ionisation
EU	European Union
EU-WFD	European Union Water Framework Directive
FLOCC	Final List of Organic Contaminants of Concern
FSH	Follicle-stimulating hormone

GF/F	Glass Fibre Filters
hAR	Human Androgen Receptor
HCl	Hydrochloric Acid
HCP	Hexachlorophene
hER	Human Estrogen Receptor
HLB	Hydrophilic-lipophilic Balanced
HPA	Hypothalamus-pituitary-adrenal
HPG	Hypothalamus-pituitary-gonad
HPLC	High-performance Liquid Chromatography
HPT	Hypothalamus-pituitary-thyroid
HRT	Hydraulic Retention Time
HTS	High-throughput Screening
KE	Key Event
KER	Key Event Relationship
LC-MS	Lliquid Chromatography-Mass Spectrometry
LD_{50}	Lethal dose at 50% of the experimental population
LMIC	Low- and Middle-income Country
LOEC	Lowest Observed Effect Concentration
MAE	Microwave-assisted Extraction
MDL	Method Detection Limit
MDMA	3,4-methylenedioxymethamphetamine
MEC	Measured Environmental Concentrations
MeOH	Methanol
MIE	Molecular-initiating Event
MoA	Mode of Action
MQL	Method Quantification Limit
MRC	Medical Research Council
MRM	Multiple Reaction Monitoring
mRNA	Messenger Ribonucleic Acid

MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NOEC	No Observed Effect Concentration
NRC	National Research Council
NRMMC	Natural Resource Management Ministerial Council
NSAID	Non-steroidal Anti-inflammatory Drug
NTMP	National Toxicity Monitoring Programme
O-6-MAM	O-6-monoacetylmorphine
OECD	Organisation of Economic Cooperation and Development
O-DMT/ODT	*O*-desmethyltramadol
OMC	Octyl Methoxycinnamate
OTC	Over The Counter
PCP	Pentachlorophenol
PEC	Predicted Environmental Concentrations
pGLV	Provisional Drinking Water Guideline Value
PGK	Phosphoglycerate Kinase
PMTCT	Prevention of Mother-to-child Transmission
PNEC	Predicted No-effect Concentration
POP	Persistent Organic Pollutant
PTFE	Polytetrafluoroethylene
PPCP	Pharmaceuticals and Personal Care Product
QSAR	Quantitative Structure-Activity Relationship
RAS	Return Activated Sludge
RQ	Risk Quotient
SAID	Steroidal Anti-inflammatory Drug
SDG	Sustainable Development Goal
SHBG	Steroid Hormone-binding Globulin
SHBP	Sex Hormone-binding Protein
SPE	Solid-phase Extraction

SPM	Solid Particulate Matter
Stats SA	Statistics South Africa
STW	Sewage Treatment Works
T_3	Triiodothyrosine
T_4	Thyroxine
TAM-EQ	Tamoxifen-equivalent Concentration
TCC	Triclocarban
TCS	Triclosan
UN	United Nations
UPLC	Ultra-performance Liquid Chromatography
UV	Ultraviolet
USEPA	United States Environmental Protection Agency
VTG	Vitellogenin
WBE	Wastewater-based Epidemiology
WFD	Water Framework Directive
WHO	World Health Organisation
WRC	Water Research Commission
WWTW	Wastewater Treatment Works
YAES	Yeast Anti-estrogen Screen
YAS	Yeast Androgen Screen
YES	Yeast Estrogen Screen

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

It is globally recognised that human activities exert significant pressure on the quality of freshwater resources through contamination by xenobiotic organic micro-pollutants. The major sources of such pollution typically originate from waste that is generated from industries, households and agriculture (Genthe et al., 2013; Patel et al., 2020). These practices introduce slow or non-degradable chemicals into water systems that can pose severe health risks to both wildlife and humans. Such chemicals are collectively referred to as contaminants of emerging concern, which are defined by the Organisation of Economic Cooperation and Development (OECD) as "a vast array of contaminants that have only recently appeared in water, or that are of recent concern because they have been detected at concentrations significantly higher than expected, or their risk to human and environmental health may not be fully understood". The latter statement is of concern, as the list of organic contaminants that are present in freshwater resources is ever-expanding without adequate understanding of their associated health risks to wildlife and humans. Apart from some known lethal toxicity risks that have been identified for many CECs, a variety of non-communicable diseases and physiological adverse health effects, such as various endocrine-disrupting outcomes, increased incidences of several cancers, spontaneous abortions, cardiovascular modulation and *in utero* physiological disorders and birth defects, have been linked to exposure to xenobiotic micropollutants (Soto and Sonnenschein, 2010; Robins et al., 2011; Zhang et al., 2020).

The introduction of CECs into natural ecosystems is exacerbated by the rapid rise in global populations and high rate of urbanisation. The situation in low- and middle-income countries, which constitute more than 80% of the global population, is even more pressing due to an increasing demand for equitable water resources, and limited improved healthcare and sanitation services. On the African continent, countries in sub-Saharan Africa are shown to face the highest rate of urbanisation in the world, with an estimated 40% of the total population currently residing in urban areas, with a rapid rise expected in the next few decades. Southern Africa is regarded as a water-scarce region that experiences limited overall access to equitable water resources for all its inhabitants. In particular, South Africa has a semi-arid climate, with seven of the nine provinces experiencing lower than average rainfall in recent years (DWS, 2019). As a result, extremely low surface water runoff is recorded for eight provinces, low levels are recorded in freshwater catchments for six provinces and the overall drought status overlook for Limpopo, the Eastern Cape, the Northern Cape and the Western Cape is declining (DWS, 2019). Such climatic factors highlight the current need for the country to safeguard existing freshwater resources and the future challenges that are faced to supply equitable water for its growing population.

Moreover, as mentioned for the rest of sub-Saharan Africa, the country also faces rapid population growth and urbanisation, with an estimated 63% of the total population of 58.8 million people (according to the mid-year population estimate for 2019 of Statistics South Africa) (Stats SA, 2019)) that reside in urban settings. This is set to increase to nearly 70% in the next 30 years (UN, 2017). As stated previously, such a rise in densified populations leads to heightened demand for the provision of equitable water and sanitation services to promote healthy living conditions for communities. However, the resultant increase in human activity and population growth elevates the use and disposal of CECs that need to be eradicated before the treated wastewater is reintroduced into freshwater supplies. For this reason, the efficient operation of WWTW and drinking water treatment works (DWTW) lies at the core to reduce the burden of anthropogenic waste products into freshwater resources, which not only impacts on the health of the natural environment, but also on the supply of clean potable and non-potable water for human use and hygiene.

1.2 IMPACT OF WASTEWATER DISCHARGE ON SURFACE WATER QUALITY

Access to clean water supplies and proper sanitation services is heavily dependent on the performance of water treatment facilities to adhere to water quality standards and to eliminate substances that may pose health concerns to the environment and humans. In South Africa, many municipal and private-sector water treatment facilities have previously been shown to lack basic chemical, microbial and physical compliance, as set out by the South African Department of Water and Sanitation (DWS) (2013). Although performance reports on the state of wastewater and drinking water treatment works in the country can be regarded as outdated (according to the latest Green Drop and Blue Drop reports published for 2013 and 2014, respectively (DWS, 2013; DWS, 2014)), they still highlight the challenges that many water treatment facilities in the country experience to safeguard freshwater resources. Moreover, current national water quality guidelines do not consider compliance in eradicating the vast amount of micropollutants present in surface waters due to the extensive cost of routine identification, the limited knowledge regarding their fate in freshwater resources and the lack of established health risks to ecosystems and humans. However, the concurrent development of sensitive and efficient analytical techniques, coupled with expanding global databases on the fate of CECs and their adverse health effects, should offer new avenues for mitigation strategies for the harmful consequences of chemical pollutants.

Numerous studies have shown that ever-increasing numbers of CECs are not being eradicated by current wastewater treatment technologies before treated water is discharged (Petrie et al., 2014; Wilkinson et al., 2017; Tran et al., 2018; Castiglioni et al., 2020). As a result of improved monitoring campaigns to identify CECs, many of the identified contaminants that persist during water treatment are shown to have some association with the development of non-communicable diseases and adverse health effects in freshwater ecosystems. Such recorded persistence of some CECs can be ascribed to the fact that WWTWs were not primarily designed to eliminate the vast amounts of pollutants that have continuously been reported in recent years. The causes of incomplete CEC removal at WWTWs can be ascribed to a variety of biotic and abiotic factors, such as the physico-chemical properties of the pollutants, the deployed treatment technologies and capacity of the water works, environmental conditions (climate, pH, hydrology, geology, etc.), and the ratio of domestic and industrial contribution to the influent. These factors lead to the large variation in the removal efficiencies of CECs at WWTWs that are reported on a global scale (Bolong et al., 2009; Ort et al., 2010; Petrie et al., 2014; Baalbaki et al., 2017; Angeles et al., 2020). As a result of the ever-increasing recorded list of potentially harmful CECs, there is a continuous effort to advance the treatment range of existing water treatment technologies to address the potential health burden caused by CECs.

It should be noted that, although there are numerous reports showing the recalcitrance of various CECs during wastewater treatment, the discharge of human waste products into environmental surface waters may not solely be a cause of inefficient WWTW performance. Estimations by the World Health Organisation (WHO) on global drinking water, sanitation and hygiene have shown that nearly 60% of the global population that is still reliant on collecting drinking water directly from surface waters resides in sub-Saharan Africa, with sanitation services that are not safely managed and with a high percentage of communities still practising open defecation (WHO, 2019). As a result, the release and exposure of pathogens in communal surroundings and their discharge, along with xenobiotic chemicals into the surrounding environment, are a regular occurrence, even at locations where no WWTW discharge is prevalent. Especially for LMICs, many rural and peri-urban communities are not supplied with basic sanitation infrastructure that directs human waste products (solid waste, greywater and sewage) to municipal landfills and/or WWTWs, where such communities rely on their own mode of waste disposal. This usually encompasses the illegal dumping of solid waste and discharge of greywater and wastewater into stormwater drainage systems. It is therefore apparent that the focus on environmental pollution should shift to include all the potential avenues that may contribute towards microbial and chemical discharge into surface and groundwater resources other than WWTW discharge.

1.3 MICROPOLLUTANT MONITORING, SURVEILLANCE AND RISK ASSESSMENT

The information regarding the presence and fate of CECs during wastewater treatment and in freshwater systems is limited for developing countries compared to developed countries in Europe, the Americas and Asia. In Africa, most of the monitoring studies have been done for South Africa, but are still restricted to certain regions in the country without follow-up or routine surveillance studies confirming the occurrence of PPCPs in the same areas over an extended period of time. For this reason, a national survey of pharmaceutical compounds present in South African waters is needed to identify pollution hotspots and to comprehend the extent of chemical pollution and associated health risks in the country's surface waters. Moreover, based on the available information on toxicological studies in South Africa and the rest of the world, several aspects still need to be addressed. A few of these topics are mentioned below and will make a significant contribution to understanding the fate and presence of chemical pathogens in environmental waters, along with their potential toxicological risks. Such interdisciplinary studies should receive high priority for future research, as they are interlinked with the larger scope of environmental water pollution investigations in the country.

1.3.1 Criteria and need for the selection of priority CECs

As it is apparent that an ever-expanding list of organic micropollutants is being recorded globally, the need exists to refine the selection of CECs that need routine surveillance based on empirical evidence of their harmful effects on ecosystems and human health. Various global regulating agencies, such as the United States Environmental Protection Agency (USEPA), the World Health Organisation, the Australian Natural Resource Management Ministerial Council (NRMMC) and the European Union Water Framework Directive (EU-WFD), have acknowledged the challenges of providing such a uniform list of priority CECs. Much advancement has been achieved in developing countries to expand water quality guidelines that include the assessment of CECs in freshwater resources (mostly drinking water quality standards), such as the WHO's guidelines for drinking water quality (WHO, 2011), USEPA's drinking water standards and health advisories tables (USEPA, 2018), the NRMMC's Australian drinking water guidelines (NHMRC-NRMMC, 2011) and guidelines for water recycling (NRMMC-NHMRC-EPHC, 2008), as well as the European Union (EU) surface water watch list under the EU's water framework directive (Loos et al., 2018).

In South Africa, some existing legislative frameworks do indeed make mention of the need for mitigation strategies to reduce and/or eliminate harmful chemicals from freshwater resources (Heath et al., 2013a; Heath et al., 2013b). However, these frameworks do not make direct mention of CECs, nor do they show congruence with international policies and directives as shown by the abovementioned international regulating bodies, mainly due to the costs associated with their detection and the lack of communication to policy level regarding their fate and toxicological risks in surface waters and the drinking water value chain. For this reason, at the core of advancing local policies on freshwater quality and pollution control is the need for South African research institutions to provide empirical evidence on the extent of CEC contamination and the associated health risk factors.

The prioritisation of CECs that require routine monitoring has indeed been proposed by some research studies in the country. A publication by Ncube et al. (2012) included a comprehensive list of CECs that should be considered for monitoring by water utilities. The authors suggested a seven-step generic protocol for the selection and prioritisation of emerging contaminants (ECs) within the drinking water value chain, whereby a Final List of Organic Contaminants of Concern was suggested. This included 100 priority CECs, comprising the following:

- Industrial chemicals (29)
- Pesticides (37)
- Disinfection by-products (13)

- Polymer residues (7)
- Cyanotoxins (9)
- Pharmaceutical and personal care products (5)

From this list, the authors confirmed the presence of 17 CECs within their drinking water value chain, including 11 pesticides, four disinfection by-products and two cyanotoxins.

A WRC study by Swartz et al. (2018) further aimed at compiling a priority list of CECs that are necessary for monitoring water resources that are destined for direct potable reuse. The authors suggested a more refined list of 20 CECs that included the following chemical classes:

- Industrial chemicals and by-products (5)
- Pesticides (4)
- Lifestyle chemicals and steroid hormones (2)
- Pharmaceuticals (7)
- Personal care products (1)
- Plasticisers (1)

Although the two priority CEC lists of Ncube et al. (2012) and Swartz et al. (2018) show some congruence in the selection of some priority CECs that need routine surveillance, it is still apparent that the focus is more on industrial products and pesticide formulations, whereby some CECs that have been recently identified as priority CECs in studies worldwide (especially pharmaceutical products) are missing. It was also acknowledged that many of the priority CECs are indeed reduced during water treatment processes to concentrations below levels that could pose a health risk to humans, except for the synthetic estrogen 17α-ethinylestradiol (Swartz et al., 2018). However, the fate of CEC degradation products and their associated health risks is still unclear. The latter concern that was indeed raised by Swartz et al. (2018) thus further highlights the need for comprehensive monitoring and risk intervention to identify whether priority CECs are indeed completely degraded during treatment processes or whether unidentified transformation products with possible heightened toxicity risks will prevail in treated potable and non-potable water resources.

1.3.2 Metabolites and transformation products of CECs

It is apparent from literature that most local and global CEC detection studies concentrate on the detection of parent CECs in contaminated water systems (Petrie et al., 2014; Petrie et al., 2016; Archer et al., 2017a). However, the toxicological health risks of CEC metabolites or transformation products in water systems may be even more detrimental than the presence of their parent compounds. It is known that some pharmaceutical compounds are rapidly metabolised in the body after administration due to the metabolite that executes the desired physiological action. For example, the analgesic compound tramadol, a persistent organic pollutant (POP) and a licit drug of abuse, will undergo hepatic metabolism by desmethylation to produce the primary metabolite *O*-desmethyltramadol (*O*-DMT), which is a more potent and persistent opioid than tramadol itself. The anti-epileptic compound carbamazepine, which is also a POP, is metabolised rapidly in the body, whereby the metabolite forms are responsible for anti-convulsant actions. These metabolites are then excreted at higher levels than the parental compounds. As a result, metabolic breakdown products are shown in some CEC monitoring studies to be predominant in wastewater treatment systems and may even show a higher extent of recalcitrance during treatment processes, making such substances a higher risk factor than the parent compound. This has been shown by the occurrence of low mass balance removal (less than 25%) and even negative mass balances (higher mass loading in treated effluent than raw influent) for various pharmaceutical metabolites at several WWTWs globally (Kasprzyk-Hordern et al., 2009; Blair et al., 2015; Petrie et al., 2016; Archer et al., 2018).

Moreover, other CECs that are not necessarily administered in humans (such as industrial chemicals, pesticides and personal care products) are also discharged into wastewater, where some CECs may be further transformed through biotic and/or abiotic factors, depending on their physico-chemical properties and, as a result, may be transformed into products that have higher toxicities than their parent counterparts. For example, the thyroid-modulating activity that is associated with exposure of the dithiocarbamate fungicide mancozeb to freshwater organisms is shown to be due to its metabolite, ethylene thiourea (USEPA, 2005; Opitz et al., 2006). Observed anti-androgenic endocrine system responses in fish through the administration of the dicarboximide fungicide vinclozolin are also shown to be due to the product's metabolites that have greater half-lives and mobility in water (Bayley et al., 2003).

Moreover, the transformation products of parent EDCs may also exert different endocrine system responses, such as the organochloride insecticide DDT, which is a known estogenic EDC, but its metabolite p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) is shown to rather have anti-androgenic activity (Mills et al., 2001). It is therefore possible that the overall toxicological profile of a contaminated water system may be under-estimated if transformation products are not considered. Moreover, even though some transformation products may have a reduced toxicological profile compared to their parent counterparts, it is still unclear whether their presence may cause physiological mixture interactions with other prevalent CECs and should thus still include the detection of recalcitrant CEC metabolites in freshwater systems.

1.3.3 Mixture interactions of environmental pollutants

It is recognised globally that numerous xenobiotic chemicals accumulate in complex mixtures in the environment. Although concentrations of CECs range from mg/ℓ to ng/ℓ levels, the chemical interaction between pollutants may be great (Carvalho et al., 2014). It is regularly found that chemical mixture studies do not always conform to conventional predicted ecotoxicological mixture interactions. Such mixture interactions are dependent on the individual chemical's general mode of action (MoA). For example, the MoA of chemicals showing gonadal endocrine-disrupting responses are grouped as being estrogenic, anti-estrogenic, androgenic or anti-androgenic (Behrends et al., 2010). In ideal mixture interactions of environmental pollutants, it is assumed that compounds with the same MoA (e.g. estrogenic + estrogenic) will generate additive mixture interactions, meaning that the chemical mixture acts jointly to generate a larger physiological or toxicological response than its individual counterparts, also known as the additivity null hypothesis (Christiansen et al., 2009). In contrast, chemicals with dissimilar MoAs (e.g. estrogenic + androgenic) are proposed to act independently from one another and may thus not impact on each other's physiological action. However, chemicals with the same MoA (e.g. estrogenic) may have dissimilar mechanisms that exert the same MoA, for example, modulating steroid receptor binding or inhibiting steroidogenic enzyme functions, potentially causing complex mixture interactions.

This complexity in mixture interactions has been highlighted in several studies (Kjærstad et al. 2010; Ermler et al., 2011; Archer and Van Wyk, 2015). Therefore, recent mixture interaction studies do not refer to the general outcome of the MoAs (estrogenic, androgenic, etc.), but to their mechanisms of action (steroid receptor agonism or antagonism, steroidogenesis inhibition or stimulation, enzyme inhibition or modulation). Bearing in mind that a vast majority of xenobiotic compounds from households, agriculture, industry and domestic waste accumulate in water systems, a large variety of compounds with both similar and dissimilar MoAs are expected to be present in the water matrix. This opens the possibility for other ecotoxicological mixture interactions to occur, such as potentiation, synergism and antagonism. Furthermore, several compounds are known to have multiple MoAs for a large variety of physiological and toxicological endpoints, therefore creating further complications in mixture interaction studies. Regardless, from the retrospective information present to date, along with continuing research being done on this topic, knowledge regarding the mixture interactions of environmental pollutants is complex and needs to be addressed.

1.3.4 Effect-based monitoring and a tiered approach for risk characterisation

Taking all environmental and socio-economic factors into account that influence freshwater pollution in the country, along with the research to date on the prevalence of an exhaustive mixture of CECs in surface waters, it may be feasible to evaluate the complete load of pollutants that are prevalent in contaminated waters. Apart from the targeted identification of CECs, much progress has been made to establish EBM tools that include bioassays, biomarkers and ecological indicators that may be used for aquatic monitoring (Wernersson et al., 2015). The establishment of an EBM toolbox is supported by the EU-WFD 2000/60/EC to streamline aquatic monitoring and risk assessment and eliminate the challenges associated with targeted chemical monitoring and risk assessment.

At the onset of the risk characterisation of CECs and contaminated waters lies the need for thorough HTS to avoid the unnecessary cost and time that is associated with conventional risk assessment protocols (Villeneuve et al., 2019). Such an approach is defined by the United States National Research Council (NRC) as assays that can be rapidly performed to evaluate either a single or a multitude of biological processes of interest that may be extrapolated to show toxicological risks on an organism level. However, a few interventions are still required for such an approach to be adopted, including the following (Villeneuve et al., 2019):

- Establishing adequate HTS bioassays
- Comparing HTS results with *in vivo* data
- Establishing conceptual extrapolation models
- Coordination and support from regulating agencies for basic and applied research
- Guidance from regulating agencies for the validation of HTS results

Several global regulatory bodies, such as the USEPA's Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and the OECD's Task Force on Endocrine Disrupters Testing and Assessment, have mandated the development and validation of testing (screening) methods for standardised assessment. The tiered approach suggested by USEPA was accepted globally and includes a battery of assays to screen or test for endocrine interactions and to identify and evaluate the potential of contaminants serving as EDCs (EDSTAC, 1998). First-tier assays were chosen to act as a MoA for HTS to identify and prioritise CECs for second-tier testing, which aids in the understanding of the specific physiological MoA. These assays are modulated by the CEC of interest (EDSTAC, 1998). As a result, second-tier screens aim to evaluate the results obtained from first-tier screens in multi-generational or long-term *in vivo* studies to gain further support for a compound or mixture of contaminants that pose health risks to wildlife and humans (EDSTAC, 1998). For this reason, the implementation of a tiered approach towards identifying and categorising risks associated with CECs still need to be expanded to advance the knowledge on 21st-century risk assessment approaches.

1.3.5 Wastewater-based epidemiology to estimate communal substance use and abuse

Apart from targeted CEC monitoring and associated risk characterisation, the investigation of target analytes at the influent of WWTWs may be used to estimate the usage patterns of CECs in communities that are connected to the investigated sewage system. This approach, called wastewater-based epidemiology, has been widely adopted to estimate the use of illicit drugs in communities, It may aid in the surveillance of substance abuse in communities, as the methods to collate information on drug use are largely limited to substance abuse treatment centres and law enforcement reports, which may lead to inaccurate or under-estimations of drug abuse.

Wastewater-based epidemiology has shown great promise to assist with such constraints by providing a near real-time profile of substance abuse on a community level (Castiglioni et al., 2016). Substance use and abuse have notable socio-economic consequences that globally impede sustainable development among communities (UNODC, 2016).

Such drugs of abuse (DoA) are not only limited to illicit substances, but include prescription and over-the-counter (OTC) medications that have the potential to cause addiction through their designed physiological mechanisms of action (such as the opioids tramadol and codeine).

As with any consumed product, DoAs are excreted in sewage either as their parental form, or as primary and secondary metabolites, depending on their metabolic pathways in the body. These substances are then transported through the connected sewage network to WWTWs. Apart from their role in the degradation of a large variety of organic pollutants, WWTWs may also serve as composite sampling sites for the chemical profiling of wastewater as a non-intrusive tool to estimate drug use and abuse within the communities connected to the sewer system (Castiglioni et al., 2014; Daughton, 2001). However, several discrepancies to this approach have been discussed (Castiglioni et al., 2016), which include the fate of the parent drug in wastewater, as well as the distinction between drug consumption and direct disposal into the recipient waters (Kasprzyk-Hordern and Baker, 2012a). For this reason, the inclusion of metabolic breakdown products as drug target residues (DTRs) were proposed to address this limitation by serving as more stable DTRs for consumption estimates, as well as to confirm whether the drug has undergone metabolic breakdown due to consumption (Petrie et al., 2016).

Apart from the benefits of establishing DoA metabolite loads in wastewater, the enantiomeric profiling of chiral DoAs may also be used to distinguish between direct disposal, consumption and manufacturing (Camacho-Muñoz et al., 2016; Emke et al., 2014; Kasprzyk-Hordern and Baker, 2012a; Kasprzyk-Hordern and Baker, 2012b; Petrie et al., 2016). Some DoAs, such as 3,4-methylenedioxymethamphetamine (MDMA) and mephedrone are manufactured in their racemic form, from which the enantiomers will follow different metabolic pathways and excretion patterns within the body. This leads to a non-racemic mixture in sewage (Castrignanò et al., 2017; Kasprzyk-Hordern and Baker, 2012a). Therefore, if the enantiomeric composition of the drug is racemic in the wastewater sample, it might indicate the direct disposal of the drug rather than its consumption (Emke et al., 2014).

In contrast, the manufacture of some chiral illicit drugs, such as methamphetamine, is primarily enantioselective (Castrignanò et al., 2017; Xu et al., 2017), as the potency and desired physiological effects differ between the chiral isoforms. For methamphetamine, the S-enantiomer is the predominant form to represent an illicit origin, which has also been confirmed during WBE (Castrignanò et al., n.d.; Xu et al., 2017). However, it has been reported that both enantiomers may also be associated with illicit methamphetamine use, depending on the method of synthesis and trafficking. For example, a racemic mixture of methamphetamine was detected in Norwegian wastewater samples in contrast to other European countries where wastewater was enriched with the S-enantiomer (Castrignanò et al., 2017). The authors highlighted that this occurrence was due to the known differences in manufacturing and trafficking the drug between countries. Establishing the enantiomeric signature of chiral DoAs in wastewater therefore provide an added value to WBE for improved drug enforcement strategies, substance abuse estimates and information to social services.

Wastewater-based epidemiology has been applied in many countries to date (Castiglioni et al., 2014; Devault et al., 2017; Emke et al., 2014; Evans et al., 2016; Lai et al., 2017; Ort et al., 2014; Petrie et al., 2016; Subedi and Kannan, 2014; Xu et al., 2017). Ironically, the value of implementing such an overarching approach to monitor drug abuse in African countries , where it may provide an effective means to fill a void left by a chronic shortage of funding and human capacity, is lacking. Given the current state of substance abuse within developing countries, as well as limited drug use statistics, assessment tools such as WBE are needed to assist with future drug use prevention strategies. Recent reports have highlighted an increase in the abuse of illicit drugs in South Africa (Dada et al., 2017; USDS, 2017), with these substances shown to be present in wastewater (Archer et al., 2017a; Archer et al., 2018).

The concept of WBE has shown promise recently to be expanded to address other health-related issues apart from substance abuse. Choi et al. (2018) summarised a variety of avenues that may be addressed in future using the WBE approach.

These include the evaluation of tobacco and alcohol use, pharmaceutical consumption, such as antibiotics, antihistamines, antidepressants and cardiovascular drugs (evaluating public health), along with biological biomarkers such as proteins and genes, which can show the extent and/or development of (non)-communicable diseases on a community level. The information for calculating per-capita mass loadings (mg per day per 1,000 inhabitants), which are used for WBE approaches, can be easily obtained, along with conventional chemical monitoring studies at WWTWs to address a variety of environmental and public health-related challenges that are faced in a rapidly urbanising world, especially for low- and middle-income countries that are shown to face major challenges in community resilience and sustainability.

1.4 PROJECT AIMS

The aims of the project were as follows:

1. Conduct a literature review on the selection of priority micropollutants of environmental concern in South Africa, including a dataset that contains all CEC-related research done in the country
2. Report on the presence and fate of novel CECs and other priority micropollutants at various South African WWTWs
3. Evaluate the environmental risk of detected CECs in wastewater discharge and surface waters
4. Conduct *in vitro* experimentation on (anti)-estrogenic endocrine-disrupting activities at various South African WWTWs
5. Evaluate the effect-based trigger values of estrogenic endocrine system responses in wastewater treatment systems and surrounding surface waters

1.5 SUMMARY OF THE REPORT

The current report is aimed at adding to the knowledge base of CECs in South Africa's freshwater ecosystems and wastewater treatment processes. The report is structured as follows:

Chapter 1	Background to the project: problem statement, future perspectives, project aims and summary of the report
Chapter 2	A survey on pharmaceuticals and steroid hormone monitoring in South African wastewater treatment systems and environmental surface waters. An overview of lethal and sub-lethal risk assessment, including discussions on the limitations of conventional health risk assessment protocols, the AOP framework and a list of predicted-no-effect concentrations of priority pharmaceuticals. The section also gives an overview of the endocrine-disrupting properties of CECs and studies in South Africa on EDCs.
Chapter 3 and 4	The chemical determination of CECs in South African WWTWs and surface waters. The study included both chiral and non-chiral mass balance estimations of target CECs at the treatment works, an environmental risk assessment of treated effluent and surface waters and an estimation of communal illicit drug use through WBE.
Chapter 5	Determination of the temporal variation of CECs at two WWTWs. The study included the quantification of priority CECs over a period of four seven-day sampling campaigns, as well as mass balance estimation, conventional WBE on illicit drug usage patterns and a novel WBE pharmaceutical usage estimation in the respective areas. An environmental health risk assessment of treated effluent was also evaluated.
Chapter 6	*In-vitro* analysis of the (anti)-estrogenic endocrine-disrupting properties at ten WWTWs in Gauteng. The study included the mass balance estimations and environmental health risks of treated effluent and surface waters using sub-lethal toxicity trigger values from literature.
Chapter 7	A final list of priority CECs based on findings of the current report and literature.
Chapter 8	Conclusions and recommendations

CHAPTER 2: EMERGING CONTAMINANTS OF CONCERN IN WATER AND HUMAN HEALTH RISK ASSESSMENTS

2.1 PHARMACEUTICALS AND STEROID HORMONES IN SOUTH AFRICAN WATER SYSTEMS

Pharmaceutical usage may vary in terms of the ratio of prescription and OTC medication issued by the private vs. public health sectors. A study by Osunmakinde et al. (2013) listed 50 of the most prescribed pharmaceuticals in both the public and private health sectors of South Africa. From these lists, the analgesic paracetamol (acetaminophen) is shown to be the most prescribed drug in both sectors. Other pharmaceutical compounds included in the list are the antibiotics amoxicillin, ampicillin, ceftriaxone, chloramphenicol, trimethoprim and sulfamethoxazole, the beta-blocker atenolol, and contraceptives containing levonorgestrel and the synthetic estrogen ethynyl-oestradiol (EE_2) (Osunmakinde et al., 2013). In the private health sector, analgesics are the most prescribed, followed by antihistamines, bronchodilators and antibiotics in second, third and fourth place, respectively (Osunmakinde et al., 2013). In the public health sector, analgesics are also the most prescribed, followed by hypotensives, antiretrovirals (ARVs) and antibiotics in second, third and fourth place, respectively (Osunmakinde et al., 2013). For both the public and private health sectors, it is shown that hypertension medication, analgesics, ARVs, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), antidiabetics and antihistamines are the most commonly prescribed medications in South Africa. Therefore, it can be expected that the country's water systems may contain a large amount of different types of pharmaceutical compounds.

Initial detection studies of EDCs in South Africa consisted of steroid hormone detection (especially estrogens) in water systems (Table 2-1). This is due to the ubiquitous usage of synthetic estrogens as contraceptives and for hormone replacement therapy by a large percentage of the population. These hormones were shown to originate from human excretions and the improper disposal of pharmaceuticals into sewage (Swart and Pool, 2007; Manickum et al., 2011; Swart et al., 2011; Manickum and John, 2014). However, it is increasingly becoming known that several types of PPCPs are accumulating in water systems to the same extent as contraceptive medications. These compounds can serve as EDCs and are not completely removed during water treatment (Ncube et al., 2012). Among these contaminants, pharmaceuticals stand out as one of the sources that might potentially cause endocrine-disrupting activities in non-target organisms.

Although it has been globally recognised that pharmaceutical compounds enter surface waters, the detection of PPCPs in water systems has only recently been done in South Africa (Table 2-1). To the authors' knowledge, this summarised table (Table 2-1) is novel on both a local and an African scale by depicting the current knowledge and research to date regarding trace levels of PPCPs and hormones in South Africa's surface waters. These detections provide valuable information regarding the presence of pharmaceutical drugs in South African waters.

Table 2-1: List of pharmaceuticals and steroid hormone concentrations (in µg/ℓ) in South African water treatment works and surface waters

Pharmaceutical	Concentration (µg/ℓ)	Location (province)	Source	Reference
Non-steroidal anti-inflammatory drugs				
Acetaminophen	5.8-58.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	5.8	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	1.0-1.7	KwaZulu-Natal	Surface water	Matongo et al., 2015
	136.9-343.6	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.04-0.2	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.02-0.2	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	21.3-119.5	North West	WWTW influent	Kanama et al., 2018
	< 0.001-11.4	North West	WWTW effluent	Kanama et al., 2018
Aspirin	2.2-10.0	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	13.7-25.4	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
Diclofenac	1.1-15.6	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	222.7	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	123.7	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	0.6 – 8.2	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
	2.7-5.6	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	2.2-2.5	Gauteng	WWTW effluent	Archer et al., 2020 (t*his report)*
	0.3-2.2	Gauteng	Surface water	Archer et al., 2020 (t*his report)*
	0.3	Gauteng	WWTW influent	Leusch et al., 2018
	0.1-10.3	North West	WWTW influent	Kanama et al., 2018
	0.1-1.6	North West	WWTW effluent	Kanama et al., 2018
	6.4-16.0	KwaZulu-Natal	WWTW influent	Madikizela and Chimuka, 2017
	1.4-2.0	KwaZulu-Natal	WWTW effluent	Madikizela and Chimuka, 2017
	1.1-1.2	KwaZulu-Natal	Surface water	Sibeko et al., 2019
Ibuprofen	0.8-18.9	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	39.8	Gauteng	WWTW influent	Amdany et al., 2014
	12.6	Gauteng	WWTW effluent	Amdany et al., 2014
	111.9	Gauteng	WWTW influent	Amdany et al., 2014
	24.6	Gauteng	WWTW effluent	Amdany et al., 2014

Pharmaceutical	Concentration (µg/ℓ)	Location (province)	Source	Reference
Ibuprofen	0.02	Gauteng	WWTW influent	Osunmakinde et al., 2013
	1.2	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	1.1	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	0.4-0.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
	62.8	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	58.7	KwaZulu-Natal	WWTW effluent	Matongo et al., 2015
	0.5-8.5	KwaZulu-Natal	Surface water	Matongo et al., 2015
	9.1-15.8	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.3-1.2	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.1-0.6	Gauteng	Surface water	Archer et al., 2020 (t*his report)*
	0.3-63.4	North West	WWTW influent	Kanama et al., 2018
	< 0.001-13.7	North West	WWTW effluent	Kanama et al., 2018
	55.0-69.0	KwaZulu-Natal	WWTW influent	Madikizela and Chimuka, 2017
	2.1-4.2	KwaZulu-Natal	WWTW effluent	Madikizela and Chimuka, 2017
	0.59-1.4	KwaZulu-Natal	Surface water	Sibeko et al., 2019
Ketoprofen	0.4-8.2	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	1.1-2.0	KwaZulu-Natal	Surface water	Madikizela et al., 2014
	1.7-6.4	KwaZulu-Natal	WWTW influent	Madikizela et al., 2014
	1.2-4.3	KwaZulu-Natal	WWTW effluent	Madikizela et al., 2014
	0.02	Gauteng	WWTW influent	Osunmakinde et al., 2013
	< 0.001	Gauteng	WWTW effluent	Osunmakinde et al., 2013
	3.2	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	0.4	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	0.4-0.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
	0.4-5.6	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.2-0.7	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.01-0.8	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	< 0.001-0.7	North West	WWTW influent	Kanama et al., 2018
	< 0.00-0.2	North West	WWTW effluent	Kanama et al., 2018

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Naproxen	55.0	Gauteng	WWTW influent	Amdany et al., 2014
	13.5	Gauteng	WWTW effluent	Amdany et al., 2014
	52.3	Gauteng	WWTW influent	Amdany et al., 2014
	20.4	Gauteng	WWTW effluent	Amdany et al., 2014
	2.9-5.5	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	1.8-2.9	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.2-1.9	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	15.0-20.0	KwaZulu-Natal	WWTW influent	Madikizela and Chimuka, 2017
	0.6-1.1	KwaZulu-Natal	WWTW effluent	Madikizela and Chimuka, 2017
	1.2-2.3	KwaZulu-Natal	Surface water	Sibeko et al., 2019
Antidepressants				
Venlafaxine	0.27-0.46	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.13-0.16	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.03-0.11	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	0.16-0.26	Western Cape	WWTW influent	Archer et al., 2020 *(this report)*
	0.15-0.21	Western Cape	WWTW effluent	Archer et al., 2020 *(this report)*
	0.05-0.14	Western Cape	Surface water	Archer et al., 2020 *(this report)*
	0.05-0.15	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.07-0.13	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.005-0.08	Gauteng	Surface water	Archer et al., 2020 *(this report)*
Antibiotics/biocides				
Ampicillin	2.5-14.5	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	6.6	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	8.9	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	3.2-5.5	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
Chloramphenicol	0.5-10.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
Erythromycin	0.6-22.6	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	0.6	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	0.2	KwaZulu-Natal	WWTW effluent	Matongo et al., 2015
	0.1-0.2	KwaZulu-Natal	Surface water	Matongo et al., 2015

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Erythromycin	0.003-0.2	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.001-0.02	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.0001-0.02	KwaZulu-Natal	Surface water	Faleye et al., 2019
Norfloxacin	0.1-1.5	North West	WWTW influent	Kanama et al., 2018
	0.02-0.4	North West	WWTW effluent	Kanama et al., 2018
	0.03-0.3	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.002-0.003	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.0005-0.001	KwaZulu-Natal	Surface water	Faleye et al., 2019
Nalidixic acid	1.7-30.8	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	29.9	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	25.2	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	12.4-23.5	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
Streptomycin	0.8-8.4	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
Sulfamethoxazole	3.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	0.1-0.2	Western Cape	WWTW influent	Hendricks and Pool, 2012
	0.08-0.1	Western Cape	STW effluent	Hendricks and Pool, 2012
	34.5	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	1.2-5.3	KwaZulu-Natal	Surface water	Matongo et al., 2015
	0.6-2.6	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	1.2-1.6	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.6-1.4	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	0.04	Gauteng	WWTW influent	Nyamukamba et al., 2019
	0.015-0.08	Gauteng	WWTW effluent	Nyamukamba et al., 2019
	0.3-9.1	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.1-0.4	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.06-0.4	KwaZulu-Natal	Surface water	Faleye et al., 2019
Tetracycline	0.6-5.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	1.1-75.8	North West	WWTW influent	Kanama et al., 2018
	0.5-3.2	North West	WWTW effluent	Kanama et al., 2018

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Triclosan	78.4	Gauteng	WWTW influent	Amdany et al., 2014
	10.7	Gauteng	WWTW effluent	Amdany et al., 2014
	127.7	Gauteng	WWTW influent	Amdany et al., 2014
	22.9	Gauteng	WWTW effluent	Amdany et al., 2014
	0.4-0.9	KwaZulu-Natal	Surface water	Madikizela et al., 2014
	2.1-9.0	KwaZulu-Natal	WWTW influent	Madikizela et al., 2014
	1.3-6.4	KwaZulu-Natal	WWTW effluent	Madikizela et al., 2014
	0.2	Gauteng	WWTW influent	Leusch et al., 2018
	< 0.001-0.4	North West	WWTW influent	Kanama et al., 2018
	< 0.001-0.3	North West	WWTW effluent	Kanama et al., 2018
Triclocarbanilide	0.06-1.8	North West	WWTW influent	Kanama et al., 2018
	< 0.001-0.5	North West	WWTW effluent	Kanama et al., 2018
Trimethoprim	0.3	KwaZulu-Natal	Surface water	Matongo et al., 2015
	4.5-11.1	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	1.2-1.6	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.3-1.1	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	0.3-8.8	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.007-0.2	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.01-0.2	KwaZulu-Natal	Surface water	Faleye et al., 2019
Tylosin	0.2-22.0	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
Ciprofloxacin	0.12-9.1	North West	WWTW influent	Kanama et al., 2018
	0.06-1.4	North West	WWTW effluent	Kanama et al., 2018
	0.7-16.9	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	27.1	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2016
	20.5	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	2.4-14.3	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
	41.0-501.6	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.1-1.1	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.06-0.7	KwaZulu-Natal	Surface water	Faleye et al., 2019

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Ofloxacin	0.7-9.7	KwaZulu-Natal	WWTW influent	Faleye et al., 2019
	0.02-0.09	KwaZulu-Natal	WWTW effluent	Faleye et al., 2019
	0.009-0.07	KwaZulu-Natal	Surface water	Faleye et al., 2019
Beta-blockers				
Atenolol	1.0-39.1	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	1.6-2.5	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.4-0.7	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.1-0.5	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	0.9	Gauteng	WWTW influent	Leusch et al., 2018
	0.4-8.3	North West	WWTW influent	Kanama et al., 2018
	0.2-3.2	North West	WWTW effluent	Kanama et al., 2018
Pindolol	0.03	Gauteng	WWTW influent	Osunmakinde et al., 2013
	< 0.001	Gauteng	WWTW effluent	Osunmakinde et al., 2013
Anti-epileptics				
Carbamazepine	0.02-0.3	Free State	Drinking water	Patterton, 2013
	0.01-0.02	KwaZulu-Natal	Drinking water	Patterton, 2013
	0.01	Gauteng	Drinking water	Patterton, 2013
	0.03-0.1	Gauteng	Drinking water	Patterton, 2013
	0.01	Gauteng	WWTW influent	Osunmakinde et al., 2013
	2.2	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	0.9	KwaZulu-Natal	WWTW effluent	Matongo et al., 2015
	0.1-3.2	KwaZulu-Natal	Surface water	Matongo et al., 2015
	0.3-0.6	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.4	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.2-0.3	Gauteng	Surface water	Archer et al., 2020 *(this report)*
	0.9	Gauteng	WWTW influent	Leusch et al., 2018
Anti-psychotics				
Clozapine	8.6	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	9.6	KwaZulu-Natal	WWTW effluent	Matongo et al., 2015
	2.2-8.9	KwaZulu-Natal	Surface water	Matongo et al., 2015

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Lipid regulators				
Bezafibrate	0.8-8.7	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2014
	0.2	KwaZulu-Natal	WWTW influent	Agunbiade and Moodley, 2015
	0.03	KwaZulu-Natal	WWTW effluent	Agunbiade and Moodley, 2016
	0.003-0.2	KwaZulu-Natal	Surface water	Agunbiade and Moodley, 2016
	1.4-3.0	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.3-0.7	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.05-0.4	Gauteng	Surface water	Archer et al., 2020 *(this report)*
Antiretrovirals				
Abacavir	3.5-14.0	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
Atazanavir	0.06-1.4	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.08-0.7	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Raltregavir	0.06-17.0	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.09-3.5	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Ribavirin	0.02	Gauteng	WWTW influent	Osunmakinde et al., 2013
	< 0.001	Gauteng	WWTW effluent	Osunmakinde et al., 2013
Ritonavir	1.6-3.2	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.46-1.5	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Famciclovir (famvir)	0.02	Gauteng	WWTW influent	Osunmakinde et al., 2013
	< 0.001	Gauteng	WWTW effluent	Osunmakinde et al., 2013
Tenofovir	0.25	Gauteng	Surface water	Wood et al., 2015
	0.16-0.19	Free State	Surface water	Wood et al., 2015
	0.1-0.3	KwaZulu-Natal	WWTW influent	Mlunguza et al., 2020
	0.1	Gauteng	Surface water	Mlunguza et al., 2020
Zalcitabine	0.07	Free State	Surface water	Wood et al., 2015
	0.03	Gauteng	Surface water	Wood et al., 2015
	0.008	Gauteng	Tap water	Wood et al., 2015
Maraviroc	0.08-0.32	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.04	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Lamivudine	0.09-0.24	Gauteng	Surface water	Wood et al., 2015
	0.84-2.2	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.13	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
	3.7-20.9	Western Cape	WWTW influent	Mosekiemang et al., 2019
Lopinavir	1.2-2.5	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	1.9-3.8	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Darunavir	0.07-43.0	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.13-17.0	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Didanosine	0.05	Free State	Surface water	Wood et al., 2015
Stavudine	0.4-0.8	Gauteng	Surface water	Wood et al., 2015
Zidovudine	0.2-0.6	Gauteng	Surface water	Wood et al., 2015
	0.5-0.1	Gauteng	WWTW effluent	Wood et al., 2015
	0.05	Gauteng/Free State	Surface water	Wood et al., 2015
	0.07	Gauteng	Tap water	Wood et al., 2015
	6.9-53.0	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.09-0.5	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
Nevirapine	0.2-1.5	Gauteng	Surface water	Wood et al., 2015
	0.7-2.8	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.5-1.9	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
	2.1	Gauteng	WWTW influent	Schoeman et al., 2015
	0.35	Gauteng	WWTW effluent	Schoeman et al., 2015
	0.05-0.19	Gauteng	WWTW influent	Schoeman et al., 2017
	0.5-0.09	Gauteng	WWTW effluent	Schoeman et al., 2017
	0.7	Western Cape	WWTW influent	Mosekiemang et al., 2019
	0.7-0.8	Western Cape	WWTW effluent	Mosekiemang et al., 2019
Lopinavir	0.28-0.31	Gauteng	Surface water	Wood et al., 2015
	0.13	Gauteng	WWTW effluent	Wood et al., 2015
Indinavir	0.26-0.59	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	0.03-0.04	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Efavirenz	24.0-34.0	KwaZulu-Natal	WWTW influent	Abafe et al., 2018
	20.0-34.0	KwaZulu-Natal	WWTW effluent	Abafe et al., 2018
	17.4	Gauteng	WWTW influent	Schoeman et al., 2015
	7.1	Gauteng	WWTW effluent	Schoeman et al., 2015
	5.5-14.0	Gauteng	WWTW influent	Schoeman et al., 2017
	4.0	Gauteng	WWTW effluent	Schoeman et al., 2017
	1.42-15.4	Western Cape	WWTW influent	Mosekiemang et al., 2019
	1.48-12.4	Western Cape	WWTW effluent	Mosekiemang et al., 2019
	1.0-26.3	KwaZulu-Natal	WWTW influent	Mlunguza et al., 2020
	3.3-37.3	KwaZulu-Natal	WWTW effluent	Mlunguza et al., 2020
Emtricitabine	31.3-172.0	Western Cape	WWTW influent	Mosekiemang et al., 2019
	0.7-41.7	Western Cape	WWTW effluent	Mosekiemang et al., 2019
	0.3-3.1	KwaZulu-Natal	WWTW influent	Mlunguza et al., 2020
	0.2-0.4	KwaZulu-Natal	WWTW effluent	Mlunguza et al., 2020
Human indicators				
Caffeine	4.5	KwaZulu-Natal	WWTW influent	Matongo et al., 2015
	0.6	KwaZulu-Natal	WWTW effluent	Matongo et al., 2015
	0.1-3.3	KwaZulu-Natal	Surface water	Matongo et al., 2015
	5.1-1,214.4	Gauteng	WWTW influent	Archer et al., 2020 *(this report)*
	0.5-3.8	Gauteng	WWTW effluent	Archer et al., 2020 *(this report)*
	0.6-6.6	Gauteng	Surface water	Archer et al., 2020 *(this report)*
Steroid hormones				
Oestrone (E_1)	0.001-0.03	KwaZulu-Natal	Surface water	Manickum and John, 2014
	0.0009-0.004	Gauteng	Surface water	Mguni et al., 2018
	0.009-0.011	Western Cape	Sewage treatment works (STW) effluent	Swart and Pool, 2007
	0.01	Western Cape	STW effluent	Swart and Pool, 2007
	0.003-0.02	KwaZulu-Natal	STW effluent	Manickum et al., 2011
	0.01-0.35	KwaZulu-Natal	WWTW influent	Manickum and John, 2014
	0.003-0.08	KwaZulu-Natal	WWTW effluent	Manickum and John, 2014
	0.02-0.02	Western Cape	STW influent	Swart et al., 2011

Pharmaceutical group/active	Concentration (µg/ℓ)	Location (province)	Source	Reference
Oestrone (E_1)	0.01-0.02	Western Cape	Surface water	Swart et al., 2011
	0.002-0.004	Gauteng	Drinking water	Van Zijl et al., 2017
	< 0.001-0.001	Western Cape	Drinking water	Van Zijl et al., 2017
	0.004-0.05	North West	WWTW influent	Kanama et al., 2018
	0.007-0.04	North West	WWTW effluent	Kanama et al., 2018
Oestriol (E_3)	0.03-1.5	North West	WWTW influent	Kanama et al., 2018
	0.01-0.5	North West	WWTW effluent	Kanama et al., 2018
Oestradiol (E_2)	0.001-0.07	KwaZulu-Natal	Surface water	Manickum and John, 2014
	0.001	Western Cape	STW effluent	Swart and Pool, 2007
	0.005	Western Cape	STW effluent	Swart and Pool, 2007
	0.01-0.02	KwaZulu-Natal	STW effluent	Manickum et al., 2011
	0.02-0.20	KwaZulu-Natal	WWTW influent	Manickum and John, 2014
	0.004-0.11	KwaZulu-Natal	WWTW effluent	Manickum and John, 2014
	0.001-0.03	Mpumalanga	Surface water	Van Wyk et al., 2014
	0.04-0.37	Gauteng	Drinking water	De Jager et al., 2013
	0.05-0.37	Western Cape	Drinking water	De Jager et al., 2013
	< 0.001	Gauteng	Drinking water	Van Zijl et al., 2017
	< 0.001	Western Cape	Drinking water	Van Zijl et al., 2017
	0.001-0.05	North West	WWTW influent	Kanama et al., 2018
	0.008-0.02	North West	WWTW effluent	Kanama et al., 2018
	0.001-0.017	Kwazulu-Natal	Surface water	Truter et al., 2015
	0.003-0.02	Western Cape	Surface water	Truter et al., 2015
	0.0007-0.03	Mpumalanga	Surface water	Truter et al. 2016
Ethynyl-oestradiol (EE_2)	0.001-0.004	KwaZulu-Natal	Surface water	Manickum and John, 2014
	0.01-0.1	KwaZulu-Natal	WWTW influent	Manickum and John, 2014
	0.001-0.008	KwaZulu-Natal	WWTW effluent	Manickum and John, 2014
	0.001-0.01	Mpumalanga	Surface water	Van Wyk et al., 2014
	< 0.001	Gauteng	Drinking water	Van Zijl et al., 2017
	0.9-9.8	North West	WWTW influent	Kanama et al., 2018
	0.5-4.6	North West	WWTW effluent	Kanama et al., 2018

Pharmaceutical group/active ingredient	Concentration (µg/ℓ)	Location (province)	Source	Reference
Ethynyl-oestradiol (EE_2)	0.0005-0.01	Mpumalanga	Surface water	Truter et al. 2016
	0.01-0.06	KwaZulu-Natal	Surface water	Manickum and John, 2014
	0.16-0.90	KwaZulu-Natal	WWTW influent	Manickum and John, 2014
	0.03	KwaZulu-Natal	WWTW effluent	Manickum and John, 2014
Testosterone	0.003-0.02	KwaZulu-Natal	Surface water	Manickum and John, 2014
	0.12-0.64	KwaZulu-Natal	WWTW influent	Manickum and John, 2014
	0.03	KwaZulu-Natal	WWTW effluent	Manickum and John, 2014

2.2 ENVIRONMENTAL HEALTH RISK CHARACTERISATION

2.2.1 Overview

Due to the complexity and sheer volume of pollutants that are present in natural water systems, global regulating bodies such as USEPA, the WHO and the European Commission (EC) have set out a framework to investigate and identify environmental pollutants in freshwater systems (USEPA, 1997; WHO, 2012; EC, 2016). These approaches consist of four main steps to be followed when doing impact assessments of water pollutants:

- Identify the hazard to the environment
- Conduct dose-response assessments
- Assess the exposure of the pollutants to non-target organisms
- Implement risk characterisation for possible pollutants entering freshwater ecosystems.

These collective approaches facilitate the management and decision-making strategies for risk reduction (Figure 2-1).

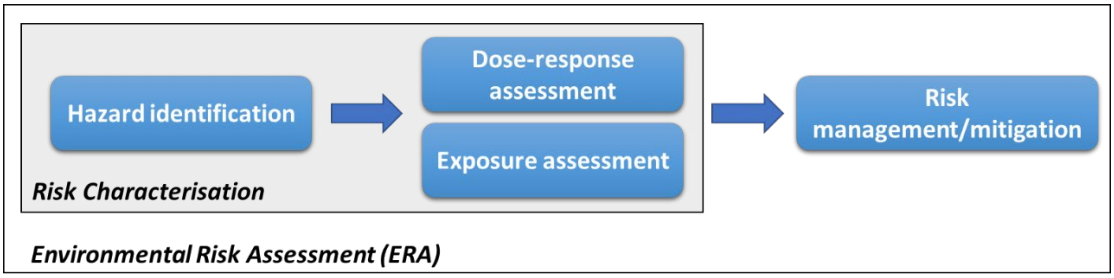


Figure 2-1: Framework for the identification and regulation of environmental pollutants in freshwater systems as set out by global environmental regulation agencies

By adhering to these approaches, the first line of investigation includes the hazard identification of environmental pollutants entering freshwater systems. It is evident from literature that the identification of problematic areas in South Africa, where water systems may be subjected to various pollutants from human sources, is much needed. Water treatment facilities also need to be a focus point for monitoring CECs as these facilities can provide information regarding the origin of freshwater pollutants in areas of interest. Regarding the pollution of our natural water resources by human activities, some insight can be obtained by observing the wellbeing (health status) of wildlife populations within contaminated waters. Wildlife species that inhabit polluted freshwater supplies are in first-line contact with environmental pollutants and can provide useful information on the presence of pathogens in environmental waters and the effects of long-term exposure. Such sentinel species serve as a valuable tool for risk characterisation.

2.2.2 Conventional environmental risk assessment

Although the presence and recalcitrance of several PPCPs have already been demonstrated within surface water systems on a global scale, the implementation of such monitoring studies to predict environmental risk needs more attention. The initial risk characterisation of CECs is based on acute and/or chronic toxicity studies, which assess toxicity towards the most sensitive organisms within ecosystems. These test organisms include several trophic levels, such as bacteria, algae, crustaceans and vertebrate species. Acute toxicity data is based on short-term toxicity effects (less than 24 hours), which are expressed as EC_{50} (concentration which shows an effect in 50% of the experimental population) or LD_{50} (lethal dose at 50% of the experimental population), whereas chronic toxicity data is based on long-term toxicity effects (more than 24 hours), which is expressed as no observed effect concentration (NOEC) or lowest observed effect concentration (LOEC) (Escher et al., 2015).

These toxicity endpoints are corrected by an assessment factor, depending on the number of test organisms or trophic levels in a battery assay, to calculate the PNEC of the emerging contaminant of interest. For risk screening, the PNEC is then compared to either predicted environmental concentrations (PEC) or measured environmental concentrations (MEC) of the CEC to obtain a Risk Quotient (RQ) as a form of assessment factor. An RQ value lower than 0.1 is considered a low risk, an RQ between 0.1 and 1.0 is considered a moderate risk, an RQ larger than 1 is considered a high risk, and an RQ larger than 10 is considered a severe risk (Figure 2-2). Extracting this knowledge, various PNECs can be derived for various classes of CECs from literature (Table 2-2).

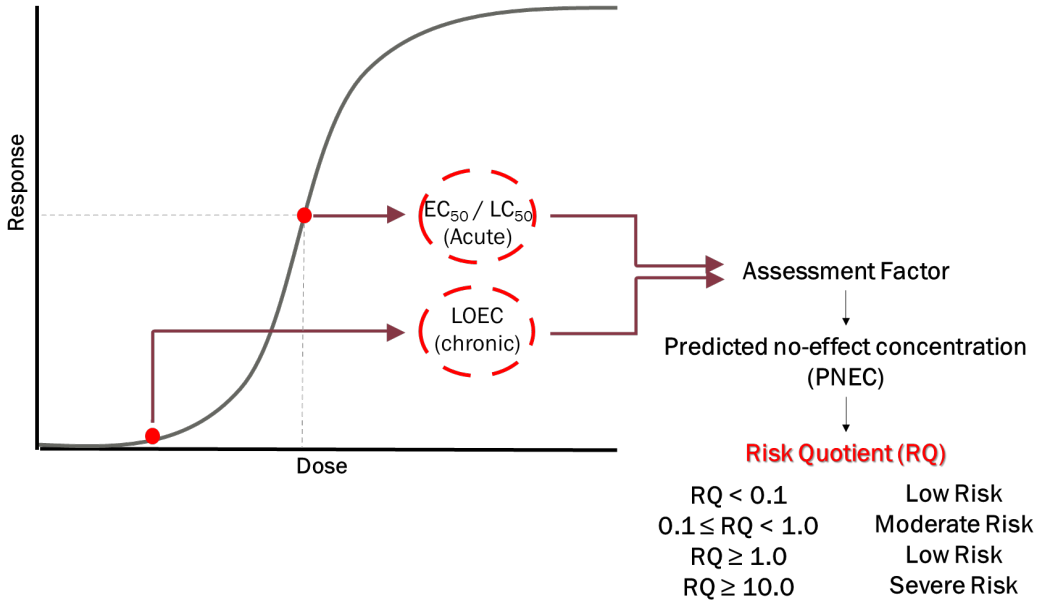


Figure 2-2: Diagram showing the pathways for estimating the PNEC of test chemicals using conventional toxicity assays of sentinel organisms

Table 2-2: Collated PNECs reported in literature on lethal, sub-lethal, acute and chronic toxicity assays of sentinel organisms for various PPCPs

Chemical	PNEC (ng/ℓ)	Description	Reference
Illicit drugs			
MDMA	216	Median algae, cladocerans and fish	Mendoza et al., 2014
	47,601	Estimated EC_{50} immobilisation in Daphnia	Aalizadeh et al., 2017
Cocaine	2,280	Median algae, Daphnia, fish	Sanderson et al., 2004
Benzoylecgonine	2,334	Estimated LD_{50} mortality in fish	Aalizadeh et al., 2017
Methamphetamine	9,736	Estimated EC_{50} immobilisation in Daphnia	Aalizadeh et al., 2017
Amphetamine	387	EC_{50} in algae	MistraPharma 2015 database; Riva et al., 2019
Methaqualone	723	Estimated IC_{50} invertebrate growth rate	Aalizadeh et al., 2017
Antiepileptics			
Carbamazepine	250	NOEC reproduction in cladocerans	Ferrari et al., 2003
	420	Median NOEC in algae, rotifers, cladocerans and fish growth/ reproduction/larval mortality	Ferrari et al., 2003
	500	EC_{50} and NOEC in algae, cyanobacteria, invertebrates and fish	Kase, 2010 (Swiss environmental quality system)
	2,276	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
	2,500	LOEC mortality and reproduction in fish	Bouissou-Schurtz et al., 2014; Bergman et al. 2011; Ferrari et al., 2004
	48,908	EC_{50} in algae	MistraPharma 2015 database, Riva et al., 2019
	100,000	EC50 growth inhibition in algae	Minguez et al., 2014
10,11-dihydro-11-hydroxycarbamazepine	2,388	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
Gabapentin	118,370	Esimated EC_{50} Daphnia immobilisation	Aalizadeh et al., 2017
Antibiotics			
Chloramphenicol	2,729	IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Tetracycline	498	IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Trimethoprim	56,010	EC_{50} growth inhibition in algae	Minguez et al., 2014
	120,000	NOEC in Daphnia	Vestel et al., 2016
	255,000	Based on algae growth	Eguchi et al., 2004

Chemical	PNEC (ng/ℓ)	Description	Reference
Sulfamethoxazole	200	NOEC in Daphnia	Vestel et al., 2016
	520	EC_{50} in algae	USEPA ECOTOX database 2015; Riva et al., 2019
	590	IC_{50} cyanobacteria growth	Ferrari et al., 2004
	1120	EC_{50} growth inhibition in algae	Minguez et al., 2014
	1,795	Worst-case aquatic	Straub, 2016
	3,080	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
	8,975	EU chronic-based aquatic animals	Straub, 2016
Azithromycin	19	NOEC in blue-green algae	Vestel et al., 2016
	500	EC_{50} growth inhibition in algae	Minguez et al., 2014
Clarithromycin	46	Based on EC_{50} of algae	USEPA ECOTOX database 2015; Riva et al., 2019
	62	Based on reproduction in Daphnia	Yamashita et al., 2006
	230	EC_{50} growth inhibition in algae	Minguez et al., 2014
Mirtazapine	414	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Sulfasalazine	56	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Anti-inflammatories			
Diclofenac	100	EC_{50} waterflea reproduction	Ferrari et al., 2004
	144	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
	1,720	Lowest predicted LC_{50} mortality value of fish	Slobodnik et al., 2012
	3,300	Toxicity to algae	Escher et al., 2011
	10,000	Chronic invertebrate NOEC	Carlsson et al., 2006
	21,300	EC_{50} growth inhibition in algae	Minguez et al., 2014
	22,703	EC_{50} in crustaceans	USEPA ECOTOX database 2015; Riva et al., 2019
	32,000	NOEC in fish	Vestel et al., 2016
	71,900	EC_{50} in green algae	Vestel et al., 2016
	116,000	Median NOEC in algae, rotifers, cladocerans and fish growth/ reproduction/larval mortality	Ferrari et al., 2003
Ketoprofen	2,000	Based on EC_{50} of algae	USEPA ECOTOX database 2015; Riva et al., 2019

Chemical	PNEC (ng/ℓ)	Description	Reference
Naproxen	1,853	Estimated LD_{50} in Daphnia	Aalizadeh et al., 2017
	4,000	NOEC for toxicity of cnidarian	Quinn et al., 2008
	6,600	EC_{50} Daphnia growth inhibition	Molander et al., 2009; Orias and Perrodin, 2013
	37,000	Invertebrate EC_{50}	Carlsson et al., 2006
	42,000	EC_{50} in algae	USEPA ECOTOX database 2015; Riva et al., 2019
	44,400	EC_{50} growth inhibition in algae	Minguez et al., 2014
Ibuprofen	6,600	EC_{50} in algae	Escher et al., 2011
	40,000	EC_{50} of crustaceans	USEPA ECOTOX database 2015; Riva et al., 2019
	200,000	EC_{50} Daphnia reproduction	Han et al., 2006
Antidepressants			
Citalopram	3,030	EC_{50} growth inhibition in algae	Minguez et al., 2014
	8,000	Based on waterflea reproduction	Henry et al., 2003
Venlafaxine	47,580	EC_{50} growth inhibition in algae	Minguez et al., 2014
Analgesics			
Acetaminophen	367	EC_{50} of crustaceans	MistraPharma 2015 database; Riva et al., 2019
	1,000	EC_{50} in fish, Ecological Structure Activity Relationships (ECOSAR)	Verlicchi et al., 2012
	6,920	LC_{50} invertebrate	Molander et al., 2009; Orias and Perrodin, 2013
	12,900	LC_{50} of Daphnia	Bouissou-Schurtz et al., 2014
	15,820	Estimated EC_{50} immobilisation in Daphnia	Aalizadeh et al., 2017
Codeine	60	Median algae, daphnia, fish	Sanderson et al., 2004
	7,186	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
	216,000	NOEC in fish	Vestel et al., 2016
Tramadol	73,000	EC_{50} in fish	Vestel et al., 2016
Antihistamines			
Cetirizine	410	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
	21,580	EC50 growth inhibition in algae	Minguez et al., 2014
Cimetidine	839	Estimated IC_{50} fish growth rate	Aalizadeh et al., 2017

Chemical	PNEC (ng/ℓ)	Description	Reference
Cimetidine	176,000	NOEC in Daphnia	Vestel et al., 2016
Fexofenadine	53	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Antidiabetics			
Bezafibrate	100,000	EC_{50} growth inhibition in algae, EC_{50} in Daphnia	Minguez et al., 2014; Vestel et al., 2016
Gemfibrozil	7,000	EC_{50} growth inhibition in algae	Minguez et al., 2014
	890,000	Chronic toxicity in fish	Deo et al., 2014
Metformin	100,000	EC_{50} in green algae	Vestel et al., 2016
Antiretrovirals			
Efavirenz	26	EC_{50} in green algae	Vestel et al., 2016
	200.8	Estimated LD_{50} in fish	Aalizadeh et al. 2017
	300	Quantitative Structure-Activity Relationship (QSAR)	Daouk et al., 2015
	2,600	NOEC in green algae	Vestel et al., 2016
Emtricitabine	23,765	Estimated IC_{50} invertebrate growth rate	Aalizadeh et al. 2017
Cardiovascular			
Atenolol	100,000	EC_{50} growth inhibition in algae, NOEC in Daphnia	Minguez et al., 2014; Vestel et al., 2016
	148,000	EC_{50} in fish	Vestel et al., 2016
	200,000	EC_{50} in crustaceans	MistraPharma 2015 database; Riva et al., 2019
Propanolol	20	NOEC in fish	Vestel et al., 2016
Irbesartan	191,000	EC_{50} in Daphnia	Vestel et al., 2016
	704,000	NOEC in fish	Vestel et al., 2016
Valsartan	27,000	QSAR	Daouk et al., 2015
Human markers			
Caffeine	50.0	NOEC invertebrate growth inhibition	Pires et al., 2016
	13,759	Estimated IC_{50} Daphnia growth rate	Aalizadeh et al., 2017
	46,004	EC_{50} in algae	Sanderson et al., 2003; Riva et al., 2019
	57,2300	LD_{50} in crustaceans	Wilkins and Metcalfe, 1993
1,7-dimethylxanthine	4.0	Geometric mean LOEC and NOEC in green algae	Rodriguez-Gill et al., 2018
	15.0	EC_{50} in green algae	Rodriguez-Gill et al., 2018

Chemical	PNEC (ng/ℓ)	Description	Reference
Nicotine	242	EC_{50} in crustaceans	USEPA ECOTOX database 2015; Riva et al., 2019
Cotinine	28,045	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Anticancer			
Tamoxifen	9.2	EC_{50} in green algae	Morrow et al., 2001; Riva et al., 2019
UV filters and preservatives			
Benzophenone-3	360	EC_{50} in algae	Rodil et al., 2009; Riva et al., 2019
	1,800	96-hour EC_{50} of *C. vulgaris*, 48-hour LD_{50} of *D. magna*, 96-hour LD_{50} of *B. rerio*	Du et al., 2017
Benzophenone-4	4,896	LOEC in fish	Kunz et al., 2006; Riva et al., 2019
	470,000	96-hour EC_{50} of *C. vulgaris*, 48-hour LD_{50} of *D. magna*, 96-hour LD_{50} of *B. rerio*	Du et al., 2017
Methylparaben	29,111	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Ethylparaben	13,292	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Propylparaben	8,192	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Butylparaben	5,064	Estimated IC_{50} cladocerans growth rate	Aalizadeh et al., 2017
Plasticizer			
Bisphenol-A	158	EC_{50} in fish	Brian et al., 2005, Riva et al., 2019
	2,005	Estimated LD_{50} in fish	Aalizadeh et al., 2017
Stain repellents/flame retardants			
Perfluorooctane sulfonic acid	1,080	EC_{50} in fish	Hagenaars et al., 2011, Riva et al., 2019
Perfluorooctanoic acid	113,041	EC_{50} in fish	Hagenaars et al., 2011, Riva et al., 2019
Non-ionic surfactants			
4-nonylphenol	190	EC_{50} in crustaceans	Comber et al., 1993, Riva et al., 2019
4-tert-octylphenol	48	EC_{50} in fish	Brian et al., 2005, Riva et al., 2019
Anti-corrosive/de-icing agent			
Benzotriazole	60,000	NOEC subchronic reproduction in Daphnia	Breedveld et al., 2002

Although the conventional approach is valuable to assess toxicity risk factors and “alarming” triggers in the environment, such conventional ERA has been proven to cause severe under-estimations, as it does not include sub-lethal endpoints that could lead to long-term health effects. Such limitations include the following:

- The relevance for selecting only specific test organisms to calculate PNEC in the environment is an under-estimation of the total ecological impacts of the emerging contaminants.
- Using animal models is timely and not cost-effective to calculate risk for most CECs on the market and in environmental waters.
- There are ethical concerns in using animal bio-indicators for toxicity testing.
- Sub-lethal and chronic (long-term; multigenerational) toxicity such as (neuro)-endocrine disruption, oxidative stress, DNA damage and various other physiological effects and non-communicable diseases.
- The prediction of environmental risk is also unknown for pollutants in highly complex chemical mixtures in the environment, which leads to the need to establish more defined ERAs that are not chemical-specific (combined moderate/low RQs of chemicals may have a combined high to severe health risk).

Due to these constraints that are shown for conventional ERA, it is necessary to construct more accurate and thorough models for risk assessment, constituting both lethal and sub-lethal toxicity. Recent advancements in EBM, molecular screening technologies and bioinformatics are just a few examples to update existing risk assessment strategies and decision-making processes for predictive ecotoxicology. For this reason, more avenues of CEC risk factors need to be considered, such as endocrine disruption and triggers for other non-communicable diseases.

2.2.3 Endocrine disruption as a sub-lethal toxicity endpoint

Several CECs have been linked to potentially causing a large variety of health effects in both invertebrate and vertebrate sentinel organisms (Daughton and Ternes, 1999; McKinlay et al., 2008; Bolong et al., 2009). Selected pollutants have been suggested to interact with the endocrine system pathways of vertebrates and are collectively referred to as endocrine-disrupting contaminants. The USEPA defines an EDC as “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behaviour” (USEPA, 1997).

Man-made compounds most frequently implicated as EDCs include pesticides, PPCPs and industrial by-products. Classic examples of environmental endocrine disruption include studies showing the feminisation of male fish and the widespread occurrence of anti-androgenic ligands within UK rivers that receive effluent from connected WWTWs (Liney et al., 2006; Jobling et al., 2009). Guillette et al. (1996, 1999) published a series of accounts confirming the disruption of the male reproductive system in juvenile male alligators in several lakes (especially Lake Apopka) in Florida, USA. Reproductive deformities, ranging from reduced penis size to altered plasma testosterone levels, were associated with the extensive agricultural use of the insecticide DDT and other POPs that lead to non-point source pollution in water systems flowing into lakes (Guillette et al., 1996; Guillette et al., 1999). Along with concerns about the general disruption of the human reproductive system, which leads to various detrimental effects such as ovarian cancer, breast cancer and declined sperm quality, international concerns were raised regarding the potential subtle disruption of the endocrine systems of humans and wildlife in the organisational window during development (Colborn et al. 1993). Documented reports on the occurrence of endocrine disruption within natural wildlife populations have raised international awareness of the harmful effects that man-made pollutants can exert on surface water quality for reuse.

EDCs are known to modulate any one of the three major axes of the endocrine system: the hypothalamus-pituitary-gonad (HPG), the hypothalamus-pituitary-thyroid (HPT) and the hypothalamus-pituitary-adrenal (HPA) axes (Figure 2-3). Within these pathways, several hormones, metabolic enzymes and receptors are responsible for the dispersal, activity and function of various physiological traits in vertebrates.

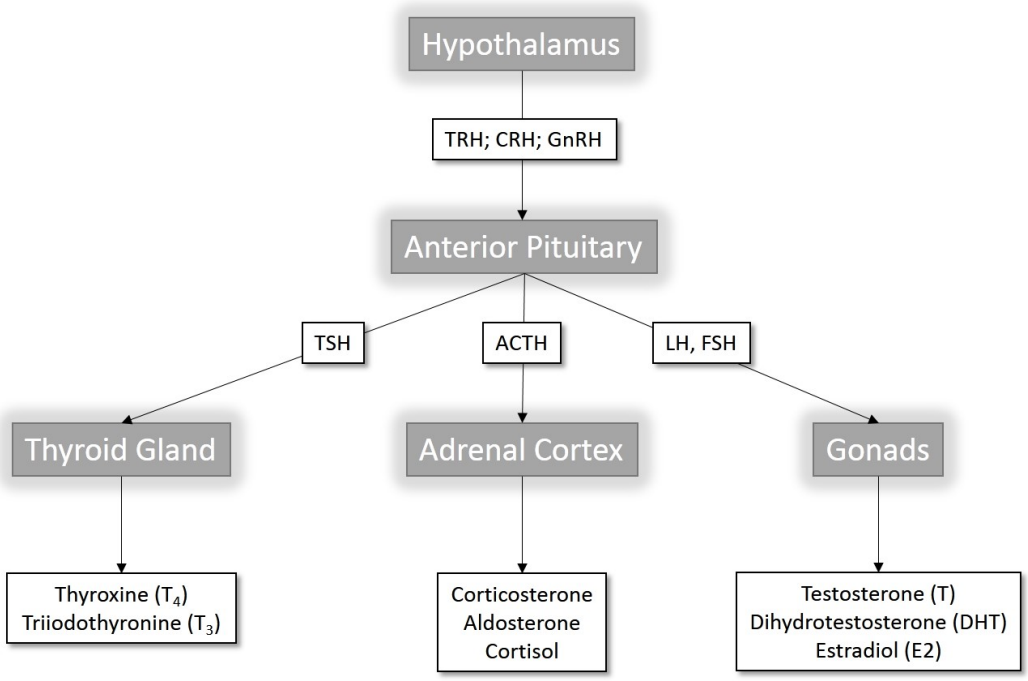


Figure 2-3: Basic representation of the three major endocrine system axes mediated by hormonal signalling from the hypothalamus and anterior pituitary gland in the brain

Due to the vast cross-talk between endocrine system axes, the disruption of a particular component within one endocrine axis may cause the modulation of other endocrine systems. It is therefore evident that a cocktail of EDCs present in the environment can have a range of negative effects on vertebrate health by modulating various endocrine system pathways. Environmental contaminants that cause disruption of the reproductive endocrine system have been the focus of many EDC studies around the world, including South Africa, where varying concentrations of contaminants with known estrogenic endocrine-disrupting effects have been found in surface waters (Aneck-Hahn et al., 2009; Bornman et al., 2007; Slabbert et al., 2007; Genthe et al., 2013). Such studies may only be the tip of the iceberg as increasing numbers of emerging contaminants are shown to have endocrine-disrupting effects. Although termed “emerging contaminants”, many of these contaminants have only recently been screened for their presence in the environment, despite being used for years. Therefore, the full extent of their presence and associated risk is not fully understood. Studies on gonadal abnormalities in wildlife living within polluted water systems have been done in South Africa, similar to those done at Lake Apopka in the USA.

The presence of intersexuality in the sharptooth catfish (*Clarias gariepinus*) has been observed at two impoundments at the Rietvlei Nature Reserve in Gauteng (Barnhoorn et al., 2004; Kruger et al., 2013). Among the intersexual fish, the presence of testicular oocytes was observed, in which the possible cause was linked to the presence of an industrial pollutant, p-nonylphenol, in the water. The endocrine-disrupting activity of this pollutant has been linked to its lipophilic properties and persistence in the environment (Lech et al., 1996; Folmar et al., 2002). Since the detection of endocrine disruption in freshwater fish, as well as the presence of POPs in the Rietvlei Nature Reserve, this area has been identified as a national priority area to monitor the presence of EDCs (Bornman and Bouwman, 2012). Further examples include populations of *C. gariepinus* in the Hartbeespoort Dam in Gauteng, where testicular abnormalities in male fish have been linked to the presence of POPs detected in the dam (Wagenaar et al., 2012).

Intersex fish were also found in Mozambican tilapia populations (*Oreochromis mossambicus*) at three impoundments in Limpopo, which are also situated within an area that is intensively sprayed with DDT to combat malaria transmission (Barnhoorn et al., 2010).

Sampling of *O. mossambicus* in the Loskop Dam (Mpumalanga), which receives water from the Olifants River (a highly polluted river system), showed elevated plasma thyroxine (T_4) hormone levels and enlarged thyroid gland follicles, indicating potential thyroid-modulating EDCs in the water. In African clawed frog populations (*Xenopus laevis*), the presence of testicular ovarian follicles was observed in male frogs caught in the north-eastern region of South Africa, which encompasses areas of high agricultural pesticide usage (Du Preez et al., 2009). Male *X. laevis* frogs collected within impoundments in the Western Cape, also situated near agricultural practices, also showed modulations of testicular spermatogenic development and altered plasma steroid and thyroid hormone levels (Van Wyk et al., 2014). As these studies only aimed to link the presence of endocrine disruption in wildlife to pesticide contamination in water systems, the presence of other contaminants, such as PPCPs or synthetic steroid hormones, was most probably overlooked. It is apparent that CECs can exert a range of physiological effects at both molecular and/or cellular level, according to the *in vitro* and *in vivo* studies shown in Table 2-3.

Table 2-3: Endocrine-disrupting effects of contaminants of emerging concern through molecular-initiating events and key events

Chemical	Molecular-initiating or key events	Assay/test species	LOEC (µg/ℓ)	Reference
Anti-epileptic				
Carbamazepine	• Reduced steroid hormone levels, elevated steroid hormone-binding globulin (SHBG) levels in male and female patients	Human patients	-	Rättyä et al., 2001; Herzog et al., 2005; Svalheim et al., 2009
	• Decrease 11-ketotestosterone levels in male fish	Zebrafish (*Danio rerio*)	0.5	Galus et al., 2013
Non-steroidal anti-inflammatories				
Diclofenac	• Elevated levels of the enzyme cytochrome P450 and vitellogenin (VTG) protein; estrogenic effects	Japanese medaka (*Oryzias latipes*)	1.0	Hong et al., 2007
	• Decreased thyroid hormone levels in male and female patients	Human patients	-	Bishnoi et al., 1994
	• Decreased thyroid hormone levels in fish	Indian major carp (*Cirrhinus mrigala)*	1.0	Saravanan et al., 2014
Ibuprofen	• Increased VTG production in male fish	Japanese medaka (*Oryzias latipes*)	1,000	Han et al., 2010
	• Reduced reproduction behaviour in fish	Japanese medaka (*Oryzias latipes*)	10	Han et al., 2010
	• Increased oestradiol hormone levels and aromatase enzyme activity; decreased testosterone hormone levels • Decreased egg fertilization in fish.	Human adenocarcinoma cell line (H295R) Florida flagfish (*Jordanella floridae*)	2,000 0.1	Han et al., 2010 Nesbitt, 2011
	• Disruption of thyroid hormone-mediated reprogramming in tadpoles	North American bullfrog (*Rana catesbeiana*)	1.5	Veldhoen et al., 2014
Naproxen	• Decreased thyroid hormone levels in male and female patients	Human patients	-	Bishnoi et al., 1994
	• Decreased egg fertilization in fish	Florida flagfish (*Jordanella floridae*)	0.1	Nesbitt, 2011
Antidepressants				
Fluoxetine	• Increased uterine weight in female rats	Wistar rats	-	Müller et al., 2012
	• Estrogenic response, proliferation of cells	MCF-7 breast cancer cells	-	Müller et al., 2012
	• Upregulation of CYP11β2 gene expression	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
	• Modulation of testicular structure, induced VTG production in male fish	Fathead minnows (*Pimephales promelas*)	0.03	Schultz et al., 2011

Chemical	Molecular-initiating or key events	Assay/test species	LOEC (µg/ℓ)	Reference
Biocides				
Triclosan	• Decreased T_4 hormone levels in female rats	Long-Evans rats		Crofton et al., 2007
	• Increased VTG gene expression, decreased sperm counts in male fish	Western Mosquitofish (*Gambusia affinis*)	101.3	Raut and Angus, 2010
	• Decreased T_3 hormone levels and other thyroid-related gene expressions in tadpoles	North American bullfrog (*Rana catesbeiana*)	0.03	Veldhoen et al., 2006
	• Increased hepatic VTG in male fish	Japanese medaka (*Oryzias latipes*)	20.0	Ishibashi et al., 2004
	• Decreased hatchability and time of hatching of fertilized eggs in fish	Japanese medaka (*Oryzias latipes*)	313.0	Ishibashi et al., 2004
	• Antagonistic activity for oestradiol/testosterone-dependent activation of estrogen/androgen-responsive gene expression	Recombinant human ovarian cancer cells (BG1Luc4E2, estrogen receptor (Erα)-positive), recombinant human cells ($T_4$7D-ARE)	-	Ahn et al., 2008
	• Enhanced testosterone-dependent activation of androgen-responsive gene expression	MDA-kb2 breast cancer cell line	-	Christen et al., 2010
Triclocarban	• Induction of CYP2B6 and CYP1B1 mRNA expression; activated estrogen receptor target genes in female ovaries	ERα-positive MCF7 breast cancer cells, humanised UGT1 mice	-	Yueh et al., 2012
	• Enhanced testosterone action through interaction with the androgen receptor	Castrated male rats, cell-based human androgen-responsive mediated bioassay	-	Chen et al., 2008
	• Enhanced oestradiol/testosterone-dependent activation of estrogen/androgen-responsive gene expression	Recombinant human ovarian cancer cells (BG1Luc4E2, ERα–positive), recombinant human cells ($T_4$7D-ARE)	-	Ahn et al., 2008
	• Enhanced testosterone-dependent activation of androgen-responsive gene expression	MDA-kb2 breast cancer cell line	-	Christen et al., 2010
Antibiotics				
Amoxicillin	• Upregulation of CYP19 and CYP17 gene expression and oestradiol hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007

Chemical	Molecular-initiating or key events	Assay/test species	LOEC (µg/ℓ)	Reference
Antibiotics				
Erythromycin	• Upregulation of CYP11β2 gene expression and progesterone/oestradiol hormone levels; downregulation of testosterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Cephalexin	• Upregulation of CYP19 gene expression; downregulation of testosterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Oxytetracycline	• Upregulation of CYP19 and 3βHSD2 gene expression; increased oestradiol hormone levels and aromatase enzyme activity	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007 Ji et al., 2010
Sulfathiazole	• Upregulation of CYP17 and CYP19 gene expression • Increased oestradiol hormone levels and aromatase enzyme activity; increased oestradiol hormone levels in male fish	Human adenocarcinoma cell line (H295R), Japanese medaka (*Oryzias latipes*)	-	Ji et al., 2010
Doxycycline	• Upregulation of CYP19 gene expression	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Tylosin (veterinary)	• Upregulation of CYP11β2 gene expression; downregulation of testosterone and oestradiol hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Steroidal anti-inflammatories				
Dexamethasone	• Upregulation of CYP11β2 gene expression; downregulation of testosterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Growth promoters				
Trenbolone	• Upregulation of CYP19 gene expression; downregulation of testosterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Painkillers				
Acetaminophen	• Upregulation of CYP11β2 gene expression and progesterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007

Chemical	Molecular-initiating or key events	Assay/test species	LOEC (µg/ℓ)	Reference
Bronchodilators				
Salbutamol	• Upregulation of CYP17 gene expression; downregulation of oestradiol hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Lipid regulators				
Bezafibrate	• Decrease in plasma 11-ketotestosterone levels in fish	Zebrafish (*Danio rerio*)	-	Velasco-Santamaría et al., 2011
Clofibrate	• Upregulation of CYP11β2 gene expression; downregulation of testosterone hormone levels	Human adenocarcinoma cell line (H295R)	-	Gracia et al., 2007
Preservatives				
Parabens	• Estrogenic and anti-androgenic effects *in vitro* and *in vivo*	Rat uterus receptor binding assay, MCF-7 breast cancer cells, transfected Chinese hamster ovary (CHO-K1) cells, recombinant yeast screens	-	Boberg et al., 2010
Parabens	• Hepatic necrosis, testicular fibrosis, induction of hepatic VTG in male fish	Common carp (*Cyprinus carpio*)	840	Barse et al., 2010
	• Increase in plasma VTG concentrations, and increase in mRNA expression of VTG subtypes and ERα in the liver of male fish	Japanese medaka (*Oryzias latipes*)	9,900	Inui et al., 2003
UV screens				
Benzophenones 4-MBC OMC	• Agonistic binding to the human estrogen receptor, induced breast cancer cell proliferation, increased uterine weight female rats	MCF-7 breast cancer cells and female Long-Evans rats	-	Schlumpf et al., 2001
	• Increase in plasma VTG concentrations, and increase in mRNA expression of VTG subtypes and ERα in the liver of male fish	Japanese medaka (*Oryzias latipes*)	9,900	Inui et al., 2003

LOEC: Lowest observed effect concentration for endocrine disruption in aquatic organisms; VTG: Yolk precursor protein vitellogenin; CYP: Cytochrome P450 enzyme; T_3: Triiodothyrosine; T_4: Thyroxine; mRNA: Messenger ribonucleic acid

The potential of these detected CECs in environmental waters to exert endocrine-disrupting effects raises concerns for their impact on environmental and human health. A further concern is that many CECs have been detected in broader environmental water systems, such as direct point sources of drinking water for human consumption. A study by Patterton (2013) detected pharmaceutical compounds in drinking water from taps in Johannesburg (Gauteng) and Bloemfontein (Free State), South Africa. In particular, the anticonvulsant drug carbamazepine was detected in 63% of tap water tested in these regions (Patterton, 2013). Anticonvulsant drugs such as carbamazepine, levetiracetam, lamotrigine and valproate have been shown to cause several reproductive endocrine system side-effects in men and women suffering from epilepsy (Table 2-3) (Rättyä et al., 2001; Svalheim et al., 2009; Harden et al., 2010), as well as in fish species exposed to carbamazepine (Galus et al., 2013).

In men using levetiracetam and valproate as treatment, it has been shown that these drugs can lead to increased testosterone and SHBG levels, which is responsible for the transport of steroid hormones in blood plasma (Rättyä et al., 2001; Harden et al., 2010). In the same studies, it was shown that men treated with carbamazepine also evidenced increased levels of SHBG, pituitary follicle-stimulating hormone and luteinising hormone (Herzog et al., 2005; Svalheim et al., 2009). Therefore, it might be possible that anticonvulsant compounds found in drinking water resources can lead to altered steroidogenesis in men. Alternatively, carbamazepine treatment in women has been shown to lead to higher sex hormone-binding protein (SHBP) levels and lower levels of progesterone and testosterone steroid hormones (Löfgren et al., 2006; Svalheim et al., 2009). These endocrine-disrupting effects of anticonvulsant drugs were shown in wildlife as well, including the modulation of steroidogenesis and ovarian malformations in ovarian follicular cells (Briggs and French, 2004; Taubøl et al., 2006).

Another group of pharmaceuticals that is frequently prescribed and detected in South African waters is the NSAIDs. A study by Amdany et al. (2014) detected varying levels of naproxen and ibuprofen in the influents and effluents of two WWTWs in Gauteng, South Africa. These compounds have been shown to alter endocrine systems in non-target vertebrate species. A full life-cycle study, exposing Japanese medaka fish (*Oryzias latipes*) to ibuprofen concentrations as low as 0.1 µg/ℓ resulted in delayed hatchling success, while a concentration of 1 mg/ℓ resulted in increased blood plasma levels of the glycoprotein vitellogenin (Table 2-3) (Han et al., 2010). This protein molecule is the precursor for egg yolk and has been validated as a biomarker to express estrogenic endocrine disruption in egg-laying vertebrate species. In the same study, the exposure of ibuprofen to a human adrenocortical carcinoma cell line (H295R) resulted in an increase in estradiol (E_2) hormone levels at concentrations of 2 and 20 mg/ℓ, and also increased aromatase enzyme activity at concentrations of 0.2 and 2 mg/ℓ (Table 2-3) (Han et al., 2010). Aromatase is the enzyme responsible for the metabolism of troponin to E_2 in steroidogenic pathways. Apart from the possible gonadal endocrine-disrupting activity of ibuprofen, exposure of *X. laevis* larvae to concentrations ranging between 30.7 and 39.9 mg/ℓ leads to malformations in the development of these larvae, indicating the teratogenic effects of ibuprofen as well (Richards and Cole, 2006).

Another NSAID that has been investigated for its endocrine-disrupting effect is diclofenac. In South Africa, diclofenac has been detected in a KwaZulu-Natal river system at concentrations varying between 1.1 and 15.6 µg/ℓ (Agunbiade and Moodley, 2014). The exposure of *X. laevis* embryos to diclofenac has been shown to cause teratogenicity at a concentration of 4 mg/ℓ (Chae et al., 2015). Furthermore, diclofenac exposure in male *O. latipes* fish showed that concentrations as low as 1 µg/ℓ can increase the gene expression for VTG in the liver, thereby showing estrogenic effects (Hong et al., 2007). Furthermore, assessment of patients using diclofenac as an NSAID has shown a reduction in serum T_3 levels (Bishnoi et al., 1994), which is the more active thyroid hormone responsible for growth, development and metabolism in the body. The other NSAID that has been found in South African waters, naproxen, has also been shown to cause a reduction in serum T_3 levels in patients taking this medication (Bishnoi et al., 1994). However, according to the authors' knowledge, little is known about the endocrine-disrupting activity of naproxen pollution into the environment and the effects of this compound on non-target organisms. The dose-dependent response of thyroid disruption by naproxen exposure still needs to be assessed in future studies.

Although some of the endocrine-disrupting effects shown above may only occur at high levels of exposure to these NSAIDs, it is important to note that a mixture of different pharmaceuticals and other contaminants might accumulate in the water system. The presence of NSAIDs such as ibuprofen, naproxen and diclofenac may contribute to endocrine disruption caused by other water pollutants as well. Furthermore, these compounds have been confirmed to be present in South African surface waters, showing that they are not completely removed from the water system after treatment. The above-mentioned studies imply that NSAIDs, such as ibuprofen, naproxen and diclofenac, are able to alter both gonadal and thyroid endocrine system pathways, and cause teratogenicity at environmentally relevant concentrations.

The PPCPs that are most frequently detected in surface waters worldwide are antibiotics and biocides. Regularly prescribed antibiotic pharmaceuticals, such as ampicillin, chloramphenicol, ciprofloxacin, erythromycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and tylosin, have all been detected in South African river systems (Agunbiade and Moodley, 2014). These compounds have all been shown to have endocrine-disrupting effects. The semi-synthetic macrolide antibiotic tylosin, which is used in veterinary medicine, has been shown to increase the expression of the aldosteronogenic gene CYP11β2, and decrease the production of testosterone and E_2 at a concentration of 3 mg/ℓ in an H295R steroidogenic assay, showing that this chemical can serve as both an anti-estrogenic and an anti-androgenic EDC (Gracia et al., 2007). In the same study, another macrolide antibiotic, erythromycin, showed an increase in the expression of CYP11β2 and a reduction in testosterone production at a concentration of 3 mg/ℓ, but caused increased production of E_2 and progesterone in the assay (Table 2-3) (Gracia et al., 2007).

Exposure of erythromycin in a recombinant YES showed that this compound may be a minor mimic of E_2 in binding to the estrogen receptor (ER) in a dose-dependent manner, therefore having estrogenic effects (Archer et al., unpublished). This shows that, although tylosin and erythromycin share the same macrolide ring in their chemical composition, the endocrine-disrupting effect differs between these two compounds, and therefore complicates environmental endocrine disruption studies if, for example, both these types of chemicals are present in environmental samples. A study by Garcia et al. (2007) also showed that tetracyclines, exposed at a concentration of 81 µg/ℓ to H295R cells, can increase the expression of CYP19 enzymes and 3βHSD2 genes (Table 2-3), which are responsible for testosterone-E_2 metabolism and the production of progesterone, respectively.

Although these antibiotics were not detected in the range that showed endocrine system modulation in an *in-vitro* assay, their effect on wildlife through long-term exposure within environmental waters is currently unknown. Furthermore, due to the extensive use of antibiotics in both humans and livestock, the expected concentrations of these chemicals in the environment may be under-estimated and may also have a cumulative endocrine-disrupting effect in the water if they accumulate in mixtures with other pollutants. It is therefore evident that antibiotic chemical pollutants should receive high priority in environmental screening in water systems and water treatment facilities in South Africa. Apart from the regularly prescribed antibiotic pharmaceuticals detected in environmental waters, it is shown that compounds in personal care products can also have endocrine-disrupting properties.

One of the most well documented compounds is the biocide triclosan, which is used as a disinfectant in soap, detergent, toothpaste, mouthwash and other products (Raut and Angus, 2010). This compound also shows a high partition coefficient (K_{ow}) value (Log K_{ow} 4.66; KOWWIN Version. 1.67, EPI Suite), which indicates that triclosan is highly lipid-soluble and does not readily dissolve in water. For this reason, it can be regarded as a POP, which can accumulate in the fat tissue of exposed organisms and can be transported in water bodies over great distances. This has been shown in a study demonstrating high levels of triclosan in the breast milk of pregnant Swedish women (Allmyr et al., 2006). The pollution of triclosan in the environment can therefore be assessed in a similar way as the exposure of organochloride insecticides in environmental waters, such as DDT and endosulfan, which are also shown to accumulate in the fat tissue of both wildlife and humans.

Although the use of triclosan has been phased out in several personal care products in developed countries, it is still found in South African consumer products, and therefore detected in surface waters (Amdany et al., 2014; Madikizela et al., 2014). Amdany et al. (2014) showed varying levels of triclosan in influents and effluents from two WWTWs in Gauteng, South Africa. These levels ranged from 78.4 to 127.7 µg/ℓ in influent samples, and 10.7 to 22.9 µg/ℓ in effluent samples (Amdany et al., 2014). Although the concentrations of triclosan are significantly reduced after water treatment, these levels are still high if sub-lethal effects are considered. Exposure of North American bullfrog tadpoles (*Rana catesbeiana*) to triclosan showed that concentrations as low as 0.3 µg/ℓ can significantly lower tadpole body mass and decrease thyroid hormone receptor gene expression (Veldhoen et al., 2006). Exposure of triclosan at 20 µg/ℓ has also been shown to induce hepatic VTG levels in male Japanese medaka (*Oryzias latipes*) (Table 2-3) (Ishibashi et al., 2004). Exposure of mature male western mosquitofish (*Gambusia affinis*) to triclosan at 101.3 µg/ℓ can cause decreased sperm counts and elevate VTG gene expression (Table 2-3) (Raut and Angus, 2010).

Triclosan exposure in MDA-kb2 breast cancer cells showed that a concentration of 289 µg/ℓ significantly induces cell proliferation, and a concentration as low as 290 ng/ℓ causes an elevated androgenic response when treated along with dihydrotestosterone (DHT), which is a more metabolically active androgen than testosterone (Table 3.2) (Christin et al., 2012). These results show that triclosan serves as an androgen agonist by binding to the androgen receptor in a human cell-based bioassay (Christin et al., 2012). These concentrations of endocrine disruption are either equivalent to or lower than levels observed in South African waters. Therefore, wildlife species living either upstream or downstream of WWTWs may be affected by levels of triclosan in the environment. Bearing these studies in mind, it is possible that exposure to low concentrations of triclosan over a long period of time (chronic exposure) may modulate both gonadal and thyroid endocrine systems in humans and other wildlife species at concentrations currently detected in environmental waters.

Due to the regular detection and known endocrine-disrupting effect of triclosan, it is important to investigate other compounds found in personal care products and detected in South African waters. Based on chemical analyses and endocrine disruption studies done elsewhere in the world, it is evident that compounds used as preservatives, disinfectants and ultraviolet (UV) filters have not received much attention as priority environmental pollutants and EDCs in South Africa. Preservatives such as parabens (methylparaben, propylparaben and octylparaben), other biocides such as triclocarban, and UV filters in sunscreens, such as benzedrone (4-MBC) and octyl methoxycinnamate (OMC), have all been shown to accumulate in wastewater systems and cause potential endocrine disruption. Several paraben compounds, as well as their metabolites, have been shown to have both estrogenic and anti-androgenic effects *in vitro* and *in vivo* (Table 3.2). These studies imply that contaminants such as parabens can affect multiple endocrine pathways and are therefore of environmental concern.

Biocides such as triclocarban are regularly included in several cosmetic and personal care products to deter microbial organisms. Although the endocrine-disrupting effect of the biocide triclosan has been well documented and found at high concentrations in the environment (Halden and Paull, 2005), limited data is available on the endocrine-disrupting effect of triclocarban. Exposure to triclocarban in human cell-based bioassays, and exposure of rodents to triclocarban, indicated that the biocide does not have endocrine-disrupting activity on its own, but rather enhances the action and binding affinity of steroid hormones (Table 3.2) (Ahn et al., 2008; Chen et al., 2008; Christen et al., 2010; Yueh et al., 2012). This shows a potentiating mechanism of endocrine disruption, as well as an alternative mode of endocrine disruption other than the direct modulation of endocrine pathways.

Several compounds used as UV filters in sunscreens, such as 4-MBC and OMC, have been shown to agonistically bind to the hER in human cell-based bioassays, and to increase VTG production in female rats and male fish species (Table 2-3).

These compounds are therefore regarded as estrogenic contaminants, which might persist in the environment for long periods of time due to their low water solubility. The abovementioned compounds are all used as either “wash-off” or “application” personal care products. Therefore, it can be assumed that products containing biocides, UV screens and preservatives will either be washed down in drain water or will be absorbed through the skin after application.

These compounds thus have multiple routes of exposure to either humans or other non-target organisms in water. Since these chemicals are also regularly used in personal care products, and their compounds have low solubility in water, their presence and persistence in the environment can be high. Paraben concentrations as high as 11 mg/ℓ have been detected in a UK river system, with concentrations as high as 30 mg/ℓ in wastewater influents (Kasprzyk-Hordern et al., 2009). Triclocarban concentrations of 6 µg/ℓ have been documented in a US river system (Halden and Paull, 2005). Environmental concentrations of UV filters as high as 13 mg/ℓ have been reported in wastewater influents (Kasprzyk-Hordern et al., 2009), with 6 mg/ℓ in wastewater effluents (Kasprzyk-Hordern et al., 2009), 266 ng/ℓ in swimming pools (Cuderman and Heath, 2007), and 3.3 µg/ℓ in seawater (Sánchez-Rodríguez et al., 2015). These compounds have not been screened for their presence in South African water systems, which highlights the importance of screening for these chemicals to evaluate their fate within water treatment facilities.

Although the harmful effects of man-made pollutants on wildlife species are well documented (Heath and Claassen, 1999; Barnhoorn et al., 2004; Bornman et al., 2009; Wagenaar et al., 2012; Kruger et al., 2013; Van Wyk et al., 2014), no national monitoring programmes or water quality guidelines have been implemented in South Africa to assess and monitor the occurrence and frequency of pollutants affecting the endocrine pathways of non-target organisms (Jooste et al., 2008). The clinical implications of EDC contamination in surface waters have also received little attention in South Africa, and the importance of using sentinel species as bio-indicators of water pollution is regularly overlooked, especially assessing the health of these organisms upstream and downstream of water treatment processes.

It is evident that most studies link the endocrine-disrupting effect observed in wildlife and water sources to the use of agricultural pesticides. This assumption is supported by the fact that agriculture comprises a large percentage of a country’s gross produce, and therefore also utilises large quantities of available surface water. Because of the notable dependence of food production on pesticides, various point (identifiable) or non-point (diffuse) pollution sources for surface water and groundwater are anticipated. However, the presence of PPCPs is regularly overlooked. These chemicals are used daily for improved healthcare, personal hygiene and/or as daily supplements. It can therefore only be assumed that the presence of PPCPs in environmental waters may contribute even more to EDC pollution in freshwater resources than pesticides used in households or agriculture. Although it is globally recognised and recorded that several classes of PPCPs are present in environmental waters (Kasprzyk-Hordern et al., 2009; Petrie et al., 2015; Blair et al., 2015), not many studies have been done on PPCP pollutants present in South African waters (especially PPCPs acting as EDCs), and this needs to be addressed in future studies.

2.2.4 The adverse outcome pathway network

To add to the understanding of evolving 21st-century toxicity testing and risk assessment, an AOP framework has been proposed (Ankley et al., 2010; Villeneuve et al., 2014; Villeneuve et al., 2019; Garcia-Reyero, 2015). This framework is structured upon existing knowledge based on the relationships between physiological pathways, spanning from molecular initiating events, which, in turn, cause a perturbation in normal biological functioning, therefore impairing a sequence of measurable key events, ranging from the cellular to the organism level (Edwards et al., 2016). Each key event is further linked to key event relationships (KERs), based on a weight-of-evidence approach.

This downstream series of key events is then coupled with an adverse outcome (AO) on a population level, which can be used for regulatory decision-making (Figure 2-4). The AOP framework has been well described by several authors (Edwards et al., 2016; Villeneuve et al., 2014; Garcia-Reyero, 2015), highlighting the advances made since its establishment by Ankley et al. (2010). Advancements of the AOP framework are still ongoing, which includes the broader AOP Knowledge Base (AOP-KB)[1], which contains the AOP Wiki[2]. This initiative is led by several global regulating bodies, namely the USEPA, the OECD, the EU's Joint Research Centre, and the US Army Engineer Research and Development Centre.

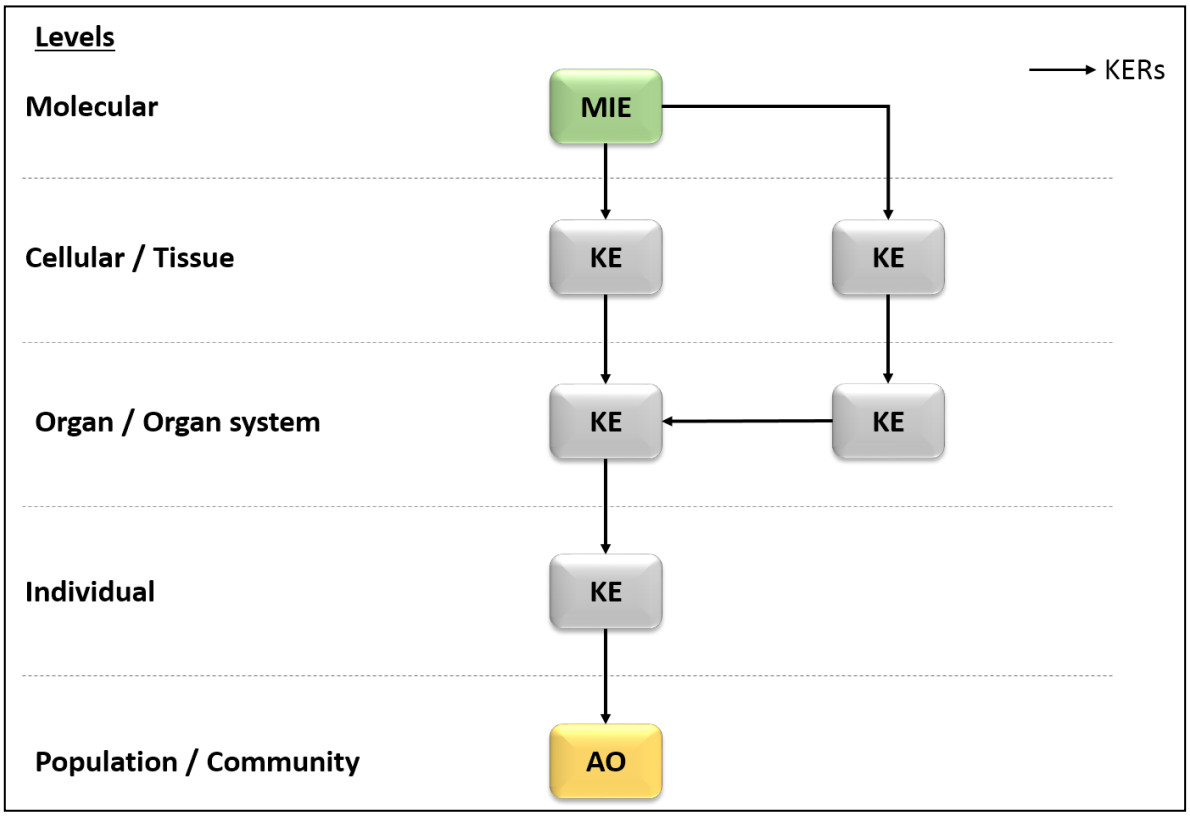


Figure 2-4: Basic representation of an AOP. A specific molecular initiating event is linked to several key events by means of key event relationships from a cellular to an organism level, leading to an adverse outcome observed within a population or community

Several AOPs can be connected into an AOP network through several MIEs and KEs leading to the same AO. Figure 2-4 is based on the template from the AOP Wiki. Although the AOP framework is not considered to be part of risk assessment, nor is it constructed to show chemical-specific outcomes, it helps with visualising downstream events that may be triggered by MIEs or KEs, which are shown to be modulated by various stressors (Coady et al., 2019). Moreover, this initiative can do the following:

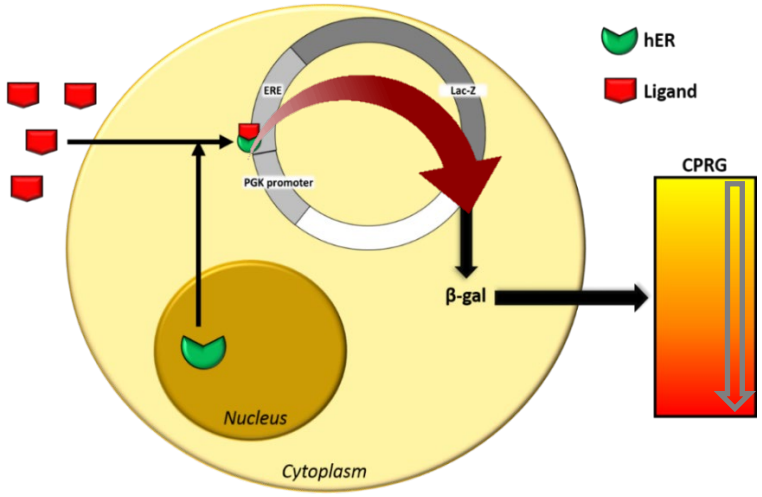
- Inform the decision making of EBM assays that are needed to provide clear relationships with a specific AOP network
- Create defined approaches to meet the specific regulatory needs of decision makers
- Provide clear endpoints that can be met for ecological risk assessment approaches
- Support decision making during emergency spills through HTS methodologies

[1] https://aopkb.org/
[2] https://aopwiki.org/wiki/index.php/Main_Page

Much progress has been made with the AOP approach since the construction of the AOP knowledge base in 2012, with various American and European stakeholders and monitoring agencies driving this approach for 21st-century toxicity assessment (Villeneuve et al., 2019). However, input from African countries is still limited. Incorporating ERAs and the AOP framework in a local context could aid in the re-establishment of the National Toxicity Monitoring Programme, which only concentrates on a few pollutants and does not address more recent "emerging contaminants" such as PPCPs (Jooste, 2008). To contribute to a more thorough national programme, more information is needed on the chronic, sub-lethal level of toxicity using wildlife species and several other biomarkers for more accurate ERA analysis. This can be achieved by adopting a tiered screening approach to quantify and organise or categorise both lethal and sub-lethal toxicity data in both *in-vitro* and *in-vivo* screening approaches.

2.2.5 An effect-based monitoring assay to assess endocrine disruption in wastewater

The YES has been widely adopted as a Category 1 bioassay that targets the affinity of an analyte to bind to the hER. Therefore, the assay is used to estimate whether organic micropollutants may mimic the activities of the natural estrogen 17-beta estradiol. This assay uses a recombinant *Saccharomyces cerevisiae* strain, which contains an integrated gene within its genome that codes for the human estrogen receptor. The binding between an exogenous estrogenic ligand (E_2 or E_2-mimicing) and the hER is carried to an expression plasmid carrying a phosphoglycerate kinase (PGK) promoter gene, an estrogen-responsive element (ERE), and a *Lac-Z* reporter gene (Figure 2-5). The active ligand-hormone complex binds to the ERE, which further initiates the expression of the *Lac-Z* gene, leading to the production of the enzyme beta galactosidase (β-gal). The production of β-gal is therefore directly dependent on the number of estrogenic ligands in the sample. The secreted β-gal from the yeast cell then metabolises chlorophenol red-b-D-galactopyranoside (CPRG) in the assay medium (yellow) into galactose and chlorophenol red (red). This colour change can be read by a spectrophotometer, which is then used to show the affinity of the ligand under investigation to bind to the hER in a dose-dependent manner.



hER: Human estrogen receptor; ERE: Estrogen-responsive element; β-gal: Beta galactosidase; CPRG: Chlorophenol red-b-D-galactopyranoside

Figure 2-5: Representation of the recombinant Yeast Estrogen Screen. Figure adopted from Roultledge and Sumpter, 1996.

Several organic micropollutants have been determined to have an affinity to bind to the hER in a dose-dependent manner (Routledge and Sumpter, 1996; Sohoni and Sumpter, 1998; Fent et al., 2006; Isidori et al., 2009). Since the development of the YES, several advancements came about to assess the binding of ligands to the hAR as well (Sohoni and Sumpter, 1998).

Wastewater from paper and pulp mill effluents have shown androgenicity in the Yeast Androgen Screen (YAS) (Svenson and Allard, 2004), as well as the glucocorticoid receptor agonist dexamethasone, which is used for various allergic and inflammatory disorders, as well as skin conditions (Bovee et al., 2007). Moreover, such assays are not only used to determine agonistic binding to the respective hormone receptors, but also to assess whether ligands may antagonise an active hormone-receptor complex (i.e. anti-estrogens and anti-androgens).

For example, the anti-epileptic drugs valproate and carbamazepine have been shown to inhibit testosterone-mediated androgen receptor activity in a dose-dependent manner (Death et al., 2005; Archer et al., unpublished), as have various antifungal pesticides and insecticides (Chatterjee et al., 2007; Archer and Van Wyk, 2015). This has led to the use of such yeast-based steroid hormone receptor binding assays to investigate the presence of (anti)-estrogenic and (anti)-androgenic ligands in environmental waters. For example, a monitoring study in the upper Olifants River catchment of South Africa showed high levels of anti-androgenicity in river water samples compared to observed estrogenicity and androgenicity in a YES and YAS, respectively, which may have originated from various anthropogenic sources (Truter et al., 2016). This bioassay therefore shows promise as a first-tier approach to investigate the total load of analytes in surface waters, which may modulate a specific endocrine system molecular-initiating event, irrespective of its chemical composition.

It has been highlighted that the advancement in sensitive analytical techniques, as well as high throughput extraction techniques (such as solid-phase extraction), has allowed for extremely sensitive outputs to assess the levels of analytes in water. This has led to detectable levels of endocrine-disrupting analytes in water samples even at low nanograms per litre (parts per trillion) levels. However, these detections do not necessarily mean that the samples are, in fact, of a high adverse health risk (Escher et al., 2018).

2.3 SUMMARY

Although there has been limited information linking these contaminants to wildlife and human health disorders locally, the mechanism of physiological action is well established for all these compounds. A worrying factor from these studies is that these contaminants are still being detected after wastewater treatment, as well as in environmental waters. Such trends of persistence of priority emerging contaminants after wastewater treatment are also recorded globally for PPCPs (Kasprzyk-Hordern et al., 2009; Petrie et al., 2015; Blair et al., 2015). Contaminants that are not removed from water treatment processes are destined to end up back in the environment (therefore affecting wildlife), or might end up in drinking water sources, as shown in reports by De Jager et al. (2013) and Patterton (2013). This emphasises the need to conduct comprehensive monitoring studies in South African surface water systems to report on the fate of priority emerging contaminants to assist with both environmental and human health risk assessment.

Various approaches to environmental risk assessment have been implemented over the years, based on acute and/or chronic toxicity data of the analytes under investigation. However, when dealing with an environmental sample, the total adverse outcome (e.g. toxicity, endocrine disruption) of all the compounds in the water sample is compared with a bioanalytical equivalent concentration (BEQ) of a known reference chemical. For example, *in vitro* assays showing estrogenicity as an adverse outcome usually compare the total estrogenicity of a water sample with a BEQ of E_2, termed an EEQ concentration.

Various *in vivo* studies have aimed to express the relevance of EEQ concentrations in environmental samples to known toxicological endpoints to elucidate the potential endocrine-disrupting activities observed in analytical techniques. For example, Caldwell et al. (2012) established a PNEC for E_2 of 2 ng/ℓ, which may modulate fish reproduction. Similarly, Brion et al. (2004) showed that a concentration of 5 ng/ℓ of E_2 may increase the production of VTG in adult male zebrafish. However, such adverse outcomes are still only representative of acute and/or sub-chronic exposure periods and, as such, do not necessarily reflect adverse outcomes over prolonged exposure periods (continued exposure throughout the organism's lifetime and future generations). For this reason, EBTVs have also been proposed (Escher et al., 2018).

Such trigger values are calculated based on various factors derived for known estrogenic compounds, including their potencies on endocrine system pathways, their pharmacokinetics for bioavailability, their acceptable or tolerable daily intake, as well as a default daily drinking water consumption and allocation value per human adult. This has led to estimated upper and lower EBTVs of 3.8 ng/ℓ (Brand et al., 2014) and 0.7 ng/ℓ EEQ concentrations (Genthe et al., 2009) for estrogenicty in drinking water sources. Although environmental surface water and treated wastewater are not necessarily allocated for potable reuse, South Africa is currently experiencing severe pressure on freshwater security, which is driven by climatic factors and an exponentially growing population. For this reason, new avenues are being considered to directly or indirectly assign reclaimed wastewater for potable reuse. It is thus becoming necessary to assess the quality of environmental surface water and reclaimed wastewater according to drinking water quality guidelines for such endocrine-disrupting endpoints to not only assess their possible commercial reuse, but also to safeguard aquatic ecosystems.

CHAPTER 3: FATE AND RISK ASSESSMENT OF SELECTED CHEMICALS OF EMERGING CONCERN IN WASTEWATER TREATMENT WORKS[3]

3.1 INTRODUCTION

The aim of this study was to undertake a CEC profiling study at two sampling locations (WWTW and in environmental waters) in Gauteng. This allowed for the investigation of temporal changes in CEC loads, as well as investigating temporal removal efficiencies using internationally recognised sampling and sample processing methodologies for CEC quantification during wastewater treatment. Moreover, the WBE approach was adopted, allowing for the estimation of per capita usage rates of illicit drugs and other pharmaceuticals at the two study locations. The ERA estimations and the alignment of the CEC concentration results with the AOP network will be discussed.

3.2 MATERIALS AND METHODS

3.2.1 Chemicals and consumables

The study included the multi-residue quantification for a selected list of DoA using analytical methods described elsewhere (Castrignanò et al., 2016). The following internal standards were included in the water samples to enable quantification: cocaine-d3, benzoylecgonine-d8, amphetamine-d5, methamphetamine-d5, mephedrone-d3, MDA-d5, MDMA-d5, cotinine-d3, EDDP-d3, heroin-d9, codeine-d6, oxycodone-d6, hydrocodone-d6, methadone-d9, ketamine-d4, norketamine-d4 and 1S,2R-(+)-ephedrine-d3. Hyper-grade methanol (MeOH, 98%) and ultra-pure water (Millipore) were used for cleaning glassware and for solid-phase extraction (SPE). All glassware was deactivated using 5% dimethyldichlorosilane (DMDCS) in toluene, followed by two wash steps in toluene and three wash steps in MeOH.

3.2.2 Study site and sample collection sites

The study site was at a WWTW situated in Gauteng (Figure 3-1). Treated wastewater effluent is discharged into a nearby river, which joins other streams that eventually feed into a major dam that supplies around 6% of the total municipal drinking water to the surrounding communities. Sampling was done over five consecutive days during July 2015 (Monday to Friday). Influent and effluent samples were taken each day at 60-minute intervals (100 mℓ per hour) from the WWTW influent (after grit screens) and effluent (after chlorination), upon which a final composite sample (100 mℓ) was obtained for each sampling location per day. The influent and effluent samples were taken concurrently and not matched to the hydraulic retention time (HRT) of the WWTW. Grab samples of river surface water (100 mℓ) were taken at a location upstream (100 m) and downstream (3.5 km from the point of discharge) of the WWTW (Figure 3-1). All samples were transported on ice from the sampling sites to the laboratory and were kept cold (± 4 ºC) and in the dark until analyte extraction, which was done within a maximum period of 10 hours.

[3] The full report of this study has been published in *Chemosphere*: Archer, E., Petrie, B., Kasprzyk-Hordern, B. and Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine-disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere* 174, 437-446. The study was co-funded by the Engineering and Physical Sciences Research Council (EPSRC) in collaboration with researchers from the Department of Chemistry, University of Bath (UK), and the East Rand Water Care Company (ERWAT), Kempton Park, Gauteng (South Africa).

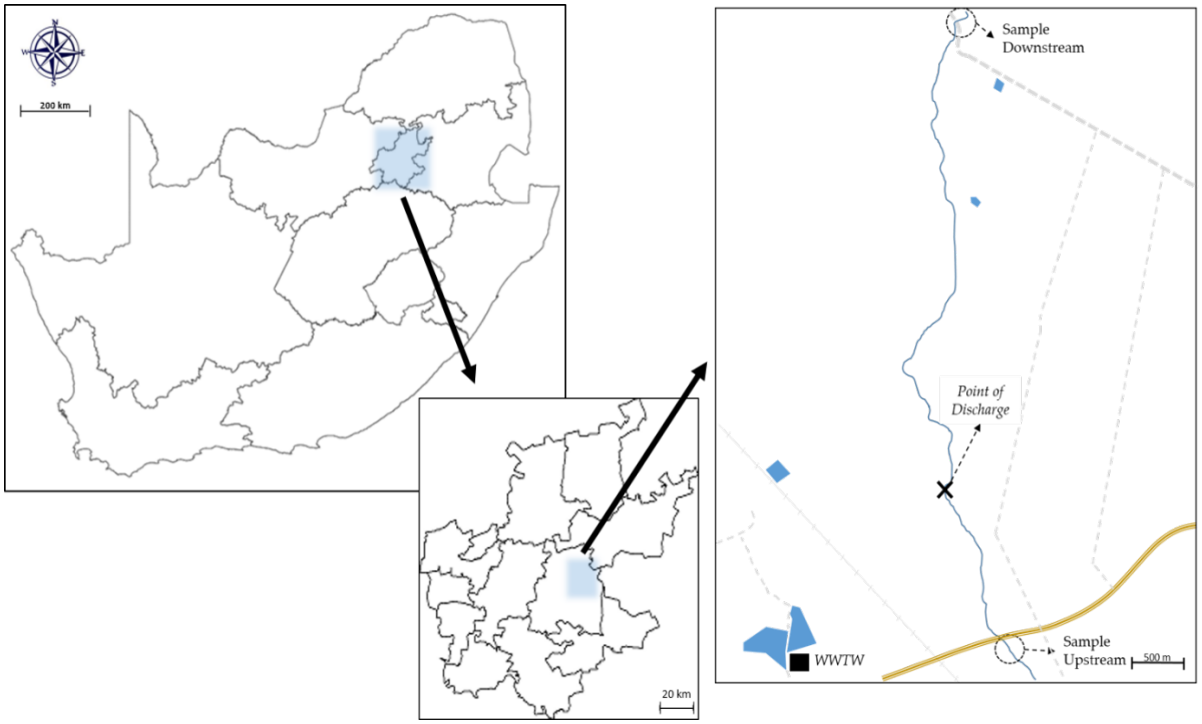


Figure 3-1: **Location map of the WWTW, point of discharge into the receiving waters, and sampling locations upstream and downstream from the plant**

3.2.3 Sample extraction procedures

The collected water samples were filtered using 0.45 µm pore size polytetrafluoroethylene (PTFE) filters prior to SPE. A 50 mℓ water sample from the various locations was extracted in duplicate. Each sample included 50 ng of each of the deuterated PPCP internal standards and was mixed well before extraction. The water samples were extracted using Oasis® hydrophilic-lipophilic balanced (HLB) (3 cc, 60 mg) SPE cartridges (Waters; Microsep). The cartridges were conditioned with 2 mℓ MeOH, followed by 2 mℓ ultrapure water (Millipore) at a flow rate of 1 mℓ per minute. After conditioning, the water samples (50 mℓ) were passed through the SPE cartridges at a flowrate of 5 mℓ per minute and allowed to run dry for a minimum period of 15 minutes. The dried cartridges were kept frozen (-20 ^{0}C) until all sampling had been completed. The cartridges were then sent to the University of Bath (UK) for chemical analysis within 10 days of extraction. Upon arrival at the University of Bath, the cartridges were dried and eluted with 4 mℓ MeOH using a manifold at a flow rate of 1 mℓ per minute. The extracts were dried under a gentle stream of nitrogen using a TurboVap evaporator (Caliper, UK, 40 ^{0}C, N2, < 5 psi) and the evaporated samples were re-suspended in 500 µℓ of a H_2O:MeOH (80:20) solvent, giving a 100x concentrated sample, and transferred to polypropylene mass spectrometry (MS) vials (Waters, Manchester, UK). Quantification of the target analytes was achieved using a method developed for ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS-MS) (Petrie et al., 2016; Archer et al., 2017a).

3.2.4 Sample analysis by chiral liquid chromatography coupled with tandem mass spectrometry

The method parameters and conditions used are described elsewhere (Castrignanò et al., 2016). Briefly, the analytes in the processed samples were separated using a Waters ACQUITY UPLC® system (Waters, Manchester, UK) equipped with a CHIRALPAK® CBH HPLC column, 5 µm particle size, L × I.D. 10 cm × 2.0 mm (Chiral Technologies, France) and a Chiral-CBH guard column 10 × 2.0 mm, 5 µm particle size (Chiral Technologies, France). The ACQUITY UPLCTM autosampler was kept at 4 ºC, and the column temperature was set at 25 ºC. All samples were injected at 20 µℓ. The mobile phase (1 mM ammonium acetate/methanol, 85:15, v/v) was injected at 0.1 mℓ.min^{-1} under isocratic conditions.

The separated analytes were quantified using a triple quadrupole mass spectrometer (Xevo TQD, Waters, Manchester, UK) with an electrospray ionisation (ESI) source, which was managed in the multiple reaction monitoring (MRM) mode. Two to three MRM transitions were created for each compound, which assisted with confirmation and quantification. Spiked quality control standards containing the deuterated and non-deuterated analytes were incorporated throughout the analytical procedure. The quality and quantification of the analytes in the samples were determined using the method detection limits (MDL) and method quantification limits (MQL) as set out in Castrignanó et al. (2016), as well as quality control criteria according to the EC's Council Directive 2002/657/EC (EC, 2002).

3.2.5 Data analysis

For each target analyte within raw wastewater and final effluent, a mass load (gram per day) was calculated to compensate for the variation in daily WWTW flow rates at the sewage inlet (influent) and outlet (effluent) using the following equation:

$$\text{Mass loads (g/day)} = \text{concentration (ng/ℓ)} * \text{WWTW}_{inf/eff} \text{ flow rate (Mℓ/day)} / 1{,}000$$

The calculated mass load estimate was then used to calculate the removal of the analytes during wastewater treatment. For removal estimates, the following equation was used:

$$\text{Removal (\%)} = [(M\ell_{inf} - M\ell_{eff}) / M\ell_{inf}] * 100$$

where $M\ell_{inf}$ and $M\ell_{ef}$ refer to the daily mass load (in gram per day) measured for each analyte within raw wastewater and treated effluent.

For each removal estimation, the HRT was considered. Both WWTWs are recorded to have an HRT of ± 24 hours, which led to removal estimates using the Mℓ of influent wastewater for the one day (for example, Monday) and the Mℓ of the effluent wastewater recorded for the next day (for example, Tuesday).

For the WBE approach to estimate drug use patterns in the different communities that are served by the WWTW, population-normalised drug loads (mg per day per 1,000 inhabitants) of the selected target analytes were calculated using Equation 3-1:

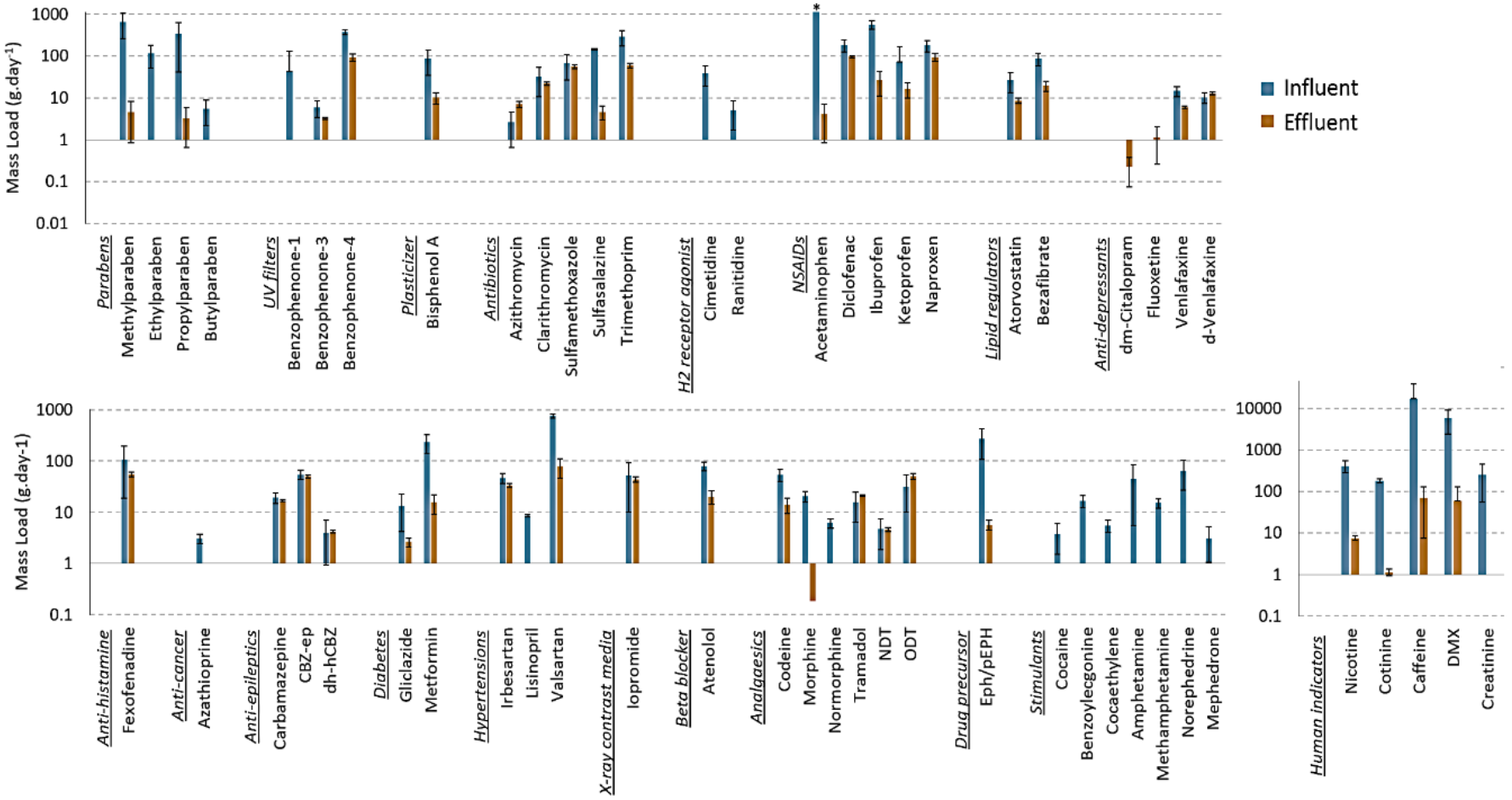
$$\text{Population-normalised drug loads (mg/day/1,000 inhabitants)} = \frac{M\ell_{inf}\,(\text{g/day}) * CF * 1 \times 10^6}{Pop.\ Est.} \qquad \text{Eq. 3-1}$$

where $M\ell_{inf}$ refers to the calculated mass loads (grams per day) of the target drug analytes, *CF* refers to the correction factor calculated by dividing the most recent excretion rates of each compound by the molar mass ratios between the parent drug and the target illicit drug, and *Pop. Est.* refers to the population estimate (*de facto* number of individuals) for each WWTW as calculated by chemical oxygen demand (COD) measurements that were taken during the same time as the sampling campaigns (1 person = 128 mg COD per day).

3.3 RESULTS AND DISCUSSION

3.3.1 Indicative removal of CECs during wastewater treatment

A total of 55 CECs was detected in wastewater influent, and 41 CECs in effluent samples, which represented 19 classes of PPCPs, human indicators, illicit drugs and metabolites (Figure 3-2) (Archer et al., 2017a). The human indicators contained chemicals that are associated with endogenous products of human metabolism or that are used for population equivalent estimates.



NSAIDs: Non-steroidal anti-inflammatory drugs; EPH/pEPH: Ephedrine/pseudoephedrine; dh-hCBZ: 10,11-dihydro-10-hydroxycarbamazepine; CBZ-ep: Carbamazepine-10,11-epoxide; DMX: 1,7-dimethylxantine; *I*: N-desmethyltramadol; ODT: *O*-desmethyltramadol

Figure 3-2: Removal efficiencies (%) of the detected compounds at the WWTW during the sampling period. Standard deviations indicate variation in between sampling days. Figure extracted from Archer et al. (2017a).

Although flow data (Mℓ/day) from each day was incorporated to calculate the mass loads of the CECs within the WWTW during the sampling period, the variation in the mass loads of the CECs between sampling days may be attributed to several factors, such as the variation in the daily human usage of these compounds, pollution events from surface water sources leading to the plant, the retention time of wastewater within the plant, or the overall performance of the treatment processes within the sampling period.

The plant showed varying removal efficiencies of the detected CECs (Figure 3-2) (Archer et al., 2017a) and conforms to similar removal data of PPCPs reported in other studies (Verlicchi et al., 2012; Bahlmann et al., 2014; Petrie et al., 2014; Petrie et al., 2016). By comparing the average mass loads of the CECs at the influent and effluent, it was calculated that 28% of all detected CECs was removed by less than 50%, and 18% of all CECs was removed by less than 25%. A significant increase in final effluent concentration of the pharmaceutical metabolite desmethylvenlafaxine (DMV) (from venlafaxine; $p = 0.039$) was measured compared to influent wastewater (negative mass balance). Negative mass balances were also calculated based on the average mass load concentrations for the pharmaceutical metabolites 10,11-dihydro-10-hydroxycarbamazepine (dh-hCBZ) and *O*-DMT, as well as the parent compound tramadol (Figure 3-2). However, statistical analysis deemed these negative mass balances non-significant due to the variation between sampling days ($p > 0.05$).

3.3.2 Presence of CECs in environmental surface water samples

A total of 40 CECs was detected in surface waters located upstream and downstream of the plant during the sampling period (Table 3-1) (Archer et al., 2017a). The levels of diclofenac, ibubrofen, ketoprofen, sulfamethoxazole and bezafibrate were also analysed in other South African surface waters, and were detected at higher concentrations than in the current case study (Agunbiade and Moodley, 2016). It should be noted that the PPCP levels in the downstream location may not be solely from WWTW discharge. For example, methylparaben, bisphenol-A, nicotine, cotinine, caffeine, and 1,7-dimethylxantine were removed with moderate to high efficiency by the WWTW (Figure 3-2), with the average concentrations of these compounds being higher in downstream samples compared to the WWTW effluent. However, it should also be mentioned that the mode of sampling was different between WWTW sample collection (24-hour composites) and river water collection (grab).

Although the loads of the above-mentioned chemicals were significantly reduced during wastewater treatment, these compounds can still be regarded as being pseudo-persistent (low concentrations being continuously discharged) and may accumulate downstream. Conversely, various anthropogenic activities (agriculture, domestic housing and industries) are found along the river leading towards the downstream sampling site, which may also suggest that there may be other direct discharge points between the WWTW effluent and the river sampling point. Regardless of the source of these CECs at the downstream site, it was shown that 26 out of the 40 detected emerging contaminants in surface waters (65%) were found to be twofold or higher in downstream samples, with codeine even detected higher than 10-fold (Table 3-1). More evaluation of the potential adverse health risks associated with the environmental samples is discussed in the next section.

Table 3-1: Mean concentrations (ng/ℓ) of detected PPCPs, metabolites, illicit drugs and human indicator compounds at sampling localities located upstream and downstream of WWTW-1. Standard deviation indicates variation between sampling days for the compounds. Table extracted from Archer et al., 2017a.

	Upstream		Downstream				Upstream		Downstream		
	Average	Standard deviation	Average	Standard deviation	Fold change		Average	Standard deviation	Average	Standard deviation	Fold change
Parabens						***Anti-epileptic***					
Methylparaben	58.68	29.21	146.06	107.28	2.49	Carbamazepine	157.06	11.47	279.47	24.12	1.78
Propylparaben	31.76	17.4	136.7	76.81	4.3	CBZ-ep	398.77	27.89	752.2	69.35	1.89
						dh-hCBZ	22.69	1.58	56.88	8.94	2.51
UV filters						***Diabetes***					
Benzophenone-3	56.24	1.72	64.32	5.95	1.14	Gliclazide	43.2	2.36	53.88	22.25	1.25
Benzophenone-4	441.07	23.76	1076.51	389.58	2.44	Metformin	73.33	7.18	174.59	81.69	2.38
Plasticiser						***Hypertensions***					
Bisphenol A	239.01	72.07	396.42	208.14	1.66	Irbesartan	311.11	28.73	554.37	120	1.78
						Valsartan	263.73	24.6	924.7	50.16	3.51
Antibiotics						***Anti-depressants***					
Azithromycin	24.55	0	6.36	3.37	0.26	Fluoxetine	34.39	22.06	109.24	125.6	3.18
Clarithromycin	76.16	13.4	235.5	66.12	3.09	Venlafaxine	35.36	3.71	94.61	19.62	2.68
Sulfamethoxazole	757.42	83.22	1013.15	294.17	1.34	Desvenlafaxine	49.99	7.51	174.88	53.8	3.5
Sulfasalazine	37.6	3.35	52.99	13.02	1.41						
Trimethoprim	382.96	42.15	898.74	303.02	2.35						
Non-steroidal anti-inflammatories						***Analgaesics***					
Acetaminophen	20.84	4.54	63.74	76.08	3.06	Codeine	11.25	6.71	128.89	65.43	11.46
Diclofenac	467.43	176.17	1461.45	508.73	3.13	EDDP	14.54	0.09	14.64	0.1	1.01
Ibuprofen	153.32	39.48	312.13	204.6	2.04	Tramadol	97.74	11.2	299.92	73.16	3.07
Ketoprofen	642.15	0	330.34	318.97	0.51	*N*-DMT	15.95	9.85	74.01	8.13	4.64
Naproxen	224.3	31.14	1112.83	518.3	4.96	*O*-DMT	207.61	32.17	577.34	149.75	2.78

	Upstream		Downstream				Upstream		Downstream		
	Average	**Standard deviation**	**Average**	**Standard deviation**	**Fold change**		**Average**	**Standard deviation**	**Average**	**Standard deviation**	**Fold change**
Lipid regulators						***Drug precursors***					
Atorvostatin	74.02	5.16	150.63	55.73	2.03	Eph/pEPH	38.79	8.01	80.36	28.37	2.07
Bezafibrate	54.93	8.29	234.37	116.75	4.27						
Antihistamines						***Stimulants***					
Fexofenadine	368.38	36.72	887.03	172.02	2.41	Cocaine	8.55	0.17	8.73	0.19	1.02
						Amphetamine	27.14	22.56	37.04	22.63	1.36
						Methamphetamine	-	-	24.9	-	-
X-ray contrast media						***Human indicators***					
Iopromide	265.81	11	598.26	235.37	2.25	Nicotine	154.32	78.7	245.52	67.57	1.59
						Cotinine	25.45	3.3	31.65	11.67	1.24
						Caffeine	812.18	146.3	2077.49	259.71	2.56
						DMX	479.36	357.58	957.62	728.56	2
Beta-blockers											
Atenolol	156.18	34.43	271.98	154.57	1.74						

EPH/pEPH: Ephedrine/pseudoephedrine; dh-10-hCBZ: 10,11-dihydro-10-hydroxycarbamazepine: CBZ-ep: Carbamazepine-10,11-epoxide;
DMX: 1,7-dimethylxantine: *N*-DMT: *N*-desmethyltramadol; *O*-DMT: *O*-desmethyltramadol

3.3.3 Environmental risk assessment

Conventional risk assessment approaches were done based on calculated PNEC values from literature for the analytes of interest, from which RQ estimations were calculated. In a similar manner to the conventional risk assessment, the risk of the detected micropollutants was also estimated considering the knowledge of their potential to cause sub-lethal toxicological endpoints. Such an approach further establishes a micropollutant as a priority substance based on its potential to cause adverse health effects at environmentally relevant concentrations. Table 3-2 lists some RQ estimations based on the detections of the PPCPs during the scoping monitoring study for both lethal and sub-lethal toxicological endpoints.

Collectively, the loads of the antibiotic sulfamethoxazole and clarithromycin, the NSAIDs diclofenac and naproxen, as well as the opioids codeine and tramadol all showed a high toxicological risk based on PNEC values (Table 3-2). Furthermore, the NSAIDs diclofenac, naproxen and ibuprofen all showed a high risk when sub-lethal endpoints were also considered. For the recalcitrant anti-epileptic drug carbamazepine, a moderate risk for both lethal and sub-lethal toxicity endpoints was estimated (Table 3-2). These estimations therefore further established these compounds as priority micropollutants of concern.

Table 3-2: Environmental risk estimates from emerging contaminants detected in South African WWTWs Risk quotients are based on conventional ERA and modulation of molecular-initiating events and key events. Table extracted from Archer et al. (2017a).

	MEC (ug/ℓ)		Environmental risk assessment					Molecular-initiating events and key events				
Compound	**Effluent**	**River water[#]**	**PNEC (µg/ℓ)**	**Reference**	**RQ_{eff}**	**RQ_{rw}**		**Event**	**Concentration (µg/ℓ)**	**Reference**	**RQ_{eff}**	**RQ_{rw}**
Diclofenac	2.31	0.96	0.10	Bergmann et al., 2011	23.1***	10.0***		Increased VTG gene expression in fish (MIE)	1.0	Hong et al., 2007; Gröner et al., 2017	2.31***	0.96**
								Decreased thyroid hormone levels in fish (KE)	1.0	Saravanan et al., 2014	2.31***	0.96**
Ibuprofen	0.66	0.23	7.10	Carlsson et al., 2006	0.13*/**	< 0.1*		Lower thyroid-mediated mRNA transcripts in tadpoles (MIE)	1.5	Veldhoen et al., 2014	0.44**	0.15**
								Decreased egg fertilization in fish (KE)	0.1	Nesbitt, 2011	6.60***	2.30***
Naproxen	2.30	0.67	3.30	Bergmann et al., 2011	0.7**	0.2*/**		Decreased egg fertilization in fish (KE)	0.1	Nesbitt, 2011	23.0***	6.7***
Carbamazepine	0.41	0.22	2.50	Ferrari et al., 2003	0.16*/**	< 0.1*		Lower keto-testosterone hormone levels in fish (KE)	0.5	Galus et al., 2013	0.82**/***	0.40**
Azithromycin	0.17	0.015	4.80	Bergmann et al., 2011	< 0.1*	< 0.1*						
Iopromide	1.06	0.43	6800.0	Bergmann et al., 2011	< 0.1*	< 0.1*						
Sulfamethoxazole	1.34	0.89	0.59	Bergmann et al., 2011	2.27***	1.51***		Induction of VTG in fish	1,000.0	Kang et al., 2006	< 0.1*	< 0.1*
Acetaminophen	0.10	0.04	0.24	Bergmann et al., 2011	0.42**	0.17*/**						
Clarithromycin	0.54	0.16	0.2	Bergmann et al., 2011	2.7***	0.8**/***						
Irbesartan	0.82	0.43	100.0	Minguez et al., 2016	< 0.1*	< 0.1*						
Valsartan	1.93	0.59	100.0	Minguez et al., 2016	< 0.1*	< 0.1*						
Ketoprofen	0.41	0.39	3.10	Bergmann et al., 2011	0.13*/**	0.13*/**						
Bezafibrate	0.48	0.14	1.20	Bergmann et al., 2011	0.4**	0.12*/**						
Tramadol	1.3	1.6	0.32	Bergmann et al., 2011	4.1***	5.0***						
Venlafaxine	0.14	0.09	0.32	Minguez et al., 2016	0.44**	0.28**						
Methamphetamine	0.69	0.48	2.30	ECOSAR	0.3**	0.21**						
Morphine	0.0001	0.05	0.09	Mendoza et al., 2014	< 0.1*	0.56**						

	MEC (ug/ℓ)		Environmental risk assessment					Molecular-initiating events and key events				
Compound	**Effluent**	**River water[#]**	**PNEC (µg/ℓ)**	**Reference**	**RQ_{eff}**	**RQ_{rw}**		**Event**	**Concentration (µg/ℓ)**	**Reference**	**RQ_{eff}**	**RQ_{rw}**
MDMA	0.006	0.002	0.22	Mendoza et al., 2014	< 0.1*	< 0.1*						
Cocaine	0.001	0.0009	2.28	Mendoza et al., 2014	< 0.1*	< 0.1*						
Benzoylecgonine	0.04	0.02	4.90	ECOSAR, n.d.	< 0.1*	< 0.1*						
Codeine	0.35	0.08	0.06	ECOSAR, n.d.	5.83***	1.33***						
Benzophenone*	1.16	0.41	6.00	ECOSAR, n.d.	0.19*/**	< 0.1*						
Methylparaben	0.11	0.11	11.2	Carlsson et al., 2006	< 0.1*	< 0.1*		Increase VTG and lower GSI in fish	8,400.0	Barse et al., 2010	< 0.1*	< 0.1*
Propylparaben	0.08	0.08						Increased VTG in fish	9.0	Scott, 2014	< 0.1*	< 0.1*
Chloramphenicol			0.019	Bergmann et al., 2011								
Amphetamine	0.09	0.03	0.98	Bergmann et al., 2011	< 0.1*	< 0.1*						
Bisphenol A	0.25	0.32	1.0	ECOSAR, n.d.	0.25**	0.32**		Induction of VTG in fish	10.0	Villeneuve et al., 2011	< 0.1*	< 0.1*
Atenolol	0.49	0.21	100.0	Minguez et al., 2016	< 0.1*	< 0.1*						
Trimethoprim	1.45	0.64	20.0	Bergmann et al., 2011	< 0.1*	< 0.1*						
Nicotine	1.8	0.90	0.014	Bergmann et al., 2011	128.6***	64.29***						
Ketamine	0.02	0.008	0.90	ECOSAR, n.d.	< 0.1*	< 0.1*						

* Low risk (RQ < 0.1), ** Median risk (0.1 < RQ < 1.0), *** High risk (RQ > 0.1) as indicated by Hernando et al., 2006.

[#] Average measured concentration of the compounds in river water samples (upstream and downstream from the WWTW)

MEC: Measured environmental concentration (based on concentrations in the current study);
PNEC: Predicted no-effect concentration (acute or chronic lethal toxicity outcomes)

3.3.4 Application of the AOP framework in risk assessment

Most of the sub-lethal toxicity endpoints presented in Table 3-2 were determined by the modulation of the production and/or molecular expression of the protein vitellogenin in exposed organisms. Currently, eight AOPs are associated with the key event of reduction in plasma VTG concentrations (KE 221) for both male and female sentinel organisms, where this key event is associated with other upstream key events and key event relationships, along with cascading downstream events on the cell, organ, individual and population level (see example in Figure 3-3). The increase in VTG production in male sentinel organisms may also serve as a standalone biomarker of effect, as this indicates some form of increased aromatase activity, leading to increased conversion of testosterone to estradiol, hence an estrogenic endpoint.

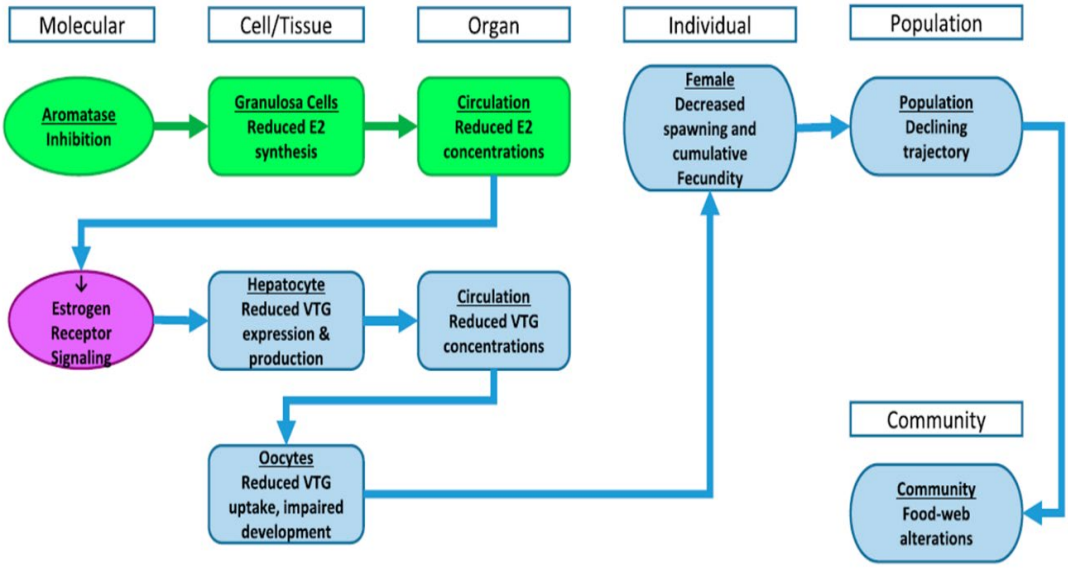


Figure 3-3: Example of an adverse outcome pathway network that is associated with a molecular-initiating event and resulting key event that leads to impaired female reproduction outcomes

Other AOPs that received less attention in the past were the modulation of thyroid-associated endocrine disruption. Decreased thyroid hormone levels, as shown for the NSAID diclofenac in a sentinel organism (KE 771), is associated with various cascading events towards adverse outcomes such as altered iodine pump activity that leads to follicular cell adenomas and carcinomas in rodents (AOP 110). The most likely sourced route of decreased thyroid hormone levels in serum may be derived from an MIE of altered thyroperoxidase activity (the enzyme responsible for thyroid hormone synthesis), leading to further downstream adverse outcomes such as subsequent neurodevelopmental disorders in mammals (AOP 42), impaired fertility in fish (AOP 271) and altered amphibian metamorphosis (AOP 175), all of which are adverse health effects that may be triggered during the early onset of foetal/embryo development. Although such AOP-cascading events cannot be solely ascribed to the exposure of diclofenac alone, such information regarding chemical-specific toxicological relationships with complex biochemical pathways provides added priority for the selection of this compound as both a CEC and an EDC. Although the authors note that this approach of risk prediction is not without its limitations, the inclusion of risk prediction of sub-lethal toxicological endpoints is vital when the potential health risk for future generations is taken into consideration. For example, it has been shown that the anti-epileptic drug carbamazepine can affect the placental barrier in mice through the administration of pregnant females in water spiked with environmentally relevant concentrations of the drug, which are proposed to cause neurological disorders and teratogenesis in the offspring (Kaushik et al., 2016).

Apart from such potentially adverse health effects, the primary mode of action of carbamazepine is blockage of the sodium channels to prevent prolonged firing of an action potential. Considering the inhibition of sodium channels from an AOP perspective, such an MIE may lead to subsequent adverse outcomes, such as reduced predator avoidance and feeding behaviour in fish (Event 588 and Event 587), all of which may be linked to neurological modulation.

3.4 SUMMARY

In this case study, 55 PPCPs were detected in WWTW influent surface water, 41 emerging contaminants were detected in effluent, and 40 CECs were detected in environmental waters located upstream and downstream of the plant. From this list of detected emerging contaminants, it could be observed that several classes of PPCPs are shown to persist throughout wastewater treatment processes, even at the current WWTW, which utilise a large set of biological nutrient removal treatment processes. Antibiotic compounds, NSAIDs, antidepressants, anti-epileptics and analgesics were selected to be of particular risk due to their overall persistence, as well as these compounds being consumed in communities. A list of CECs persisted through the WWTW process, with 28% of all detected emerging contaminants removed by less than 50%, and 18% of all CECs removed by less than 25%. Negative mass balances of some pharmaceuticals and metabolites were observed within the WWTW, suggesting back-transformation of CECs during wastewater treatment.

Some hypotheses for the occurrence of such events have been considered: Persistent CECs may accumulate in aggregates within the WWTW, leading to subsequent dissolution through biotic or abiotic processes. More likely, metabolites and/or conjugate forms of CECs that enter WWTW influents unnoticed are subsequently back-transformed or deconjugated into parental compounds through either biotic or abiotic processes (Blair et al., 2015; Verlicchi et al., 2012). For example, conjugate forms of ethynyl-estradiol, carbamazepine and diclofenac have been shown to be deconjugated by bacterial cultures (Aris et al., 2014; Lee et al., 2012; Vieno et al., 2007), while metabolites of the antibiotic sulfamethoxazole were shown to be transformed to the parent compound by photolytic processes (Bonvin et al., 2013).The same trend has been shown in several global WWTW monitoring studies, in which negative removal of the antidepressant venlafaxine and its primary metabolite were also estimated. The prevalence of antibiotic substances within surface waters also raises concern over their contribution to antimicrobial resistance (AMR) development, as compounds such as sulfamethoxazole and trimethoprim persisted somewhat through wastewater treatment and were subsequently detected in the high ng/ℓ to low µg/ℓ level in the recipient river water. It is suggested that urban wastewater may provide a hotspot for the proliferation of AMR bacteria and resistance genes at WWTWs (Rizzo et al., 2013; Guo et al., 2017). However, the ability of low-level antibiotics to drive AMR development is less known, whereby such a drive in AMR development may well also derive from alternative human activities.

CHAPTER 4: ENANTIOMERIC PROFILING OF CHIRAL PHARMACEUTICALS AND ILLICIT DRUGS OF ABUSE IN SOUTH AFRICAN WATER SYSTEMS[4]

4.1 INTRODUCTION

The aim of this study was to undertake a CEC profiling study at two sampling locations (WWTW and in environmental waters) in Gauteng. This allowed for the investigation of temporal changes in CEC loads, as well as investigating temporal removal efficiencies using internationally recognised sampling and sample processing methodologies for CEC quantification during wastewater treatment. Moreover, the WBE approach was adopted, allowing for the estimation of per capita usage rates of illicit drugs and other pharmaceuticals at the two study locations.

4.2 MATERIALS AND METHODS

4.2.1 Study sites and sampling locations

The study comprised two WWTWs that were sampled over a consecutive seven-day campaign: WWTW-1 is in the northern interior (Gauteng), and WWTW-2 is located near the coastline of the Western Cape (Figure 4-1). WWTW-1 is situated 3 km from a lifestyle estate and 4 km from the nearest populated area, with a treatment capacity of 63 Mℓ per day. It receives a proportion of 60:40 domestic:industrial wastewater. The treatment consists of biological nutrient removal (BNR) modules, whereby the total treated effluent is directly discharged into the receiving river system after disinfection. WWTW-2 is situated within proximity of a populated area with a treatment capacity of 105 Mℓ per day. It receives a proportion of 80:20 domestic:industrial wastewater. The treatment consists of conventional activated sludge treatment modules, followed by clarification and chlorination. The water is then discharged into either maturation ponds or the receiving river system.

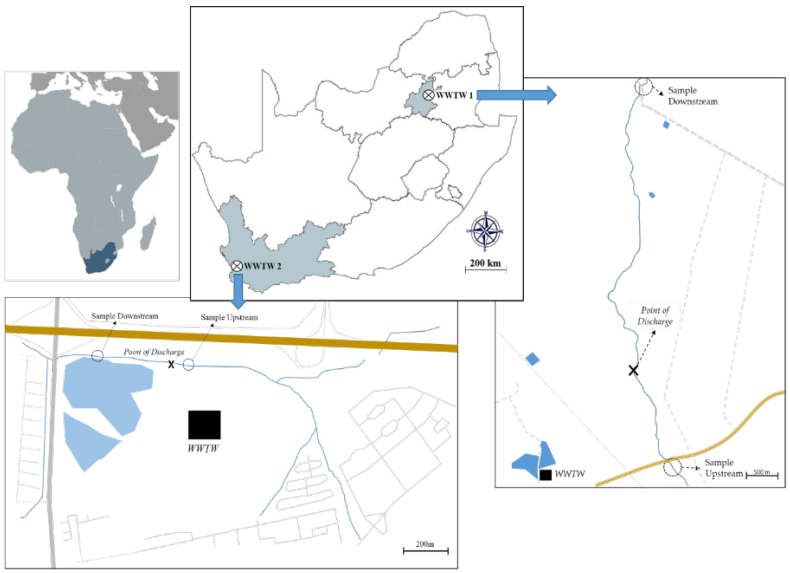


Figure 4-1: Map showing the sampling locations of WWTW-1 (Gauteng) and WWTW-2 (Western Cape)

[4] The full report for this study has been published in *Science of the Total Environment*: Archer, E., Castrignanò, E., Kasprzyk-Hordern, B. and Wolfaardt, G.M., 2018. Wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters. *Science of the Total Environment* 625, 792-800. The study was co-funded by the EPSRC in collaboration with researchers from the Department of Chemistry, University of Bath (UK). Assistance for the acquisition of wastewater and surface water sampling was provided by scientific staff and wastewater controllers at ERWAT, Kempton Park, Gauteng, and the City of Cape Town Municipality, Scientific Services, Athlone, Western Cape.

4.2.2 Sample collection and preparation

Sampling was done over seven consecutive days at the WWTW, which included samples from the raw sewage (after grit screens), final effluent (after chlorination), return activated sludge (RAS) and river water collected 50 m upstream and 50 m downstream of the plants. For WWTW-1, the downstream sampling point was located 3.5 km from the point of discharge due to inaccessibility closer to the point of discharge. Time-proportional composite samples (100 mℓ) were taken every 10 minutes from raw sewage and final effluent, and then combined to obtain a 24-hour sample of the treatment steps. Grab samples (250 mℓ) were taken for the RAS and river water. The samples were kept cold during sampling and transportation to the laboratory, from which further sample filtration and extraction were immediately completed.

The RAS samples (100 mℓ) were centrifuged (5,000 rpm, 4 °C, 15 minutes), from which the supernatant was collected to separate the liquid phase (RAS_{liquid}) from the solid phase (RAS_{solid}, sludge pellet). The freeze-dried RAS_{solid} pellet was subjected to a standard protocol of microwave-assisted extraction (MAE) (Evans et al., 2015), with slight modifications. Briefly, a 0.25 g dried sludge sample was spiked with the internal standard mixture (5 µl of a 1 $\mu g.m\ell^{-1}$ concentration) and left for 30 minutes. The samples were then suspended in 30 mℓ of a 50:50 mixture of MeOH and ultrapure water. The suspension was transferred to the MAE tubes and extracted using a Mars5 MAE machine (CEM Corporation). The temperature was increased over nine minutes and held at 121 ºC for 30 minutes. The samples were then allowed to cool down to room temperature and then filtered through 0.7 µm glass fibre filters (GF/F) (Whatman, GF/F). The filtered samples were diluted in 270 mℓ ultrapure water to obtain a concentration of MeOH less than 5%. All sample types (influent, effluent, river, RAS_{solid}, RAS_{liquid}) were then filtered using 0.7 µm glass fibre filters and a vacuum manifold, and continued to standard SPE procedures.

Samples were extracted using Oasis® HLB cartridges (Waters; 3 cc, 60 mg), and Oasis® MAX cartridges (Waters; 3 cc, 60 mg) for the RAS_{solid} samples using the following protocol: The Oasis® HLB cartridges were conditioned with 2 mℓ MeOH, followed by 2 mℓ of ultrapure water, and the Oasis® MAX cartridges with 4 mℓ MeOH and 4 mℓ ultrapure water, where the solvents were allowed to pass by gravity. Each sample was then divided into duplicates, which included a 50 mℓ sample for raw sewage, final effluent and RAS_{liquid}, 100 mℓ for river water upstream and downstream, and 300 mℓ for the RAS_{solid}. The extraction was carried out using a vacuum manifold (Supelco Visiprep). The samples were passed through the cartridges at a rate of 6 $m\ell.min^{-1}$ and allowed to run dry for at least 30 minutes. The dried cartridges were then transported refrigerated for elution and analysis. Upon arrival, the cartridges were eluted with 4 mℓ MeOH into 5 mℓ salinised glass vials and dried under a gentle stream of nitrogen (5-10 psi, 40 ºC) using a TurboVap evaporator (Caliper, UK). The dried samples were then reconstituted in 0.5 mℓ of the mobile phase used during liquid chromatography mass spectrometry (LC/MS) (1 mM ammonium acetate:methanol, 85:15, v/v), after which the suspended samples were vortexed and filtered through 0.2 µm PTFE filters (Whatman, Puradisc, 13 mm) using 3 mℓ syringes. The filtered samples were then placed in polypropylene plastic vials fitted with pre-slit PTFE/silicone septa (Waters, UK) for chemical analysis.

4.2.3 Sample analysis by chiral liquid chromatography coupled with tandem mass spectrometry

The method parameters and conditions used are described elsewhere (Castrignanò et al., 2016). Briefly, the analytes in the processed samples were separated using a Waters ACQUITY UPLC® system (Waters, Manchester, UK) equipped with a CHIRALPAK® CBH HPLC column, 5 µm particle size, L × I.D. 10 cm × 2.0 mm (Chiral Technologies, France) and a Chiral-CBH guard column 10 × 2.0 mm, 5 µm particle size (Chiral Technologies, France). The ACQUITY UPLCTM autosampler was kept at 4 ºC, and the column temperature was set at 25 ºC. All samples were injected at 20 µℓ. The mobile phase (1 mM ammonium acetate/methanol, 85:15, v/v) was injected at 0.1 $m\ell.min^{-1}$ under isocratic conditions. The separated analytes were quantified using a triple quadrupole mass spectrometer (Xevo TQD, Waters, Manchester, UK) with an ESI source, which was managed in the MRM mode.

Two to three MRM transitions were created for each compound, which assisted with confirmation and quantification. Spiked quality control standards containing the deuterated and non-deuterated analytes were incorporated throughout the analytical procedure. The quality and quantification of the analytes in the samples were determined using the MDL and MQL as set out in Castrignanó et al. (2016), as well as quality control criteria according to the EC's Council Directive 2002/657/EC (EC, 2002).

4.2.4 Data analysis

For each target analyte within raw wastewater and final effluent, a mass load (grams per day) was calculated to compensate for the variation in daily WWTW flow rates at the sewage inlet (influent) and outlet (effluent) using the following equation:

$$\text{Mass loads (g/day)} = \text{Concentration (ng/ℓ)} * \text{WWTW}_{inf/eff} \text{ flow rate (Mℓ/day)} / 1{,}000$$

The calculated mass load estimate was then used to calculate the removal of the analytes during wastewater treatment. For removal estimates, the following equation was used:

$$\text{Removal (\%)} = [(M\ell_{inf} - M\ell_{eff}) / M\ell_{inf}] * 100$$

where $M\ell_{inf}$ and $M\ell_{ef}$ refer to the daily mass load (in gram per day) measured for each analyte within raw wastewater and treated effluent.

For each removal estimation, the HRT was considered. Both WWTWs are recorded to have an HRT of ± 24 hours, which led to removal estimates using the Mℓ of influent wastewater for the one day (for example, Monday) and the Mℓ of the effluent wastewater recorded for the next day (for example, Tuesday).

For the WBE approach to estimate drug use patterns in the different communities that are served by the WWTW, population-normalised drug loads (mg per day per 1,000 inhabitants) of the selected target analytes were calculated using Equation 4-1:

$$\text{Population-normalised drug loads (mg/day/1,000 inhabitants)} = \frac{M\ell_{inf}\,(\text{g/day}) * CF * 1 \times 10^{6}}{Pop.\ Est.} \qquad \text{Eq. 4-1}$$

where $M\ell_{inf}$ refers to the calculated mass loads (grams per day) of the target drug analytes, *CF* refers to the correction factor calculated by dividing the most recent excretion rates of each compound by the molar mass ratios between the parent drug and the target illicit drug, and *Pop. Est.* refers to the population estimate (*de facto* number of individuals) for each WWTW as calculated by COD measurements that were taken during the same time of the sampling campaigns (1 person = 128 mg COD per day).

To distinguish between the illicit or pharmaceutical origin of the chiral drugs, or to assist with distinction between direct disposal and consumption of the DTR within the study areas, the enantiomeric fraction (EF) of the selected chiral compounds in wastewater was calculated using Equation 4-2:

$$\text{Enantiomeric fraction (EF)} = \frac{(+)}{[(+) + (-)]} \quad or \quad \frac{E1}{[E1 + E2]} \qquad \text{Eq. 4-2}$$

where (+) and (-) refer to the concentrations (ng/ℓ) of the enantiomers in wastewater influent or E1 and E2 as the mass load concentrations (grams per day) of the first and second eluted enantiomer, respectively.

An EF equal to 1 or 0 represents an enantiomerically pure substance, whereas an EF equal to 0.5 represents a racemic mixture of the drug.

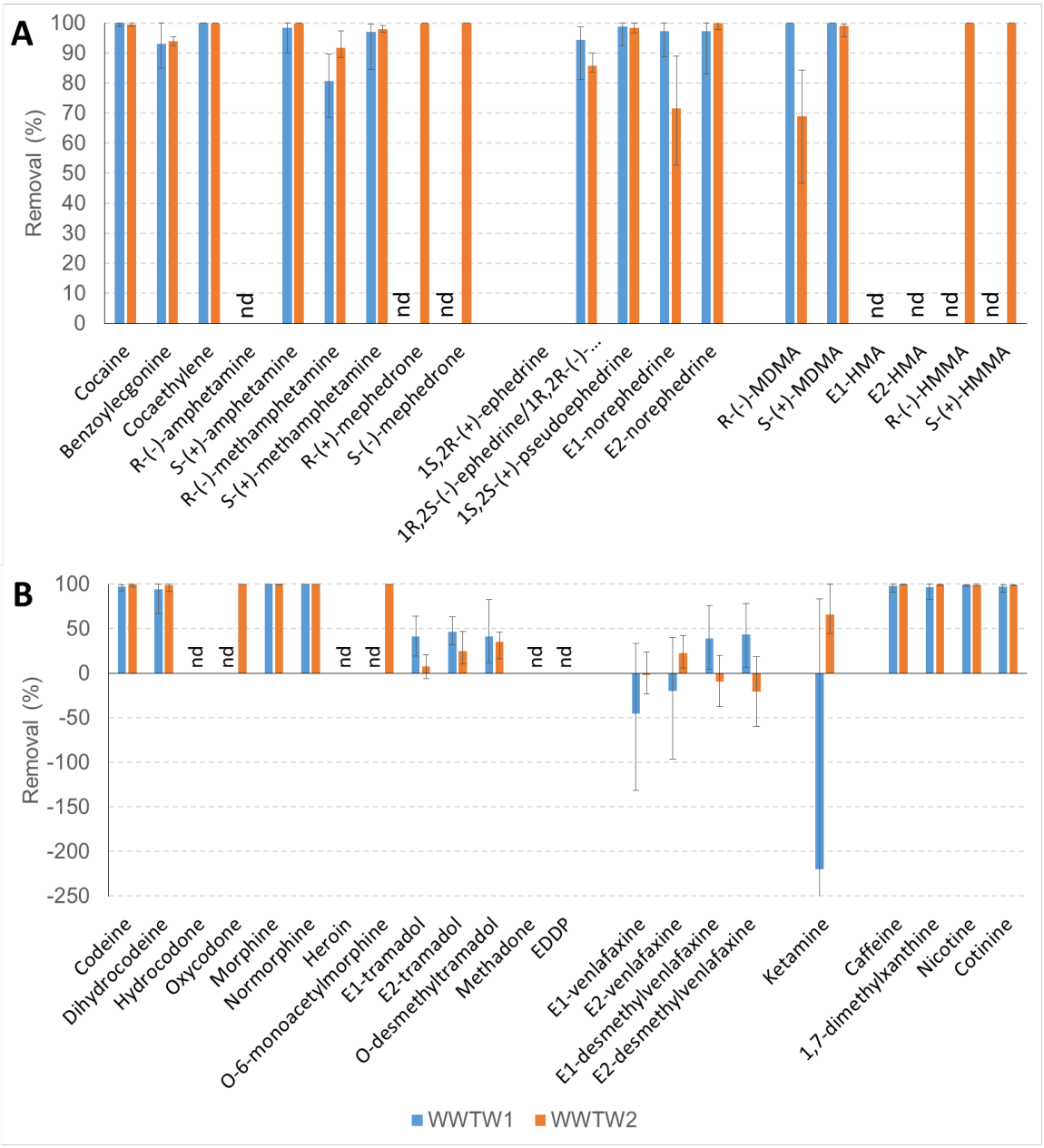
4.3 RESULTS AND DISCUSSION

4.3.1 Indicative removal of CECs during wastewater treatment

A total of 38 CECs, including illicit stimulants and hallucinogens, precursor drugs, opioids, antidepressants, anaesthetics and four human chemical markers, were screened in the aqueous phase of wastewater. All the illicit compounds and their associated metabolites were removed with high efficiency at both WWTWs (Figure 4-2A). Removal estimates for (±)-methamphetamine was shown to be stereo-selective at both WWTWs, whereby *R*-(-)- methamphetamine was removed less than *S*-(+)-methamphetamine (Figure 4-2A). This has been proven in both micro- and macroscale biodegradation studies, confirming an enrichment of *R*-(-)-methamphetamine during biological treatment (Bagnall et al., 2013; Xu et al., 2017). The same was shown for MDMA, whereby *R*-(-)-MDMA was enriched in treated wastewater effluent at WWTW-2; therefore, it was less effectively removed during treatment (Figure 4-2A).

This result has been confirmed in microcosm studies where stereoselective biodegradation was shown to favour the degradation of the *S*-enantiomer (Evans et al., 2016). Ketamine showed moderate to negative removal during WWTW treatment at both study sites (Figure 4-2B), which is in agreement with other studies (Baker and Kasprzyk-Hordern, 2011) although the loads varied significantly during sampling days. Mass load ratios of cocaine compared to its primary metabolite, benzoylecgonine, in raw wastewater ranged from 1.8 to 4.0 at WWTW-1, and from 1.9 to 3.8 at WWTW-2. These ratios suggest that a large proportion of cocaine is present within sewage in its unmetabolised form, suggesting possible disposal rather than consumption (benzoylecgonine/cocaine < 5) (Karolak et al., 2010). However, the overall higher loads of benzoylecgonine compared to cocaine in the raw samples does indeed confirm hepatic metabolism of the drug, thereby confirming its consumption within the domestic community connected to the sewage system.

The two pharmaceutical compounds, which ranged from moderate to negative removal during the current study, include the opioid tramadol, the antidepressant venlafaxine, and their primary metabolites (Figure 4-2B). Both tramadol and its primary metabolite, *O*-DMT, were moderately removed at WWTW-1, whereas the removal of tramadol at WWTW-2 was stereo-selective, but generated the same removal profile for *O*-DMT between the study sites (Figure 4-2). Venlafaxine showed an overall negative mass balance during treatment at WWTW-1, whereas its metabolite, DMV, was moderately removed (Figure 4-2B). Removal of venlafaxine and DMV at WWTW-2 showed an opposite result, whereby venlafaxine removal was slightly negative to low, and DMV was negatively removed on average during the sampling campaign (Figure 4-2B).



Nd: Not determined

Figure 4-2: Removal (percentage) of illicit drugs and precursors (A) and other drugs of abuse and human indicators (B) during wastewater treatment. Error bars represent the lowest and highest recorded removal for each compound during sampling.

In total, 31 of the target compounds were quantified within raw wastewater at WWTW-1, and 35 of the compounds were quantified at WWTW-2 (Table 4-1). These results confirm the use of illicit drugs, as well as other OTC and prescription DoAs by the surrounding populations. Overall, most of the CECs were detected at higher concentrations in WWTW-2 due to its wastewater source having a larger proportion of domestic sewage, as well as its larger holding capacity and estimated population being 1.7-fold and 2.4-fold higher, respectively, than that recorded for WWTW-1.

Table 4-1: Concentrations (ng/ℓ; min – max) calculated during the seven-day sampling campaign at the WWTWs

Compound	WWTW-1 (Gauteng)		WWTW-2 (Cape Town)	
	Influent	Effluent	Influent	Effluent
Stimulants				
Cocaine	40.9-145.1	0.6	107.8-349.7	0.7-1.7
Benzoylecgonine	129.8-346.3	9.0-52.6	409.6-1084.2	28.0-52.6
R-(-)-amphetamine	-	-	-	-
S-(+)-amphetamine	13.1-20.7	-	50.9-128.9	-
R-(-)-methamphetamine	55.7-121.8	26.4-52.9	94.7-183.5	26.8-30.7
S-(+)-methamphetamine	248.5-773.9	2.3-148.4	1,587.4-3,226.4	96.6-151.9
R-(+)-mephedrone	-	-	1.6-6.4	-
S-(-)-mephedrone	-	-	1.1-5.7	-
Precursors				
1S,2R-(+)-ephedrine	-	-	-	-
1R,2S-(-)-ephedrine/*1R,2R*-(-)-pseudoephedrine	964.6-1,749.9	26.8-217.5	274.1-461.1	46.2-63.8
1S,2S-(+)-pseudoephedrine	822.1-1,299.7	73.3	430.4-742.2	11.1-20.2
E1-norephedrine	44.2-61.4	0.6-5.0	5.7-27.7	4.0-6.1
E2-norephedrine	69.1-100.8	10.4	19.1-48.1	-
Hallucinogens				
R-(-)-MDMA	1.7-2.5	0.3	7.6-57.5	2.9-9.3
S-(+)-MDMA	0.7-1.9	-	3.9-34.4	1.1
E1-HMA	-	-	-	-
E2-HMA	-	-	-	-
R-(-)-HMMA	4.9	-	7.3-16.0	-
S-(+)-HMMA	7.2-8.0	-	8.6-19.2	-
Opioids				
Codeine	1,372.4-2,619.5	10.9-149.4	1,663.2-2,0567.7	22.6-93.5
Dihydrocodeine	6.2-13.7	4.2	10.5-59.7	4.6
Hydrocodone	-	21.1-40.4	-	59.9-167.1
Oxycodone	3.2-6.8	-	4.8-6.6	-
Morphine	291.8-407.2	-	761.0-9379.7	62.1
Normorphine	88.9-157.2	-	131.8-173.8	-
Heroin	-	-	-	-
O-6-monoacetylmorphine	-	-	28.2-66.0	-
E1-tramadol	143.5-577.7	193.7-402.1	682.8-950.5	656.0-821.3
E2-tramadol	353.9-507.9	153.3-285.4	645.7-870.4	498.8-692.7

Compound	WWTW-1 (Gauteng)		WWTW-2 (Cape Town)	
	Influent	Effluent	Influent	Effluent
O-desmethyltramadol	742.2-1,122.3	157.1-905.1	1,901.9-2,382.4	1,141.0-1,949.5
Methadone	-	-	-	-
EDDP	-	-	0.6-4.6	-
Antidepressants				
E1-venlafaxine	26.4-75.8	43.6-85.7	77.0-127.3	90.9-112.5
E2-venlafaxine	26.9-75.1	34.6-63.2	83.4-132.7	68.1-98.0
E1-desmethylvenlafaxine	342.9-531.5	111.1-444.4	274.9-424.9	312.6-572.8
E2-desmethylvenlafaxine	204.4-334.4	56.3-246.6	162.5-251.0	188.7-382.4
Anaesthetics				
Ketamine	1.9-399.0	6.6-61.4	4.9-17.1	2.8-5.4
Human biomarkers				
Caffeine	230,741-458,704	96-23,732	8,717-104,776	145.8-864.4
1,7-dimethylxanthine	106,914-208,044	252-27,964	178,205-316,160	1,232.6-4,250.9
Nicotine	13,423-40,987	186.7-637.7	64,497-90,513	282.2-1,751.9
Cotinine	3,177.5-3,876.0	27.6-331.0	4,038.7-5,666.8	27.5-104.3

Within the aqueous phase of RAS samples (RAS_{liquid}), the concentrations of hydrocodone were higher than for codeine, ranging from 40.8 to 60.2 ng/ℓ at WWTW-1 and from 89.7 to 318.2 ng/ℓ at WWTW-2, as opposed to average concentrations of codeine calculated at 2.8-274.6 ng/ℓ and 6.6-15.1 ng/ℓ at WWTW-1 and WWTW-2, respectively (Archer et al., 2018). Enantiomeric profiling in the aqueous phase of RAS showed a racemic mixture for venlafaxine (average EF = 0.5 ± 0.02; Figure 4-3A and 4-3B), and slight stereoselectivity for DMV (average EF = 0.6 ± 0.01; Figure 4-3C and 4-3D) at both WWTWs.

On average, the concentrations of a racemic mixture of DMV compared to venlafaxine were 3.9 times more in RAS_{liquid} samples for WWTW-1 and 4.2 times more for WWTW-2. In raw wastewater samples, DMV/venlafaxine ratios were 8.3 and 3.0 for WWTW-1 and WWTW-2, respectively, suggesting further desmethylation metabolic processes during activated sludge treatment for WWTW-2, but high possible desmethylation processes within sewage prior to treatment at WWTW-1 (in-sewer degradation). This is supported by the fact that a racemic DMV concentration was similar between raw influent and RAS_{liquid} samples for WWTW-1, but 1.8 times higher in RAS_{liquid} samples than raw influent wastewater for WWTW-2.

These results imply that, although recalcitrant compounds such as venlafaxine and its metabolite are returned into the treatment steps (hence increasing residence time for their removal), their continued persistence and regular input into raw sewage will compromise their removal during the overall treatment process.

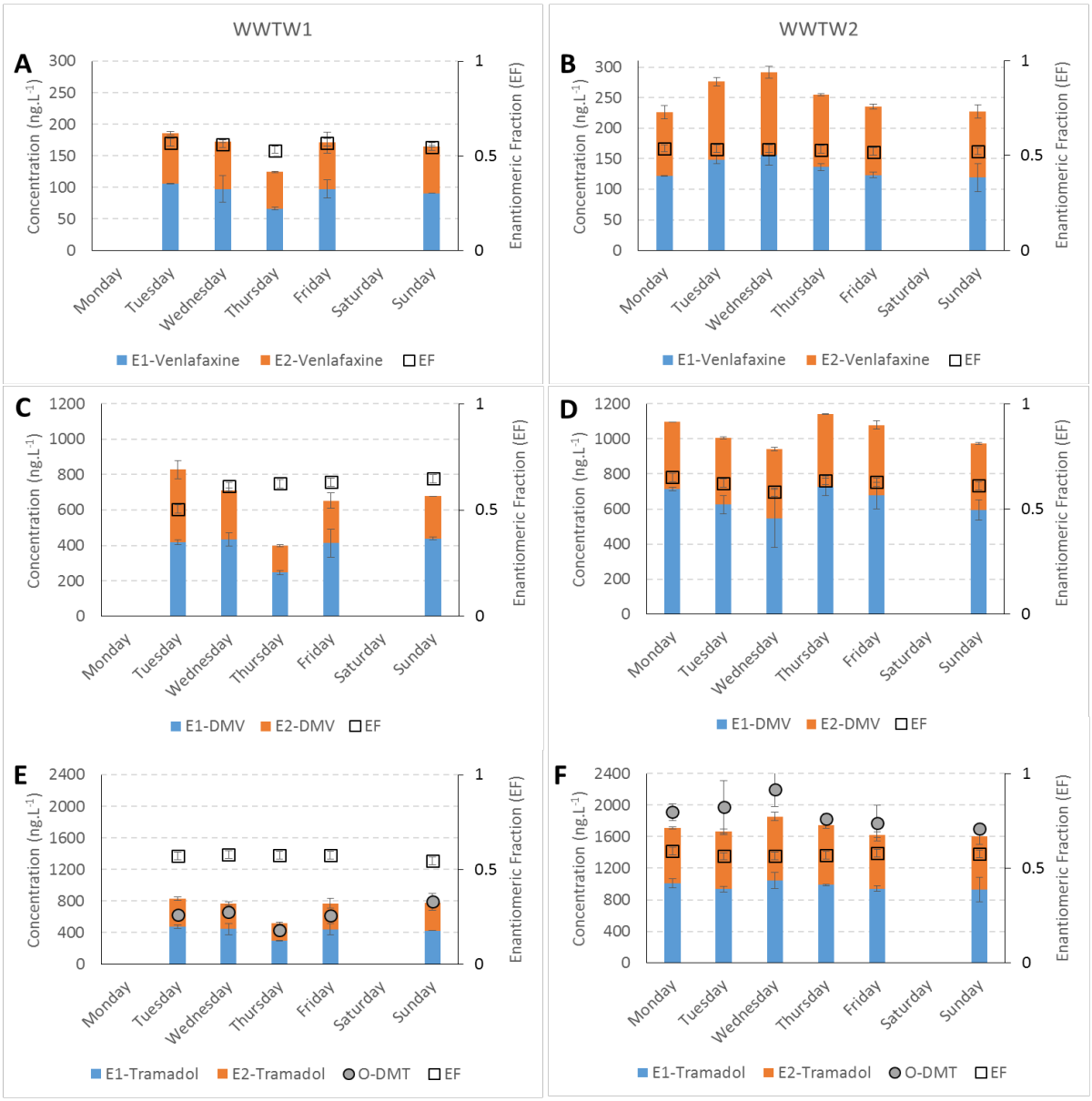


Figure 4-3: Concentrations (ng/ℓ) and enantiomeric fractions of venlafaxine (A and B), DMV (C and D) and tramadol (E and F) within the aqueous phase of RAS

The chiral signature for tramadol and venlafaxine were both racemic for influent and effluent samples at the two WWTWs (Figure 4-4), suggesting no enantio-selective degradation during treatment. The EF for DMV did not differ between influent and effluent samples for both study sites either (Figure 4-4). However, the chiral signature for DMV showed slight stereoselectivity, with EFs of 0.62 (± 0.01) and 0.64 (± 0.01) in influent and effluent samples, respectively, at WWTW-1, and 0.63 (± 0.02) and 0.61 (± 0.02) in influent and effluent samples, respectively, for WWTW-2 (Figure 4-4). This suggests that DMV degradation is also not enantio-selective during wastewater treatment. However, the enantio-selective toxicity for these recalcitrant CECs should be established for refined risk assessment purposes.

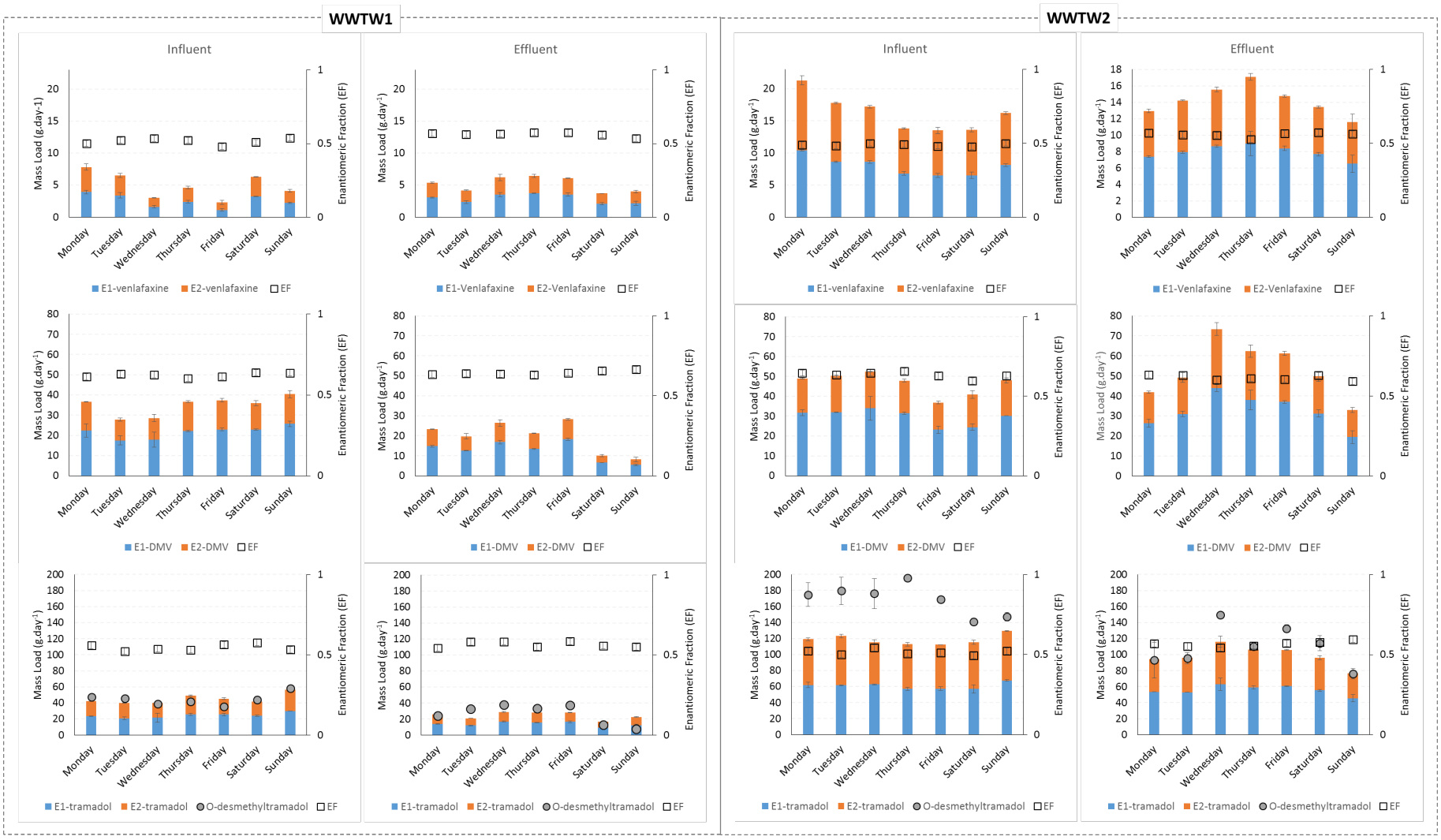


Figure 4-4: Mass loads (g.day^{-1}) and enantiomeric fractions (of venlafaxine, DMV, tramadol and *O*-DMT within raw influent and treated effluent wastewater samples from the two study sites

4.3.2 Detection of CECs in environmental surface waters

Compounds that were removed with high efficiency at WWTW-1 and thus discharged at low concentrations from treated wastewater, such as the human markers caffeine, nicotine and their metabolites, were detected at higher concentrations overall in downstream samples during the sampling period (Table 4-2). The average concentration of *S*-(+)-methamphetamine during the sampling campaign was shown to be 18 times higher in the downstream samples than in the upstream river water (Table 4-2), from which *S*-(+)-methamphetamine was removed with relatively high efficiency during wastewater treatment (see Figure 4-2A).

As this compound is known to be the primary enantiomer of illicit drug use, the current results suggest that this compound is either excreted or directly disposed from an additional source downstream from the WWTW. Interestingly, the same result of higher CEC concentrations in downstream, rather than treated effluent discharge, was shown in the previous case study in Chapter 3.3, from which sampling was done in a different year when the WWTW did not experience any treatment failures (Archer et al., 2017a). Overall, 25 of the 38 target compounds screened during the current study were detected in river water associated with WWTW-1, whereby 23 of the compounds were detected at concentrations two-fold and higher in downstream samples (Table 4-2). This further suggests additional pollution activities downstream from the point of WWTW discharge.

It is worth mentioning that WWTW-2 discharges a large percentage of its treated effluent into maturation ponds for reuse due to the ongoing droughts in the region, although the ratio of treated wastewater discharge into maturations ponds compared to river discharge could not be verified. The distance between upstream and downstream sampling sites at WWTW-2 was much shorter than for WWTW-1 (300 m as opposed to 4 km), thus there is less time for the degradation of the target analytes between these two sampling points. The CECs that were detected at higher concentrations in downstream than upstream samples were the more enantio-stable R-(-)-methamphetamine and R-(-)-MDMA, as well as the reported recalcitrant CECs venlafaxine and DMV (Table 4-2).

However, both tramadol and O-DMT, which also showed low to moderate removal during wastewater treatment, were not detected at higher concentrations in downstream samples (Table 4-2), which may suggest other contributing factors that may lead to their sorption onto sediment rather than persisting in the aqueous phase of environmental waters. Another interesting observation was the detection of the minor metabolite of heroin, O-6-MAM, during two sampling days in upstream water, along with higher concentrations of S-(+)-methamphetamine (the less stable enantiomer), in the measured treated sewage effluent (Table 4-2). Therefore, like the results observed for river water associated with WWTW-1, the results suggest alternative pollution or possible dumping or excretion of sewage into the river system that is not associated with WWTW discharge, as the river system associated with WWTW-2 passes through both formal and informal domestic housing upstream from the plant.

Table 4-2: Concentrations (ng/ℓ) of the CECs within river water located upstream and downstream from the wastewater treatment processes

Chemical	River associated with WWTW-1 (Gauteng)					River associated with WWTW-2 (Cape Town)				
	Upstream		Downstream		Fold change*	Upstream		Downstream		Fold change*
	Average	Min - Max	Average	Min - Max		Average	Min-Max	Average	Min - Max	
Illicit drugs										
Cocaine	ND		5.2		-	1.0	0.6-1.6	0.8	0.4-2.1	0.8
Benzoylecgonine	3.3	2.7-3.7	10.7	5.4-34.9	3.2	20.6	14.2-28.7	20.1	13.2-31.0	1.0
R-(-)-methamphetamine	4.8	3.2-5.8	20.0	10.7-33.1	4.2	14.1	6.1-25.9	18.5	9.6-28.3	1.3
S-(+)-methamphetamine	1.6	0.8-5.0	29.0	1.7-178.8	18.1	218.7	137.9-465.2	113.3	73.5-206.1	0.5
Methamphetamine-rac	**6.3**	**4.2-10.8**	**49.0**	**12.4-210.5**	**7.8**	**232.7**	**144.2-491.1**	**131.8**	**81.3-234.4**	**0.6**
R-(-)-MDMA	ND		ND		-	0.7	0.4-1.2	2.1	0.7-4.6	3.0
S-(+)-MDMA	ND		ND		-	0.3	0.2-0.4	ND		-
rac-MDMA	**-**		**-**		**-**	**0.9**	**0.6-1.5**	**-**		**-**
Precursors										
1S,2R-(+)-ephedrine	0.6	0.7-1.1	1.8	1.2-3.1	3.0	ND		ND		-
1R,2S-(-)-ephedrine/*1R,2R*-(-)-pseudoephedrine	19.4	16.9-22.9	42.6	20.1-142.6	2.2	28.8	26.2-35.9	30.9	24.3-35.1	1.0
1S,2S-(+)-pseudoephedrine	3.9	3.1-5.6	19.2	5.3-46.2	4.9	12.5	9.2-20.1	13.1	11.6-14.4	1.0
E1-Norephedrine	ND		3.1	1.9-5.4	-	ND		1.7	0.7-2.4	-
E2-Norephedrine	ND			ND	-	ND			ND	-
Opioids										
O-6-MAM	ND		ND		-	14.6	11.7-17.5	ND		-
Morphine	ND		ND		-	64.1	54.8-80.0	30.2	19.5-55.1	0.5
Codeine	5.6	4.1-9.4	50.3	4.3-217.5	9.0	42.1	29.4-51.4	27.6	16.3-35.8	0.7
Hydrocodone	4.7	4.3-5.2	ND		-	16.6	13.7-18.6	ND		-
E1-tramadol	37.6	21.3-54.3	128.1	55.6-196.2	3.4	1,137.2	881.4-1,458.9	705.7	494.7-974.8	0.6
E2-tramadol	30.8	15.3-44.6	98.6	38.6-149.3	3.2	829.8	594.0-1,076.7	505.1	368.5-647.5	0.6

Chemical	River associated with WWTW-1 (Gauteng)					River associated with WWTW-2 (Cape Town)				
	Upstream		Downstream		Fold change*	Upstream		Downstream		Fold change*
	Average	Min - Max	Average	Min - Max		Average	Min-Max	Average	Min - Max	
Tramadol-rac	**68.4**	**36.6-98.9**	**226.7**	**94.2-345.5**	**3.3**	**1,967.0**	**1,475.4-2,535.6**	**1,210.7**	**863.2-1,622.3**	**0.6**
O-DMT	61.1	51.9-73.2	257.3	62.9-410.1	4.2	2,573.5	2,221.5-2,824.7	1,753.7	1,394.4-2,100.6	0.7
Antidepressants										
E1-venlafaxine	6.2	2.5-8.9	27.9	14.4-47.9	4.5	35.0	24.7-46.8	61.1	37.2-104.5	1.7
E2-venlafaxine	5.6	2.5-7.8	21.7	10.9-37.2	3.9	41.1	31.4-54.6	49.4	26.9-83.4	1.2
Venlafaxine-rac	**11.8**	**5.0-16.7**	**49.6**	**25.3-85.1**	**4.2**	**76.1**	**56.1-101.4**	**110.6**	**64.1-187.9**	**1.5**
E1-DMV	36.5	12.1-52.2	107.4	24.0-187.3	2.9	227.5	110.9-347.8	256.2	153.8-423.4	1.1
E2-DMV	24.2	9.0-34.4	64.1	14.8-106.2	2.6	108.7	48.3-173.0	135.2	84.3-241.0	1.2
DMV-rac	**60.7**	**21.1-85.7**	**171.5**	**39.5-293.5**	**2.8**	**336.1**	**159.2-506.9**	**391.3**	**238.1-664.4**	**1.2**
Anaesthetics										
Ketamine	2.3	1.6-2.9	13.5	3.8-28.3	5.9	7.8	6.6-9.1	5.9	3.9-7.9	0.8
Human markers										
Caffeine	541.2	104.4-1,100.0	3,289.0	113.0-21,130.9	6.1	2,293.7	1,313.4-4,316.1	1,105.6	187.3-1,894.4	0.5
1.7-DMX	592.5	165.1-1,298.5	3,632.3	216.5-21,464.9	6.1	9,654.9	4,196.1-15,027.0	4,089.8	1,308.5-6,130.5	0.4
Nicotine	139.1	16.6-312.4	241.4	60.1-438.2	1.7	1,645.9	553.3-4,332.2	1,956.4	280.6-7,007.5	1.2
Cotinine	28.6	14.8-48.5	71.6	24.0-268.4	2.5	293.2	169.6-473.4	156.2	46.3-297.3	0.5

* Fold change is the differences in the average concentrations of the analytes between upstream and downstream samples. Fold change > 1 = higher concentration in downstream water.

O-6-MAM: O-6-monoacetylmorphine; *O-DMT:* O-desmethyltramadol; *DMV:* Desmethylvenlafaxine; *1,7-DMX:* 1,7-dimethylxanthine; *ND*: Not detected

4.3.3 Using the WBE approach to estimate substance use and abuse

The mass loads calculated for cocaine in the aqueous phase of raw wastewater showed a slight increase during the weekend period (Saturday and Sunday) for WWTW-2, but not for WWTW-1 (Figure 4-5). However, both the metabolites of cocaine, benzoylecgonine (BEG) and cocaethylene (CE) showed a significant increase in their loads within raw wastewater during the weekend period for both WWTWs (ANOVA, $P < 0.05$) (Figure 4-5). This can be attributed to the known recreational use of this drug. The BEG loads ranged from 5.59 to 17.26 grams per day at WWTW-1, and between 33.59 and 76.98 grams per day at WWTW-2, whereas the cocaine loads ranged between 2.12 and 7.23 grams per day at WWTW-1, and between 8.84 and 39.97 grams per day at WWTW-2 (Figure 4-5).

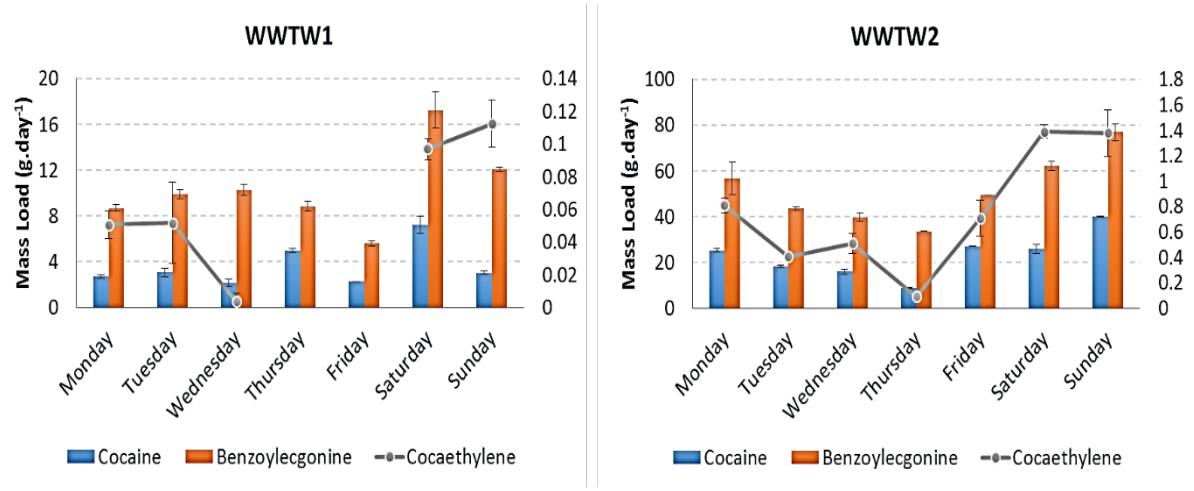


Figure 4-5: Daily mass loads (g/day) estimated for cocaine, BEG (1º y-axis) and CE (2º y-axis) within raw wastewater entering WWTW-1 and WWTW-2

The observed higher mass loads for BEG compared to cocaine in raw wastewater samples therefore assumes its consumption rather than direct disposal into wastewater. For WWTW-1, the cocaine/BEG ratio varied between 0.2 and 0.6 (median 0.3), and for WWTW-2, it varied between 0.3 and 0.5 (median 0.4), which is below the suggested cut-off ratio of 0.75 that is indicative of human consumption (Van Nuijs et al., 2009). However, these ratios were higher than suggested in a more recent study to assume *in vivo* cocaine metabolism, which was set at 0.1 or lower (Castiglioni et al., 2011). This may then suggest some direct disposal of the drug or other factors affecting these ratios, such as the route of administration of street drugs containing cocaine and co-administration with other substances. The detection of CE in wastewater highlights a few considerations that should be addressed when WBE is applied for cocaine consumption estimates. This metabolite is formed when cocaine is co-administered with alcohol, which is shown to lead to a decrease in the hepatic metabolism of the parent drug (Parker and Laizure, 2010).

It has been shown that the percentage of excretion products during the co-administration of cocaine and alcohol over a 24-hour period was 4.6% for cocaine, 21.1% for BEG and 0.7% for CE (De la Torre et al., 1991). Although only eight subjects for the study were used, these excretion values are very different from case studies where cocaine was administered alone (Khan and Nicell, 2011). Although cocaine is shown to be less stable in wastewater than BEG (Castiglioni et al., 2016), the increased levels of the parent drug cannot be considered to be a sole result of direct disposal, but may also be as a result of co-administration with alcohol. Co-administration of cocaine with alcohol may thus supress hepatic metabolism of BEG and instead cause increased excretion of the parent drug. This implies that re-adjustment of the correction factors should be considered in future studies. This will include refinement of parent/metabolite ratios to compensate for simultaneous alcohol intake by users.

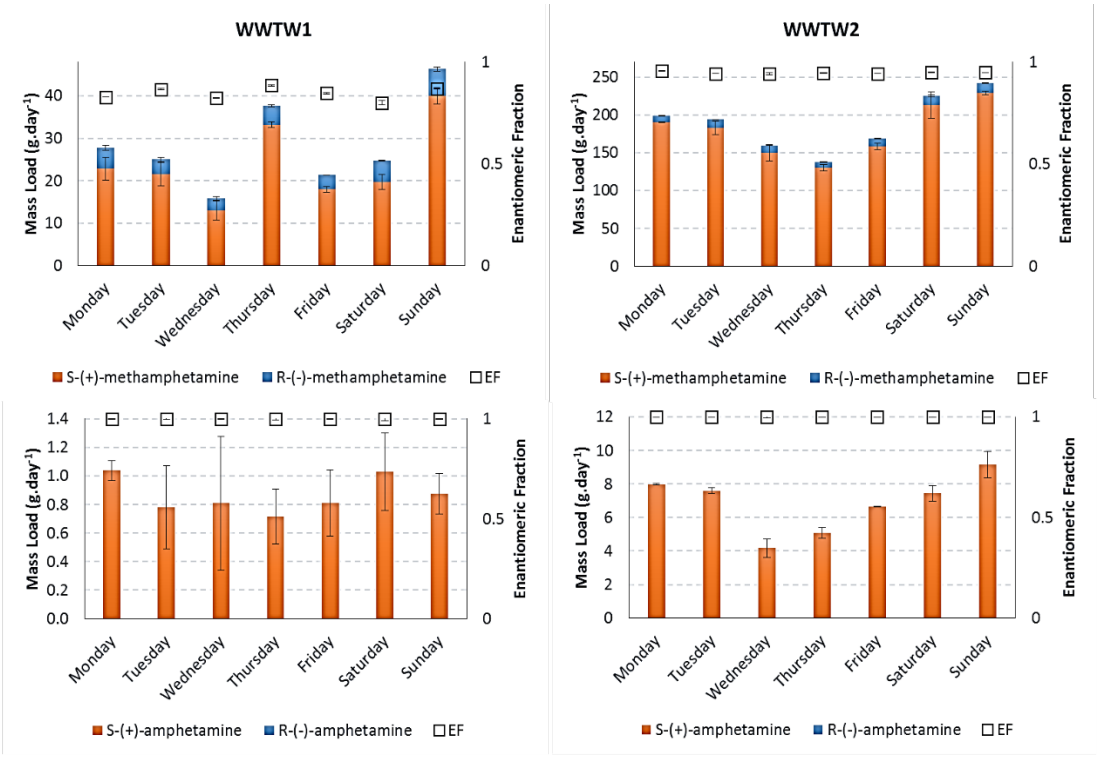
Average population-normalised mass loads for cocaine (using BEG) for WWTW-1 were estimated at 155.8 mg per day per 1,000 inhabitants during weekdays (Monday to Friday), and 263.8 mg per day per 1,000 inhabitants over the weekend (Saturday and Sunday) (Table 4-3). For WWTW-2, mean consumption of cocaine during the week was estimated at 342.0 mg per day per 1,000 inhabitants and 533.0 mg per day per 1,000 inhabitants over the weekend (Table 4-3). Although it is reported that cocaine is considered a secondary DoA in South Africa (Dada et al., 2017), the use estimates fell within the range of cocaine consumption estimated in several European cities during a 2016 monitoring campaign (Table 4-3), but were lower than estimates in South American studies and some European countries such as Belgium and England (EMCDDA, 2016).

Table 4-3: Drug use estimates (mg per day per 1,000 inhabitants; ± standard deviation) of selected illicit drugs at WWTW-1 and WWTW-2 based on the detected loads of parental and metabolite compounds in raw wastewater. Selected drug target residues were used to estimate the use of the illicit drugs.

WWTW-1								
Drug	**DTR**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Cocaine	BEG	156.4 ± 5.1	178.4 ± 77.5	184.9 ± 7.9	158.9 ± 8.0	100.6 ± 3.5	310.7 ± 28.7	216.9 ± 3.2
MDMA	MDMA	2.2 ± 0.2	2.6 ± 0.2	-	-	-	-	4.9 ± 0.7
	HMMA	6.2 ± 1.5	-	-	-	7.7 ± 0.5	-	10.4 ± 0.3
Methamphetamine	Methamphetamine	319.4 ± 38.1	287.5 ± 37.7	181.9 ± 31.3	433.1 ± 10.9	245.2 ± 8.9	284.7 ± 21.5	532.5 ± 28.6
Mephedrone	Mephedrone	-	-	-	-	-	-	-
WWTW-2								
Drug	**DTR**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Cocaine	BEG	435.6 ± 54.3	333.1 ± 6.7	305.1 ± 13.4	257.3 ± 0.6	378.7 ± 0.1	476.3 ± 15.9	589.6 ± 27.5
MDMA	MDMA	36.7 ± 0.04	15.2 ± 0.6	15.3 ± 1.1	9.0 ± 0.1	13.1 ± 1.7	33.1 ± 0.7	61.6 ± 0.7
	HMMA	19.9 ± 1.2	15.0 ± 2.3	8.3 ± 0.9	7.9 ± 0.6	7.7 ± 0.1	8.5 ± 0.3	26.6 ± 1.4
Methamphetamine	Methamphetamine	975.0 ± 5.4	948.4 ± 45.4	781.5 ± 52.9	675.0 ± 20.5	825.6 ± 22.6	1100.3 ± 93.4	1184.8 ± 13.5
Mephedrone	Mephedrone	-	-	-	-	7.6 ± 0.3	-	10.4 ± 0.9
EMCDDA-SCORE 2016*								
Cocaine	**113.8** (*Munich, Gemany*), **138.4** (*Oslo, Norway*), **169.6** (*Paris, France*), **390.4** (*Bristol, UK*), **409.6** (*Antwerp, Belgium*), **484.7** (*Geneva, Switzerland*), **699.1** (*Barcelona, Spain*)							
Methamphetamine	**58.3** (*Oslo, Norway*), **83.4** (*Helsinki, Finland*), **89.5** (*Espoo, Finland*), **136.7** (*Dresden, Germany*), **261.9** (*Budweis, Czech Republic*), **310.2** (*Piestany, Slovenia*), **671.8** (*Bratislava, Slovenia*)							
MDMA	**2.5** (*Athens, Greece*), **4.5** (*Milan, Italy*), **10.8** (*Porto, Portugal*), **17.2** (*Paris, France*), **34.2** (*Helsinki, Finalnd*), **51.2** (*Bristol, UK*), **59.3** (*Zurich, Switzerland*)							

* Examples of daily means of population-normalised mass loads estimated for several European cities as reported by the EMCDDA-SCORE initiative for 2016.

Mass loads of methamphetamine calculated in the current study were the highest when compared to other detected illicit drugs, highlighting its use as a primary abused substance in the study areas. The chiral signature for methamphetamine showed, almost exclusively, the presence of *S*-(+)-methamphetamine at both WWTWs (EF 0.8 to 1.0) (Figure 4-6), which is similar to monitoring studies in Europe (Castrignanò et al., 2017; Evans et al., 2016) and China (Xu et al., 2017). At WWTW-1, mass loads varied between 2.90 and 6.30 grams per day for *R*-(-)-methamphetamine, and between 12.92 and 40.01 grams per day for *S*-(+)-methamphetamine (Figure 4-6). At WWTW-2, mass loads were higher for both enantiomers, which varied between 7.77 and 11.94 grams per day for *R*-(-)-methamphetamine, and between 130.17 and 229.07 grams per day for *S*-(+)-methamphetamine (Figure 4-6). The mass loads for the racemic methamphetamine mixture also increased significantly during the weekend period at WWTW-2 (ANOVA, $P < 0.05$) (Figure 4-6), which is not surprising, as this plant is located in an area where adolescent and recreational use of the drug is high (Asante and Lentoor, 2017; Pluddemann et al., 2010).



EF = Enantiomeric fraction

Figure 4-6: Daily mass loads (g/day) of methamphetamine and *S*-(+)-amphetamine in raw wastewater entering WWTW-1 and WWTW-2

Clandestine manufacturing of illicit methamphetamine usually aims to synthesise the *S*-enantiomer due to its more desired physiological effect over *R*-enantiomer (Xu et al., 2017). The chiral signature for methamphetamine during the current study suggests an illicit origin rather than a resultant breakdown from licit pharmaceuticals, given the history of high methamphetamine abuse in the country (Arfer et al., 2017; Asante and Lentoor, 2017; Dada et al., 2017; Pluddemann et al., 2010). Although *R*-(-)-methamphetamine loads were lower than *S*-(+)-methamphetamine in raw wastewater for both WWTWs, the loads were not negligible and could possibly have been derived from licit pharmaceuticals, for example treatments for Parkinson's disease containing selegiline.

However, a lack of national prescription data limits the possible profiling to distinguish between the licit or illicit origin of *R*-enantiomer. In contrast, no enantiomeric study has been conducted in the country to verify the chiral signature and purity of "street" methamphetamine, which is mostly synthesised using ephedrine precursors and may yield variable enantiomerically pure drugs between various clandestine laboratories. For this reason, the racemic loads of methamphetamine were still deemed feasible to be used for consumption estimates. The population-normalised methamphetamine loads ranged from 181.9 to 532.5 mg per day per 1,000 inhabitants at WWTW-1, and from 675.0 to 1184.8 mg per day per 1,000 inhabitants at WWTW-2 (Table 4-3). These estimates correlate well with reports in other countries where high methamphetamine use is reported, such as Slovakia and the Czech Republic (EMCDDA, 2016), China (Xu et al., 2017), Australia (Thai et al., 2016) and New Zealand (Lai et al., 2017), and highlights the need for further profiling to more accurately estimate its consumption and manufacturing within the country.

For amphetamine, it has been highlighted that the presence of the drug in wastewater cannot solely be ascribed to abuse, nor can it be pinpointed to originate from a single source. Amphetamine may originate from several prescription medications, which may either include *S*- and/or *R*-enantiomer, or a mixture of both.

In South Africa, amphetamine-type stimulants (ATS) are registered to be used in several prescription medications, including attention-deficit hyperactivity disorder (ADHD) medications, NSAIDs, Parkinson's disease treatment and appetite suppressants. However, prescription data is not available in the country, along with few reports on the abuse of ATS medications. In the current study, amphetamine was detected exclusively as *S*-(+)-amphetamine in raw sewage (EF = 1), which ranged between 0.71 and 1.04 grams per day at WWTW-1, and between 4.17 and 9.15 grams per day at WWTW-2 (Figure 4-6). This finding differs from monitoring studies where amphetamine in raw wastewater was detected to be enriched with *R-(-)*-amphetamine, which was concluded to have originated from the use of amphetamine itself rather than as a breakdown product from other compounds (Kasprzyk-Hordern and Baker, 2012a; Kasprzyk-Hordern and Baker, 2012b).

In contrast, other studies have also detected an enrichment of *S*-(+)-amphetamine in raw sewage (Evans et al., 2016; Xu et al., 2017). Several factors have led to the conclusion that the presence of *S*-(+)-amphetamine in the wastewater samples analysed during the current study were derived from the reduction of methamphetamine rather than amphetamine use. First, the use and abuse of amphetamine itself may be low in the country due to access to other more easily available illicit substances. Second, given the more rapid metabolism of *S*-(+)-amphetamine over *R*-(-)-amphetamine during human metabolism and in wastewater (Kasprzyk-Hordern and Baker, 2012a), it would be assumed that raw wastewater samples would have at least contained traces of *R*-(-)-amphetamine, or mostly be enriched with this more stable enantiomer. Third, the breakdown of *S*-(+)-methamphetamine will exclusively lead to the formation of *S*-(+)-amphetamine (Bagnall et al., 2013). The higher load of *S*-(+)-methamphetamine in wastewater at both study sites (EF = 0.9 ± 0.1) (Figure 4.6) may lead to a higher fraction of breakdown of *S*-enantiomer than *R*-enantiomer. The low levels of *R*-(-)-methamphetamine in wastewater then simply lead to a lower fraction of *R*-(-)-amphetamine, which was below detection.

4.3.4 Environmental risk assessment

From the assessed target analytes during the current study, the compounds that showed the most pronounced lethal toxicity risk were tramadol, codeine and nicotine, having high RQs for both treated wastewater effluent and river water at both study sites (Table 4-4). For venlafaxine, a medium risk was calculated for the surface waters and treated effluent wastewater from both study sites, whereas the other analytes were considered a low risk for lethal toxicity endpoints (Table 4-4).

It needs to be stressed that such RQ estimations do not imply that a direct risk will be observed in nature. Most toxicity assays are done under controlled laboratory conditions, where test water is normally cleaned and only one substance of interest is added for the experimentation. However, large complex mixtures exist in nature with a variation of dose-dependent mixture interactions. For this reason, risk assessment approaches such as RQ estimations should be trigger values or early warning systems for further intervention rather than an established threshold of toxicity. As mentioned before, these PNEC-derived risk values do not consider adverse health effects at chronic, sub-lethal and/or multi-generational level.

Apart from reports showing lethal toxicity risks, the potential of the target analytes to exert sub-lethal, adverse health effects is discussed below. These outcomes are more representative of the potential long-term health effects of the pollutants through continued daily exposure rather than sporadic events that may lead to acute and/or chronic lethal toxicity. As an effect, sub-lethal toxicity endpoints are also more useful to represent toxicity for higher vertebrates other than using invertebrate and/or microbial test organisms.

Table 4-4: Environmental risk screening based on acute PNEC toxicity data on the most sensitive evaluated test species in literature (algae, cladocerans or fish). Risk quotients were measured for the minimum and maximum measured environmental concentration (MEC, ng/ℓ, range, minimum and maximum) determined for each analyte within WWTW effluent (*eff*) and surrounding environmental waters (*rw*)

Compound	PNEC (ng/ℓ)	Reference	WWTW1				WWTW2			
			MEC_{eff} (ng/ℓ)	RQ_{eff}	MEC_{rw} (ng/ℓ)#	RQ_{rw}	MEC_{eff} (ng/ℓ)	RQ_{eff}	MEC_{rw} (ng/ℓ)#	RQ_{rw}
Tramadol	320.0	Bergmann et al., 2011	511.7 (341.6-714.8)	1.1-2.2***	147.6 (36.6-345.5)	0.1-1.1**/***	1,308.8 (1,142.8-1,646.4)	3.6-5.1***	1,588.9 (863.2-2,535.6)	2.7-7.9***
Methamphetamine	1,970.0	Mendoza et al., 2014	66.4 (28.7-212.2)	< 0.1*	27.7 (4.2-210.5)	< 0.1*	143.7 (118.3-182.5)	< 0.1*	182.2 (81.3-491.1)	< 0.1*
Venlafaxine	322.0	Minguez et al., 2016	108.1 (78.2-148.9)	0.2-0.5**	30.7 (5.0-85.1)	< 0.1-0.2*/**	191.3 (182.4-206.9)	0.6**	93.3 (56.1-187.9)	0.2-0.6**
Ketamine	720.0	ECOSAR, n.d.	23.9 (6.0-63.6)	< 0.1*	7.9 (1.6-28.3)	< 0.1*	3.7 (2.1-6.0)	< 0.1*	6.8 (3.9-9.1)	< 0.1*
Morphine	93.0	Mendoza et al., 2014	-	-	-	-	0.1	< 0.1*	47.1 (19.5-80.0)	0.2-0.9**
Cocaine	2280.0	Mendoza et al., 2014	0.6	< 0.1*	5.4	< 0.1*	1.1 (0.6-2.7)	< 0.1*	0.9 (0.4-2.1)	< 0.1*
Benzoylecgonine	4,900.0	ECOSAR, n.d.	19.0 (9.0-54.0)	< 0.1*	7.6 (2.7-34.9)	< 0.1*	40.1 (27.7-57.8)	< 0.1*	20.4 (13.2-31.0)	< 0.1*
Codeine	60.0	ECOSAR, n.d.	61.4 (8.4-150.6)	0.1-2.5**/***	30.0 (4.1-217.5)	0.1-3.6**/***	53.6 (14.3-96.3)	0.2-1.6**/***	34.8 (16.3-51.4)	0.3-0.9**
Nicotine	14.0	Bergmann et al., 2011	340.1 (73.3-715.3)	5.2-51.1***	190.2 (16.6-438.2)	1.2-31.3***	932.5 (246.5-1,759.5)	17.6-125.7***	1,801.2 (280.6-7,007.5)	20.0-500.5***
MDMA	216.0	Mendoza et al., 2014	0.3	< 0.1*	-	-	5.8 (2.8-11.0)	< 0.1*	1.5 (0.6-4.6)	< 0.1*
Caffeine	490,000.0	Deo et al., 2014	3,837.2 (96.0-23,732.5)	< 0.1*	1,915.1 (104.4-21,330.9)	< 0.1*	393.4 (171.0-864.4)	< 0.1*	1.699.7 (187.3-4316.1)	< 0.1*
Cotinine	520,000.0	Deo et al., 2014	92.5 (27.6-331.0)	< 0.1*	50.1 (14.8-268.4)	< 0.1*	56.5 (27.5-104.3)	< 0.1*	224.7 (46.3-473.4)	< 0.1*
Ephedrine	3,620.0	Mendoza et al., 2014	41.8 4.2-217.5)	< 0.1*	14.6 (0.7-142.6)	< 0.1*	40.3 (11.1-63.8)	< 0.1*	21.3 (9.2-35.9)	< 0.1*
O-6-MAM	1,340.0	Mendoza et al., 2014	-	-	-	-	-	-	14.6 (11.7-17.5)	< 0.1*

* Low risk; ** Median risk; *** High risk based on RQ values (Hernando et al., 2006)

Average measured concentration of the compounds in river water samples (upstream and downstream from the WWTW)

4.4 SUMMARY

This study included a week-long monitoring campaign at two South African WWTWs, one in the East Rand district of Gauteng and one in Cape Town in the Western Cape. Raw influent, treated effluent, RAS and environmental surface waters sampled upstream and downstream of the plants were screened for the presence of selected CECs. Most compounds analysed were removed with high efficiency at both treatment plants, except for the opioid drug tramadol and the antidepressant venlafaxine and their metabolites, confirming the results of Chapter 3. The enantiomeric signature of methamphetamine in raw wastewater suggests an illicit origin (Castrignanò et al., 2017; Xu et al., 2017), as all raw wastewater samples were enriched with S-enantiomer. A minor, yet exclusive metabolite of heroin, O-6-monoacetylmorphine (O-6-MAM), was detected in raw influent of WWTW-2 only, confirming the presence of heroin use within the communities served by WWTW-2.

For prescription and OTC drugs (including ketamine), distinctions could not be made between their general use apart from their potential abuse within communities due to the unavailability of a unified pharmacological prescription database for the country (currently divided into the public and private health sector (Osunmaakinde et al., 2013)). Regardless of this, influent wastewater samples revealed high concentrations of the opiates codeine and morphine up to µg/ℓ levels. These concentrations were even shown to be higher than detections in other parts of the world (Thai et al., 2016). These results highlight the extent of codeine use and potential abuse within the current study areas, as confirmed by the South African Medical Research Council (MRC).

As discussed before, various factors may drive negative removal of target analytes during wastewater treatment. The current study further suggests that such recalcitrant compounds, which are known to have a strong association with solids, are constantly recirculated within RAS, from which desorption from the solid matrix and increased contact time with anaerobic digestion may contribute to its pseudo-persistence throughout sampling days. Both tramadol and venlafaxine undergo desmethylation into their primary metabolites, which is shown to be driven by anaerobic digestion during activated sludge treatment (Baalbaki et al., 2017; Falås et al., 2016; Gasser et al., 2012; Kasprzyk-Hordern and Baker, 2012a). However, significant degradation under anaerobic conditions mostly require long residence times (14 days), which are not necessarily feasible for WWTW treatment.

The average negative removal of venlafaxine during WWTW-1 treatment may well be due to a difference in sludge maturity and residence times. As desmethylation does, in fact, lead to the degradation of venlafaxine, it may inversely lead to the build-up of DMV during treatment, which is shown by the negative mass balances of DMV estimated for WWTW-2. However, as both venlafaxine and tramadol remain within the wastewater treatment system (within RAS), their pseudo-persistence may compromise daily estimations of removal.

During the sampling campaign at WWTW-1 (Gauteng), the plant experienced a treatment downtime on the Saturday of sampling due to an unexpected electricity load-shedding event by the national power utility. As a result, the treatment processes at the plant were not operational during the day, which is proposed to have led to inefficient degradation of the target analytes. This was observed by a clear spike in the mass loads of the cocaine metabolite benzoylecgonine, S-(+)-methamphetamine, ephedrine, codeine, caffeine, 1,7-dimethylxanthine and cotinine on the following Sunday in the treated wastewater effluent (supplementary data in Archer et al., 2018), which are all CECs that are shown to be well removed during the normal operating conditions of the WWTW. Interestingly, the loads of *O*-DMT and DMV were lower in treated effluent during Saturday and Sunday compared to the other sampling days, but not for the parent compounds tramadol and venlafaxine. This may suggest a decrease in desmethylation metabolic activities in the activated sludge modules during the treatment downtime, leading to reduced degradation of parent compounds into their primary metabolites. On the other hand, both tramadol and venlafaxine are known to have a high sorption affinity to solids (Baalbaki et al., 2017; Boix et al., 2016).

Because of the treatment module downtime, the solid particulate matter (SPM) within activated sludge treatment settled down, which may have retained these compounds, explaining why they were not detected at higher concentrations in treated effluent.

Considering the investigation of the presence and fate of various CECs within two WWTWs, the studies also aimed to identify the presence of these compounds in surrounding environmental surface waters located upstream and downstream from the point of WWTW discharge. A total of 42 emerging contaminants were detected during the first case study (Chapter 3), consisting of 17 classes of PPCPs and illicit substances. As expected, a long list of emerging contaminants was present within the river waters. Interestingly, emerging contaminants that showed moderate to high removal at the WWTW were again detected at high concentrations in downstream water samples, which was confirmed during the other monitoring study using the same plant (WWTW-1) as the one in this study. This occurrence was explained by the distant sampling location of the downstream sample from the WWTW discharge, highlighting that additional pollution sources may exist further downstream.

During the second scoping study, the focus was placed on the detection of persistent pharmaceuticals and DoAs. The detection of the target analytes in river water samples located upstream and downstream of the WWTWs showed converse results, with WWTW-1 showing higher overall levels of analytes in downstream samples, and WWTW-2 showing higher levels of analytes in upstream water samples. Again, the higher-fold change of analytes detected in downstream samples from WWTW-1 was attributed to the distant sampling location from the WWTW discharge. Interestingly, high loads of illicit drugs were detected within river samples that are not directly linked to WWTW discharge, which further suggests the direct discharge of sewage from communities that are not connected to the sewage network. This hypothesis is supported by the detection of increased loads of human lifestyle chemicals such as caffeine and nicotine, which are well removed during wastewater treatment.

This was also shown for the river system located next to WWTW-2, where the average concentrations of caffeine and its primary metabolite were detected at higher levels upstream from the WWTW. However, the known recalcitrant compounds, such as the antidepressant venlafaxine and its metabolites, may well originate from WWTW discharge, as these compounds also showed low removal during both case studies. Venlafaxine has been detected exceeding 100 ng/ℓ in UK river systems, and its metabolite DMV has been detected exceeding 200 ng/ℓ (Evans et al., 2017). The same trend was found in the current study for both WWTWs, in which concentrations of DMV were more than two-fold higher in the river water samples compared to the parent compound. These results further warrant the prioritisation of venlafaxine and its metabolite as a recalcitrant micropollutant within urban surface waters. Other compounds, such as antibiotics, the plastisiser bisphenol-A, UV filters, parabens, NSAIDs, opioids and the anti-epileptic carbamazepine again showed that these substances should receive priority, as they are still pseudo-persistent within surface waters and may be contributing factors to environmental and human health.

It is clear from the results presented here that setting out a universal priority list of CECs may prove difficult, as each location is impacted on by varying types of wastewater and surrounding human activity. Nevertheless, from the investigation of the loads of CECs detected downstream, it becomes clear that more information is required in future studies on the adverse health risks of pharmaceutical breakdown products for more advanced risk assessment approaches. Moreover, evaluating the health status of a water system by only considering a select list of target analytes has its limitations, as it does not consider whether the detected analytes or the complex mixtures of various other unidentified analytes do, in fact, pose an adverse risk to environmental and human health. Regardless, targeted chemical determination during water treatment and in surface water shows more strength in providing information to assess WWTW performance, as well as novel insights on the chemical use patterns of the surrounding communities through a WBE approach.

CHAPTER 5: TEMPORAL VARIATION OF CEC LOADS WITHIN TWO SOUTH AFRICAN WASTEWATER TREATMENT WORKS

5.1 INTRODUCTION

The recent water scarcity that was experienced in the Western Cape has raised awareness of the potential use of treated wastewater for both potable and non-potable purposes. However, the known recalcitrance of certain priority organic CECs may compromise the quality of such reclaimed water resources, as it is becoming more evident that several of these pollutants pose a risk to aquatic ecosystems and human health. Along with such regional challenges, South Africa is currently facing exponential population growth, aligned with rapid urbanisation. This places pressure on both an increased demand for the allocation of sanitary water supplies to communities, and a higher contribution of wastewater containing persistent micro-pollutants, which needs to be treated by WWTWs. Furthermore, due to the recent drought that was experienced in the province (between 2015 and 2018), WWTWs are faced with sewage that is not necessarily mixed with other wastewater sources (greywater), placing pressure on their operating capacities and overall treatment performance. The current case study includes a monitoring campaign at two major WWTWs in Cape Town, which incorporates the detection of persistent pharmaceutical and consumer compounds within raw wastewater influent and treated wastewater effluent.

Information regarding the presence and fate of organic CECs in South African WWTWs has increased in the past decade. However, such information is sourced from Gauteng and KwaZulu-Natal (Archer et al., 2017). As a result, the current study aimed to broaden the information regarding the fate of CECs during wastewater treatment in the Western Cape, particularly in Cape Town. The Western Cape has been experiencing a severe drought period since 2016. As a result, water consumption for residential use has decreased, leading to lower sewage levels entering WWTWs. This may have a severe effect on treatment performance, as required water parameters are not met. During July 2018, the province received normal rainfall again, which ended the three-year drought.

The aim of this study was to undertake a CEC profiling study at two Cape Town WWTWs with similar conventional activated sludge treatment technologies, but alternative treatment capacities and disinfection or tertiary treatment steps. This allowed for the investigation of temporal changes in CEC loads at the WWTWs, as well as an investigation of temporal removal efficiencies using internationally recognised sampling and sample processing methodologies for CEC quantification during wastewater treatment. Moreover, the WBE approach was adopted, allowing for the estimation of per capita usage rates of illicit drugs and other pharmaceuticals at the two study locations. The environmental risk assessment estimations and the alignment of the CEC concentration results with the AOP network will be discussed.

5.2 MATERIALS AND METHODS

5.2.1 Chemicals and consumables

The study included the multi-residue quantification for a selected list of DoAs using analytical methods described elsewhere (Castrignanò et al., 2016). The following internal standards were included in the water samples to enable quantification: cocaine-d3, benzoylecgonine-d8, amphetamine-d5, methamphetamine-d5, mephedrone-d3, MDA-d5, MDMA-d5, cotinine-d3, EDDP-d3, heroin-d9, codeine-d6, oxycodone-d6, hydrocodone-d6, methadone-d9, ketamine-d4, norketamine-d4 and 1S,2R-(+)-ephedrine-d3. Hyper-grade methanol (98%) and ultra-pure water (Millipore) were used to clean glassware and for solid-phase extraction. All glassware was deactivated using 5% DMDCS in toluene, followed by two wash steps in toluene and three wash steps in MeOH.

5.2.2 Study area and sample collection

Two WWTWs that are managed by the City of Cape Town were selected for the study (Figure 5-1). WWTW-1 operates at a capacity of 200 Mℓ per day, serving a population of around 700,000 people. WWTW-2 operates at a capacity of 50 Mℓ per day, serving a population of around 200,000 people. The population estimates were based on COD measurements for the raw inflow at the plant during the sampling period (1 person = 128 mg COD per day). Both plants operate using conventional primary treatment (coarse and grit screening followed by primary sedimentation), secondary treatment (biological nutrient removal through activated sludge treatment followed by secondary clarification) and the disinfection of treated wastewater through chlorination. WWTW-1 discharges the treated wastewater into maturation ponds after chlorination, whereas WWTW-2 discharges secondary clarified wastewater into maturation ponds prior to chlorination.

Aqueous samples were collected at the raw inlet (after grit screens) and final effluent (after chlorination) at the two WWTWs over a period of seven consecutive days (Wednesday to Tuesday) during the months of February and July 2018/19 (four sampling events). The daily samples comprised 100 mℓ wastewater taken every 10 minutes (from 10:00 to 10:00) using a 24-hour time-proportional composite sampler (Aquacell, Aquamatic Ltd., UK). The composite samples were collected in plastic bottles and transported to the laboratory on ice for same-day processing. Each daily sample from the raw and effluent wastewater source of the treatment plants was split into two 50 mℓ duplicates for further processing for chemical determination.

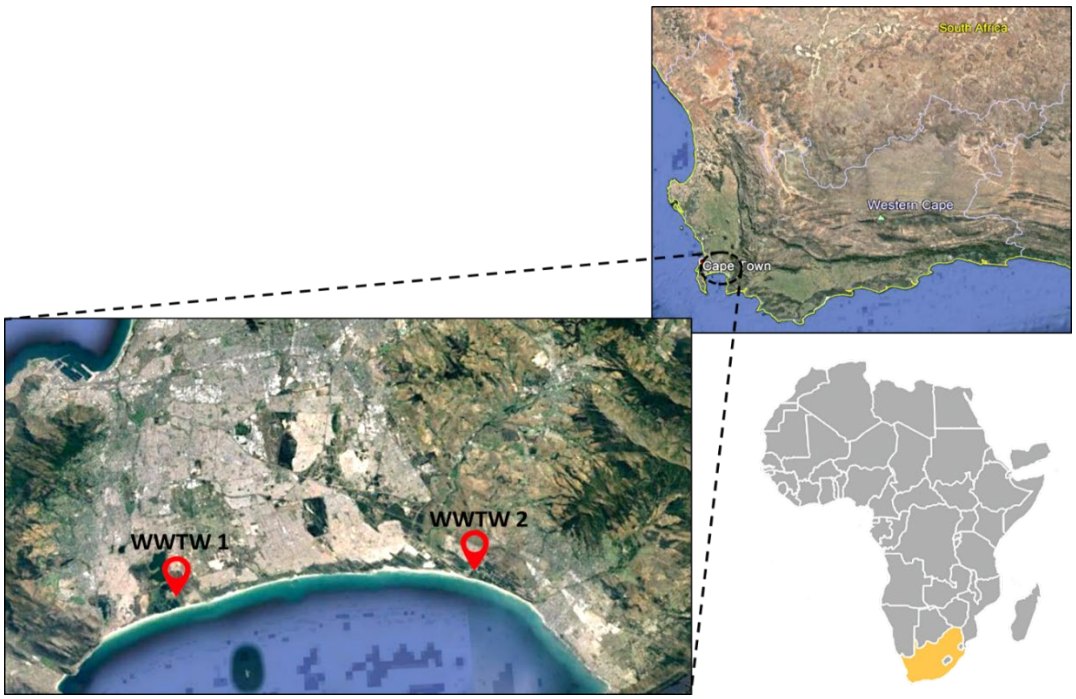


Figure 5-1: Sampling locations of the two WWTWs located in Cape Town, Western Cape

5.2.3 Sample preparation for analysis

Generally, all sample processing and extraction methodologies were followed as stipulated by a best-practice protocol set out by members of the European Monitoring Centre for Drugs and Drug Addiction's Sewage CORe analysis Europe group (EMCDDA-SCORE) (Castiglioni et al., 2016) for the wastewater analysis of emerging micropollutants. Raw wastewater was centrifuged first (5,000 rpm for 15 minutes, 4 °C) to separate the solid particulate matter from the aqueous matrix prior to pre-filtration and spiking.

All 50 mℓ samples were spiked with 50 µℓ of a 1 mg/mℓ stock of deuterated internal standards to account for analyte loss during sample processing (see Table 5-1), as well as matrix suppression and analyte recovery during the chemical analysis. The spiked samples were then pre-filtered using 0.7 um glass fibre filters (Whatman, GF/F) with a vacuum-filtering device. They then proceeded to a standard protocol of SPE for sample clean-up, analyte retention and pre-concentration.

The filtered water samples were extracted using Oasis® HLB cartridges (3 cc, 60 mg; Waters), which retains polar organic and slightly non-polar organic compounds. The cartridges were pre-conditioned with 2 mℓ high-performance liquid chromatography (HPLC)-grade MeOH followed by 2 mℓ de-ionised water (Milli-Q). All cartridge pre-conditioning was performed under gravity. The aqueous samples were then loaded through the cartridges using a 12-channel SPE vacuum manifold (SUPELCO, VISIPREP™, Sigma-Aldrich) at a flow rate of 5 mℓ per minute and dried for 30 minutes under vacuum. Elution of the samples was done using 4 mℓ HPLC-grade MeOH into DMDCS-silanised glass vials and evaporated under a gentle stream of nitrogen and a heating mantle (35 °C). The dried samples were then reconstituted in a volume of 1 mℓ HPLC-grade MeOH in LC-MS vials, giving a 50x concentrated WWTW sample for analyte quantification using LC-MS.

5.2.4 Sample analysis using liquid chromatography-mass spectrometry

Chromatography was acquired using a UPLC (Waters AQUITY). Separation of the target analytes was achieved using de-ionised water (MilliQ) containing 0.1% formic acid (mobile phase-A) and 100% HPLC-grade MeOH (mobile phase-B). Starting conditions were 100% mobile phase-A, which were maintained for 0.2 minutes and then reduced to 10% mobile phase-A over 6.8 minutes and to 0% mobile phase-A over 0.1 minutes. This was returned to 100% mobile phase-A over a period of 0.4 minutes and maintained for 2.5 minutes to allow for re-equilibration. The total run time was 10 minutes, allowing for the ion separation of 21 chemicals (including reference deuterated standards) (Table 5-1).

Table 5-1: Details of the chromatographic retention times and mass spectrometry parameters used in the LC-MS method to estimate the concentrations of CECs in wastewater and river water samples

Target analyte	RT (min)	Precursor ion m/z	Product ion 1 (m/z)	CV (V)	CE (eV)	Product ion 2 (m/z)	CV (V)	CE (eV)	Internal standard
Standards									
Acetaminophen	2.14	152.0	110.0	20	25	93.0	20	25	Methamphetamine-d5
Emtricitabine	2.26	247.9	130.2	15	15	-			Emtricitabine-13C5
Codeine	2.30	300.0	215.0	40	25	152.0	40	40	Cocaine-d3
3,4-methylenedioxymethamphetamine	2.61	194.1	163.1	20	25	105.1	20	25	MDMA-d5
Methamphetamine	2.70	150.0	91.0	25	20	119.0	25	10	Methamphetamine-d5
Caffeine	3.01	195.0	138.0	38	15	110.0	38	23	Methamphetamine-d5
Benzotriazole	3.24	120.0	65.0	30	20	92.0	30	15	Naproxen-d3
Sulfamethoxazole	3.33	254.0	156.0	20	25	147.0	20	25	Sulfamethoxazole-13C6
Benzoylecgonine	3.34	290.0	168.0	20	19	105.0	20	30	Cocaine-d3
Cocaine	3.43	304.0	182.0	40	20	82.0	40	30	Cocaine-d3
10-hydroxy-10,11-dihydrocarbamazepine	4.73	255.0	194.0	20	25	-			Carbamazepine-d10
Carbamazepine	5.21	237.0	194.0	20	25	179.0	40	38	Carbamazepine-d10
Methaqualone	5.40	251.1	132.0	30	30	91.0	30	35	Methamphetamine-d5
Naproxen	5.94	231.0	185.0	20	10	-			Naproxen-d3
Diclofenac	6.66	296.0	250.0	15	15	215.0	15	15	Naproxen-d3
Efavirenz	6.80	316.0	232.4	25	15	243.8	25	20	Efavirenz-d4
Deuterated/13C-labeled standards									
Emtricitabine-^{13}C5	2.26	251.0	133.1	15	15	100.6	15	20	-
MDMA-d5	2.61	199.1	165.1	20	15	-			-
Methamphetamine-d5	2.71	155.0	92.0	25	15	121.0	25	15	-
Cocaine-d3	3.44	307.0	185.0	10	15	85.0	10	25	-
Sulfamethoxazole-^{13}C6	3.35	260.0	162.0	20	15	114.0	20	20	-
Carbamazepine-d10	5.16	247.0	204.0	30	25	-			-
Naproxen-d3	5.92	234.0	188.0	20	10	170.0	20	20	-
Efavirenz-d4	6.80	320.2	171.8	25	25	246.1	25	20	-

This method used a reversed-phase BEH C18 column (Waters AQUITY, 1.7 µm pore size, 2.1 x 100 mm) equipped with a 0.2 µm inline column filter. The column temperature was maintained at 50 °C. The flow rate of the mobile phases was set at 0.4 mℓ per minute with a sample injection volume of 2 µL. The UPLC was coupled with a triple quadrupole mass spectrometer (Xevo TQ-MS, Waters AQUITY) equipped with an ESI source. All the analytes were determined using an ESI^+ mode. Nitrogen was used as both nebulising and desolvation gas, and argon as collision gas.

The acquisition of the LC-MS data was achieved using an MRM mode with two fragment ions for each compound where possible (the first fragment as quantifier and the second fragment as qualifier). The optimised MS/MS parameters for the target analytes are shown in Table 5-1. Linearity of a reference standard calibration curve for each target analyte was achieved using a 10-point concentration range ranging from 1 to 750 µg/ℓ in the same solvent as the reconstituted water samples (HPLC-grade MeOH). Inter- and intra-day assay precision was determined by duplicate injections of the standard curve each day during three separate days. The integration of the analyte standard curves and surface water sample concentrations was determined using TargetLynx software (Version 4.1, Waters).

To assess the extent of analyte loss during sample processing, as well as potential sample matrix effects on ionisation during LC-MS acquisition, a matrix recovery study was done. Reference and internal standards of the list of compounds in Table 5-1 were spiked into ultrapure (MilliQ) water, river water, effluent wastewater and influent wastewater at a low and high concentration (50 ng/ℓ and 500 ng/ℓ, respectively). Water sample matrices were also spiked with only the internal standard to account for any residual analytes that were present in the sample matrices. The applied UPLC-MS/MS method showed good analyte recoveries for most of the CECs and the method was deemed sufficient for target analyte quantification in experimental samples.

5.2.5 Data analysis

The flow-proportional mass loads (Mℓ; g/day), WWTW removal estimates (percentage) and population-normalised mass loads (mg per day per 1,000 inhabitants) were calculated in a similar manner as described in Chapter 3.2.5. These calculations allowed for the temporal investigation of CEC loads during wastewater treatment for risk characterisation and treatment plant CEC removal evaluation, along with a WBE approach to investigate substance use/abuse in communities and usage patterns of other target analytes such as opioids, antibiotics and NSAIDs.

5.3 RESULTS AND DISCUSSION

5.3.1 Temporal CEC loads and WWTW removal

Both WWTWs in the current study utilise conventional activated sludge treatment for biological nutrient removal, but differ in their treatment capacity and the location of their maturation ponds after the final clarification step. WWTW-1 utilises chlorination directly after final clarification, whereby the treated wastewater is discarded into maturation ponds. WWTW-2's final clarification permeate is first discarded into maturation ponds, upon which the treated water from the maturation ponds is disinfected by chlorine and then discharged. Although the raw influent was sampled after grit screening for both WWTWs, the final effluent samples were taken after chlorination (before maturation ponds) for WWTW-1 and after chlorination (after maturation ponds) for WWTW-2. The presence and fate of the target CECs will be discussed here based on the flow-proportional mass loads (g/day) of the target CECs.

The results showed significant removal of the overall load of CECs at the two WWTWs (Figure 5-2). The total mass loads of the CECs were almost double in WWTW-1 compared to WWTW-2 during all the sampling campaigns due to this plant serving a larger population with sanitary services.

Overall, the treatment performance of WWTW-2 was better for the target analytes during the study, except for some recalcitrant contaminants that showed similar removal profiles at both treatment works. In particular, the loads of the ARV emtricitabine were as high as the total combined mass loadings of the other CECs during the study (Figure 5-2). This can be attributed to the high prescription rates of ARVs in the country, as it is recorded that South Africa has the highest antiretroviral treatment (ART) programme in the world, along with a large percentage of its citizens being diagnosed with HIV.

Removal of emtricitabine was higher than efavirenz for WWTW-2 at least, which could be attributed to various physico-chemical differences between the drugs such as emtricitabine having a much lower partitioning coefficient than efavirenz (Kow = -1.4 and 4.6, respectively). As a result, efavirenz may be more likely to partition in solid particulate matter or sludge and may show a pseudo-persistence during wastewater treatment. The stability of the two drugs may also vary, leading to efavirenz being much more persistent during activated sludge treatment. It should be noted that, although it seemed as if the total loads of CECs were well removed at both WWTWs, the results may be suppressed by the high loads of the lifestyle chemical caffeine and the analgesic acetaminophen in the raw influent (Figure 5-2), which are both well removed during wastewater treatment (Figure 5-2).

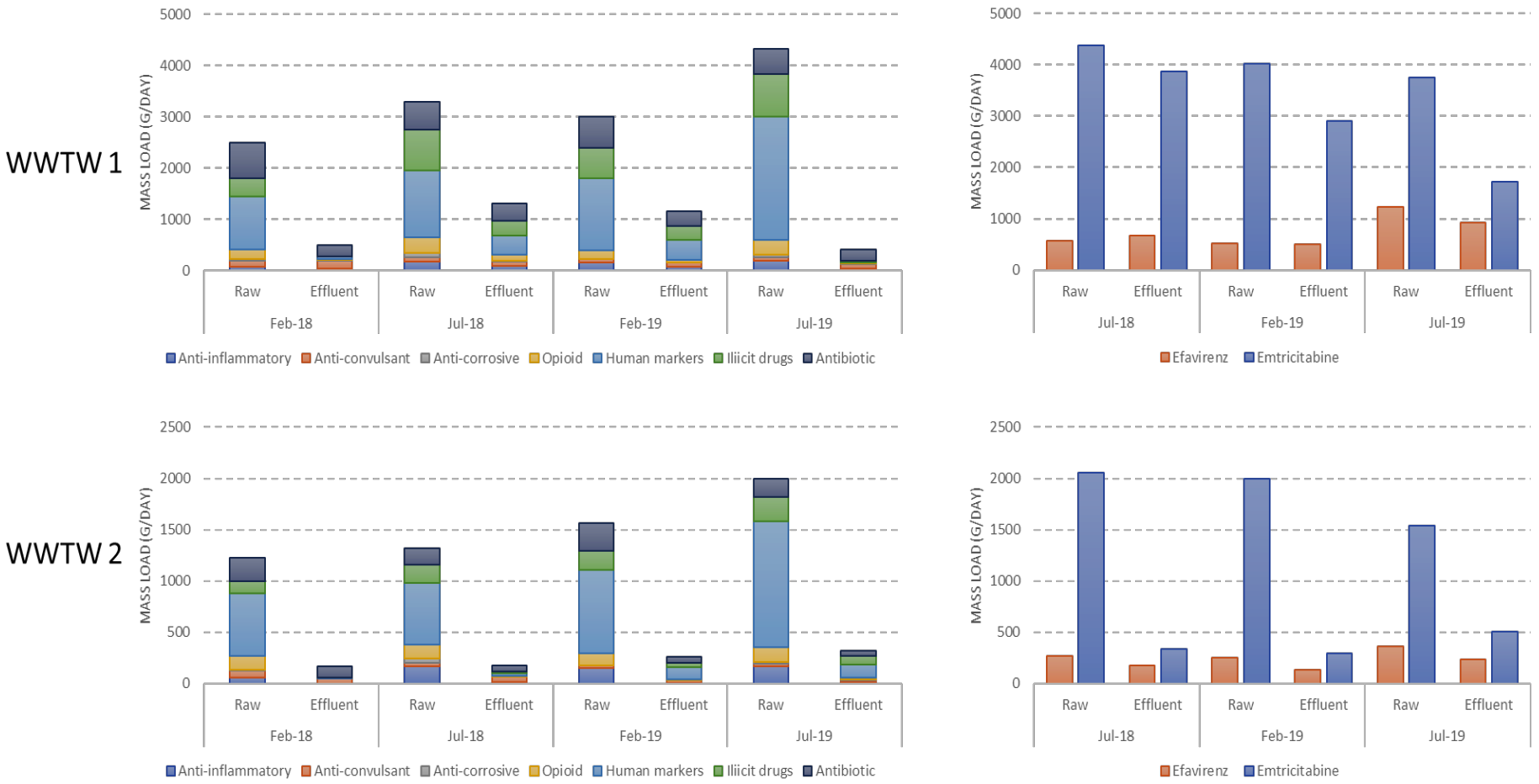


Figure 5-2: Flow-proportional mass loads (g/day) of the target analytes in the raw influent (after grit screens) and treated effluent (after chlorination) measured during the two-year sampling campaign for WWTW-1 and WWTW-2

Overall, the CECs that showed moderate to low removal profiles were diclofenac, 10,11-dihydro-hydroxycarbamazepine, benzotriazole, methaqualone, sulfamethoxazole, emtricitabine and efavirenz (Figure 5-3). Negative removals were once again calculated for carbamazepine at both WWTWs, confirming the extent of recalcitrance of this drug.

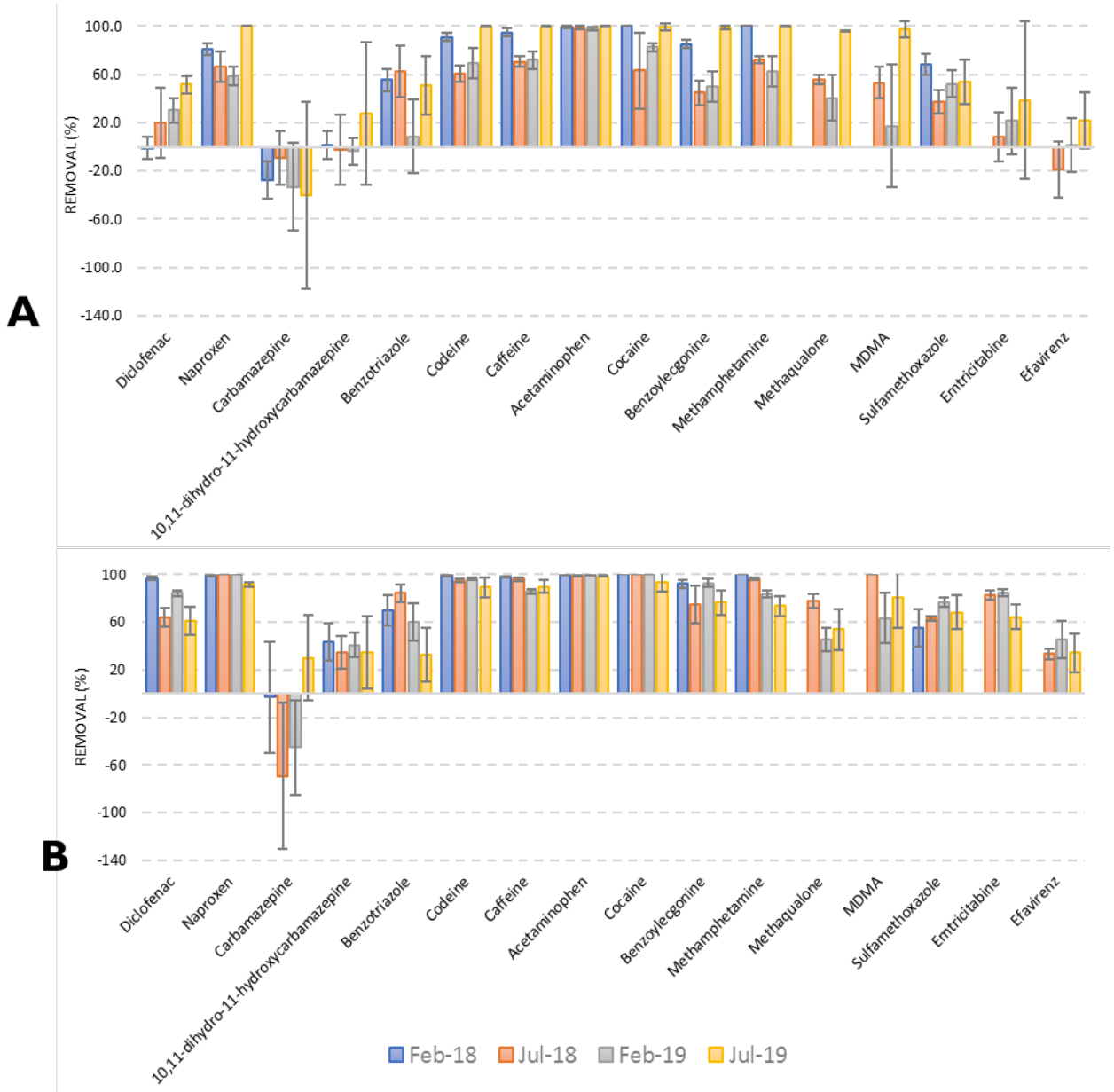


Figure 5-3: Removal (percentage) of the selected target analytes measured during the two-year sampling campaign for WWTW-1 (A) and WWTW-2 (B)

Temporal variations in the CEC loads during the two-year sampling campaign were also investigated to see whether changes in CEC usage could be determined across seasons (Figure 5-4 and 5-5). The influent loads for some target analytes, such as diclofenac, naproxen, benzotriazole, codeine and acetaminophen, were slightly lower during the February 2018 sampling period for both WWTWs.

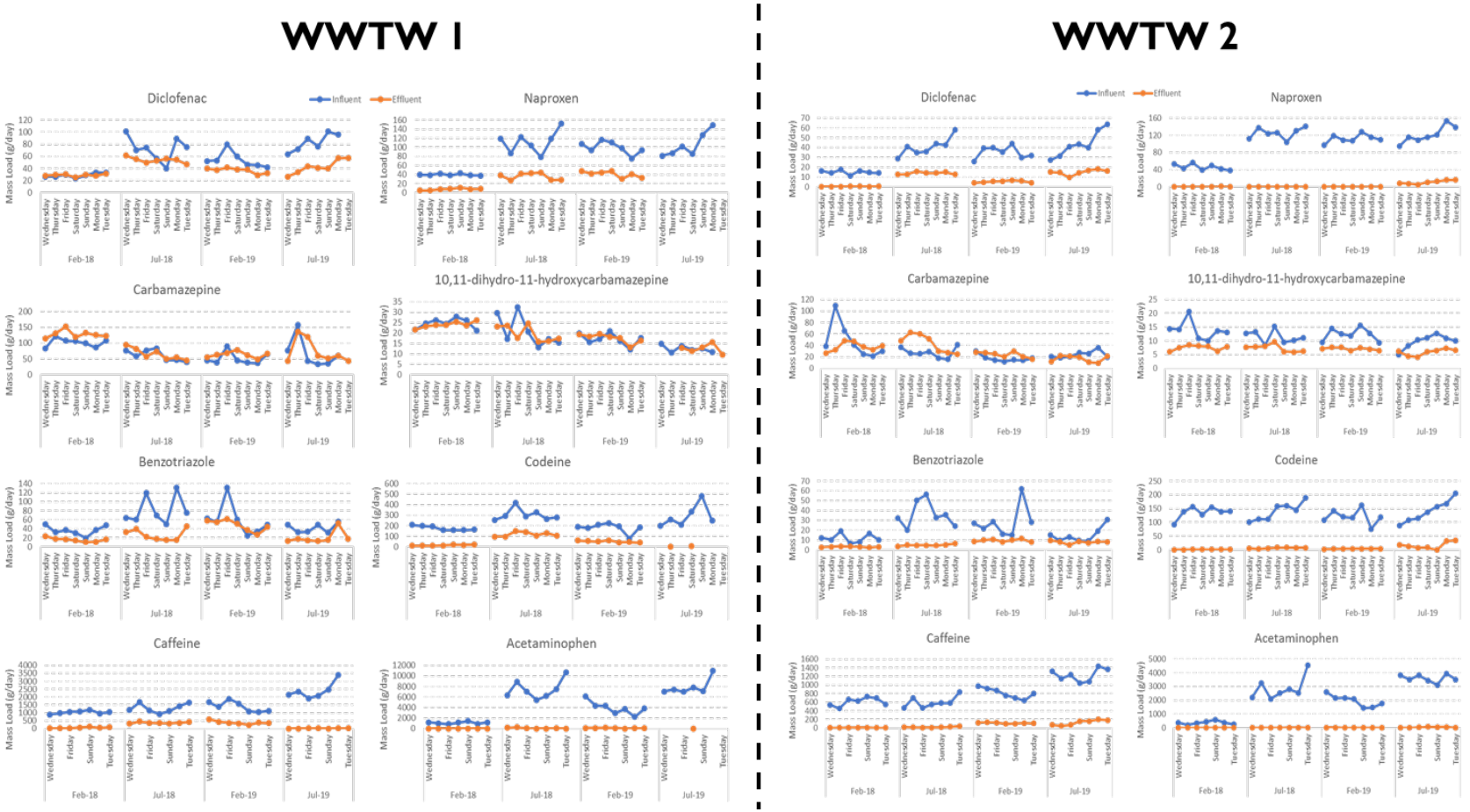


Figure 5-4: Part I: Temporal variation in flow-proportional mass loads (g/day) of the selected list of target analytes measured during the two-year sampling campaign for WWTW-1 and WWTW-2

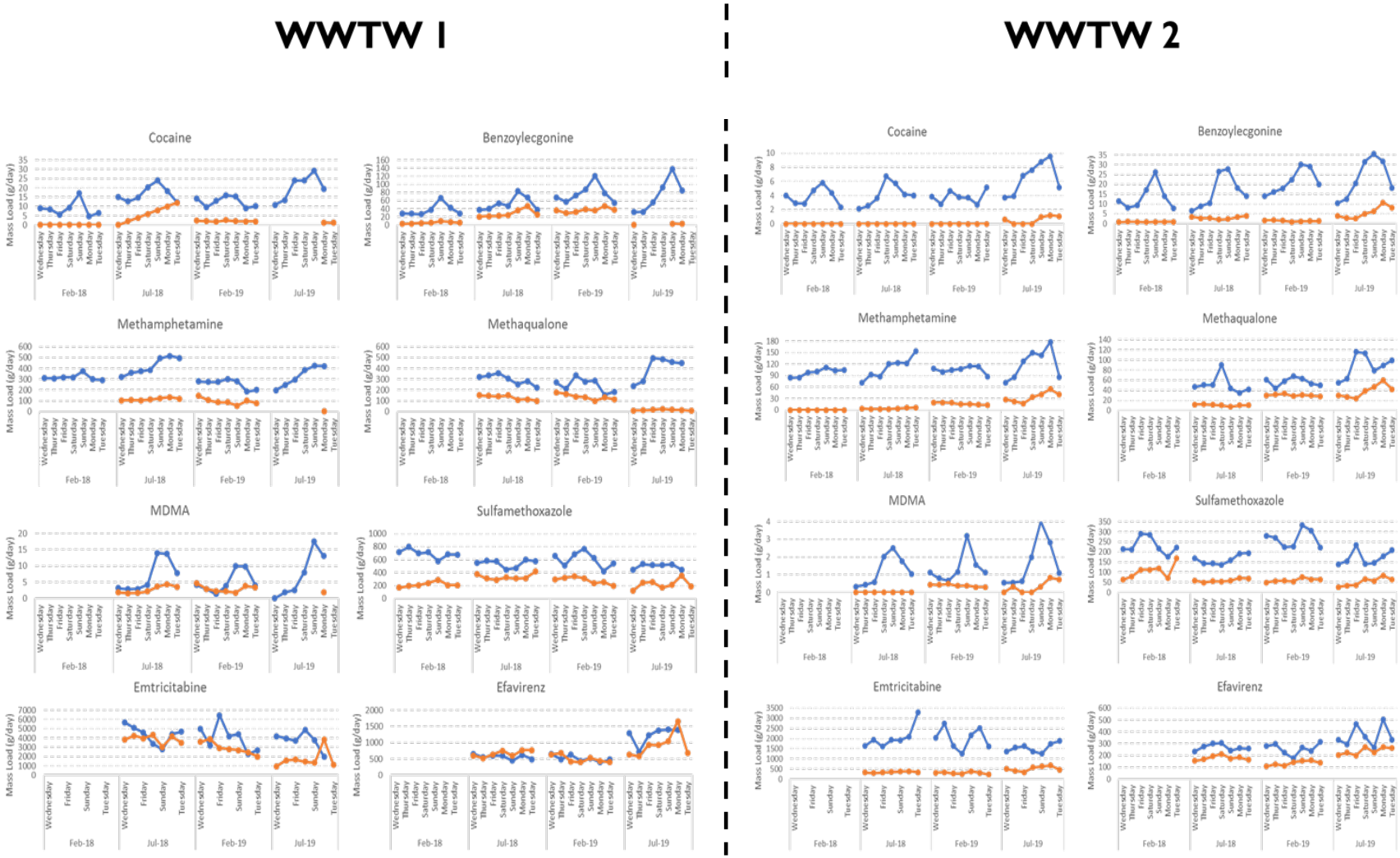


Figure 5-5: Part II: Temporal variation in flow-proportional mass loads (g/day) of the selected list of target analytes measured during the two-year sampling campaign for WWTW-1 and WWTW-2

During this period, severe drought was recorded for the Western Cape, where daily domestic water usage was also substantially lowered. However, such lower usage of domestic water could not have skewed the results, as the estimation of mass loadings of the CECs at the WWTWs (as opposed to measured concentrations; ng/ℓ) compensated for the lower influx of wastewater at the treatment facilities. This is further supported by the mass loading estimations of the illicit drugs, where similar patterns were shown throughout the two-year sampling campaign with slight increases in the loads of methaqualone (mandrax) and cocaine during the 2019 sampling, along with a confirmed recreational use of cocaine and MDMA in the areas.

The lower loading of some CECs during the drought period could be due to other biotic and/or abiotic factors in the sewage system that may influence the fate of these CECs in the sewage system due to their physico-chemical characteristics. However, this needs to be further investigated to justify this hypothesis. Another interesting observation was the constant load of the antibiotic sulfamethoxazole during the 2018/19 sampling period, as it was initially expected that antibiotic usage would fluctuate during the year (higher expected usage over the winter months). However, sulfamethoxazole (along with trimethoprim) is a broad-spectrum antibiotic that is prescribed for various health conditions. For example, people on ART are given sulfamethoxazole-trimethoprim prophylaxis to prevent opportunistic infections due to their compromised immune systems. For this reason, sulfamethoxazole usage will be continuous throughout the year and may not show temporal usage fluctuations, highlighting that this CEC will have continuous pseudo-persistence in WWTW discharge due to its moderate to low removal.

5.3.2 Using the WBE approach to monitor substance use in communities

5.3.2.1 Monitoring illicit substance abuse in communities

A recreational use trend for both cocaine and MDMA was confirmed for both locations, with slightly higher use trends in cocaine at WWTW-2, but similar use trends at both areas for MDMA (Figure 5-6 and 5-7, respectively). Cocaine and MDMA population-normalised mass loads were somewhat higher during the 2019 sampling period and the usage levels compared to those calculated for Germany, Belgium, Austria, Finland, France and Slovakia (2018 data from the EMCDDA-SCORE[5]).

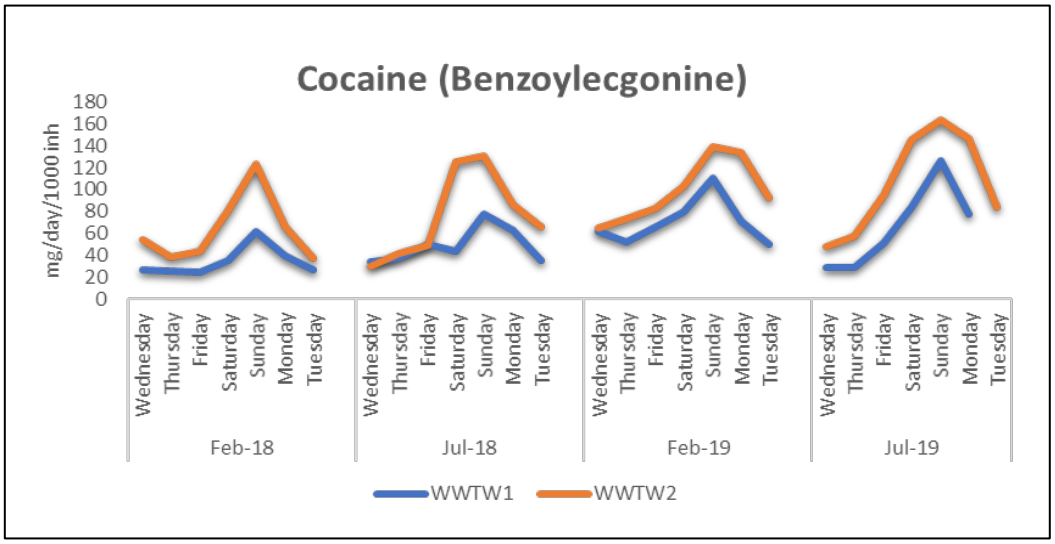


Figure 5-6: Population-normalised mass loads (grams per day per 1,000 inhabitants) of codeine, quantified from the raw wastewater at the two WWTWs during the study period

[5] http://www.emcdda.europa.eu/topics/pods/waste-water-analysis

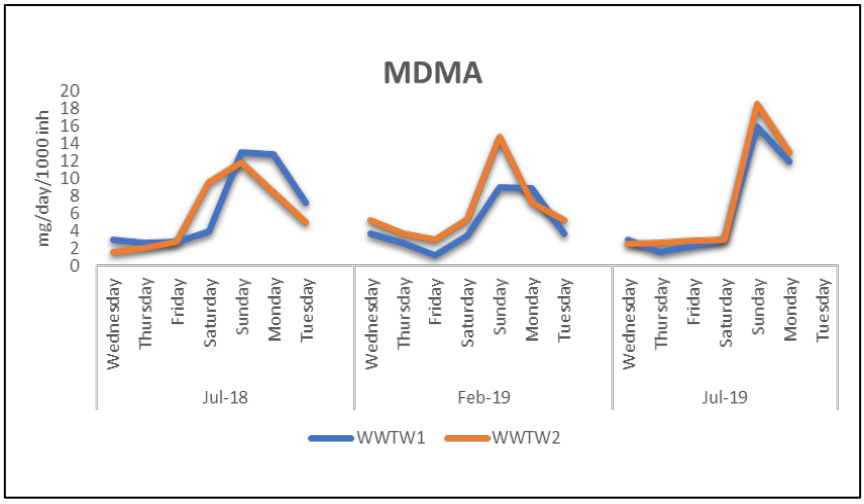
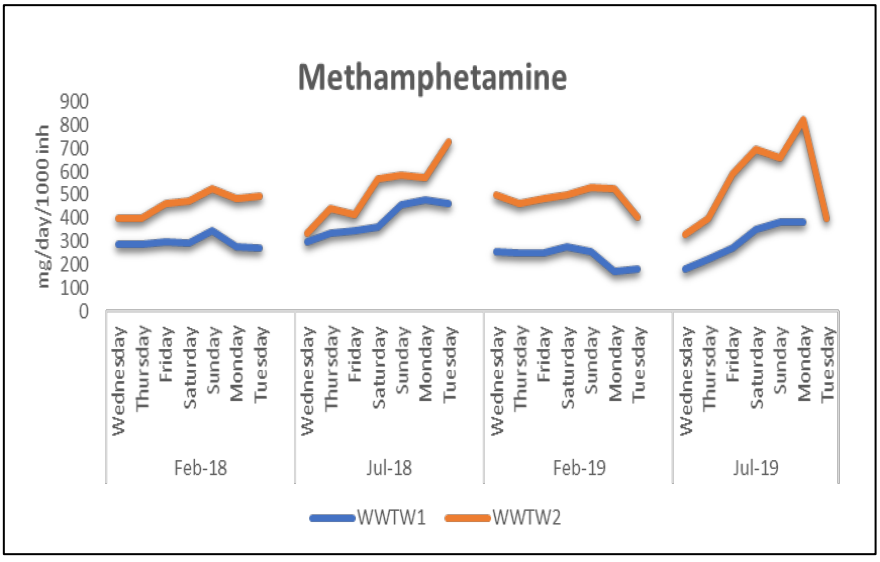


Figure 5-7: Population-normalised mass loads (grams per day per 1,000 inhabitants) of MDMA, quantified from the raw wastewater at the two WWTWs during the study period

Substance use patterns of methamphetamine (tik) and methaqualone (mandrax) did not show a recreational use trend as for the other DoAs, with both drugs showing similar use levels at both WWTW areas (methamphetamine use was slightly lower at WWTW-1) (Figure 5-8 and 5-9, respectively). This is the first study to estimate methaqualone use trends using WBE, so no international data could be used for comparison. However, the population-normalised mass loads for methamphetamine for both study sites showed two to three times higher values than estimated for any European country (Germany = 211.3 mg per day per 1,000 inhabitants out of 70 European cities; 2018 data from the EMCDDA-SCORE). These results highlight the extent of methamphetamine and methaqualone abuse in South Africa compared to various European countries. South African substance abuse data from treatment centres confirms this.[6] However, such treatment centre data does not include the total community that is subjected to substance abuse and may not show regional substance abuse data. For this reason, the application of WBE in the country can serve as a support system to evaluate communal substance abuse trends in the country.



[6] http://www.mrc.ac.za/sites/default/files/attachments/2018-03-02/SACENDUupdateJan2018.pdf

Figure 5-8: Population-normalised mass loads (grams per day per 1,000 inhabitants) of methamphetamine (tik), quantified from the raw wastewater at the two WWTWs during the study period

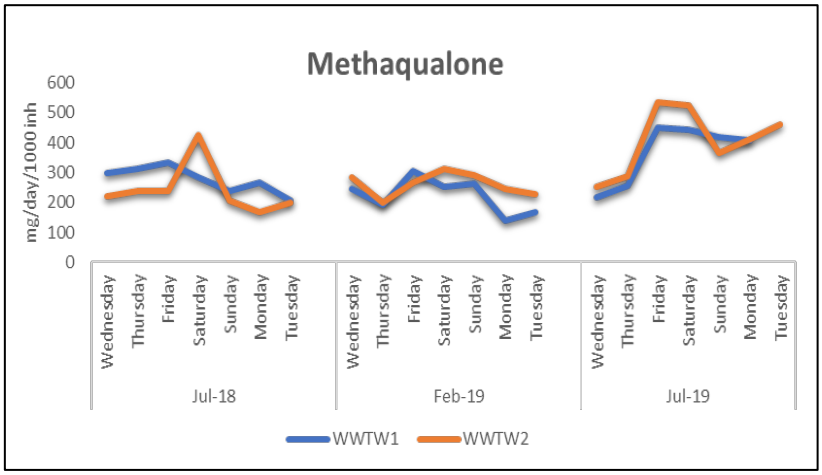


Figure 5-9: Population-normalised mass loads (grams per day per 1,000 inhabitants) of methaqualone (mandrax), quantified from the raw wastewater at the two WWTWs during the study period

5.3.2.2 Monitoring pharmaceutical substances use

The evaluation of other human-related health markers during the study showed higher usage trends of the antibiotic sulfamethoxazole at WWTW-2 (Figure 5-10), even though this plant receives less wastewater influent and has a smaller estimated population. This implies that a larger percentage of the population is using this antibiotic, and is also using it in a different manner as slightly higher loads were shown during the summer months compared to a constant load of the drug at WWTW-1 (Figure 5-10). The usage data for the opioid codeine and the ARVs emtricitabine and efavirenz confirmed that a larger percentage of the population associated with WWTW-2 is using these drugs, along with a slight increase in the use of efavirenz at both study locations during 2019 (Figure 5-10).

Population-normalised mass loads (grams per day per 1,000 inhabitants) of the antibiotic sulfamethoxazole, the opioid codeine and the ARVs emtricitabine and efavirenz were quantified from the raw wastewater at the two WWTWs during the study period.

The data presented here provides an example of the value of WBE to compare substance use and abuse trends between communities and over periods of time. Although the authors acknowledge that much still needs to be done to refine WBE for human health-related markers regarding their in-sewer stability and excretion patterns, the results presented still allow for comparisons between communities and raises the need for other environmental chemistry research groups to adopt this methodology in their future and ongoing research.

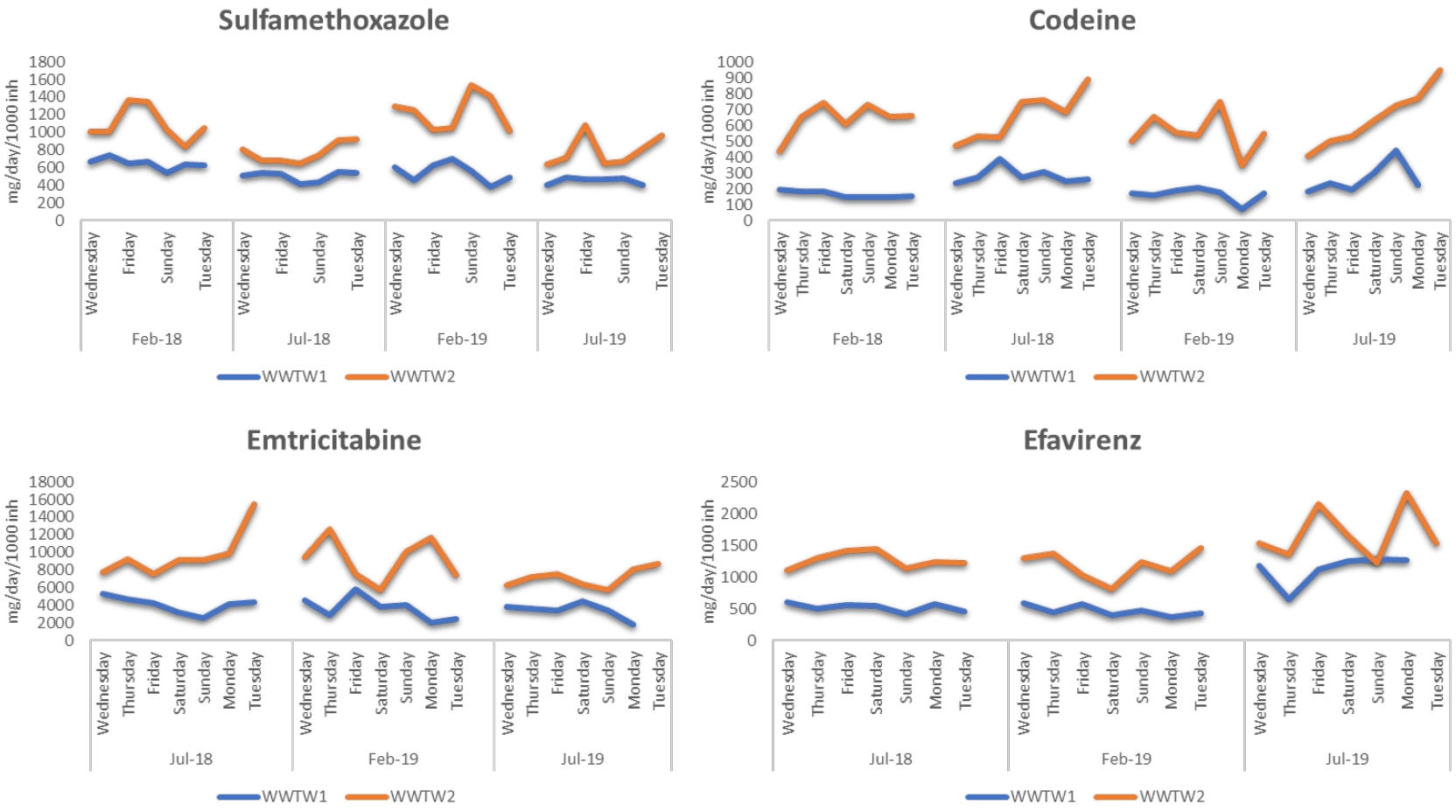


Figure 5-10: Population-normalised mass loads (grams per day per 1,000 inhabitants) of selected pharmaceutical substances, quantified from the raw wastewater at the two WWTWs during the study period

5.3.3 Environmental risk assessment

The results generated for the two-year monitoring study at two Western Cape WWTWs for the selected target analytes were evaluated using current PNEC values obtained from the NORMAN Network database (Table 5-2). The calculated PNECs from this network are derived from either predicted QSAR models and/or experimental results for various anthropogenic pollutants. The estimated RQs were calculated according to the toxicity endpoints for each target CEC listed below:

Table 5-2: PNECs of the target CECs extracted from the NORMAN Network database (www.norman-network.net)

Chemical	PNEC (ng/ℓ)	Description
Diclofenac	144	Estimated IC_{50} *D. magna* growth rate
Naproxen	1,853	Estimated LD_{50} in *D. magna*
Carbamazepine	2,276	Estimated IC_{50} *D. magna* growth rate
10,11-dihydro-11-hydroxycarbamazepine	2,388	Estimated IC_{50} *D. magna* growth rate
Benzotriazole	7,765	Estimated IC_{50} *S. capricornutum* growth rate
Codeine	7,186	Estimated IC_{50} *D. magna* growth rate
Caffeine	13,759	Estimated IC_{50} *D. magna* growth rate
Acetaminophen	15,820	Estimated EC_{50} immobilisation in *D. magna*
Cocaine	3,446	Estimated LD_{50} in *P. promelas*
Benzoylecgonine	2,334	Estimated LD_{50} in *P. promelas*
Methamphetamine	9,736	Estimated EC_{50} immobilisation in *D. magna*
Methaqualone	723	Estimated IC_{50} *S. capricornutum* growth rate
MDMA	47,601	Estimated EC_{50} immobilisation in *D. magna*
Sulfamethoxazole	3,080	Estimated IC_{50} D*. magna* growth rate
Emtricitabine	23,765	Estimated IC_{50} *S. capricornutum* growth rate
Efavirenz	201	Estimated LD_{50} in *P. promelas*

Based on the results presented in Table 5-3, high to severe risk was estimated for the NSAID diclofenac, the illicit drug methaqualone, the antibiotic sulfamethoxazole and the ARVs emtricitabine and efavirenz. Moreover, moderate risk is still predicted for the anti-convulsant carbamazepine and its metabolite, the opioid codeine and the lifestyle chemical caffeine. The results across the entire sampling period (February 2018 to July 2019) did not show much temporal variation in the toxicological risks for the various CECs. This could be ascribed to the treatment plant that needs to maintain constant operating conditions over time, especially for WWTW-2, which operates with a maturation pond, allowing for extended residence time of treated secondary effluent before disinfection. Regardless of this extended maturation of treated effluent, the levels of some CECs remained within the range to show similar toxicological risks for a plant that was not sampled after its maturation ponds (WWTW-1).

Table 5-3: Risk quotients estimated for the target CECs in treated WWTW-1 and WWTW-2 effluent during the two-year monitoring study

WWTW-1	February 2018							July 2018							February 2019							July 2019						
	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue
Diclofenac	3.1	3.2	3.1	2.9	3.2	3.1	3.5	4.9	4.5	4.1	4.2	4.9	4.6	4.0	3.7	3.6	3.9	3.7	4.3	4.3	4.3	2.0	2.6	3.3	3.1	3.0	4.4	4.4
Naproxen	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.4	0.3	0.5	0.3							
Carbamazepine	0.4	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.4	0.4	0.5	0.4	0.5	0.6	0.2	0.7	0.6	0.3	0.3	0.3	0.2
10,11-dihydro-11-hydroxycarbamazepine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1			0.1	0.1	0.1	0.1	0.0
Benzotriazole	0.0	0.0		0.0		0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Codeine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.0		0.0			
Caffeine	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.6	0.4	0.4	0.3	0.3	0.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetaminophen		0.0	0.0			0.0		0.2	0.3	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1				0.0			
Cocaine								0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						0.0	0.0
Benzoylecgonine	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.4	0.3	0.0				0.0	0.0	
Methamphetamine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2						0.0	
Methaqualone								2.4	2.4	2.3	2.4	1.9	1.9	1.7	3.3	3.1	2.6	2.6	2.2	3.9	3.0	0.1	0.2	0.3	0.4	0.3	0.2	0.1
MDMA								0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0						0.0	
Sulfamethoxazole	0.7	0.8	0.8	1.0	1.2	0.9	0.8	1.4	1.2	1.1	1.2	1.3	1.2	1.7	1.3	1.4	1.5	1.4	1.2	1.8	1.2	0.4	0.9	0.9	0.6	0.8	1.3	0.7
Emtricitabine								1.6	1.8	1.7	1.7	1.3	1.8	1.5	2.0	2.2	1.7	1.7	1.8	2.2	1.6	0.4	0.7	0.8	0.7	0.6	1.8	0.5
Efavirenz								40.2	35.8	43.7	49.6	42.9	53.2	54.8	42.6	45.8	28.7	27.8	42.4	46.2	37.7	35.0	31.9	51.2	50.9	56.8	90.9	37.5

WWTW-2	February 2018							July 2018							February 2019							July 2019						
	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue
Diclofenac	3.0	2.8	2.7	3.2	3.2	2.9	2.6	4.8	4.6	5.5	4.8	5.3	5.2	4.5	1.6	1.8	2.1	2.4	2.6	2.5	1.8	5.2	4.8	3.6	4.5	5.8	6.1	
Naproxen	0.1	0.1	0.1	0.1	0.1	0.2	0.0															0.2	0.2	0.2	0.3	0.3	0.4	0.4
Carbamazepine	0.8	0.8	1.0	1.0	0.9	0.9	0.8	1.2	1.4	1.3	1.1	0.7	0.6	0.6	0.7	0.6	0.6	0.5	0.7	0.5	0.4	0.3	0.5	0.5	0.4	0.2	0.2	0.4
10,11-dihydro-11-hydroxycarbamazepine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Benzotriazole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.0
Codeine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.2
Caffeine	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.2	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.3	0.2	0.3	0.5	0.6	0.7	0.6
Acetaminophen				0.0				0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.1	0.0
Cocaine															0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0
Benzoylecgonine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.2
Methamphetamine								0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2
Methaqualone								0.9	0.9	0.8	0.7	0.6	0.7	0.7	2.3	2.3	2.4	2.3	2.4	2.4	2.3	2.0	1.7	1.7	2.4	3.2	4.0	2.6
MDMA															0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0			0.0	0.0	0.0
Sulfamethoxazole	1.1	1.1	1.3	1.6	1.9	0.8	1.8	1.1	0.8	0.9	0.9	1.0	1.1	1.1	0.9	1.0	1.0	1.0	1.4	1.2	1.2	0.4	0.5	0.6	1.0	0.9	1.3	0.9
Emtricitabine								0.6	0.5	0.6	0.6	0.8	0.7	0.6	0.7	0.7	0.6	0.6	0.9	0.8	0.6	1.0	0.8	0.7	1.1	1.3	1.4	0.9
Efavirenz								48.1	51.0	55.4	59.9	52.6	53.1	48.1	30.0	33.3	28.9	41.3	42.7	46.1	40.6	49.3	52.3	52.6	62.6	55.9	65.5	58.4

Low risk: RQ < 0.1; green; Median risk: 0.1 < RQ < 1.0; orange; High risk: RQ > 0.1; red, as indicated by Hernando et al., 2006.

Values highlighted in red and yellow text indicate a severe risk (RQ > 10)

These results further confirm the prioritisation of recalcitrant or high-load pseudo-persistence of the CECs diclofenac, sulfamethoxazole, methaqualone, carbamazepine and the ARVs emtricitabine and efavirenz to receive further intervention of the following:

- Their potential ecological risk on other sentinel indicators
- Their interaction with various biochemical pathways (potential sub-lethal toxicity responses)
- The persistence of their breakdown products and/or partitioning profiles to the aqueous, solid matrices and bio-accumulation in freshwater biota

5.4 SUMMARY

This study investigated the temporal presence and fate of selected CECs at two Western Cape WWTWs. Treated effluent for WWTW-2 showed an overall higher removal profile, which may be attributed to the lower loads recorded to be received by the plant, as well as the fact that the treated effluent is discharged into a maturation pond (extended hydraulic retention time) prior to disinfection and discharge. Regardless, the anti-convulsant carbamazepine still showed an overall negative removal (increased loads at the effluent samples) for both WWTWs, which further highlights the need for more in-depth monitoring and investigation into the adverse health outcomes of both the parent drug and its primary or secondary metabolites. Although the primary metabolites of all the target CECs were not analysed in this study, future studies should consider analysing for such contaminants, as the current results still do not distinguish whether the target CECs are “removed” by the plant, or merely transformed to primary and secondary metabolites, the toxicological risk of which is currently unknown. Alternatively, the need for EBM approaches may overcome the uncertainty regarding whether the treatment plant, in fact, removes the toxicological risk of the complex mixtures of wastewater.

Using WBE, the current study investigated substance use (and abuse) trends in a community in the Western Cape. This is the first ever report to show the presence and fate of the illicit drug methaqualone (mandrax) in a wastewater monitoring study, and the first study for South Africa on the loads of the corrosion inhibitor compound benzotriazole. The illicit use of methaqualone is high in South Africa, but not for countries in the rest of the world, which explains why this drug is normally ignored in global monitoring studies. However, given its high loadings in WWTWs (like methamphetamine) and its moderate persistence through wastewater treatment, this drug should receive priority to establish its potentially adverse health effect in recipient surface waters. For the other CECs, moderate to low removal of carbamazepine (and a metabolite), diclofenac, sulfamethoxazole, emtricitabine and efavirenz is recorded for both WWTWs, also highlighting the need for these analytes to be monitored for their potentially adverse health risks.

Wastewater reclamation has become crucial in recent times to sustain water security in the drought-stricken Western Cape. More recently, other parts of the country, including the Northern Cape, Eastern Cape and Gauteng, are also facing drought impacts due to various climatic and anthropogenic influences. For this reason, the surveillance of CECs in water bodies destined for potable and/or non-potable reuse should receive greater priority in the country. The current study produced data for four months of samples over different seasons and daily screenings (over seven consecutive days) each month. This approach resulted in a targeted analysis of 28 raw influent and 28 treated effluent samples for each WWTW during the study period. The addition of flow-proportional mass loadings of the target analytes (grams per day), along with the addition of labelled internal standards, allowed for better evaluation of WWTW performance and analyte quantification in changing sample matrices. Although no clear temporal variation was shown for any of the CECs in the study at both WWTWs, changes in CEC mass balances over time allowed for the evaluation of WWTW performance over an expanded period of time, along with verification of the recalcitrance of priority CECs that need to be considered for further risk assessment.

CHAPTER 6: *IN-VITRO* ANALYSIS OF ESTROGENICITY AT SOUTH AFRICAN WASTEWATER TREATMENT WORKS – CASE STUDY[7]

6.1 INTRODUCTION

The aim of this study was to use an *in-vitro* recombinant yeast receptor binding assay to assess the level and persistence of estrogenicity at several South African WWTWs that have received good accreditation from the DWS's Green Drop initiative[8]. The seasonal and daily variation of estrogenicity within WWTW influent and effluent, as well as the associated river waters upstream and downstream of the plants, were compared and considered in the context of potential adverse health risks associated with the measured estrogenic concentrations. The influence of compounds that may antagonise estrogen-mediated receptor binding (anti-estrogens) in the bioassay used for the present study was also investigated to further elaborate on the potential endocrine-disrupting risk that treated wastewater may pose on surface waters spanning multiple modes of action.

6.2 MATERIALS AND METHODS

6.2.1 Site of study and sampling

The study site was situated in the East Rand of Gauteng. This region of the country experiences its rainfall during summer (December to February), whereas a colder, but drier climate is experienced in winter (June to August). Ten WWTWs were selected for the study, which vary in operating capacity, treatment processes and sources of wastewater (Table 6-1). The 10 WWTWs were all sampled during summer 2015/16 (December) and winter 2016 (June) for five consecutive days (Monday to Friday). No rainfall was recorded at any of the study sites during the summer 2015/16 and winter 2016 sampling campaigns.

Table 6-1: Information on the sample sites during the current study

Source	Load (domestic:industrial)	Capacity (Mℓ/day)	Population estimate[#]
WWTW-1	70:30	105.0	528,942[*]
WWTW-2	60:40	55.0	260,448[*]
WWTW-3	100:0	0.4	4,516
WWTW-4	100:0	16.0	23,273
WWTW-5	90:10	35.0	275,929
WWTW-6	60:40	32.0	229,674[*]
WWTW-7	83:17	36.0	73,574
WWTW-8	96:4	83.0	330,473
WWTW-9	100:0	10.0	35,397
WWTW-10	96:4	155.0	1,092,297

[#] Based on COD measurements generated from raw sewage influent from each WWTW. Plants marked with an asterisk should receive attention for population estimates, as a significant load of industrial wastewater could contribute towards COD counts in wastewater influent.

[7] The full report for this study has been accepted for publication in *Environmental Pollution*: Archer, E., Wolfaardt, G.M., Van Wyk, J.H., and Van Blerk, N., 2020. Investigating (anti)-estrogenic activities within South African wastewater and receiving surface waters: Implication for reliable monitoring. *Environmental Pollution*. Date of acceptance: 19 March 2020.
[8] http://dwa.gov.za

Grab samples (200 mℓ) were collected from both the influent (after grit screens) and effluent (after chlorination) each morning. Samples were acidified to pH 3 using hydrochloric acid (HCl) and stored at 4 ^{0}C. These daily samples were topped up until the final sampling day (Friday) when analyte extraction took place, giving a five-day semi-composite sample for each WWTW. Four WWTWs were selected and further sampled during summer 2016/17 (December) for seven consecutive days (Monday to Sunday). High rainfall was recorded for the whole study region during this period. Grab samples (200 mℓ) of both influent and effluent wastewater were collected each morning, followed by immediate filtration and sample processing, giving a daily grab sample for each of the four WWTWs.

For all the sampling campaigns (December 2015, December 2016 and July 2016), grab samples of surface water (200 mℓ) in connected rivers were also taken at locations upstream (50 m) and downstream (50 m) of the respective points of discharge of the WWTWs. However, daily sampling of river water was not possible due to logistical constraints. Therefore, samples were collected on a single day during the week, acidified (pH 3) using HCl and kept cold (± 4 ^{0}C) during transportation and storage until analyte extraction.

6.2.2 Sample extraction

Water samples were filtered using glass fibre filters (1.2 µm pore size; Munktell) with a glass vacuum filtering system (Millipore) to get rid of solids in the samples. The filtrate was then extracted by SPE using Oasis® HLB cartridges (6 cc, 200 mg; Waters). The cartridges were conditioned with 4 mℓ MeOH, followed by 4 mℓ of ultrapure water (Millipore) and allowed to pass through the column by gravity. After conditioning, the water samples (200 mℓ) were passed through using a manifold (Supelco, Visiprep) at a flow rate of 5 mℓ per minute and allowed to run dry for a minimum period of 30 minutes. The dried cartridges were eluted with 5 mℓ MeOH (HPLC grade; Sigma) by gravity and then dried under a gentle stream of nitrogen. The evaporated samples were then re-suspended in 400 µℓ MeOH, giving a 500x concentrated water sample. All samples were stored in 2 mℓ amber glass vials (CNW Technologies, Stargate Scientific) and stored at -20 ºC until the bioassays were done.

6.2.3 *In-vitro* analysis procedure

The YES protocol adopted for this study is described by Sohoni and Sumpter (1998). The assay medium was prepared and the yeast was incubated in an assay medium containing no CPRG for 48 hours under 26 ºC on an orbital shaker. The concentrated wastewater extracts (500x) were serially diluted and 10 µℓ was spiked into the 96-well sterile flat-bottomed plates with low evaporation lids (Costar, 3370, Sigma). The previously incubated yeast culture was then included in a new assay medium containing CPRG at a concentration of approximately 8 x 10^{5} cells per mℓ. The seeded assay medium was then added at 200 µℓ per well into the assay plate to provide a final concentration of the water extracts, ranging from 50x to 1.56x. A concentration of 1x was depicted as an unconcentrated water sample. For the raw wastewater samples, serial dilutions of the samples were made with MeOH to obtain a concentration range for each sample ranging from 12.5x to 0.39x in the assay due to cytotoxicity observed in the 50x and 25x concentrated samples.

For the final effluent and river water samples, serial dilutions of the samples were made with MeOH to obtain a concentration range for each sample ranging from 50x to 6.25x due to the lower observed estrogenicity in these samples compared to raw wastewater samples. All samples were analysed in triplicate in the same assay plate, and each assay was repeated twice. A standard curve for the steroid hormone 17β-estradiol (CAS 50-28-2; Sigma) was included for each assay plate in 12 serial dilutions, ranging from 1.0 to 2,700.0 ng/ℓ. Blank wells were also included in each assay plate containing only the assay medium and 10 µℓ of evaporated MeOH without any hormone spike or water sample extracts. The assay plates were then allowed to incubate on a shaker for 72 hours at 30 ºC under dark conditions.

The Yeast Anti-estrogen Screen (YAES) was performed for the treated wastewater effluent samples during two sampling campaigns (winter 2016 and summer 2016/17) in the same manner as described for the YES, with minor modifications. Each well in the 96-well assay plate was spiked with a submaximal E_2 concentration of 450 ng/ℓ prior to the addition of the concentrated water extracts (final concentration of 75x, 50x and 25x). Apart from an E_2 standard curve as used in the YES, the YAES contained a positive control of the estrogen receptor antagonist tamoxifen (TAM) (CAS 10540-29-1; Sigma) in 12 serial dilutions (0.91 to 1,857.6 mg/ℓ in the assay). The blank wells were separated into two sets; one set containing the assay medium and yeast with a submaximal E_2 spike (six wells) and one set containing blank wells with only the assay medium and yeast (six wells). The assay plates were then allowed to incubate on a shaker for 72 hours at 30 °C under dark conditions.

Upon the 72 hours of incubation, the YES and YAES assay plates were measured for colour change using a spectrophotometer. The absorbance was measured at 570 nm for colour change of CPRG caused by steroid hormone-mediated β–galactosidase production, and 620 nm for turbidity change and cytotoxicity. The turbidity change calculations (620 nm) were necessary to assess potential false negative or positive results generated for the colour change calculations (570 nm), as a loss in turbidity (caused by cytotoxic analytes in the sample) leads to less viable yeast cells in the assay to produce β-galactosidase. The threshold for cytotoxicity in the samples was determined using Equation 6-1. Samples that were below this threshold were excluded from further calculations.

$$\text{Cytotoxicity} = \text{Median Blank}_{620nm} - (3*\text{stdev of Blank}_{620nm}) \quad \text{[Eq. 6-1]}$$

To correct for turbidity in the wells, a corrected absorbance (CA) was calculated for each sample in the assay using Equation 6-2. This calculation compensated for background absorbance from the yeast suspension and allowed for more accurate measurement of the colour change in the assay medium.

$$\text{Corrected absorbance (CA)} = (OD_{570nm} - [OD_{620nm} - \text{Blank}_{620nm}]) \quad \text{[Eq. 6-2]}$$

where OD_{570nm} and OD_{620nm} refer to the optical density of the sample measured at 570 nm and 620 nm, respectively, and Blank_{620nm} refers to the median optical density measured for the blank wells in each assay plate at 620 nm.

Water samples were only considered for further analysis if the corrected absorbance was above a detection threshold using Equation 6-3:

$$\text{Detection} = \text{Median blank}_{CA} + (3*\text{stdev of Blank}_{CA}) \quad \text{[Eq. 6-3]}$$

For the YES, the CA of water samples above the detection threshold of the assay were log-transformed and expressed as a percentage of the maximum log-absorbance value calculated in the E_2 standard curve using Equation 6-4:

$$\text{Log \% max } E_2 \text{ (sample/standard curve)} = (\text{log-CA}_{sample} / \text{log-CA}_{E2\ max}) * 100 \quad \text{[Eq. 6-4]}$$

A non-linear calibration curve was then constructed for the E_2 standard curve of each individual assay plate by plotting the calculated log % max E_2 of the E_2 dilution series against its known concentration (in ng/ℓ). An E_2 EEQ (ng/ℓ) for each water sample was then calculated from the generated trend line of the calibration curve and corrected for their dilution factors to obtain the final EEQ concentration of each water sample (in ng/ℓ).

The calculated EEQ values were then used to estimate their mass loads within raw wastewater and treated effluent to compensate for the variation in flow rates of the various treatment plants between the daily and seasonal sampling campaigns using Equation 6-5:

$$\text{Mass load (g/day)} = EEQ_{inf/eff} * FR_{inf/eff} * 1/1000 \quad \text{[Eq. 6-5]}$$

where $EEQ_{inf/eff}$ refers to the EEQ concentration (in ng/ℓ) of the samples in the YES for influent and effluent wastewater samples, and $FR_{inf/eff}$ refers to the flow rate (Mℓ per day) of the influent and effluent wastewater for each WWTW during the time period of the sampling campaigns.

To estimate the removal efficiency of estrogenicity at the WWTW, removal percentage from each raw wastewater sample and the corresponding treated effluent sample were calculated using Equation 6-6:

$$\text{Removal (\%)} = (M\ell_{inf} - M\ell_{eff}) / M\ell_{inf} * 100 \quad \text{[Eq. 6-6]}$$

where $M\ell_{inf/eff}$ refers to the mass loads (in grams per day) of the samples calculated from Equation 5-5 for influent and effluent wastewater samples at the various WWTWs.

For the YAES, the CA of the water extracts was compared with the CA measured for the blank wells containing the submaximal E_2 spike to evaluate the percentage change of the sample extracts from the submaximal E_2 spike. Samples that were above the E_2 spike threshold (above 100%) were considered to have a masking effect of inert estrogenicity supressing the anti-estrogen response, whereas samples below the E_2 spike threshold (below 100%) were considered to contain analytes that significantly supress the binding of E_2 to the hER in the assay, showing an anti-estrogenic response. The samples that successfully supressed E_2-mediated receptor binding were considered for the quantification of a TAM EEQ (µg/ℓ), calculated in a similar manner as was calculated for the EEQ calculations in the YES.

6.3 RESULTS AND DISCUSSION

6.3.1 Estrogenic activity in wastewater effluents

Raw influent wastewater showed varying levels of estrogenic activity during the various sampling campaigns, which ranged from 4.4 to 45.4 ng/ℓ EEQ during the summer periods (2015/16 and 2016/17) (Table 6-2 and 6-3) and 6.6 to 31.5 ng/ℓ EEQ during the winter 2016 sampling (Table 6-2). WWTW-2, WWTW-7 and WWTW-10 showed the highest levels of estrogenicity during summer 2015/16 (Table 6-2), and WWTW-1, WWTW-3 and WWTW-5 showed the highest levels during winter 2016 (Table 6-3). However, by taking the variations in plant flow rates into consideration, EEQ mass loads (grams per day) showed that WWTW-1, WWTW-2, WWTW-8 and WWTW-10 had the highest levels during summer 2015/16, compared to WWTW-1, WWTW-2, WWTW-5 and WWTW-10, which had the highest levels during winter 2016 (Figure 6-1). As it is expected that the estrogenic response in the YES will most likely be from natural and synthetic steroids (associated with domestic wastewater), the WWTWs that received the largest estrogenic loads were those that predominantly receive domestic sewage, apart from WWTW-2, which receives a large proportion of industrial wastewater. For the selected WWTWs screened during summer 2016/17, the EEQ estimations within raw wastewater did not differ significantly between sampling days for WWTW-2, WWTW-9 or WWTW-10 (ANOVA, $P > 0.05$) (Table 6-3). However, EEQ values for WWTW-8 showed a significant variation in estrogenicity between sampling days (ANOVA, $P < 0.05$), with values on Tuesday and Wednesday being significantly lower than the weekend samples (Tukey, $P < 0.001$).

Table 6-2: Estradiol-equivalent concentrations (ng/ℓ ± standard deviation) measured within the various water matrices from the WWTWs and environmental waters during December 2015 (summer) and June 2016 (winter)

Site	Summer 2015/2016					Winter 2016				
	Influent	Effluent	Removal (%)	Upstream	Downstream	Influent	Effluent	Removal (%)	Upstream	Downstream
WWTW-1	16.8 ± 4.1****	0.4 ± 0.2*	98	11.8 ± 2.3****	6.5 ± 1.2****	30.1 ± 4.9****	0.5 ± 0.01*	99	6.3 ± 0.2****	1.9 ± 0.01**
WWTW-2	34.1 ± 0.4****	0.3 ± 0.1	99	0.2 ± 0.1	0.8 ± 0.4**	23.5 ± 2.1****	0.9 ± 0.3**	96	0.2 ± 0.03	0.2 ± 0.01
WWTW-3	14.9 ± 3.4****	0.4 ± 0.1*	97	1.3 ± 0.7**	0.8 ± 0.3**	31.5 ± 2.8****	0.5 ± 0.01	98	-	0.6 ± 0.01*
WWTW-4	11.8 ± 1.3****	-	-	1.5 ± 0.5**	0.3 ± 0.1	13.5 ± 0.1****	-	-	0.8 ± 0.06**	-
WWTW-5	4.4 ± 1.1***	0.7 ± 0.4**	84	-	0.4 ± 0.2*	29.4 ± 5.4****	1.4 ± 0.03**	95	-	0.6 ± 0.1*
WWTW-6	5.3 ± 2.2****	0.9 ± 0.3**	84	-	1.3 ± 0.8*	7.0 ± 1.6****	1.4 ± 0.04**	81	0.3 ± 0.02	1.6 ± 0.1**
WWTW-7	29.4 ± 6.3****	0.3 ± 0.1	99	2.5 ± 0.9***	0.5 ± 0.3*	11.4 ± 2.0****	0.2 ± 0.01	98	-	-
WWTW-8	8.8 ± 2.7****	2.6 ± 1.3***	70	1.2 ± 0.5**	0.5 ± 0.1*	6.6 ± 0.7****	4.9 ± 0.2***	26	-	5.0 ± 0.1****
WWTW-9	7.8 ± 2.5****	6.9 ± 0.3****	13	7.5 ± 0.2****	9.5 ± 0.7****	15.1 ± 0.9****	1.3 ± 0.03**	91	18.9 ± 0.2****	11.3 ± 1.9****
WWTW-10	22.8 ± 4.9****	1.1 ± 0.04**	95	0.6 ± 0.3*	0.4 ± 0.1*	15.3 ± 3.8****	0.9 ± 0.03**	94	1.2 ± 0.01**	0.6 ± 0.06*

* EEQ trigger value (0.4 ng/ℓ) of effluent estrogenicity on long-term fish exposure (Jarošová et al., 2014)

** Estimated trigger value (0.7 ng/ℓ) for E_2 in humans (Genthe et al., 2013)

*** Predicted no-effect concentration (2 ng/ℓ) for E_2 to modulate fish reproduction (Caldwell et al., 2012)

**** Concentration of E_2 (5 ng/ℓ) showing increased VTG production in adult male zebrafish (Brion et al., 2004)

Table 6-3: Daily variation in estradiol-equivalent concentrations (ng/ℓ) using the YES for the various WWTWs during December 2016/17 (summer)

Day	WWTW-2			WWTW-8			WWTW-9			WWTW-10		
	Influent	**Effluent**	**Removal**	**Influent**	**Effluent**	**Removal**	**Influent**	**Effluent**	**Removal**	**Influent**	**Effluent**	**Removal**
Mon	15.7 ± 1.6****	1.1 ± 0.3**	93	14.3 ± 2.3****	1.3 ± 0.6**	91	28.9 ± 4.0****	0.9 ±0.4**	97	21.0 ± 2.6****	1.5 ± 0.4**	95
Tue	13.9 ± 4.3****	2.6 ± 0.3***	81	6.9 ± 0.9****	1.5 ± 0.3**	78	27.9 ± 3.3****	1.7 ±0.2**	94	45.4 ± 3.0****	0.5 ± 0.3*	99
Wed	13.5 ± 0.9****	1.6 ± 0.4**	88	8.2 ± 1.1****	1.6 ± 0.3**	80	27.7 ± 2.8****	1.9 ±0.4**	93	22.3 ± 3.2****	1.1 ± 0.2**	95
Thu	-	1.2 ± 0.3**	-	9.2 ± 2.6****	3.0 ± 0.9***	67	23.8 ± 1.2****	1.2 ±0.3**	94	22.1 ± 3.9****	1.3 ± 0.1**	99
Fri	10.8 ± 2.2****	0.7 ± 0.1**	94	12.7 ± 0.8****	1.0 ± 0.2**	92	21.7 ± 3.3****	1.5 ±0.01*	92	21.2 ± 4.8****	-	-
Sat	14.3 ± 1.5****	1.4 ± 0.2**	90	13.0 ± 3.3****	1.5 ± 0.4**	88	25.4 ± 2.5****	0.6 ±0.2*	98	21.2 ± 6.4****	1.8 ± 0.2**	99
Sun	10.6 ± 3.6****	0.8 ± 0.3**	93	15.1 ± 3.4****	1.5 ± 0.8**	90	24.7 ± 11.9****	0.4 ±0.1*	98	24.4 ± 2.5****	2.1 ± 0.7***	99
Ave	**13.1 ± 2.0****	**1.3 ± 0.6****	**90**	**11.3 ± 3.2****	**1.6 ±0.6****	**84**	**26.8 ± 3.5****	**0.7 ±0.6***	**97**	**25.4 ±8.9****	**1.4 ± 0.4****	**98**

* EEQ trigger value (0.4 ng/ℓ) of effluent estrogenicity on long-term fish exposure (Jarošová et al., 2014)

** Estimated trigger value (0.7 ng/ℓ) for E_2 in humans (Genthe et al., 2013)

*** Predicted no-effect concentration (2 ng/ℓ) for E_2 to modulate fish reproduction (Caldwell et al., 2012)

**** Concentration of E_2 (5 ng/ℓ) showing increased VTG production in adult male zebrafish (Brion et al., 2004)

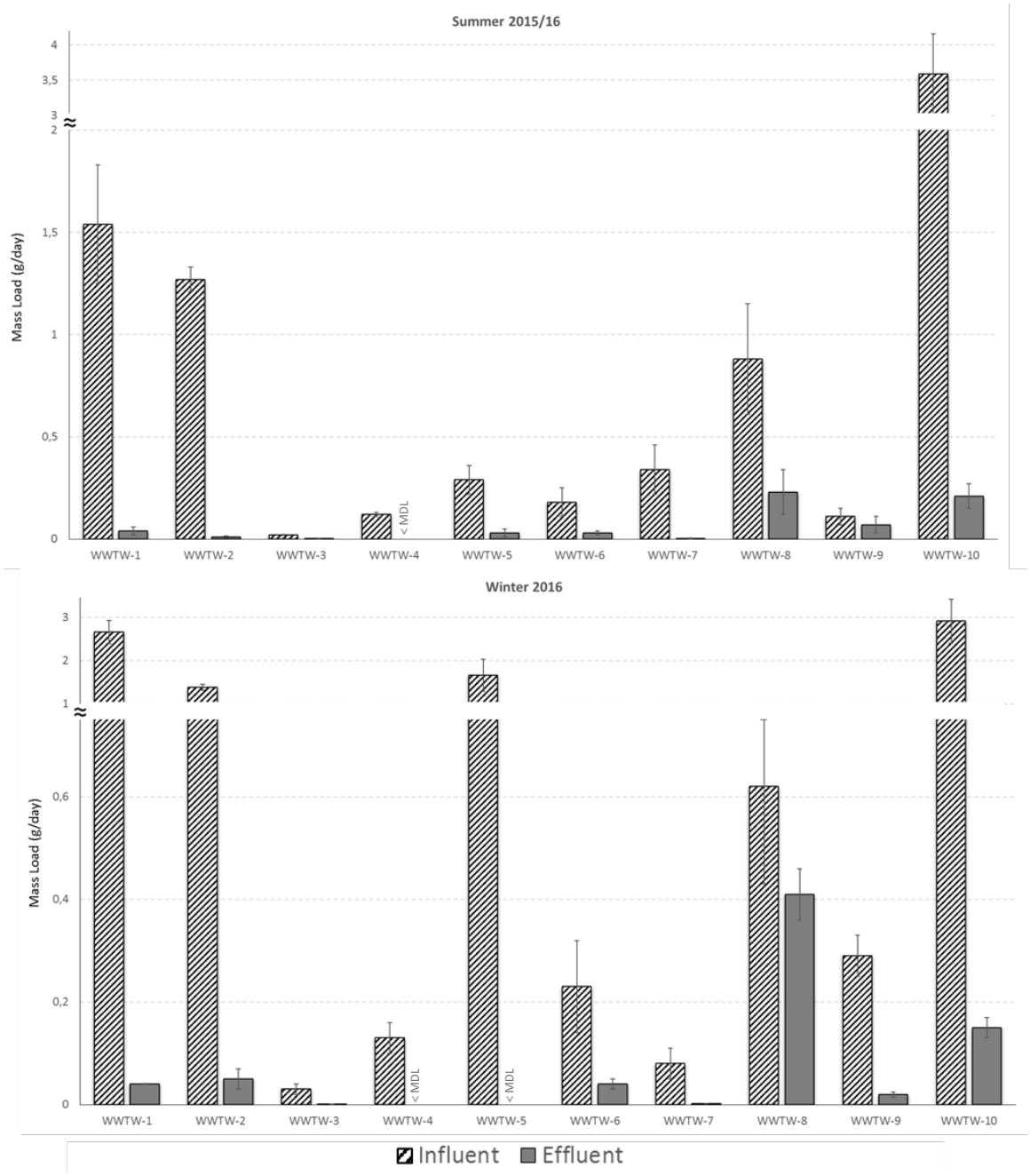
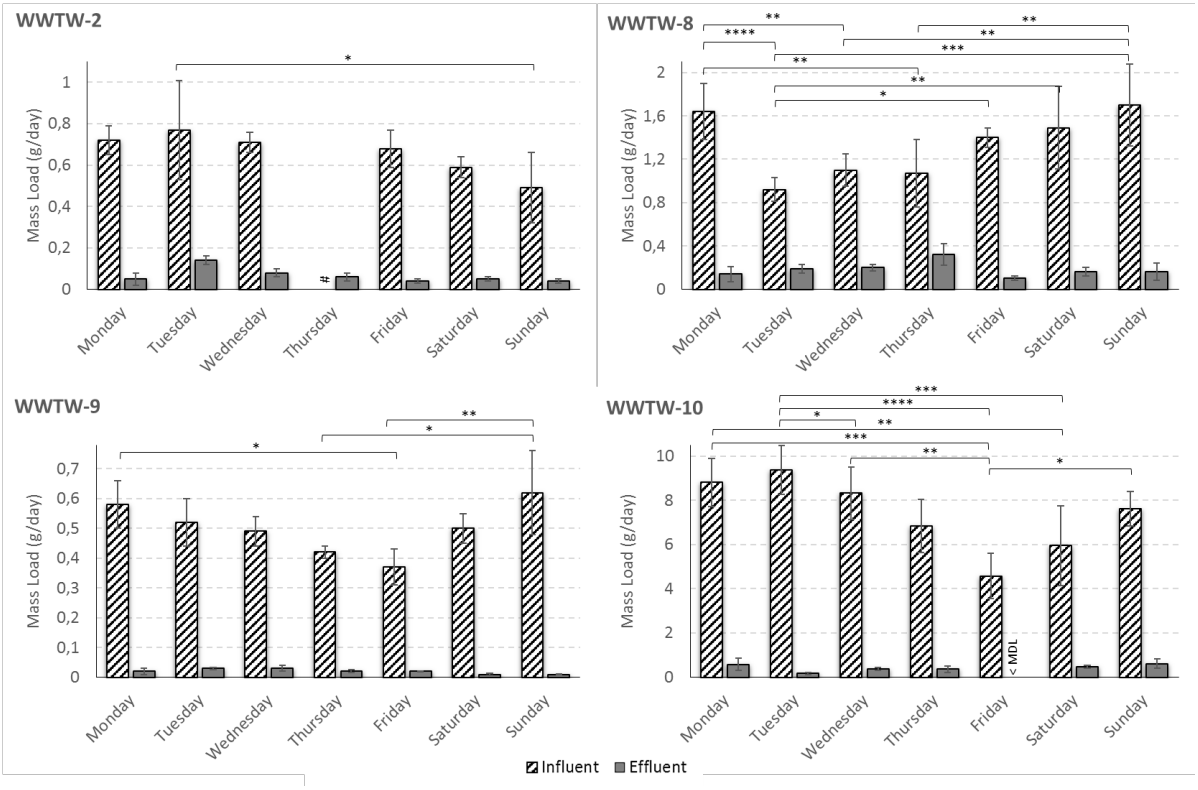


Figure 6-1: Mass load (grams per day) of EEQ measured using the YES for the various WWTWs during sampling campaigns in summer 2015/16 (December) and winter 2016 (June)

The variation in daily flow rates could also impact on the variation of net estrogenicity between sampling days. For this reason, EEQ mass loads were instead used to compare between sampling days. For WWTW-2, significant variation was only calculated between the Tuesday and the Sunday samples (ANOVA, $P = 0.033$; Tukey, $P < 0.05$) (Figure 6-2; Table 6-4). For WWTW-8, a significant increase in estrogenic load was calculated over the weekend period (Friday to Sunday), as well as for the following Monday (ANOVA, $P < 0.0001$; Tukey, $P < 0.05$) (Figure 6-2; Table 6-4). For WWTW-9, estrogenic load decreased as the week continued, and increased significantly from Friday to the weekend period (ANOVA, $P < 0.0001$; Tukey, $P < 0.05$) (Figure 6-2; Table 6-4). For WWTW-10, the same trend was observed as for WWTW-9, with a more pronounced decrease during the week, followed by an increase over the weekend period (ANOVA, $P < 0.0001$; Tukey, $P < 0.01$) (Figure 6-2; Table 6-4).

Again, WWTW-8, WWTW-9 and WWTW-10 receives domestic wastewater exclusively, whereas WWTW-2 receives a large proportion of industrial wastewater. Furthermore, the level of estimated EEQ mass loads calculated for these WWTWs (WWTW-10 > WWTW-8 > WWTW-2 > WWTW-9) (Figure 6-2) were in association with each plant's estimated population size. These results further suggest that estrogenicity is influenced by the *de facto* and *de novo* population being served by the sewage plants. As shown by the current study, the daily variation for estrogenicity in raw wastewater does not follow the same trend for all WWTWs (Figure 6-2), but highlights the fact that wastewater treatment monitoring for estrogenicity should not just be a periodic event, as significant variation in estrogenic compounds entering WWTWs may occur over time.



Statistically significant variation between sampling days is shown as:
**** = P < 0.0001; *** = P < 0.001; ** = P < 0.01; * = P < 0.05

#: Sample was lost during processing

Figure 6-2: Daily variation in mass loads (grams per day) of EEQ concentrations estimated for the various WWTWs during the summer 2016/17 sampling campaign (December)

The two summer sampling campaigns (December 2015/16 and 2016/17) allowed for comparisons between similar seasons experiencing varying climatic events, such as rainfall. The EEQ mass loads in raw wastewater influent between the two sampling campaigns did not differ significantly for WWTW-8 (t-test, p > 0.05), but were significantly different for WWTW-2, WWTW-9 and WWTW-10 (t-test, p < 0.05) (Table 6-4).

Table 6-4: Mass loads (grams per day) estimated using EEQ concentrations in the YES for both raw and effluent wastewater samples at the various WWTWs

Site	December 2015 (summer)			June 2016 (winter)			December 2016 (summer)		
	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)
WWTW-1	1.54 ± 0.29	0.04 ± 0.02	97	2.66 ± 0.27	0.04 ± 0.001	98	-	-	-
WWTW-2	1.27 ± 0.06	0.01 ± 0.004	99	1.38 ± 0.07	0.05 ± 0.02	96	0.66 ± 0.1	0.07 ± 0.04	89
WWTW-3	0.02 ± 0.003	0.0004 ± 0.0001	98	0.03 ± 0.01	0.001 ± 0.0002	98	-	-	-
WWTW-4	0.12 ± 0.01	-	-	0.13 ± 0.03	-	-	-	-	-
WWTW-5	0.29 ± 0.07	0.03 ± 0.02	90	1.66 ± 0.37	-	-	-	-	-
WWTW-6	0.18 ± 0.07	0.03 ± 0.01	83	0.23 ± 0.09	0.04 ± 0.01	83	-	-	-
WWTW-7	0.34 ± 0.12	0.003 ± 0.001	99	0.08 ± 0.03	0.002 ± 0.0001	98	-	-	-
WWTW-8	0.88 ± 0.27	0.23 ± 0.11	74	0.62 ± 0.19	0.41 ± 0.05	34	1.33 ± 0.3	0.18 ± 0.1	86
WWTW-9	0.11 ± 0.04	0.07 ± 0.04	36	0.29 ± 0.04	0.02 ± 0.005	93	0.50 ± 0.09	0.02 ± 0.01	96
WWTW-10	3.59 ± 0.57	0.21 ± 0.06	94	2.91 ± 0.51	0.15 ± 0.02	95	7.36 ± 1.69	0.37 ± 0.2	95

Higher loads were estimated during summer 2015/16 for WWTW-2 as opposed to higher loads that were recorded during summer 2016/17 for WWTW-9 and WWTW-10. To further demonstrate the complex nature of these analyses, it is worth considering the high rainfall pattern that was experienced during the summer 2016/17 sampling, which subsequently led to higher flow rate operations from the WWTWs. In particular, the flow rates for both raw influent and treated effluent at WWTW-10 increased more than two-fold. Due to the large treatment capacity of WWTW-10, raw wastewater destined for WWTW-7 and WWTW-8 is normally diverted to this plant during large runoff surges, such as rainfall events. The estimated EEQ concentrations (ng/ℓ) in raw wastewater for this plant between the two summer sampling campaigns did not differ significantly (t-test, $p > 0.05$) (Table 6-2 and 6-3), whereas EEQ mass loads (grams per day) increased by a factor of 2.1 from summer 2015/16 to summer 2016/17 (Table 6-4). As EEQ estimates were similar between summer 2015/16 and 2016/17, the two-fold increase in EEQ mass loads was merely due to a larger percentage of wastewater being treated, but not necessarily a dilution of the total estrogenic concentrations.

Estimating the exact source of estrogenicity within environmental samples may prove difficult. Although several pesticides, PPCPs and industrial by-products have been established as estrogenic EDCs, estrogenicity within waste and surface waters is primarily attributed to the presence of the synthetic estrogen EE_2, and secondarily to the presence of natural estrogen steroid hormones (Tanaka et al., 2000), merely due to steroid hormones being more potent estrogen-responsive agonists than the estrogen-mimicking EDCs. For example, plastisisers such as bisphenol-A (BPA) and phthalates are known to bind to the hER in a dose-dependent manner, although at far lower potencies than E_2 and EE_2 (Bistan et al., 2012). Levels of phthalates have also been detected at concentrations reaching mg/ℓ levels in South African surface waters (Fatoki et al., 2008), which warrants the potential of such known estrogen agonists to exert an additive mixture effect towards a net estrogenic response in bioassays such as the YES.

The EEQ mass load estimates (grams per day) were considered most useful to show the removal of estrogenicity at the various WWTWs and between sampling campaigns, rather than using raw EEQ concentrations (ng/ℓ). For example, EEQ estimations (ng/ℓ) for raw and treated effluent wastewater for WWTW-9 showed a removal of 13% during summer 2015/16, and a removal of 26% for WWTW-8 during winter 2016 (Table 6-3). By using EEQ mass load estimations, removal was 36% for WWTW-9 and 34% for WWTW-8, respectively (Table 6.3). EEQ loads showed moderate to high removal in all the WWTWs during the sampling periods (Table 6-2 and 6-3), except for the previously mentioned WWTWs. WWTW-8 showed moderate removal during summer 2015/16 and low removal during winter 2016 (Table 6.2), as well as variable removal between sampling days during summer 2016/17 (Table 6-3).

It should be stated that the wastewater residence time of each WWTW was not included for removal calculations. Therefore, such removal estimations are also considered semi-quantitative, as a raw influent and treated effluent sample taken during the same day does not reflect the time taken for the treatment of the influent sample before its effluent product is analysed in parallel. Regardless, the removal estimates from the current study show good agreement with similar studies using the YES (Murk et al., 2002). Although a moderate to significant removal of estrogenic compounds during wastewater treatment is reported (Manickum and John, 2014), the levels that will be discharged into recipient waters may still pose an environmental risk or may add to the pollutants that are also present within the surface water system.

The EEQ estimations in treated wastewater effluents from the various WWTWs varied from 0.3 to 6.9 ng/ℓ during summer 2015/16 (Table 6-2), 0.2 to 4.9 ng/ℓ during winter 2016 (Table 6-2) and 0.2 to 3.0 ng/ℓ during summer 2016/17 (Table 6-3). Mass loads of estimated EEQ concentrations, which compensated for flow variations between the WWTWs, ranged from 0.0004 to 0.23 grams per day during summer 2015/16, 0.001 to 0.41 grams per day during winter 2016, and 0.01 to 0.58 grams per day during summer 2016/17 (Table 6-4).

The estimated EEQ concentrations from the current study correlate well with similar monitoring studies done elsewhere in the world. EEQ concentrations estimated for two Canadian WWTW effluents ranged from 1.0 to 24.0 ng/ℓ (Arlos et al., 2018) and from 0.5 to 18.0 ng/L in WWTW effluent from 75 European WWTWs (Jarošová et al., 2014b). Although high EEQ concentations were estimated in the study by Jarošová et al. (2014b), it should be noted that final effluents of only two of the 75 WWTWs sampled showed EEQ concentrations above 10 ng/ℓ, most ranging between 1.0 and 4.0 ng/ℓ (Jarošová et al., 2014b).

Some studies have aimed to correlate the concentrations of steroid hormones with observed EEQ concentrations generated from assays such as the YES. A study by Truter et al. (2016) compared levels of E_2 and EE_2 with estimated EEQ values using the YES. Within a surface water sampling site, concentrations of E_2 ranged between 3.9 and 30.8 ng/ℓ, for EE_2 between 1.36 and 10.83 ng/ℓ, and for EEQ in the YES between 10.15 and 43.01 ng/ℓ. From these results, the combined load of the major estrogen analytes (E_2 and EE_2) correlated well with the EEQ concentrations generated by the YES in the same samples. However, the same was not observed for comparisons between the concentrations of known steroid hormones and EEQ estimates in various WWTW effluents, in which the EEQ values were much lower than concentrations of the known estrogenic micropollutants (Aerni et al., 2004).

The current study did not analyse the levels of steroid hormones. However, concentrations of E_2 from WWTW effluents have been shown to range from 1.0 to 20.0 ng/ℓ in South African monitoring studies (Manickum et al., 2011). Although these maximum levels of E_2 detected in WWTW influent and effluent are higher than shown for total estrogenicity in the current study, it should be noted that the current assay deals with a mixture of micro-pollutants in the water sample. Therefore, analytes that do not necessarily bind to steroid hormone receptors, but interfere with steroid receptor binding (such as anti-estrogens) may influence the results of the assay. For this reason, the presence of anti-estrogenicity was measured in treated wastewater to verify such a masking effect.

The estimated TAM-EQ concentrations ranged from 3.7 to 78.7 ug/ℓ in the treated wastewater from the ten WWTWs during the winter 2016 sampling campaign (Figure 6-3), and from 0.7 to 10.0 ug/ℓ for four WWTWs during the summer 2016/17 sampling campaign (Table 6-5). The results from the YAES indicated a masking effect by estrogenic compounds present in the water samples, whereby estrogenicity in the YAES decreased in the concentrated water extracts in a dose-dependent manner (data not shown). Higher estrogenicity was also associated with lower anti-estrogenicity for most of the WWTW effluent samples (Figure 6-3). Pollutants contributing to anti-estrogenicity may vary between the location of WWTWs, which are influenced by the type of industry in the area, as well as different agricultural practices and types of crops that necessitate different pesticide applications. Recalcitrant pollutants with the ability to antagonise estrogen receptor binding may potentially persist throughout wastewater treatment processes. For example, the bisphenol antiseptic hexachlorophene (HCP), the synthetic vitamin menadione and the pesticide/disinfectant pentachlorophenol (PCP) have all shown affinity to antagonise estrogen receptor binding and associated transcriptional activity by several *in-vitro* assays (Jung et al., 2004). Anti-estrogenicity has also been identified for several fungicides, herbicides and a neonicotinoid (Westlund and Yargeau, 2017). Fragrances and UV filters are also known anti-estrogens, all with demonstrated recalcitrance to biodegradation (Hernandez Leal et al., 2010). Furthermore, degradation processes may create undesired by-products, which may lead to anti-estrogenicity in treated wastewater. In particular, chlorination is known to increase anti-estrogenicity in wastewater by means of dissolved organic matter, creating various disinfection by-products (Wu et al., 2009). Focused studies at WWTWs that apply chlorination as the final disinfection step prior to and after the chlorination step should provide valuable information in this regard.

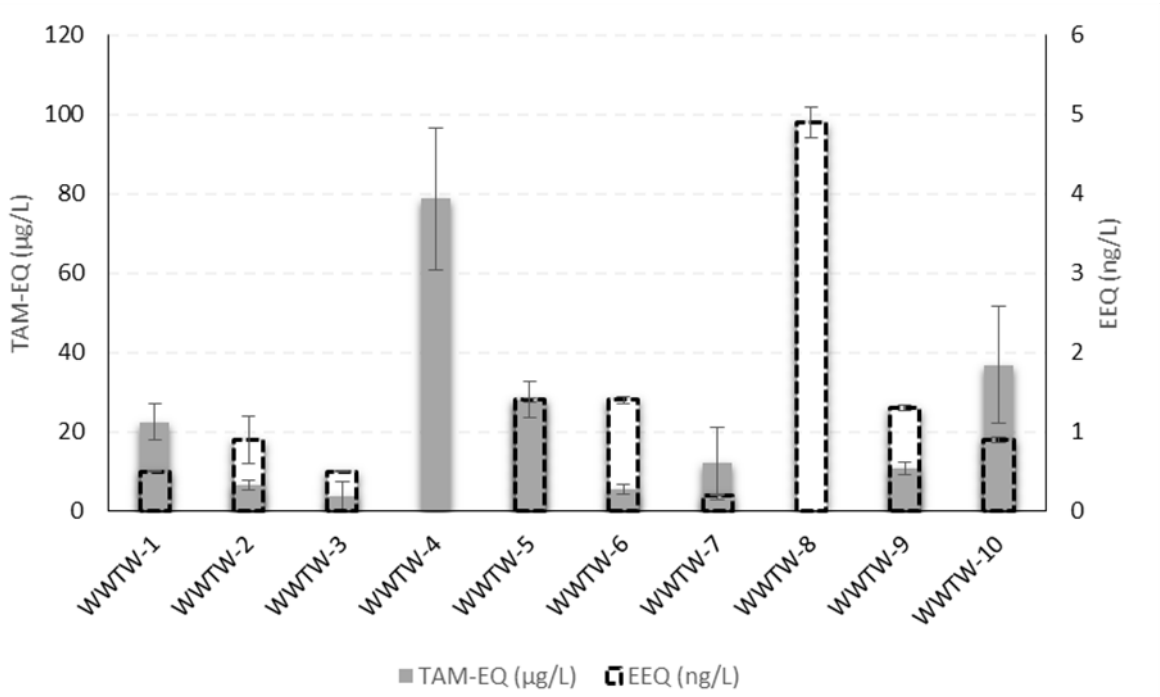


Figure 6-3: Anti-estrogenicity calculated as TAM-EQ (grey bars; 1º y-axis; µg/ℓ) and estrogenicity calculated as EEQ values (dashed bar; 2º y-axis; ng/ℓ) for treated wastewater effluent at the various WWTWs during the winter 2016 sampling campaign

Table 6-5: Daily variation in TAM-EQ (µg/ℓ) estimated for treated effluent water samples from selected WWTWs during the summer 2016 sampling campaign

Sampling day	WWTW-1	WWTW-2	WWTW-9	WWTW-10
Monday	-	1.4 ± 0.6	3.0 ± 0.3	1.3 ± 0.3
Tuesday	-	1.0 ± 0.2	-	2.2 ± 1.3
Wednesday	3.8 ± 2.2	1.3 ± 0.2	2.2 ± 0.7	-
Thursday	-	4.8 ± 2.6	1.6 ± 0.7	5.4 ± 2.0
Friday	2.3 ± 1.3	10.0 ± 3.0	2.9 ± 0.7	2.2 ± 0.7
Saturday	2.2 ± 0.6	1.9 ± 0.4	2.0 ± 0.6	2.4 ± 0.7
Sunday	0.7 ± 0.3	-	-	-
Average	**2.3 ± 1.3**	**3.4 ± 3.5**	**2.3 ± 0.6**	**2.7 ± 1.6**

6.3.2 Estrogenic activity in river water

Although the source of estrogenicity in surface waters is often proposed to primarily originate from WWTW effluent discharge, the EEQ estimates for river water located upstream from seven of the 10 WWTWs showed higher levels than downstream water samples. This was particularly shown for WWTW-9, where the average upstream EEQ value during the winter 2016 sampling was 18.9 (± 0.2) ng/ℓ as opposed to a calculated EEQ value of 15.1 (± 0.9) ng/ℓ for the raw influent of the plant. This river system has been reported to experience extreme pollution pressure from peri-urban communities located upstream from the WWTW, including direct sewage disposal into the river system.

The river system associated with WWTW-1 passes through informal settlements and industrial areas. The high loads of estrogenicity within upstream river water samples are indicative of alternative pollution sources. The river system associated with WWTW-2 serves as a main feed into a reservoir in a nature reserve, providing approximately 8% of the drinking water to a city with a population exceeding 2.1 million. This drinking water resource has previously been shown to be highly impacted on by industrial pollutants and other known EDCs (Aneck-Hahn et al., 2009; Barnhoorn et al., 2015). Alarmingly, the estimated EEQ concentrations from the downstream river sample were higher than those estimated for WWTW-2 effluent during the summer 2015/16 sampling campaign. The authors previously reported similar results for this river, showing an extensive list of PPCPs at higher concentrations in downstream river water compared to treated wastewater discharge (Archer et al., 2017). The same trend was shown in the current study for WWTW-1, WWTW-3, WWTW-6, WWTW-7 and WWTW-9 during the summer 2015/16, and WWTW-1, WWTW-3, WWTW-6 and WWTW-9 during the winter 2016 sampling.

The YES received wide recognition as a bioassay to estimate net estrogenicity in surface waters, with estimated EEQ values ranging between 0.33 and 26.5 ng/ℓ in Europe (Cargouët et al., 2004; Matthiessen et al., 2006), and between 0.07 and 6.4 ng/ℓ in Asia (Ra et al., 2011; Xiao et al., 2017). The higher levels of estrogenicity in the current study are in agreement with estimated EEQ values in another South African river system that receives domestic wastewater discharge and is surrounded by agriculture and mining activities, ranging between 10.2 ng/ℓ during winter, 14.2 ng/ℓ during spring and 43.0 ng/ℓ during summer (Truter et al., 2016). The reliance on these rivers for potable water on various scales – from small communities to large cities – highlights the need to establish the environmental risk through a refined weight-of-evidence approach.

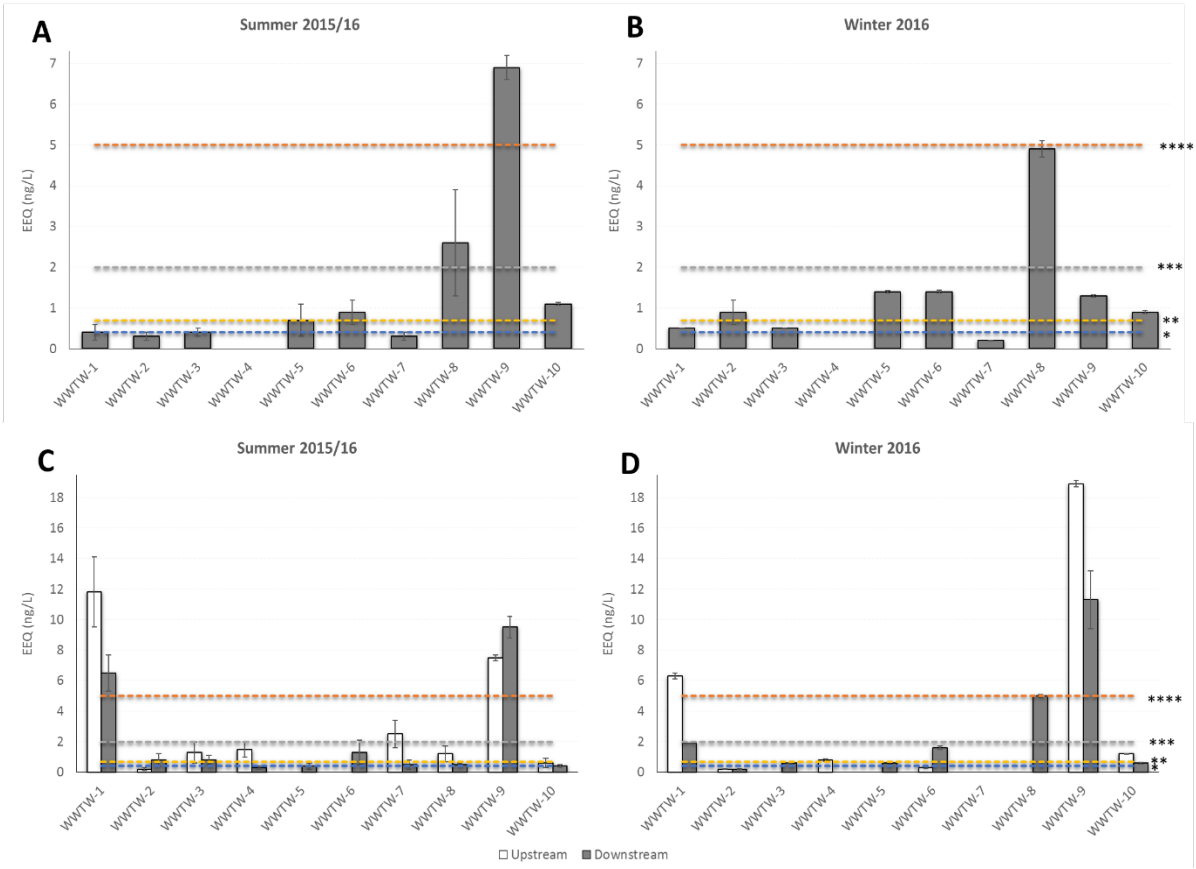
Despite the moderate to efficient removal of estrogenicity by wastewater treatment, the measured EEQ values still pose a potential adverse health risk. As conventional risk assessment approaches are focused on acute or chronic toxicity endpoints, the use of PNEC and NOEC is mostly incorporated to assess potential lethal toxicity in aquatic wildlife (Hernando et al., 2006). However, such an approach is focused on the toxicity of individual chemicals, and therefore does not consider the complex mixture interactions of environmental pollutants within a water system. The YES offers a viable option that indicates the net estrogenic potential of a water sample to modulate hormone receptor binding, with the estimated EEQ concentrations providing a semi-quantitative assessment of all compounds that may mimic an estrogenic response in a similar manner as E_2.

It is therefore possible to compare such EEQ values to other toxicological studies. For example, it has been shown that a concentration of 5 ng/ℓ E_2 may induce the production of VTG in male fish (Brion et al., 2004). This protein is a precursor of egg yolk in oviparous animals, and is considered an established biomarker of endocrine disruption, as its production is mediated by plasma steroid hormones (Aerni et al., 2004). Any pollutant in the water samples that mimics an E_2 mechanism of action (expressed as EEQ in the YES) may therefore create the same effect. As expected, nearly all the EEQ estimates of raw influent WWTW samples were above the 5 ng/ℓ threshold, showing increased estrogenicity responses in fish, as several micro-pollutants are known to mimic an estrogen response similar to E_2 (Archer et al., 2017b; McKinlay et al., 2008).

On the other hand, only one WWTW effluent sample showed an EEQ estimate above the 5 ng/ℓ threshold during the summer 2015/16 sampling campaign (Figure 6-4), with both its river water samples located upstream and downstream of the discharge to also surpass this 5 ng/ℓ level during both the summer and winter sampling campaigns (Figure 6-4). Overall, the estimated river water EEQ concentrations showed more pronounced environmental risks than treated wastewater effluent, whereby EEQ estimates for upstream and downstream river water at WWTW-1 and WWTW-9 exceeded or were close to this threshold during summer 2015/16 and winter 2016 (Figure 6-4).

Apart from the observed estrogenic limit, which shows a definite modulation of fish endocrine systems using an *in-vivo* model, a PNEC of 2.0 ng/ℓ E_2 has also been established as a baseline limit, which may modulate fish reproduction (Caldwell et al., 2012).

The EEQ estimations for treated effluent samples showed that WWTW-8 exceeded this threshold during both the summer 2015/16 and winter 2016 sampling campaigns (Figure 6-4). For river water, only the upstream sampling location from WWTW-7 was above this PNEC (Figure 6-4). Using such risk indicator concentrations for the YES suggests whether further *in-vivo* studies should proceed, as such studies are timely and costly, but still necessary to confirm areas under severe risk.



*Concentration of E_2 (5 ng/ℓ) showing increased VTG production in fish (Brion et al., 2004).
** PNEC (2 ng/ℓ) to modulate fish reproduction (Caldwell et al., 2012).
*** Estimated trigger value (0.7 ng/ℓ) for risk in drinking water (Genthe et al., 2013).
**** EEQ trigger value (0.4 ng/ℓ) of estrogenicity on long-term fish exposure (Jarošová et al., 2014).

Figure 6-4: EEQ (ng/ℓ) measured in treated effluent (A and B) and river water samples located upstream and downstream of the ten WWTWs during a month of summer 2015/16 and winter 2016

On the other hand, effect-based trigger values have also been proposed to serve as a marker for a potential risk of adverse health outcomes. An estimated E_2 trigger value of 0.7 ng/ℓ for drinking water standards has been proposed (Genthe et al., 2013), above which further monitoring should be considered to establish the identity and origin of the compounds. Jarošová et al. (2014a) proposed a lower effect-based EEQ trigger value of 0.4 ng/ℓ for long-term exposure to effluent-impacted surface waters. Several WWTW effluent and river water samples from both the summer 2015/16 and the winter 2016 sampling campaigns were above these thresholds (Figure 6-4).

6.4 SUMMARY

The results presented here highlight the complex range of factors that may influence the fate of estrogenic EDCs during wastewater treatment, and the complexity when total estrogenicity and anti-estrogenicity are assessed using a single bioassay. The calculated EEQ and TAM-EQ values for total anti-estrogenicity revealed by YES and YAES, respectively, correlate well with existing literature and highlight the value of flow-proportional EEQ mass load estimations as an approach to compare WWTWs with variable treatment capacities. This offers a more refined estimation of removal estimations at WWTWs, and the subsequent potential negative impact caused by discharge. The YES and YAES performed in this study further highlighted the complexity of environmental samples where a mixture of organic and inorganic pollutants are present. However, the potential masking of both outcomes should be considered when using such *in-vitro* assays for risk decision making, as several mechanisms other than agonistic/antagonistic receptor binding may also influence the results. Regardless of whether environmental waters contain both estrogenic and anti-estrogenic analytes, the combined response generated from the YES and YAES still provide a semi-quantitative outcome, as these assays reflect a net (anti)-estrogenic response.

Even though a notable reduction of estrogenicity by the WWTWs was measured, the effluent EEQ levels reported here remain a cause of concern because of the risk for adverse outcomes to the aquatic environment and, by extension, human health. Furthermore, despite the evidence implicating WWTWs as a major source for estrogenic endocrine disruption, the data suggests additional sources. In many parts of the developing world, alternative pollution sources include greywater from informal settlements and leakage from aging sewage systems, in addition to those also found in the developed world, among others, from pesticide run-off. Clearly, the relative contribution of these alternative sources will show great regional variation, and with building evidence of the possible negative effect of other micro-pollutants not discussed here, including micro-plastics, it is imperative to further develop and refine techniques such as YES and YAES to become affordable tools to provide realistic indications of health risks, even in remote areas. This is especially relevant to the third Sustainable Development Goal (SDG) of the United Nations: “Good Health and Well-Being”, which calls for strengthened capacity for early warning and risk reduction, especially within developing countries. Reliable early detection of EDCs in water may indeed greatly facilitate improved universal healthcare through preventative rather than reactive treatment.

CHAPTER 7: MANAGING CONTAMINANTS OF EMERGING CONCERN IN SOUTH AFRICAN WATER SYSTEMS

7.1 INTRODUCTION

From the information reported within the current report, along with emerging trends for priority pollutants, a more refined list of priority micro-pollutants could be made by taking all factors as highlighted by some global regulating bodies into consideration, such as those implemented by the EU and United Nations (UN), as well as new substances that have not been monitored extensively for their presence within surface waters and wastewater. Other South African studies have also aimed at presenting a priority list of CECs that require surveillance during wastewater treatment and within environmental surface waters, based on their regular occurrence and potential to pose adverse health effects in ecosystems and human health (Swartz et al., 2018; Ncube et al., 2012). The results from the current report and case studies show some similarities with such priority lists. However, based on the results of the current report, the authors propose alternative “emerging” contaminants based on these contaminants’ persistence during wastewater treatment, their chiral toxicological profiles and their regular use in South African communities. The list is summarised in Table 7-1 and includes the classes of antibiotics/biocides, NSAIDs, antidepressants, anticonvulsants, opioids and ARVs, as well as plasticisers, the herbicide atrazine and the anti-corrosion inhibitor benzotriazole.

From the scoping studies shown in the current report, it must be emphasised that prioritisation of micro-pollutants depend on the specific problem that should be addressed. Whether it is to improve on general water quality legislation, AMR development, or to report on the occurrence of long-term adverse health outcomes such as endocrine disruption. Each micropollutant will therefore receive priority as its combined presence will contribute to certain risk-related endpoints. Regardless, the current aim of prioritising micro-pollutants of concern was to include substances that may impact on health-related outcomes in wildlife and humans, with the focus on emerging contaminants that are persistent throughout wastewater treatment, along with their presence within environmental surface waters.

7.1.1 Antibiotics

The antibiotic class of micropollutants may be divided into pharmaceutical antibiotics and industrial or consumer product biocides. For pharmaceutical antibiotics, three compounds have been considered due to their wide use to treat several infections, their common occurrence during monitoring studies, their recalcitrance during wastewater treatment, their known veterinary use, as well as reports showing the presence of resistance genes against these compounds. These include sulfamethoxazole, trimethoprim and colistin.

Both sulfamethoxazole and trimethoprim are prescribed in combination due to their potentiating mixture interactions (such as co-trimazole). Sulfamethoxazole has been detected as high as 5,300 ng/ℓ and 1,400 ng/ℓ in surface waters located in KwaZulu-Natal and Gauteng, respectively, whereas trimethoprim has been detected as high as 300 ng/ℓ and 1,100 ng/ℓ within the same waters, respectively (Archer et al., 2017; Matongo et al., 2015). In other global monitoring campaigns, sulfamethoxazole and trimethoprim were detected at concentrations of 1.8 ng/ℓ and 22 ng/ℓ in UK surface waters, respectively (Petrie et al., 2014), 60 ng/ℓ and 2.1 ng/ℓ in Taiwan, and 38 ng/ℓ and 9 ng/ℓ in the USA, respectively (Luo et al., 2014). These results therefore show that the extent of antibiotic pollution of these compounds in South African surface waters is much higher if compared to other global monitoring campaigns, and therefore warrants their continued monitoring due to their widespread use in the country.

Following the increased resistance associated with carbapenem-resistant gram-negative infections, the use of colistin has been implemented as a last-resort treatment (Cai et al., 2012; Liu et al., 2016).

However, this drug has been shown to result in renal and neural toxicity in humans and is therefore avoided if possible. Regardless, the WHO has classified colistin as a critically important medication for human treatment due to increased resistance against third- and fourth-generation antibiotics. Furthermore, its extensive use as a veterinary medicine in the past also led to an increased risk for the possible development of resistance against this last-resort antimicrobial for human use. Recently, several colistin-resistant isolates from various bacterial strains have been detected in hospitals within the Western Cape (Newton-Foot et al., 2017). Most isolates further showed the presence of the plasmid-mediated *mcr-1* colistin-resistant gene, which is known to be stably transferred among bacterial strains and therefore poses an increased risk for further resistance development in other potential pathogens (Newton-Foot et al., 2017).

As highlighted by the WHO, unnecessary prescription and agricultural use of antibiotics pose a severe risk, which leads to the development of AMR. For this reason, monitoring for the presence and fate of colistin within wastewater and environmental surface waters should receive priority to assess the possible risk of the distribution of colistin-resistant genes into the environment. Recently, a study by Hembach et al. (2017) showed the occurrence of the *mcr-1* gene within bacterial isolates from a German WWTW effluent. The authors concluded that complex mixtures of antibiotics, biocides, disinfectants and pharmaceuticals may well provide selective pressure to promote horizontal gene transfer within the WWTW system. To the authors' knowledge, no analytical techniques have been developed for the detection of colistin in wastewater, which creates a large gap in the literature to assess the possible impact of colistin in environmental surface waters upon such AMR development.

For the priority list of biocides, triclosan, triclocarban and methylparaben were selected due to their wide usage as a preservative in consumer products. Although paraben preservatives were shown to be effectively removed during wastewater treatment, high concentrations are still being reported for these compounds in environmental surface waters. Concentrations of methylparaben up to 1,598.0 ng/ℓ, 920 ng/ℓ and 27 500 ng/ℓ were reported in river systems from Poland, China and Brazil, respectively (Czarczyńska-Goślińska et al., 2017), whereas methylparaben was detected at concentrations up to 295 ng/ℓ in a recent South African surface water monitoring study (Archer et al., 2017). These results indicate that the risk associated with methylparaben pollution may be lower in South Africa compared to other countries. However, due to the known risk of methylparaben to exert endocrine-disrupting effects, more monitoring of this compound in surface waters needs to be included, as information within South Africa is limited.

The other biocide preservatives, triclosan and triclocarban, have been shown to pose a significant risk towards the aquatic environment due to their widespread occurrence and recalcitrance in surface waters. Although most studies have focused on triclosan for its presence in both surface waters and solids (sediment and sludge), there is increased evidence and interest for the inclusion of triclocarban. Triclosan has been detected in global surface waters at concentrations of up to 101 ng/ℓ in the UK, 1,023 ng/ℓ in China, 220 ng/ℓ in Germany and 39 ng/ℓ in Greece (Luo et al., 2014; Petrie et al., 2014). In South Africa, triclosan and triclocarban have been detected at concentrations up to 8,720 ng/ℓ and 360 ng/ℓ in Gauteng surface waters, respectively (Lehutso et al., 2017), therefore highlighting the extent of pollution of these compounds into local surface waters. The highly bio-accumulative potential of both these substances in human and animal tissue underscores their environmental risk, along with their potential to modulate endocrine-system pathways (Veldhoen et al., 2006; Crofton et al., 2007; Simon et al., 2014). The removal of both triclosan and triclocarban is highly dependent on the type of wastewater treatment process (Pycke et al., 2014; Lehutso et al., 2017), which warrants more case-specific monitoring studies to evaluate a wider range of WWTW processes being managed in South Africa.

In line with the removal of both triclosan and triclocarban by WWTWs, the extent and fate of their metabolic breakdown products, which are formed during treatment processes, is still unclear, as a significant toxicological risk may also be associated with their metabolites.

Although treatment processes such as ozonation shows promise to effectively remove the loads of triclosan, the formation of several degradation by-products has been shown (Orhon et al., 2017), which further warrants their inclusion as priority micropollutants.

7.1.2 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs, such as ibuprofen, naproxen, diclofenac, ketoprofen, aspirin and acetaminophen, have been extensively screened for their presence within surface waters on a global scale due to their availability as common OTC medications. Not only are these substances regarded as posing a definite lethal toxicological risk, they have also been shown to modulate molecular and cellular events within both gonadal and thyroid endocrine system pathways (Hong et al., 2007; Han et al., 2010; Gröner et al., 2017; Veldhoen et al., 2014). It should be pointed out, though, that these endocrine-disrupting effects typically occur at concentrations that are higher than normally detected in environmental waters. Although relevant studies have mostly focused on single contaminants to date, the combined presence of substances with a similar mode of action may create complex physiological mixture interactions at concentrations that show such endocrine-disrupting effects in test organisms. For this reason, studies have also started to focus on the total amount of NSAIDs that are present in environmental surface waters.

A study by Ebele et al. (2017) highlighted the variation in NSAID loads within surface waters between several countries. One key observation that was made was the significantly higher concentrations shown for total NSAIDs detected in Nigerian surface waters (> 10,000 ng/ℓ) compared to other monitoring studies in Europe (10 to 1,000 ng/ℓ), North America (100 to 700 ng/ℓ), Australia (100 to 500 ng/ℓ) and Asia (500 to 5,000 ng/ℓ) (Ebele et al., 2017). Within the authors' monitoring studies, total NSAIDs within surface waters ranged from 1,508 to 3,281 ng/ℓ, with diclofenac detected as high as 2,182 ng/ℓ during one of the sampling periods (Archer et al., 2017). These levels are in line with monitoring studies in Asian surface waters, but lower than reported for Nigerian surface waters. However, a monitoring study by Agunbiade and Moodley (2016) reported on total NSAID concentrations ranging between 15,526 and 27,870 ng/ℓ in surface waters in KwaZulu-Natal, with diclofenac being the second-most abundant compound after aspirin. From the authors' scoping monitoring studies, the highest contribution for total NSAID loads were derived from diclofenac and naproxen, which correlate well to their known recalcitrance during wastewater treatment. Diclofenac has further also been included in the EC's first watch list (Carvalho et al., 2015), along with nine other priority substances. The authors propose that both diclofenac and naproxen should be selected as priority micropollutants of concern due to their known potential to cause adverse health effects, as well as being regarded as common OTC medications.

7.1.3 Industrial product (benzotriazole)

Benzotriazole is shown to be used in several domestic and industrial applications for its anti-corrosive properties, including consumer products, textile dying, cooling and hydraulic agents, aircraft de-icing fluids and UV filters, as well as several other applications. Additionally, the compound is incorporated into plastics to increase stability from exposure to UV, it acts as an antifogging agent in photography and it is used as an intermediate substance for the synthesis of pharmaceuticals and fungicides. For example, benzotriazole-based pharmaceuticals, such as vorozole, alizapride and tetrabromo-benzotriazole, are well known to be used in clinical therapy. Vorozole is used as an inhibitor of the aromatase enzyme, therefore inhibiting the conversion of testosterone to estradiol in the body. Alizapride is an anti-emetic, which acts as a dopamine receptor antagonist, whereas tetrabromo-benzotriazole has been proposed for anti-cancer treatment. Taking the wide use of this compound, as well as its known clinical applications, into account warrants the prioritisation of this compound for monitoring in surface waters.

To the authors' knowledge, the presence and quantity of benzotriazole has not been investigated in South African water systems. However, this substance, along with its degradation by-products and derivatives, has been considered as a priority micropollutant in several European studies. There is compelling evidence for the widespread occurrence of benzotriazole in surface waters, with one monitoring campaign showing a 94% occurrence of benzotriazole in 122 surface water samples across Europe, with concentrations reaching as high as 8,000 ng/ℓ (Loos et al., 2009). More recently, a monitoring study by Östman et al. (2017) showed a 100% detection frequency of benzotriazole in 133 surface water and sludge samples from 11 WWTWs in Sweden, with concentrations up to 13,000 ng/ℓ in treated effluent wastewater. In Switzerland, it was found that concentrations of benzotriazoles occurred at concentrations as high as 100,000 ng/ℓ in the discharge of municipal wastewater treatment plants (Giger et al., 2006; Voutsa et al., 2006).

Benzotriazoles are removed at moderate to high efficiency during wastewater treatment, depending on the specific treatment process (Hart et al., 2004; Voutsa et al., 2006; Zhao et al., 2017). The high levels of benzotriazoles detected in treated wastewater effluent are therefore concerning, and most probably pose a risk to aquatic wildlife and humans. Fish toxicity studies using rare minnows (*Gobiocypris rarus*) showed that exposure to benzotriazole at concentrations up to 5 mg/ℓ may modulate plasma E_2 concentrations in both male and female fish (Liang et al., 2014). Furthermore, benzotriazole exposure as low as 50 ug/ℓ was shown to significantly increase liver VTG levels, as well as up-regulation of several gonadal endocrine axis transcripts in the brain, gonads and liver (Liang et al., 2014). These results indicate that benzotriazole may modulate endocrine systems through molecular-initiating events, as well as impacting on downstream key events on a cellular level. Apart from its toxicological effects in wildlife, benzotriazole has also been implicated in strongly contributing to mutagenesis within surface waters (Watanabe et al., 2002).

7.1.4 Herbicide (atrazine)

The triazine herbicide atrazine is known for its wide use in agriculture and recreational spaces, such as golf courses. This compound has been withdrawn from use in EU countries due to evidence showing its potential as an endocrine disruptor, a carcinogen, as well as being highly persistent within both the aqueous and solid-phase environments. However, it is still widely used in countries such as the USA. In South Africa, it is traditionally used in the northern regions of the country for the control of weeds during maize production, although it is more recently also used for other crops. Atrazine has further been detected in several drinking water sources in South Africa, mainly in the Free State and Gauteng (Odendaal et al., 2015), which can be attributed to its known recalcitrance within surface waters throughout the year. These compounds were detected at concentrations up to 1,237 ng/ℓ, 760 ng/ℓ, 543 ng/ℓ and 453 ng/ℓ during summer, autumn, winter and spring, respectively, at a sampling site near Hartbeespoort Dam in Gauteng (Rimayi et al., 2018).

Atrazine concentrations up to 60.8 ng/ℓ have also been detected in surface waters from the Rietvlei Nature Reserve (Wooding et al., 2017), as well as concentrations up to 247 ng/ℓ in the Lower Olifants River near the eastern border of the Kruger National Park (unpublished data from the research group). Globally, atrazine is also attributed as being one of the most commonly detected pesticides in surface waters due to its wide agricultural application; for example, up to 132 ng/ℓ of atrazine was detected in 12 surface water sites in the USA (Reilly et al., 2012). A recent study also reported the presence of breakdown products of atrazine in several soil, sediment and surface water samples in Poland during a 2014 sampling campaign (Barchanska et al., 2017). This finding highlights the persistence of these compounds in the environment, as atrazine application has been restricted in EU countries since 2004. The environmental risk, which both atrazine and its metabolites may have on the aquatic ecosystem, as well as its detection in drinking water resources, raises a concern. Moreover, due to the wide use of this compound in the northern regions of South Africa, along with its known environmental recalcitrance, its potential to leach into transboundary water systems potentially poses a significant challenge for catchment management.

7.1.5 Antidepressants

The monitoring studies of the current project revealed the presence of the antidepressant venlafaxine in both wastewater and environmental surface waters. Moreover, its metabolic breakdown products were present at higher concentrations, which warrants further investigation of the toxicological risk and mixture effect of both the parent drug and its metabolites on wildlife and humans.

Another consideration is the potential added mixture effect that venlafaxine and its primary metabolite, DMV, may have on aquatic life, as its metabolic breakdown products were shown to be present at higher concentrations in surface waters. Global monitoring studies have shown venlafaxine and DMV to occur at concentrations as high as 100 ng/ℓ and 200 ng/ℓ, respectively, in UK surface waters (Evans et al., 2017), whereas concentrations of DMV in South African river water samples ranged between 21.1 and 664.4 ng/ℓ in the current case studies. These levels are close to or within the reported toxicity levels shown for venlafaxine, and within the range that shows brain monoamine and neuroendocrine modulation in fish. The toxicity of DMV has not been established in a similar manner as its parental compound in vertebrates. However, given the fact that several pharmaceutical metabolites are primarily responsible for the desired physiological effect, as well as DMV being an active ingredient of antidepressant medications, the presence of both pharmaceutical parent compounds and metabolites in surface water systems may be more deleterious than previously estimated.

During the present study, venlafaxine and its primary metabolite, DMV, were shown to be persistent CECs during wastewater treatment. Chronic exposure of male fathead minnows (*Pimephales promelas*) to venlafaxine at a concentration of 305 ng/ℓ has been shown to result in 40% mortality of the test organisms (Schultz et al., 2011). Moreover, exposure rainbow trout (*Oncorhynchus mykiss*) to venlafaxine at a concentration as low as 260 ng/ℓ led to a significant reduction in the dopamine metabolism in the brain, whereas a concentration of 1,020 ng/ℓ significantly modulates brain neuroendocrine pathways (Melnyk-Lamont et al., 2014). In *Danio rerio*, venlafaxine exposure of 500 ng/ℓ showed a reduction in plasma E_2 concentrations in female fish and a reduction in plasma 11-ketotestosterone concentrations in male fish (Galus et al., 2013a). These results highlight the possibility of venlafaxine not only modulating neuroendocrine responses in aquatic species at environmentally relevant concentrations, but also posing a risk towards the modulation of reproductive endocrine systems.

Like the discussion on the opioid drug tramadol, an additional consideration is the potential added mixture effect that venlafaxine and DMV may have on aquatic and human health, as DMV was shown to be present at higher concentrations in surface waters compared to venlafaxine during the case studies of the current report. However, the toxicity of DMV has not been established in a similar manner as for its parent compound in vertebrates. Given the fact that several pharmaceutical metabolites are primarily responsible for the desired physiological effect, the presence of both pharmaceutical parent compounds and metabolites in surface water systems should be considered for risk assessment approaches (De Jongh et al., 2012). As for tramadol risk assessment for human health, estimated provisional guideline value for a venlafaxine and DMV mixture was calculated at 19 µg/ℓ (De Jongh et al., 2012). However, this value is much higher than any combined concentrations for either treated effluent or surface waters at both study sites, which implies a low risk for human health

7.1.6 Anticonvulsants

The recalcitrance of the anti-epileptic carbamazepine during wastewater treatment, as well as in environmental waters, is well known and reported (Luo et al., 2014). Consequently, carbamazepine has been detected in several drinking water sources in South Africa (Odendaal et al., 2015), as well as other countries worldwide (Szymonik et al., 2017). Carbamazepine is usually included in monitoring campaigns due to its known persistence in the environment, as well as its recalcitrance during conventional wastewater treatment processes (Luo et al., 2014).

Carbamazepine and several other pharmaceutical products (including the antibiotic sulfamethoxazole) showed a high degree of persistence in Swedish waters, with concentrations ranging from 160 to 1,180 ng/ℓ (Galus et al., 2013a). Carbamazepine concentrations have also been shown to range from 2.0 to 17.0 ng/ℓ within a transboundary river system in France (Chonova et al., 2017), whereas carbamazepine concentrations in surface waters as high as 684 ng/ℓ in the UK, 1,194 ng/ℓ in Germany and 595 ng/ℓ in Korea have been reported (Luo et al., 2014; Nakada et al., 2017). Such high levels already surpass the known concentration to exert reproductive endocrine disruption in fish (500 ng/ℓ).

Within South African surface waters, carbamazepine has been detected up to 300 ng/ℓ and 3,200 ng/ℓ in Gauteng and KwaZulu-Natal, respectively (Matongo et al., 2015; Archer et al., 2017), which highlights more of an associated risk to pose adverse health effects in aquatic environments. Moreover, the metabolic breakdown products of carbamazepine are removed with low efficiency during WWTW processes and were even found at higher concentrations in treated wastewater than in the incoming streams. Despite the near absence of information on their toxicological effects, carbamazepine metabolites pose a significant risk to the environment and should also be included.

The recalcitrance of carbamazepine at WWTWs and in surface waters has been well recorded globally. Along with its potential lethal toxicity risk, studies have also proposed that carbamazepine may be associated with the disruption of reproductive endocrine system pathways. For example, an *in-vivo* exposure of 500 ng/ℓ carbamazepine in water was shown to cause significant reduction in plasma 11-ketotestosterone (11-KT) concentrations in male fish (Galus et al., 2013b), whereas *in-vitro* studies showed that carbamazepine may have an anti-estrogenic and anti-androgenic effect on steroid hormone binding in a dose-dependent manner (unpublished data from the research group). Moreover, carbamazepine has been shown to cross the placental barrier in mice through its administration to pregnant females through water spiked with environmentally relevant concentrations of the drug, which are proposed to cause neurological disorders and teratogenesis in the offspring (Kaushik et al., 2016). Such multiple toxicity endpoints of the drug, along with its known recalcitrance during wastewater treatment, warrants this a contaminant of high priority to be regulated in future water quality policy frameworks.

7.1.7 Opioids

The widespread use of the opioid drug tramadol for pain relief necessitates assessment of the presence and fate of this drug in surface waters on a global scale. Along with its widespread use, tramadol has been associated with a toxicological risk to aquatic ecosystems. Tramadol has been detected at a maximum concentration of 5,970 ng/ℓ in UK surface waters (Petrie et al., 2014) and as low as 2 ng/ℓ in surface waters in Switzerland (Szymonik et al., 2017). Tramadol concentrations as high as 1,603 ng/ℓ have been detected in UK WWTW effluent (Baker and Kasprzyk-Hordern, 2013), whereas tramadol was detected at concentrations as high as 540 ng/ℓ in a South African WWTW effluent, and 337 ng/ℓ in surface waters from a sampling campaign in Gauteng (Archer et al., 2017). Although tramadol itself does not lead to significant high levels in surface waters to affect aquatic vertebrate development as shown by Sehonova et al. (2016), it should be pointed out that its primary metabolites are also regularly detected in wastewater effluent and surface waters. It is still unclear, though, whether these metabolites might pose an additive toxicological effect with its parental counterpart. Furthermore, both tramadol and its metabolites show low removal during conventional wastewater treatment processes in South African monitoring studies, from which their loads in treated wastewater effluent may be even higher than in raw wastewater. Furthermore, the risk of tramadol has not only been associated with aquatic toxicology, but it is also highlighted as a legal drug of abuse. The monitoring of tramadol in wastewater therefore provides further information to show the extent of drug abuse within communities associated with the sewage network, as well as increased information based on its presence in South African surface waters.

Although conventional risk assessment shows a low environmental risk for opioids (based on lethal toxicity endpoints), sub-lethal toxicity endpoints of opioids are diverse.

A strong association with observed endocrine modulations in laboratory animals and humans has been drawn following opioid administration, especially for morphine (Vuong et al., 2010). In particular, morphine exposure to female rats caused a polycystic morphology of ovaries and decreased intact brain neurons (Karimi et al., 2017), as well as other undesired endocrine system modulations, including altered growth and thyroid-stimulating hormone levels, and decreased steroid hormone levels (Vuong et al., 2010). Even though endocrine-disrupting endpoints on aquatic wildlife have received little attention, the potential genotoxicity that could be caused by opioids should not be ignored. For example, morphine exposure in mice has been linked to the increased incidence of micro-nucleated bone marrow erythrocytes in a dose-dependent manner (Puli and Patil, 2007). Taking into account that the metabolism of codeine and heroin use also leads to the formation of morphine and its derivatives highlights the need for combined mixture risk assessment, as the combination of these opiates may have synergistic, potentiating or additive mixture interactions. This warrants the need to include these compounds as priority emerging contaminants for risk assessment monitoring, especially due to their regular detection within surface waters as highlighted in the current study. Apart from their potential adverse risks to wildlife and humans exposed to contaminated surface waters, the abuse of these substances in communities is also of socio-economic concern. In particular, the abuse of common OTC medications, such as codeine, is globally well reported to be on the rise, with South Africa being no exception (Parry et al., 2017).

Another opioid drug, tramadol, has also been associated with variable toxicological risks to aquatic ecosystems. Exposure at a concentration of 10 µg/ℓ has been shown to cause hatching retardation in *Danio rerio* and retardation in total body length development in *Cyprinus carpio,* therefore influencing the development of fish during early ontogeny (Sehonova et al., 2016). Although tramadol does not reach such high concentrations in wastewater effluent and surface waters, its recalcitrance and pseudo-persistence, as well as the high level of detection of its primary metabolite, *O*-DMT, still holds concern about whether these substance may bio-accumulate in non-target organisms to reach the level of such adverse health outcomes over time. It has been shown that *O*-DMT also has a higher affinity for opiate receptors than tramadol itself (De Jongh et al., 2012), making this a much more potent CEC than the parent compound, which warrants the need for future investigation into the possible mixture interaction of such a chemical with other recalcitrant pollutants with similar physiological modes of action.

Concerning human health risks, a study by De Jongh et al. (2012) aimed to establish a provisional drinking water guideline value for a combined mixture of tramadol and *O*-DMT by considering daily therapeutic doses, acceptable daily intake and/or tolerable daily intake estimations. The authors concluded that a threshold of 6 µg/ℓ should be considered for a combined parent/metabolite risk to human health. During the scoping studies in the current report, the highest combined tramadol/*O*-DMT concentration was 3.46 µg/ℓ in treated wastewater effluent and 5.3 µg/ℓ in recipient surface waters. This implies that river water from at least one study site was close to this threshold to potentially impact on human health. However, such surface waters are seldom used for direct potable reuse, which requires further investigation at water distribution systems to confirm whether these substances persist through drinking water treatment plants.

7.1.8 Plasticisers

Bisphenol analogues, such as BPA and bisphenol-S (BPS), are widely used in industrial and consumer products, such as polycarbonate plastics, epoxy resins, thermal paper, toys and food packaging. Their endocrine-disrupting potential has been well investigated, along with their associated risks to cause non-communicable diseases such as cancer. *In-vitro* studies have shown, for example, that BPA can induce agonistic binding to the human oestrogen receptor, which has further been shown to lead to the proliferation of breast cancer cells (Boberg et al., 2010; Schlumpf et al., 2001). Exposure of zebrafish (*Danio rerio*) to BPA at concentrations as low as 500 ng/ℓ showed a significant reduction in fecundity, increased teratogenesis in larvae, and modulation of gonadal endocrine system pathways, such as elevated plasma E_2 concentrations in male fish (Ji et al., 2013).

Although the removal of BPA during wastewater treatment has also been well reported and may vary from moderate to high efficient removal in the aqueous phase, these substances have a strong tendency to partition into solids, such as sediment and sludge (Wu et al., 2018), therefore posing a significant risk to leach into the environment through pathways other than treated wastewater. A study by Sun et al. (2017) highlighted that 12% of wastewater influent loads of bisphenol analogues may be discharged in wastewater effluent, while 3% of the influent load of bisphenol analogues may be discharged through excess sludge, with BPA still shown as the primary persistent analogue. The BPA concentrations in surface waters have been detected up to 34 ng/ℓ in the UK, 215 ng/ℓ in Germany, 162 ng/ℓ in Greece and 334 ng/ℓ in Korea (Luo et al., 2014; Petrie et al., 2014). It has also been detected at concentrations up to 67.0 ng/ℓ, 26.5 ng/ℓ and 3.4 ng/ℓ in river water from China, India and Japan, respectively, and at concentrations up to 29.0 ng/ℓ, 380 ng/ℓ and 12.0 ng/ℓ in a separate study on the same river systems (Wu et al., 2018). Concentrations of BPA up to 95.0 ng/ℓ, 79.0 ng/ℓ and 290 ng/ℓ were reported in river systems located in Poland, China and Japan, respectively (Czarczyńska-Goślińska et al., 2017). In a South African study, BPA has been detected in surface waters ranging from 167.0 to 616.0 ng/ℓ, showing a much higher occurrence than other studies globally (Archer et al., 2017). To the authors' knowledge, the presence and fate of BPS in South African surface waters has not been reported. This highlights the need to consider the inclusion of this substance as a priority micro-pollutant. The increased risk of bisphenol analogues to enter the environment through both aqueous and solid matrices, along with limited data showing their presence and fate within South African surface waters, requires further monitoring. Specific considerations for future monitoring studies should include the quantification of bisphenol analogues in solid matrices.

7.1.9 Antiretrovirals

The African continent, especially sub-Saharan Africa, has the largest percentage of people living with HIV. The most recent statistics from the United Nations shows an estimated 37.9 million people living with HIV, of which an estimated 25.6 million people are from sub-Saharan Africa (67.5% of the global estimate). In the mid-year population estimate statistics of South Africa (Stats SA, 2019), an estimated 20.4% of the total population (7.7 million) is living with HIV (UNAIDS data for 2019). To combat this high prevalence of HIV infection, South Africa has the highest ART programme in the world, with 62% of adults and 63% of children on ART in the country (UNAIDS data for 2019). Considering the high prevalence of ART in the country, along with the large dosage that needs to be taken to treat the infection, and its co-administration with other antimicrobial drugs to treat infections associated with a weakened immune response, the burden to remove excreted ARVs and such co-administered drugs may be regarded as being the highest in the world. Due to the African continent being the highest user of ART, monitoring studies to investigate the presence and fate of ARVs in the environment are mostly reliant on African studies. Although such studies were limited in the past, vast research advances have been made to show their recalcitrance during wastewater treatment (Osunmakinde et al., 2013; Schoeman et al., 2015; Schoeman et al., 2017; Abafe et al., 2018; Mosekiemang et al., 2019), their fate and distribution in environmental surface waters and drinking water resources (Wood et al., 2015; Rimayi et al., 2018) and to identify their toxicological effects on sentinel aquatic organisms (Robson et al., 2017).

During wastewater treatment, high to moderate removal of the ARVs emtricitabine, zidovudine, lamivudine, maraviroc, famciclovir and ribavirin (Osunmakinde et al., 2013; Abafe et al., 2018; Mosekiemang et al., 2019), and low to moderate removal of the ARVs efavirenz, indinavir, nevirapine, darunavir, lopinavir, ritonavir, raltregavir and atazanavir (Schoeman et al., 2015; Schoeman et al., 2017; Abafe et al., 2018; Mosekiemang et al., 2019) have been recorded in the country, where treated effluent discharge still shows concentrations in the low µg/ℓ range. In surface waters, ARVs have been detected as high as 350 and 310 ng/ℓ for efavirenz and lopinavir, respectively, and even in tap water samples (Wood et al., 2015; Rimayi et al., 2018).

However, as new HIV infections are shown to be ever-increasing, along with improvements in ART programmes (such as Prevention of Mother-to-child Transmission (PMTCT) treatments), the need for the continued surveillance and establishment of toxicological risks of ARVs and their breakdown products is of the utmost importance. As mentioned previously, the high dosage and co-administration of ARVs and other antimicrobial drugs are of concern due to their increased release into surface waters, not only through wastewater treatment discharge, but also from direct discharge of sewage and other physical waste products that are largely associated with (peri)-urban areas that lack proper sanitation infrastructure.

The measured concentrations of the ARVs emtricitabine and efavirenz were shown to be in the µg/ℓ range in treated wastewater effluent in the current report, with moderate to high removal of emtricitabine and negative to low removal of efavirenz during treatment, highlighting these substances as priority CECs based on their recalcitrance and/or loads in WWTW discharge. Although the adverse health effects of ART are well recorded in human patients undergoing treatment (Carr and Cooper, 2000), toxicity studies to assess the environmental burden of ARVs are limited. This may be ascribed to the fact that the use of ARVs is lower in developed countries (where most of the toxicity studies are done) than in Africa. Regardless, some studies have aimed to assess the adverse health effects of efavirenz and nevirapine in the Mozambican tilapia, *Oreochromis mossambicus,* at environmentally relevant concentrations (Robson et al., 2017; Nibamureke et al., 2019). It was found that efavirenz induced hepatotoxicity and higher blood leukocrit levels in acute exposure as low as 20.6 ng/ℓ of adult fish (Robson et al., 2017), but chronic exposure of nevirapine as low as 1.48 µg/ℓ did not lead to altered growth rates in juvenile fish (Nibamureke et al., 2019). However, further investigation of mixture toxicities for sentinel organisms is needed, as most ARVs are co-administered with one another and will be present in complex mixtures in the environment, where their physiological interactions are still unknown.

7.1.10 Psychoactive substances (including illicit drugs)

Illicit substances are not normally included as priority CECs for risk assessment due to these substances , which are a primary source of contamination in surface waters, being well removed during wastewater treatment. However, the results that were presented in the current WRC report (K5/2733) still showed levels of illicit substances both upstream and downstream of wastewater treatment discharge. Concentrations as low as 40 ng/ℓ showed a significant decrease in lysosomal stability and increased DNA damage in hemocytes of the zebra mussel, *Dreissena polymorpha*, where higher concentrations (220 and 10,000 ng/ℓ) led to a further increase in cytotoxicity and genotoxicity (Binelli et al., 2012).

It should also be noted that only a portion of consumed cocaine will be excreted in an unmetabolised form in sewage (1 to 9%), while the majority will be found as primary hepatic metabolites, such as benzoylecgonine (Baselt, 2004). Benzoylecgonine exposure at concentrations of 500 ng/ℓ showed an increase in the oxidative stress markers and cytotoxicity of hemocytes in *D. polymorpha* (Parolini et al., 2013). More recently, benzoylecgonine exposure as low as 11.5 ng/ℓ showed DNA damage and slight oxidative stress in zebrafish embryos (*Danio rerio*), as well as other cytogenotoxic endpoints at concentrations of 115 ng/ℓ (Parolini et al., 2017). Furthermore, the authors concluded that benzoylecgonine posed a higher cytogenotoxicity risk than cocaine in their combined analysis of *D. rerio* embryos. Even though the information is still relatively limited, there is increasing evidence that metabolic breakdown products have significant adverse health effects, apart from their parent counterparts, emphasising the need to also include them for risk assessment.

The modulation of the neuroendocrine system through illicit drug exposure has also been reported for laboratory animals and aquatic vertebrates. Exposure of rats under laboratory conditions to methamphetamine has resulted in increased corticosterone release, as well as decreased dopamine levels in the brains of exposed animals compared to controls (Herring et al., 2010).

Methamphetamine has further been shown to induce mating behaviour in male sailfin molly fish (*Poecilia latipinna*), from which such elevated sexual behaviour was attributed to the modulation of monoamine levels in the brain and disruption of other dopaminergic pathways (Ghazilou and Ghazilou, 2011). The influence of monoamines to control gonadotropin release in the vertebrate brain is well known (Waye and Trudeau, 2011). As an effect, the modulation of neurotransmitter release (including monoamines) through the environmental exposure of psychoactive stimulants, such as methamphetamine, may not only affect physiological pathways associated with cognitive function and behaviour, but also potentially modulate the early onset of the control in gonadal endocrine system pathways.

7.2 PROPOSED PRIORITY LIST OF CONTAMINANTS OF EMERGING CONCERN IN SOUTH AFRICAN WATERS

Based on the current knowledge on the presence and fate of the CECs that have been discussed in the current report, along with other priority CECs that have been characterised by other studies (Ncube et al., 2012; Swartz et al., 2018), a proposed list of priority CECs is given that are relevant in an African context (Table 7-1). Although the list may not be limited to the substances listed here, the selection is based on their regular detection in South African surface waters, their recalcitrance during wastewater treatment and/or literature showing their potential to exert adverse health effects (including both lethal and sub-lethal toxicity) in aquatic environments that may serve as bio-indicators towards human health effects.

Table 7-1: Proposed list of micropollutants that are assessed, based on their use, their recalcitrance within surface waters and water treatment, as well as their potential health risks

Chemical class	Active ingredient	Use	Risk	Removal at WWTW	Reference
Antimicrobial	Sulfamethoxazole	Pharmaceutical	AMR	Low to moderate	Luo et al., 2014; Current report
	Trimethoprim	Pharmaceutical	AMR	Low to moderate	Luo et al., 2014
	Triclosan	Personal care products	AMR, CAR, TOX	Low to high	Pycke et al., 2014; Lehutso et al., 2017
	Triclocarban	Personal care products	AMR, CAR, TOX	Low to high	Pycke et al., 2014; Lehutso et al., 2017
	Methylparaben	Personal care products	EDC, CAR	High	Archer et al., 2017
NSAID*	Naproxen	Pharmaceutical	EDC	Moderate	Luo et al., 2014; Current report
	Diclofenac	Pharmaceutical	EDC, TOX	Low to moderate	Luo et al., 2014; Current report
Anti-epileptic	Carbamazepine	Pharmaceutical	EDC	Negative to low	Luo et al., 2014; Current report
Opioid	Tramadol	Pharmaceutical	DoA, EDC	Negative to low	Archer et al., 2017
	Codeine	Pharmaceutical	DoA	Moderate to high	Petrie et al., 2016; Current report
Antidepressant	Venlafaxine	Pharmaceutical	EDC	Negative to low	Archer et al., 2017
Plasticiser	Bisphenol-A	Consumer products	EDC, CAR	Moderate to high	Luo et al., 2014
	Bisphenol-S	Consumer products	EDC, CAR	N/A	
Herbicide	Atrazine	Pesticides	EDC	Low	Luo et al., 2014
Antiretroviral	Emtricitabine	Pharmaceutical	N/A	Moderate	Current report

Chemical class	Active ingredient	Use	Risk	Removal at WWTW	Reference
	Efavirenz	Pharmaceutical	TOX	Negative to low	Current report
	Nevirapine	Pharmaceutical	N/A	Low to moderate	Abafe et al., 2018
Industrial chemicals	TDCPP and TCEP	Various	TOX, MUT, CAR	Negative to low	Zeng et al., 2015
	PFOA	Various	EDC, CAR	Low to moderate	Swartz et al., 2018
	Benzotriazole	Various	EDC, MUT	Moderate to high	Zhao et al., 2017; Current report
Disinfection by-product	NDMA	Various	TOX, CAR	Negative to low	Mitch and Sedlak, 2004

* Non-steroidal anti-inflammatory drug

AMR: Antimicrobial resistance; CAR: Carcinogen; DoA: Drug of abuse; TOX: Cytotoxic;
EDC: Endocrine-disrupting contaminant; MUT: Mutagenic; N/A: Not available

CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

The work that was presented in the current report was aimed at adding to the knowledge base of CECs in South Africa freshwater ecosystems and wastewater treatment processes. Although many initiatives have been mentioned for the compilation of a priority substance list, the identification of PPCP CECs is under-represented. The work included here revealed both well-known and novel CECs in South African freshwater ecosystems, which were further extended to include pharmaceutical metabolites and the enantio-selective fate of chiral CECs during wastewater treatment and in environmental surface waters. These monitoring results highlight the extent of previously unreported CECs in the country, whereby their potential environmental risks were evaluated using lethal and sub-lethal toxicity endpoints.

The recalcitrance of various PPCPs and metabolic breakdown products takes place during various types of wastewater treatment in the country, and thus raises concern that such CECs should receive higher priority to establish their human and environmental health risks. Moreover, the case studies found that, for many of the monitoring campaigns, CEC pollution also originates upstream of the discharge of WWTWs that were investigated in the study, raising concern about the extent of alternative pollution sources in LMICs where various communities are not connected to a municipal sanitation infrastructure.

Wastewater-based epidemiology was done for the first time on the African continent in this study, and showed a high illicit drug usage in South African communities compared to other developed and developing countries. Although such high usage trends are indeed reported on by other regulating bodies, the concept of WBE proved to supplement such databases in the country by serving as a non-intrusive tool to estimate substance use and abuse on a community level. Moreover, the concept of WBE was extended to include pharmaceutical use profiling, which is shown as an emerging discipline in the field of environmental analytical chemistry to address public health.

The study further investigated the removal potential of EDCs from various South African WWTWs by using an EBM bioassay (the YES), whereby the results showed that a large variation in wastewater treatment technologies exists to eradicate contaminants for which they were not originally designed. Furthermore, the study raised the issue of toxic masking when EBM tools are used for complex environmental monitoring, such as waste and surface waters. Although the authors acknowledge that the YES may only serve as a first-tier screening tool and only tests the affinity of EDCs to bind to the human estrogen receptor, the assay still proved useful as this assay is reported to be more robust against the toxic nature of environmental surface waters and wastewaters compared to mammalian cell lines. The results that were generated from the YES showed that EEQ concentrations of WWTW discharge and environmental surface waters regularly surpassed established effect-based trigger values that warrant the need for further intervention at the test sites. As with the results for CECs in the current report, the EEQ estimations in surface waters also confirmed higher levels of pollution upstream from WWTW discharge, which further raises the need for more intervention into addressing alternative pollution sources other than WWTW discharge alone.

On the technical side, to promote improved environmental monitoring in the country, the collection protocols of water samples and quantification for CECs using LC-MS was done in accordance with other international studies and directives. First, it was concluded that the inclusion-labelled internal standards serve as a vital component for reliable CEC quantification in wastewater and surface waters, as a high degree of matrix suppression in more contaminated water sources may prevail, which compromises adequate quantification and mass balance estimations to assess WWTW performance. The use of time- or flow-proportional composite sampling should be implemented at WWTWs where possible, such as a sampling mode apart from sporadic grab sampling, which is shown to be a vital component in reliably gaining insight into the loads of CECs that enter treatment facilities and that are discharged into recipient water bodies due to the high degree of daily and sub-daily variation in CECs in sewer systems.

From all the future needs on CEC monitoring and risk characterisation that were mentioned in the current report, it becomes apparent that environmental monitoring may be multi-faceted by addressing various challenges that are associated with the contamination of freshwater resources with CECs. It is also clear that various environmental and public health challenges, along with the evaluation of water treatment operations, can be addressed when monitoring campaigns are well planned and executed, along with WWTW operating staff and management, allowing for multiple research and health-related topics that can be addressed, such as the following:

- Expanding the knowledge database of CECs at various locations of interest
- Identifying the fate of CECs in surface waters and water treatment
- Conducting a mass balance estimation to investigate water treatment performance
- Performing environmental risk characterisation that includes both lethal and sub-lethal health outcomes, either through effect-based monitoring or through targeted CEC quantification
- Substance use and abuse profiling on a community level using wastewater-based epidemiology

It is also evident that there is a large source of information available, which can aid in the prioritisation of emerging contaminants for environmental risk assessment. Several key points were discussed here and should be prioritised in future studies to ensure the sustainability of our freshwater resources and the overall health status of natural ecosystems and inhabitants.

REFERENCES

ABAFE OA, SPATH J, FICK J, JANSSON S, BUCKLEY C, STARK A, PIETRUSCHKA B and MARTINCIGH BS (2018) LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. Chemosphere **200** 660–670. https://doi.org/10.1016/j.chemosphere.2018.02.105.

AERNI HR, KOBLER B, RUTISHAUSER BV, WETTSTEIN FE, FISCHER R, GIGER W, HUNGERBÜHLER A, MARAZUELA MD, PETER A, SCHÖNENBERGER R, VÖGELI AC, SUTER MJF and EGGEN RIL (2004) Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. Analytical and Bioanalytical Chemistry **378** 688–696. http://doi.org/10.1007/s00216-003-2276-4.

AGUNBIADE F and MOODLEY B (2016) Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa. Environmental Toxicology and Chemistry **35 (1)** 36–46. http://doi.org/10.1002/etc.3144.

AGUNBIADE FO and MOODLEY B (2014) Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment **186 (11)** 7273–7291. http://doi.org/10.1007/s10661-014-3926-z.

AHN K-C, ZHAO B, CHEN J, CHEREDNICHENKO G, SANMARTI E, DENISON MS, LASLEY B, PESSAH IN, KÜLTZ D, CHANG DPY, GEE, SJ and HAMMOCK BD (2008) In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: Receptor-based bioassay screens. Environmental Health Perspectives **116 (9)** 1203–1210. https://doi.org/10.1289/ehp.11200.

AL AUKIDY M, VERLICCHI P and VOULVOULIS N (2014) A framework for the assessment of the environmental risk posed by pharmaceuticals originating from hospital effluents. Science of the Total Environment **493 (1)** 54–64. https://doi.org/10.1016/j.scitotenv.2014.05.128.

ALLMYR M, ADOLFSON-ERICI M, MCLACHLAN MS and SANDBORGH-ENLUND G (2006) Triclosan in plasma milk from Swedish nursing mothers and their exposure via personal care products. Science of the Total Environnent **372 (1)** 87–93. https://doi.org/10.1016/j.scitotenv.2006.08.007.

AMDANY R, CHIMUKA L and CUKROWSKA E (2014) Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application. Water SA **40 (3)** 407–415.

ANECK-HAHN NH, BORNMAN MS and DE JAGER C (2009) Oestrogenic activity in drinking waters from a rural area in the Waterberg district, Limpopo province, South Africa. Water SA **35 (3)** 245–252.

ANGELES LF, MULLEN RA, HUANG IJ, WILSON C, KHUNJAR W, SIROTKIN HI, MCELROY AE and AGA DS (2020) Assessing pharmaceutical removal and reduction in toxicity provided by advanced wastewater treatment systems. Environmental Science: Water Research and Technology **6** 62–77.

ANKLEY GT, BENNETT RS, ERICKSON RJ, HOFF DJ, HORNUNG MW, JOHNSON RD, MOUNT DR, NICHOLS JW, RUSSOM CL, SCHMIEDER PK, SERRRANO JET and VILLENEUVE DL (2010) Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry **29 (3)** 730–741. https://doi.org/10.1002/etc.34.

ARCHER E and VAN WYK JH (2015) The potential anti-androgenic effect of agricultural pesticides used in the Western Cape: In vitro investigation of mixture effects. Water SA **41 (1)** 129–137. https://doi.org/10.4314/wsa.v41i1.16.

ARCHER E, CASTRIGNANO E, KASPRZYK-HORDERN B and WOLFAARDT GM (2018) Wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters. Science of the Total Environment **625** 792–800. https://doi.org/10.1016/j.scitotenv.2017.12.269.

ARCHER E, PETRIE B, KASPRZYK-HORDERN B and WOLFAARDT GM (2017a) The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere **174** 437–446. http://doi.org/10.1016/j.chemosphere.2017.01.101.

ARCHER E, WOLFAARDT GM and VAN WYK JH (2017b) Review: Pharmaceutical and personal care products (PPCPs) as endocrine disrupting contaminants (EDCs) in South African surface waters. Water SA **43** 684–706.

ARFER KB, TOMLINSON M, MAYEKISO A, BANTJES J, VAN HEERDEN A and ROTHERAM-BORUS MJ (2017) Criterion validity of self-reports of alcohol, cannabis, and methamphetamine use among young men in Cape Town, South Africa. International Journal of Mental Health and Addiction 16 (1) 45–52. http://doi.org/10.1007/s11469-017-9769-4.

ARIS AZ, SHAMSUDDIN AS and PRAVEENA SM (2014) Occurrence of 17α-ethynylestradiol (EE2) in the environment and effect on exposed biota: A review. Environment International **69** 104–119. https://doi.org/10.1016/j.envint.2014.04.011.

ARLOS MJ, PARKER WJ, BICUDO JR, LAW P, MARJAN P, ANDREWS SA and SERVOS MR (2018) Multi-year prediction of estrogenicity in municipal wastewater effluents. Science of the Total Environment **610–611** 1103–1112. http://doi.org/10.1016/j.scitotenv.2017.08.171.

ASANTE KO and LENTOOR AG (2017) Use of crystal methamphetamine among male adolescents in Cape Town, South Africa: Caregivers' experiences. Substance Abuse Treatment, Prevention, and Policy **12** 18. http:/doi.org/10.1186/s13011-017-0102-9.

BAALBAKI Z, SULTANA T, METCALFE C and YARGEAU V (2017) Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics. Chemosphere **178** 439–448. http://doi.org/10.1016/j.chemosphere.2017.03.070.

BAGNALL J, MALIA L, LUBBEN A and KASPRZYK-HORDERN B (2013) Stereoselective biodegradation of amphetamine and methamphetamine in river microcosms. Water Research **47** 5708–5718. http://doi.org/10.1016/j.watres.2013.06.057.

BAKER DR and KAASPRZYK-HORDERN B (2013) Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. Science of the Total Environment **454–455** 442–456.

BAKER DR, BARRON L and KASPRZYK-HORDERN B (2014) Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: Chemical analysis and drug use estimates. Science of the Total Environment **487** 629–641. http://doi.org/10.1016/j.scitotenv.2013.11.107.

BAKER DR and KASPRZYK-HORDERN B (2011) Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive

electrospray ionisation tandem mass spectrometry. Journal of Chromatography A **1218** 1620–1631. http://doi.org/10.1016/j.chroma.2011.01.060.

BARCHANSKA H, SAJDAK M, SZCZYPKA K, SWIENTEK A, TWOREK M and KUREK M (2017) Atrazine, triketone herbicides, and their degradation products in sediment, soil and surface water samples in Poland. Environmental Science and Pollution Research **24** 644–658. http://doi.org/10.1007/s11356-016-7798-3.

BARNHOORN IEJ, VAN DYK JC, PIETERSE GM and BORNMAN MS (2010) Intersex in feral indigenous freshwater Oreochromis mossambicus, from various parts in the Luvuvhu River, Limpopo province, South Africa. Ecotoxicology and Environmental Safety **73 (7)** 1537–1542. https://doi.org/10.1016/j.ecoenv.2010.07.026.

BARNHOORN IEJ, BORNMAN MS, PIETERSE GM and VAN VUREN JHJ (2004) Histological evidence of intersex in feral sharptooth catfish (Clarias gariepinus) from an estrogen-polluted water source in Gauteng, South Africa. Environmental Toxicology **19** 603–608. http://doi.org/10.1002/tox.20068.

BARNHOORN IEJ, DYK JC, VAN GENTHE B, HARDING WR, WAGENAAR GM and BORNMAN MS (2015) Organochlorine pesticide levels in Clarias gariepinus from polluted freshwater impoundments in South Africa and associated human health risks. Chemosphere **120** 391–397. http://doi.org/10.1016/j.chemosphere.2014.08.030.

BARSE AV, CHAKRABARTI T, GHOSH TK, PAL AK, KUMAR N, RAMAN RP and JADHAO SB (2010) Vitellogenin induction and histo-metabolic changes following exposure of Cyprinus carpio to methyl paraben. Asian-Australian Journal of Animal Sciences **23 (12)** 1557–1565. https://doi.org/10.5713/ajas.2010.10118.

BASELT RC (2004) *Disposition of toxic drugs and chemicals in man*, 7th ed., Biomedical Publications.

BAYLEY M, LARSEN PF, BÆKGAARD H and BAATRUP E (2003) The effects of vinclozolin, an anti-androgenic fungicide, on male Guppy secondary sex characters and reproductive success. Biology of Reproduction **69 (6)** 1951–1956. https://doi.org/10.1095/biolreprod.103.017780.

BEHRENDS T, URBATZKA R, KRACKOW S, ELEPFANDT A and KLOAS W (2010) Mate calling behaviour of male South African clawed frogs (Xenopus laevis) is suppressed by the antiandrogenic endocrine disrupting compound flutamide. General and Comparative Endocrinology **168 (2)** 269–274. https://doi.org/10.1016/j.ygcen.2010.01.017.

BERGMANN A, FOHRMANN R and WEBER F-A (2011) Zusammenstellung von monitoringdaten zu umweltkonzentrationen von arzneimitteln. Umweltforschungsplan des bundesministeriums für umwelt, naturschutz und reaktorsicherheit. Forschungskennzahl **360 14**. http://www.uba.de/uba-info-medien/4188.html.

BIJLSMA L, BOTERO-COY AM, RINCÓN RJ, PEÑUELA GA and HERNÁNDEZ F (2016) Estimation of illicit drug use in the main cities of Colombia by means of urban wastewater analysis. Science of the Total Environment **565** 984–993. http://doi.org/10.1016/j.scitotenv.2016.05.078.

BINELLI A, PEDRIALI A, RIVA C and PAROLINI M (2012) Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model Dreissena polymorpha. Chemosphere **86** 906–911. http://doi.org/10.1016/j.chemosphere.2011.10.056.

BISHNOI A, CARLSON HE, GRUBER BL, KAUFMAN LD, BOCK JL and LIDONNICI K (1994) Effects of commonly prescribed nonsteroidal anti-inflammatory drugs on thyroid hormone measurements. The American Journal of Medicine **96 (3)** 35–238. https://doi.org/10.1016/0002-9343(94)90148-1.

BISTAN M, PODGORELEC M, LOGAR RM and TIŠLER T (2012) Yeast estrogen screen assay as a tool for detecting estrogenic activity in water bodies. Food Technology and Biotechnology **50** 427–433.

BLAIR B, NIKOLAUS A, HEDMAN C, KLAPER R and GRUNDL T (2015) Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere **134** 395–401. http://doi.org/10.1016/j.chemosphere.2015.04.078.

BOBERG J, TAXVIG C, CHRISTIANSEN S and HASS U (2010) Possible endocrine disrupting effects of parabens and their metabolites. Reproductive Toxicology **30** 301–312. doi:10.1016/j.reprotox.2010.03.011

BOLONG N, ISMAIL AF, SALIM MR and MATSUURA T (2009) A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination **239** 229–246. http://doi.org/10.1016/j.desal.2008.03.020.

BONVIN F, OMLIN J, RUTLER R, SCHWEIZER WB, ALAIMO PJ, STRATHMANN TJ, MCNEILL K and KOHN T (2013) Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: Evidence for abiotic back-transformation. Environmental Science and Technology **47 (13)** 6746–6755. https://doi.org/10.1021/es303777k.

BORNMAN MS, VAN VUREN JHJ, BOUWMAN H, DE JAGER C, GENTHE B and BARNHOORN IEJ (2007) *Endocrine disruptive activity and the potential health risk in an urban nature reserve*. WRC Report No. 1505/1/07, Water Research Commission, Pretoria, South Africa.

BORNMAN R and BOUWMAN H (2012) Environmental pollutants and diseases of sexual development in humans and wildlife in South Africa: Harbingers of impact on overall health? Reproduction in Domestic Animals **47 (4)** 327–332. https://doi.org/10.1111/j.1439-0531.2012.02094.x.

BRIGGS DE and FRENCH JA (2004) Levetiracetam safety profiles and tolerability in epilepsy patients. Expert Opinion on Drug Safety **3 (5)** 415–424. https://doi.org/10.1517/14740338.3.5.415.

BRION F, TYLER CR, PALAZZI X, LAILLET B, PORCHER JM, GARRIC J and FLAMMARION P (2004) Impacts of 17beta-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (Danio rerio). Aquatic Toxicology **68** 193–217. http://doi.org/10.1016/j.aquatox.2004.01.022.

CAI Y, CHAI D, WANG R, LIANG B and BAI N (2012) Colistin resistance of Acinetobacter baumannii: Clinical reports, mechanisms and antimicrobial strategies. Journal of Antimicrobial Chemotherapy **67 (7)** 1607–1615.

CALDWELL DJ, MASTROCCO F, ANDERSON PD, LÄNGE R and SUMPTER JP (2012) Predicted-no-effect concentrations for the steroid estrogens estrone, 17beta-estradiol, estriol, and 17beta-ethinylestradiol. Environmental Toxicology and Chemistry **31** 1396–1406. http://doi.org10.1002/etc.1825.

CAMACHO-MUÑOZ D, PETRIE B, CASTRIGNANÒ E and KASPRZYK-HORDERN B (2016) Enantiomeric profiling of chiral pharmacologically active compounds in the environment with the usage of chiral liquid chromatography coupled with tandem mass spectrometry. Current Analytical Chemistry **12** 303–314.

CARGOUËT M, PERDIZ D, MOUATASSIM-SOUALI A, TAMISIER-KAROLAK S and LEVI Y (2004) Assessment of river contamination by estrogenic compounds in Paris area (France). Science of the Total Environment **324** 55–66. http://doi.org/10.1016/j.scitotenv.2003.10.035.

CARLSSON C, JOHANSSON A-K, ALVAN G, BERGMAN K and KÜHLER T (2006) Are pharmaceuticals potent environmental pollutants? Part I: Environmental risk assessments of selected active pharmaceutical ingredients. Science of the Total Environment **364** 67–87.

CARVALHO RN, CERIANI L, IPPOLITO A and LETTIERI T (2015) *Development of the first watch list under the environmental quality standards directive*. Directive 2008/105/EC, as amended by Directive 2013/39/EU, in the field of water policy, European Commission. http://doi.org/10.2788/101376.

CASTIGLIONI S, BAGNATI R, MELIS M, PANAWENNAGE D, CHIARELLI P, FANELLI R and ZUCCATO E (2011) Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. Water Research **45** 5141–5150. http://doi.org/10.1016/j.watres.2011.07.017.

CASTIGLIONI S, BIJLSMA L, COVACI A, EMKE E, HARMAN C, HERNÁNDEZ F, KASPRZYK-HORDERN B, ORT C, VAN NUIJS ALN, DE VOOGT P and ZUCCATO E (2016) Estimating community drug use through wastewater-based epidemiology. Assessing illicit drugs in wastewater: Advances in wastewater-based drug epidemiology. EMCDDA Insights **22**. http://doi.org/10.2810/017397.

CASTIGLIONI S, THOMAS KV, KASPRZYK-HORDERN B, VANDAM L and GRIFFITHS P (2014) Testing wastewater to detect illicit drugs: State of the art, potential and research needs. Science of the Total Environment **487** 613–620. http://doi.org/10.1016/j.scitotenv.2013.10.034.

CASTIGLIONI S, ZUCCATO E, FATTORE E, RIVA F, TERZAGHI E, KOENIG R, PRINCIPI P and DI GUARDO A (2020) Micropollutants in Lake Como water in the context of circular economy: A snapshot of water cycle contamination in a changing pollution scenario. Journal of Hazardous Materials **384** 121441.

CASTRIGNANÒ E, LUBBEN A and KASPRZYK-HORDERN B (2016) Enantiomeric profiling of chiral drug biomarkers in wastewater withthe usage of chiral liquid chromatography coupled with tandem massspectrometry. Journal of Chromatography A **1438** 84–99. http://doi.org/10.2174/1573411012666151009195039.

CASTRIGNANÒ E, MARDAL M, RYDEVIK A, MISEREZ B, RAMSEY J, SHINE T, PANTOS GD, MEYER B and KASPRZYK-HORDERN B (2017) A new approach towards biomarker selection in estimation of human exposure to chiral chemicals: A case study of mephedrone. Scientific Reports **7**.

CASTRIGNANÒ E, YANG Z, BADE R, BAZ-LOMBA JA, CASTIGLIONI S, CAUSANILLES A, COVACI A, GRACIA-LOR E, HERNANDEZ F, KINYUA J, MCCALL A-K, VAN NUIJS ALN, ORT C, PLOSZ BG, RAMIN P, ROUSIS NI, RYU Y, THOMAS KV, DE VOOGT P, ZUCCATO E and KASPRZYK-HORDERN B (n.d.) Enantiomeric profiling of chiral illicit drugs in a pan-European study. Water Research **130** 151–160.

CAUSANILLES A, RUEPERT C, IBÁÑEZ M, EMKE E, HERNÁNDEZ F and DE VOOGT P (2017) Occurrence and fate of illicit drugs and pharmaceuticals in wastewater from two wastewater treatment plants in Costa Rica. Science of the Total Environment **599** 600–698. http://doi.org/10.1016/j.scitotenv.2017.04.202.

CHAE J-P, PARK MS, HWANG Y-S, MIN B-H, KIM S-H, LEE H-S and PARK M-J (2015) Evaluation of developmental toxicity and teratogenicity of diclofenac using Xenopus embryos. Chemosphere **120 (1)** 52–58. https://doi.org/10.1016/j.chemosphere.2014.05.063.

CHEN J, CHANG AHN K, GEE NA, AHMED MI, DULEBA AJ, ZHAO L, GEE SJ, HAMMOCK BD and LASLEY BL (2008) Triclocarban enhances testosterone action: A new type of endocrine disruptor? Endocrinology **149 (3)** 1173–1179. https://doi.org/10.1210/en.2007-1057.

CHOI PM, TSHARKE BJ, DONNER E, O'BRIEN JW, GRANT SC, KASERZON SL, MACKIE R, O'MALLEY E, CROSBIE ND, THOMAS KV and MUELLER JF (2018) Wastewater-based epidemiology biomarkers: Past, present and future. Trends in Analytical Chemistry **105** 453–469. https://doi.org/10.1016/j.trac.2018.06.004.

CHONOVA T, LABANOWSKI J, COURNOYER B, CHARDON C, KECK F, LAURENT E, MONDAMERT L, VASSELON V, WIEST L and BOUCHEZ A (2017) River biofilm community changes related to pharmaceutical loads emitted by a wastewater treatment plant. Environmental Science and Pollution Research http://doi.org/10.1007/s11356-017-0024-0.

CHRISTEN V, CRETTAZ P, OBERLI-SCHRÄMMLI A and FENT K (2010) Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro. Chemosphere **81 (10)** 1245–1252. https://doi.org/10.1016/j.chemosphere.2010.09.031.

CHRISTIANSEN S, SCHOLZE M, DALGAARD M, VINGGAARD AM, AXELSTAD M, KORTENKAMP A and HASS U (2009) Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environmental Health Perspectives **117 (12)** 1839–1846. https://doi.org/10.1289/ehp.0900689.

COLBORN T, VOM SAAL FS and SOTO AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives **101** 378–384. http://doi.org/10.1016/0195-9255(94)90014-0.

CROFTON KM, PAUL KB, DEVITO MJ and HEDGE JM (2007) Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine. Environmental Toxicology and Pharmacology **24** 194–197.

CUDERMAN P and HEATH E (2007) Determination of UV filters and antimicrobial agents in environmental water samples. Analytical and Bioanalytical Chemistry **387 (4)** 1343–1350. https://doi.org/10.1007/s00216-006-0927-y.

CZARCZYŃSKA-GOŚLIŃSKA B, ZGOLA-GRZEŚKOWIAK A, JESZKA-SKOWRON M, FRANKOWSKI R and GRZEŚKOWIAK T (2017) Detection of bisphenol A, cumylphenol and parabens in surface waters of Greater Poland Voivodeship. Journal of Environmental Management **204** 50–60.

DADA S, BURNHAMS NB, ERASMUS J, PARRY C, BHANA A, TIMOL F, NEL E, KITSHOFF D, WEIMANN R and FOURIE D (2017) *South African Community Epidemiology Network on Drug Use (SACENDU) report, monitoring alcohol, tobacco and other drug abuse treatment admissions in South Africa.*

DAUGHTON CG and TERNES TA (1999) Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives **107 (6)** 907–938. https://doi.org/10.1289/ehp.99107s6907.

DAUGHTON CG (2001) Emerging pollutants,and communicating the science of environmental chemistry and mass spectrometry: Pharmaceuticals in the environment. Journal of the American Society fro Mass Spectrometry **12** 1067–1076. http://doi.org/10.1016/S1044-0305(01)00287-2.

DE JAGER C, ANECK-HAHN NH, SWART P, TRUEBODY B and VAN ZIJL MC (2013) *Estrogenic activity and endocrine disrupting chemical (EDC) status in water obtained from selected distribution points in Pretoria and Cape Town.* WRC Report No. KV317/13, Water Research Commission, Pretoria, South Africa.

DE LA TORRE R, FARRE M, ORTUNO J, CAMI J and SEGURA J (1991) The relevance of urinary cocaethylene following the simultaneous administration of alcohol and cocaine [2]. Journal of Analytical Toxicology **15** 223.

DEPARTMENT OF WATER AND SANITATION (DWS) (2013) *Green Drop Report of 2013*. DWS, Pretoria, South Africa. http://www.dwa.gov.za/Dir_WS/GDS/Docs/DocsDefault.aspx.

DEPARTMENT OF WATER AND SANITATION (DWS) (2014). *Blue Drop Report for 2014*. DWS, Pretoria, South Africa. https://www.green-cape.co.za/assets/Water-Sector-Desk-Content/DWS-2014-Blue-Drop-report-national-overview-part-1-of-2-2016.pdf.

DEVAULT DA, LÉVI Y and KAROLAK S (2017) Applying sewage epidemiology approach to estimate illicit drug consumption in a tropical context: Bias related to sewage temperature and pH. Science of the Total Environment **584–585** 252–258. http://doi.org/10.1016/j.scitotenv.2017.01.114.

DU PREEZ LH, KUNENE N, HANNER R, GIESY JP, SOLOMON KR, HOSMER A and VAN DER KRAAK GJ (2009) Population-specific incidence of testicular ovarian follicles in Xenopus laevis from South Africa: A potential issue in endocrine testing. Aquatica Toxicology **95 (1)** 10–16. https://doi.org/10.1016/j.aquatox.2009.07.018.

EBELE AJ, ABDALLAH MA-E and HARRAD S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminates **3** 1–16.

ECOLOGICAL STRUCTURE ACTIVITY RELATIONSHIPS (ECOSAR) (n.d.) ECOSAR Predictive Model. United States Environmental Protection Agency (USEPA), Washington, DC, USA. https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model.

EDWARDS SJ and KJELLERUP BV (2013) Applications of biofilms in bioremediation and biotransformation of persistent organic pollutants, pharmaceuticals/personal care products, and heavy metals. Applied Microbiology and Biotechnology **97 (23)** 9909–9921. https://doi.org/10.1007/s00253-013-5216-z.

EDWARDS SW, TAN Y, VILLENEUVE DL, MEEK ME and MCQUEEN CA (2016). Adverse outcome pathways − organizing toxicological information to improve decision making. Minireviews. Journal of Pharmacology and Experimental Therapeutics **356** 170–181.

EMKE E, EVANS S, KASPRZYK-HORDERN B and DE VOOGT P (2014) Enantiomer profiling of high loads of amphetamine and MDMA in communal sewage: A Dutch perspective. Science of the Total Environment **487** 666–672. http://doi.org/10.1016/j.scitotenv.2013.11.043.

Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) (1998) *EDSTAC Final Report*. United States Environmental Protection Agency (USEPA), Washington, DC, USA. http://www.epa.gov/endo/pubs/edspoverview/finalrpt.htm.

ERMLER S, SCHOLZE M and KORTENKAMP A (2011) The suit-ability of concentration addition for predicting the effects of multi-component mixtures of up to 17 anti-androgens with varied structural

features in an in vitro AR antagonist assay. Toxicology and Applied Pharmacology **257 (2)** 189–197. https://doi.org/10.1016/j.taap.2011.09.005.

ESCHER BI, NEALE PA and LEUSCH FDL (2015) Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. Water Research **81** 137–148.

ESSACK SY, CONNOLLY C and STURM AW (2005) Antibiotic use and resistance in public-sector hospitals in KwaZulu-Natal. South African Medical Journal **95 (11)** 865–870.

European Commission (EC) (2016) *Technical guidance document on risk assessment*. EUR 20418 EN/1, EC Joint Research Centre, Ispra, Italy.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2011) *Report on the risk assessment of mephedrone in the framework of the Council Decision on new psychoactive substances*. European Union, Brussels, Belgium. http://doi.org/10.2810/40800.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2016) *EMCDDA sewage analysis CORe group Europe (SCORE). Wastewater analysis and drugs – a European multi-city study.* European Union, Brussels, Belgium. http://www.emcdda.europa.eu/topics/pods/waste-water-analysis.

EVANS S, BAGNALL J and KASPRZYK-HORDERN B (2017) Enantiomeric profiling of a chemically diverse mixture of chiral pharmaceuticals in urban water. Environmental Pollution **230** 368–377. http:/doi.org/10.1016/j.envpol.2017.06.070.

EVANS SE, BAGNALL J and KASPRZYK-HORDERN B (2016) Enantioselective degradation of amphetamine-like environmental micropollutants (amphetamine, methamphetamine, MDMA and MDA) in urban water. Environmental Pollution **215** 154–163. http://doi.org/10.1016/j.envpol.2016.04.103.

EVANS SE, DAVIES P, LUBBEN A and KASPRZYK-HORDERN B (2015) Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Analytica Chimica Acta **882** 112–126. http://doi.org/10.1016/j.aca.2015.03.039.

FALÅS P, WICK A, CASTRONOVO S, HABERMACHER J, TERNES TA and JOSS A (2016) Tracing the limits of organic micropollutant removal in biological wastewater treatment. Water Research **95** 240–249. http://doi.org/10.1016/j.watres.2016.03.009.

FALEYE AC, ADEGOKE AA, RAMLUCKAN K, FICK J, BUX F and STENSTRÖM (2019) Concentration and reduction of antibiotic residues in selected wastewater treatment plants and receiving waterbodies in Durban, South Africa. Science of the Total Environment **678** 10–20. https://doi.org/10.1016/j.scitotenv.2019.04.410.

FATOKI O, OLUJIMI O, ODENDAAL J and GENTHE B (2008) Health risk assessment of plasticizer in wastewater effluents and receiving freshwater systems. Recent Advances in Plasticizers. http://doi.org/10.5772/35950.

FERRARI B, MONS R, VOLLAT B, FRAYSSE B, PAXÉUS N, LO GIUDICE R, POLLIO A and GARRIC J (2004) Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environmental Toxicology and Chemistry **23** 1344–1354.

FLEMMING HC, WINGENDER J, SZEWZYK U, STEINBERG P, RICE SA and KJELLEBERG S (2016) Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology **14 (9)** 563–575. https://doi.org/10.1038/nrmicro.2016.94.

FOLMAR LC, HEMMER MJ, DENSLOW ND, KROLL K, CHEN J, CHEEK A, RICHMAN H, MEREDITH H and GRAU EG (2002) A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. Aquatic Toxicology **60 (1–2)** 101–110. https://doi.org/10.1016/S0166-445X(01)00276-4.

FRENCH CE and BRUCE NC (1994) Purification and characterization of morphinone reductase from Pseudomonas putida M10. Biochemical Journal **301** 97–103.

GALUS M, JEYARANJAAN J, SMITH E, LI H, METCALFE C and WILSON JY (2013a) Chronic effects of exposure to a pharmaceutical mixture and municipal wastewater in zebrafish. Aquatic Toxicology **132–133** 212–222.

GALUS M, KIRISCHIAN N, HIGGINS S, PURDY J, CHOW J, RANGARANJAN S, LI H, METCALFE C and WILSON JY (2013b) Chronic, low concentration exposure to pharmaceuticals impacts multiple organ systems in zebrafish. Aquatic Toxicology **132–133**, 200–211.

GARCIA-REYERO N (2015) Are adverse outcome pathways here to stay? Environmental Science and Technology **49** 3–9. https://doi.org/10.1021/es504976d.

GASSER G, PANKRATOV I, ELHANANY S, WERNER P, GUN J, GELMAN F and LEV O (2012) Field and laboratory studies of the fate and enantiomeric enrichment of venlafaxine and O-desmethylvenlafaxine under aerobic and anaerobic conditions. Chemosphere **88** 98–105. http://doi.org/10.1016/j.chemosphere.2012.02.074.

GENTHE B, ROUX WJ LE SCHACHTSCHNEIDER K, OBERHOLSTER PJ, ANECK-HAHN NH and CHAMIER J (2013) Health risk implications from simultaneous exposure to multiple environmental contaminants. Ecotoxicology and Environmental Safety **93** 171–179. http://doi.org/10.1016/j.ecoenv.2013.03.032.

GERBA CP and SMITH JE (2005) Sources of pathogenic microorganisms and their fate during land application of wastes. Journal of Environmental Quality **34 (1)** 42–48.

GHADAKPOUR M, BESTER E, LISS SN, GARDAM M, DROPPO I, HOTA S and WOLFAARDT GM (2014) Integration and proliferation of Pseudomonas aeruginosa PA01 in multispecies biofilms. Microbiology Ecology **68 (1)** 121–131. https://doi.org/10.1007/s00248-014-0398-1.

GIGER W, SCHAFFNER C and KOHLER HP (2006) Benzotriazole and tolytriazole as aquatic contaminants. 1. Input and occurrence in rivers and lakes. Environmental Science and Technology **40** 7186–7192.

GLOBAL WATER RESEARCH COALITION (GWRC) (2003) *Endocrine disrupting compounds: An overview of the sources and biological methods for measuring EDCs.* BTO 2003.008, GWRC, London, UK.

GRACIA T, HILSCHEROVA K, JONES PD, NEWSTED JL, HIGLEY EB, ZHANG X, HECKER M, MURPHY MB, YU RMK, LAM PKS, WU RSS and GIESY JP (2007) Modulation of steroidogenic gene expression and hormone production of H295R cells by pharmaceuticals and other environmentally active compounds. Toxicology and Applied Pharmacology **225 (2)** 142–153. http://doi.org/10.1016/j.taap.2007.07.013.

GRÖNER F, HÖHNE C, KLEINER W and KLOAS W (2017) Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in Nile tilapia (Oreochromis niloticus). Chemosphere **166** 473–481.

GUILLETTE Jr. LJ, WOODWARD AR, CRAIN DA, PICKFORD DB, ROONEY AA and PERCIVAL HF (1999) Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. General and Comparative Endocrinology **116 (3)** 356–372. https://doi.org/10.1006/gcen.1999.7375.

GUILLETTE LJ, PICKFORD DB, CRAIN DA, ROONEY AA and PERCIVAL HF (1996) Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. General and Comparative Endocrinology **101** 32–42.

GUO J, LI J, CHEN H, BOND PL and YUAN Z (2017) Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements. Water Research **123** 468–478.

HALDEN RU and PAULL DH (2005) Co-occurrence of triclocarban and triclosan in US water resources. Environmental Science and Technology **39 (6)** 1420–1426. https://doi.org/10.1021/es049071e.

HAN S, CHOI K, KIM J, JI K, KIM S, AHN B, YUN J, CHOI K, KHIM JS, ZHANG X and GIESY JP (2010) Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (Oryzias latipes) and freshwater cladocerans Daphnia magna and Moina macrocopa. Aquatic Toxicology **98** 256–264.

HARDEN CL, NIKLOV B, KANDULA P, LABAR D and PANNULLO S (2010) Effect of levetiracetam on testosterone levels in male patients. Epilepsia **51 (11)** 2348–2351. https://doi.org/10.1111/j.1528-1167.2010.02732.x.

HART DS, DAVIS LC, ERICKSON LE and CALLENDER TM (2004) Sorption and partitioning parameters of benzotriazole compounds. Microchemical Journal **77 (1)** 9–17.

HEATH RGM and CLAASSEN M (1999) *An overview of the pesticide and metal levels present in populations of the larger indigenous fish species of selected South African rivers*. WRC Report No. 428/1/99, Water Research Commission, Pretoria, South Africa.

HEATH R, BOYD L, MALETE O and MASOABI D (2013a) *Introduction to the management of EDCs: Volume I.* WRC Report No. TT 560/13, Water Research Commission, Pretoria, South Africa.

HEATH R, BOYD L, MALETE O and MASOABI D (2013b) *Guideline for the management of EDCs in a catchment: Volume V.* WRC Report No. TT 562/13, Water Research Commission, Pretoria, South Africa.

HEMBACH N, SCHMID F, ALEXANDER J, HILLER C, ROGALL ET and SCHWARTZ T (2017) Occurrence of the mcr-1 colistin resistance gene and other clinically relevant antibiotic resistance genes in microbial populations at different municipal wastewater treatment plants in Germany. Frontiers in Microbiology **8** 1282.

HENDRICKS R and POOL EJ (2012) The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues. Journal of Environmental Science and Health Part A **47 (2)** 289–297. https://doi.org/10.1080/10934529.2012.637432.

HERNANDEZ LEAL L, VIENO N, TEMMINK H, ZEEMAN G and BUISMAN CJN (2010) Occurrence of xenobiotics in gray water and removal in three biological treatment systems. Environmental Science and Technology **44** 6835–6842. http://doi.org/10.1021/es101509e.

HERNANDO MD, MEZCUE M, FERNÀNDEZ-ALBA AR and BARCELÒ D (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta **69** 334–342.

HERZOG AG, DRISLANE FW, SCHOMER DL, PENNELL PB, BROMFIELD EB, DWORETZKY BA, FARINA EL and FRYE CA (2005) Differential effects of antiepileptic drugs on sexual function and hormones in men with epilepsy. Neurology **65 (7)** 1016–1020. https://doi.org/10.1212/01.wnl.0000178988.78039.40.

HONG HN, KIM HN, PARK KS, LEE S-K and GU MB (2007) Analysis of the effects diclofenac has on Japanese medaka (Oryzias latipes) using real-time PCR. Chemosphere **67** 2115–2121.

HOTA S, HIRJI Z, STOCKTON K, LEMIEUX C, DEDIER H, WOLFAARDT G and GARDAM MA (2009) Outbreak of multidrug-resistant Pseudomonas aeruginosa colonization and infection secondary to imperfect intensive care unit room design. Infection Control and Hospital Epidemiology **30 (1)** 25–33. https://doi.org/10.1086/592700.

INUI M, ADACHI T, TAKENAKA S, INUI H, NAKAZAWA M, UEDA M, WATANABE H, MORI C, IGUCHI T and MIYATAKE K (2003) Effect of UV screens and preservatives on vitellogenin and choriogenin production in male medaka (Oryzias latipes). Toxicology **194** 43–50. https://doi.org/10.1016/S0300-483X(03)00340-8.

ISHIBASHI H, MATSUMURA N, HIRANO M, MATSUOKA M, SHIRATSUCHI H, ISHIBASHI Y, TAKAO Y and ARIZONO K (2004) Effects of triclosan on early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. Aquatic Toxicology **67 (2)** 167–179. https://doi.org/10.1016/j.aquatox.2003.12.005.

JAROŠOVÁ B, BLÁHA L, GIESY JP and HILSCHEROVÁ K (2014a) What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? Environment International **64** 98–109. http://doi.org/10.1016/j.envint.2013.12.009.

JAROŠOVÁ B, ERSEKOVÁ A, HILSCHEROVÁ K, LOOS R, GAWLIK BM, GIESY JP and BLÁHA L (2014b) Europe-wide survey of estrogenicity in wastewater treatment plant effluents: the need for the effect-based monitoring. Environmental Science and Pollution Research **21** 10970–10982. http://doi.org/10.1007/s11356-014-3056-8.

JI K, CHOI L, LEE S, PARK S, KHIM JS, JO EH, CHOI K, ZHANG X and GIESY JP (2010) Effects of sulfathiazole, oxytetracycline and chlortetracycline on steroidogenesis in the human adrenocarcinoma (H295R) cell line and freshwater fish Oryzias latipes. Journal of Hazardous Material **182 (1-3)** 494–502. https://doi.org/10.1016/j.jhazmat.2010.06.059.

JI K, HONG S, KHO Y and CHOI K (2013) Effects of bisphenol S exposure on endocrine functions and reproduction of Zebrafish. Environmental Science and Technology **47** 8793–8800.

JOBLING S, BURN RW, THORPE K, WILLIAMS R and TYLER C (2009) Statistical modelling suggests that antiandrogens in effluents from wastewater treatment works contribute to widespread sexual disruption in fish living in English rivers. Environmental Health Perspectives **117 (5)** 797–802. https://doi.org/10.1289/ehp.0800197.

JOOSTE S, BOLLMOHR S and THWALA M (2008) *National toxicity monitoring program: Report on Phase 3: Pilot implementation and testing of the design*. Report No. N/0000/REQ1008, Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa.

JUNG J, ISHIDA K and NISHIHARA T (2004) Anti-estrogenic activity of fifty chemicals evaluated by in vitro assays. Life Sciences **74** 3065–3074. http://doi.org/10.1016/j.lfs.2003.10.030.

KANAMA KM, DASO AP, MPENYANA-MONYATSI L and COETZEE MAA (2018) Assessment of pharmaceuticals, personal care products, and hormones in wastewater treatment plants receiving inflows from health facilities in North West province, South Africa. Journal of Toxicology **2018**. https://doi.org/10.1155/2018/3751930.

KANG HJ, CHOI K, KIM M-Y and Kim P-G (2006) Endocrine disruption induced by some sulfa drugs and tetracyclines on Oryzias latipes. Korean Journal of Environmental Health Sciences **32** 227–234.

KARIMI R, KARAMI M and NADOUSHAN MJ (2017) Rat's polycystic ovary due to intraventromedial hypothalamus morphine injection. Reproductive Sciences 193371911769858. http://doi.org/10.1177/1933719117698581.

KASPRZYK-HORDERN B, DINSDALE RM and GUWY AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Research **43 (2)** 363–380. https://doi.org/10.1016/j.watres.2008.10.047.

KASPRZYK-HORDERN B and BAKER DR (2012a) Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environnemental Science and Technology **46** 1681–1691. http://doi.org/10.1021/es203113y.

KASPRZYK-HORDERN B and BAKER DR (2012b) Estimation of community-wide drugs use via stereoselective profiling of sewage. Science of the Total Environment **423** 142–150. http://doi.org/10.1016/j.scitotenv.2012.02.019.

KASPRZYK-HORDERN B, KONDAKAL VVR and BAKER DR (2010) Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry. Journal of Chromatography A **1217** 4575–4586. http://doi.org/10.1016/j.chroma.2010.04.073.

KAUSHIK G, HUBER DP, AHO K, FINNEY B, BEARDEN S, ZARBALIS KS and THOMAS MA (2016) Maternal exposure to carbamazepine at environmental concentrationscan cross intestinal and placental barriers. Biochemical and Biophysical Research Communications **474** 291–295. http://dx.doi.org/10.1016/j.bbrc.2016.04.088.

KHAN U and NICELL JA (2011) Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environment International **37** 1236–1252. http://doi.org/10.1016/j.envint.2011.05.009.

KJÆRSTAD MB, TAXVIG C, ANDERSEN HR and NELLEMANN C (2010) Mixture effects of endocrine disrupting compounds in vitro. International Journal of Andrology **33 (2)** 425–433. https://doi.org/10.1111/j.1365-2605.2009.01034.x.

KÖNIG M, ESCHER BI, NEALE PA, KRAUSS M, HILSCHEROVÁ K, NOVÁK J, TEODOROVIĆ I, SCHULZE T, SEIDENSTICKER S, KAMAL HASHMI MA, AHLHEIM J and BRACK W (2017) Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environmental Pollution **220** 1220–1230. http://doi.org/10.1016/j.envpol.2016.11.011.

KRUGER T, BARNHOORN I, JANSEN VAN VUREN J and BORNMAN R (2013) The use of the urogenital papillae of male feral African sharptooth catfish (Clarias gariepinus) as indicator of exposure

to estrogenic chemicals in two polluted dams in an urban nature reserve, Gauteng, South Africa. Ecotoxicology and Environmental Safety **87** 98–107. https://doi.org/10.1016/j.ecoenv.2012.10.004.

LAI FYIN, WILKINS C, THAI P and MUELLER JF (2017) An exploratory wastewater analysis study of drug use in Auckland, New Zealand. Drug and Alcohol Review **36 (5)** 597–601. http://doi.org/10.1111/dar.12509.

LECH JJ, LEWIS SK and REN L (1996) In vivo estrogenic activity of nonylphenol in rainbow trout. Toxicological Sciences **30 (2)** 229–232. https://doi.org/10.1093/toxsci/30.2.229.

LEHUTSO RF, DASO AP and OKONKWO JO (2017) Occurrence and environmental levels of triclosan and triclocarban in selected wastewater treatment plants in Gauteng province, South Africa. Emerging Contamination (in press). http://dx.doi.org/10.1016/j.emcon.2017.07.001.

LEUSCH FDL, NEALE PA, ARNAL C, ANECK-HAHN NH, BALAGUER P, BRUCHET A, ESCHER bi, ESPERANZA M, GRIMALDI M, LEROY G, SCHEURER M, SCHLICHTING R, SCHRIKS M nad HERBERT A (2018) Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. Water Research **139** 10–18. https://doi.org/10.1016/j.watres.2018.03.056.

LEVY SB (1998) The challenge of antibiotic resistance. Scientific American **278 (3)** 46–53. https://doi.org/10.1038/scientificamerican0398-46.

LIANG X, WANG M, CHEN X, ZHA J, CHEN H, ZHU L and WANG Z (2014) Endocrine disrupting effects of benzotriazole in rare minnow (Gobiocypris rarus) in a sex-dependent manner. Chemosphere **112** 154–162.

LINEY KE, HAGGER JA, TYLER CR, DEPLEDGE MH, GALLOWAY TS and JOBLING S (2006) Health effects in fish of long-term exposure to effluents from wastewater treatment works. Environmental Health Perspectives **114 (1)** 81–89.

LIU YY, WANG Y, WALSH TR, YI LX, ZHANG R, SPENCER J, DOI Y, TIAN G, DONG B, HUANG X, YU LF, GU D, REN H, CHEN X, LV L, HE D, ZHOU H, LIANG Z, LIU JH and SHEN J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. The Lancet Infectious Diseases **165 (2)** 161–168.

LÖFGREN E, TAPANAINEN JS, KOIVUNEN R, PAKARINEN A and ISOJÄRVI JI (2006) Effects of carbamazepine and oxcarbazepine on the reproductive endocrine function in women with epilepsy. Epilepsia **47** 1441–1446. http://doi.org10.1111/j.1528-1167.2006.00506.x.

LOOS R, GAWLIK BM, LOCORO G, RIMAVICIUTE E, CONTINI S and BIDOGLIO G (2009) EU-wide survey of polar organic persistent pollutants in European river waters. Environmental Pollution **157 (2)** 561–568.

LOOS R, MARINOV D, SANSEVERINO I, NAPIERSKA D and LETTIERI T (2018) *Review of the first watch list under the water framework directive and recommendations for the second watch list.* EUR 29173 EN, European Union, Brussels, Belgium. http://doi.org/10.2760/614367, JRC111198.

LUO Y, GUO W, NGO HH, NGHIEM LD, IBNEY HAI FI, ZHANG J, LIANG S and WANG XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environment **473-474** 619–641.

MADIKIZELA LM and CHIMUKA L (2017) Simultaneous determination of naproxen, ibuprofen and diclofenac in wastewater using solid-phase extraction with high performance liquid chromatography. Water SA **43 (2)** 264–274.

MADIKIZELA LM, MUTHWA SF and CHIMUKA L (2014) Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high-performance liquid chromatography. South African Journal of Chemistry **67** 143–150.

MALETZ S, FLOEHR T, BEIER S, KLÜMPER C, BROUWER A, BEHNISCH P, HIGLEY E, GIESY JP, HECKER M, GEBHARDT W, LINNEMANN V, PINNEKAMP J and HOLLERT H (2013) In vitro characterization of the effectiveness of enhanced sewage treatment processes to eliminate endocrine activity of hospital effluents. Water Research **47 (4)** 1545–1557. https://doi.org/10.1016/j.watres.2012.12.008.

MANICKUM T and JOHN W (2014) Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Science of the Total Environment **468–469** 584–597. http://doi.org/10.1016/j.scitotenv.2013.08.041.

MANICKUM T, JOHN W and TERRY S (2011) Determination of selected steroid estrogens in treated sewage effluent in the Umsunduzi (Duzi) River water catchment area. Hydrology: Current Research **2** 1–7. http://doi.org/10.4172/2157-7587.1000117.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015) Pharmaceutical residues in water and sediment of Msunduzi river, KwaZulu-Natal, South Africa. Chemosphere **134** 133–140. http://doi.org/10.1016/j.chemosphere.2015.03.093.

MATTHIESSEN P, ARNOLD D, JOHNSON AC, PEPPER TJ, POTTINGER TG and PULMAN KGT (2006) Contamination of headwater streams in the United Kingdom by oestrogenic hormones from livestock farms. Science of the Total Environment **367** 616–630. http:/doi.org/10.1016/j.scitotenv.2006.02.007.

MCKINLAY R, PLANT JA, BELL JNB and VOULVOULIS N (2008) Endocrine disrupting pesticides: Implications for risk assessment. Environment International **34** 168–183. http://doi.org/10.1016/j.envint.2007.07.013.

MELNYK-LAMONT N, BEST C, GESTO M and VIJAYAN MM (2014) The antidepressant venlafaxine disrupts brain monoamine levels and neuroendocrine responses to stress in rainbow trout. Environmental Science and Technology **59 (22)** 13434–13442.

MENDOZA A, RODRÍGUEZ-GIL JL, GONZÁLEZ-ALONSO S, MASTROIANNI N, LÓPEZ DE ALDA M, BARCELÓ D and VALCÁRCEL Y (2014) Drugs of abuse and benzodiazepines in the Madrid region (central Spain): Seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. Environment International **70** 76–87.

MGUNI SB, SCHOEMAN C, MARAIS SS, CUKROWSKA E and CHIMUKA L (2018) Determination of oestrogen hormones in raw and treated water samples by reverse phase ultra-fast liquid chromatography mass spectrometry – a case study in Johannesburg south, South Africa. Water SA **44 (1)** 111–117. http://dx.doi.org/10.4314/wsa.v44i1.13.

MILLS LJ, GUTJAHR-GOBELL RE, HAEBLER RA, HOROWITZ DJB, JAYARAMAN S, PRUELL RJ, MCKINNEY RA, GARDNER GR and ZAROOGIAN GE (2001) Effects of estrogenic (o,p'-DDT; octylphenol) and anti-androgenic (p,p'-DDE) chemicals on indicators of endocrine status in juvenile male summer flounder (Paralichthys dentatus). Aquatic Toxicology **52** 157–176.

MINGUEZ L, PEDELUCQ J, FARCY E, BALLANDONNE C, BUDZINSKI H and HALM-LEMEILLE M-P (2016) Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environmental Science and Pollution Research **23** 4992–5001.

MITCH WA and SEDLAK DL (2004) Characterization and fate of N-nitrosodimethylamine precursors in municipal wastewater treatment plants. Environmental Science and Technology **38** 1445–1454.

MLUNGUZA NY, NCUBE S, MAHLAMBI PN, CHIMUKA L and MADIKIZELA LM (2020) Determination of selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre liquid phase microextraction and liquid chromatography – tandem mass spectrometry. Journal of Hazardous Material **382** 121067. https://doi.org/10.1016/j.jhazmat.2019.121067.

MOSEKIEMANG TT, STANDER MA and DE VILLIERS A (2019) Simultaneous quantification of commonly prescribed antiretroviral drugs and their selected metabolites in aqueous environmental samples by direct injection and solid phase extraction liquid chromatography – tandem mass spectrometry. Chemosphere **220** 983–992. https://doi.org/10.1016/j.chemosphere.2018.12.205.

MÜLLER JC, IMAZAKI PH, BOARETO AC, LOURENÇO ELB, GOLIN M, VECHI MF, LOMBARDI NF, MINATOVICZ BC, SCIPPO M-L, MARTINO-ANDRADE AJ and DALSENTER PR (2012) In vivo and in vitro estrogenic activity of the antidepressant fluoxetine. Reproductive Toxicology **34 (1)** 80–85. https://doi.org/10.1016/j.reprotox.2012.04.001.

MURK AJ, LEGLER J, VAN LIPZIG MMH, MEERMAN JHN, BELFROID AC, SPENKELINK A, VAN DER BURG B, RIJS GBJ and VETHAAK D (2002) Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environmental Toxicology and Chemistry **21** 16–23. http://doi.org/10.1002/etc.5620210103.

MURRAY GE, TOBIN RS, JUNKINS B and KUSHNER DJ (1984) Effect of chlorination on antibiotic resistance profiles of sewage-related bacteria. Applied and Environmental Microbiology **48 (1)** 73–77.

NAKADA N, HANAMOTO S, JÜRGENS MD, JOHNSON AC, BOWES MJ and TANAKA H (2017) Assessing the population equivalent and performance of wastewater treatment through the ratios of pharmaceuticals and personal care products present in a river basin: Application to the river Thames basin, UK. Science of the Total Environment **575** 1100–1108.

NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL, NATIONAL RESOURCE MANAGEMENT MINISTERIAL COUNCIL (NHMRC-NRMMC) (2011) *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. NHMRC-NRMMC, Commonwealth of Australia, Canberra, Australia.

NATIONAL INTEGRATED WATER INFORMATION SYSTEM (NIWIS) (2019) *Drought Status and Management Information System*. NIWIS, Department of Water and Sanitation, Pretoria, South African. http://niwis.dws.gov.za/niwis2/.

NATURAL RESOURCE MANAGEMENT MINISTERIAL COUNCIL, NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL. ENVIRONMENT PROTECTION AND HERITAGE COUNCIL (NRMMC-NHMRC-EPHC) (2008) *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 2). Augmentation of Drinking Water Supplies.* NRMMC-NHMRC-EPHC, Commonwealth of Australia, Canberra, Australia.

NCUBE E, VOYI K and PREEZ H (2012) Implementing a protocol for selection and prioritisation of organic contaminants in the drinking water value chain: Case study of Rand Water, South Africa. Water SA **38** 487–504.

NESBITT R (2011) *Effects of chronic exposure to ibuprofen and naproxen on Florida flagfish (Jordanella floridae) over one complete lifecycle.* Master's thesis, University of Ontario Institute of Technology, Oshawa, Canada. https://ir.library.dc-uoit.ca/bitstream/10155/176/1/Nesbitt_Richard.pdf.

NEWTON-FOOT M, SNYMAN Y, RERATILWE M, MALOBA B and WHITELAW AC (2017) Plasmid-mediated mcr-1 colistin resistance in Escherichia coliand Klebsiella spp. clinical isolates from the Western Cape region of South Africa. Antimicrobial Resistance and Infection Control **6** 78.

NYAMUKAMBA P, MOLOTO MJ, TAVENGWA N and EJIDIKE IP (2019) Evaluating physicochemical parameters, heavy metals, and antibiotics in the influents and final effluents of South African wastewater treatment plants. Polish Journal of Environmental Studies **28 (3)** 1305–1312. https://doi.org/10.15244/pjoes/85122.

ODENDAAL C, SEAMAN MT, KEMP G, PATTERTON HE and PATTERTON H-G (2015) An LC-MS/MS based survey of contaminants of emerging concern in drinking water in South Africa. South African Journal of Science **111 (9/10)**. http://dx.doi.org/10.17159/sajs.2015/20140401.

ODJADJARE EE, IGBINOSA EO, MORDI R, IGERE B, IGELEKE CL and OKOH AI (2012) Prevalence of multiple antibiotics resistant (MAR) Pseudomonas species in the final effluents of three municipal wastewater treatment facilities in South Africa. International Journal of Environmental Research and Public Health **9 (6)** 2092–2107. https://doi.org/10.3390/ijerph9062092.

OPITZ R, HARTMANN S, BLANK T, BRAUNBECK T, LUTZ I and KLOAS W (2006) Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in Xenopus laevis tadpoles. Toxicological Sciences **90 (2)** 337–348. https://doi.org/10.1093/toxsci/kfj083.

ORHON KB, ORHON AK, DILEK FB and YETIS U (2017) Triclosan removal from surface water by ozonation – Kinetics and by-products formation. Journal of Environmental Management **204 (1)** 327–336.

ORT C, LAWRENCE MG, RIECKERMANN J and JOSS A (2010) Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: Are your conclusions valid? A critical review. Environmental Science and Technology **44** 6024–6035. http://doi.org/10.1021/es100779n.

ORT C, VAN NUIJS ALN, BERSET JD, BIJLSMA L, CASTIGLIONI S, COVACI A, DE VOOGT P, EMKE E, FATTA-KASSINOS D, GRIFFITHS P, HERNÁNDEZ F, GONZÁLEZ-MARIÑO I, GRABIC R, KASPRZYK-HORDERN B, MASTROIANNI N, MEIERJOHANN A, NEFAU T, ÖSTMAN M, PICO Y, RACAMONDE I, REID M, SLOBODNIK J, TERZIC S, THOMAIDIS N and THOMAS KV (2014) Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction **109** 1338–1352. http://doi.org/10.1111/add.12570.

ÖSTMAN M, LINDBERG RH, FICK J, BJÖRN E and TYSKLIND M (2017) Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater. Water Research **115** 318–328.

OSUNMAKINDE CS, TSHABALALA OS, DUBE S and NINDI MM (2013) *Verification and validation of analytical methods for testing the levels of PPHCPs (pharmaceutical and personal health care products) in treated drinking water and sewage*. WRC Report No. 2094/1/13, Water Research Commission, Pretoria, South Africa.

PARKER RB and LAIZURE SC (2010) The effect of ethanol on oral cocaine pharmacokinetics reveals an unrecognized class of ethanol-mediated drug interactions. Drug Metabolism and Disposition **38** 317–322. http://doi.org/10.1124/dmd.109.030056.

PAROLINI M, GHILARDI A, DELLA TORRE C, MAGNI S, PROSPERI L, CALVAGNO M, DEL GIACCO L and BINELLI A (2017) Environmental concentrations of cocaine and its main metabolites modulated antioxidant response and caused cyto-genotoxic effects in zebrafish embryo cells. Environmental Pollution **226** 504–514. http://doi.org/10.1016/j.envpol.2017.04.046.

PAROLINI M, PEDRIALI A, RIVA C and BINELLI A (2013) Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel Dreissena polymorpha. Science of the Total Environment **444** 43–50. http://doi.org/10.1016/j.scitotenv.2012.11.076.

PARRY CDH, RICH E, VAN HOUT MC and DELUCA P (2017) Codeine misuse and dependence in South Africa: Perspectives of addiction treatment providers. South African Medical Journal **107** 451. http://doi.org/10.7196/SAMJ.2017.v107i5.12242.

PATEL N, KHAN AA, SHAHANE S, RAI D, CHAUHAN D, KANT C and CHAUDHARY VK (2020) Emerging pollutants in aquatic environment: Source, effect, and challenges in biomonitoring and bioremediation – a review. Pollution **6 (1)** 99–113.

PATTERTON H-G (2013) *Scoping study and research strategy development on currently known and emerging contaminants influencing drinking water quality.* WRC Report No. 2093/1/13. Water Research Commission, Pretoria, South Africa.

PETRIE B, BARDEN R and KASPRZYK-HORDERN B (2014) A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Research **72** 3–27. http://doi.org/10.1016/j.watres.2014.08.053.

PETRIE B, YOUDAN J, BARDEN R and KASPRZYK-HORDERN B (2015) Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry. Journal of Chromatography A **1431** 64–78. http://doi.org/10.1016/j.chroma.2015.12.036.

PETRIE B, YOUDAN J, BARDEN R and KASPRZYK-HORDERN B (2016) New framework to diagnose the direct disposal of prescribed drugs in wastewater – a case study of the antidepressant fluoxetine. Environmental Science and Technology **50** 3781–3789. http://doi.org/10.1021/acs.est.6b00291.

PETROVIC M, ELJARRAT E, LOPEZ DE ALDA MJ and BARCELO D (2004) Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. Analytical and Bioanalytical Chemistry **378** 549–562. http://doi.org/10.1007/s00216-003-2184-7.

PLUDDEMANN A, FLISHER AJ, MCKETIN R, PARRY CD and LOMBARD CJ (2010) A prospective study of methamphetamine use as a predictor of high school non-attendance in Cape Town, South Africa. Substance Abuse Treatment, Prevention, and Policy **5** 25. http://doi.org/10.1186/1747-597X-5-25.

PULI LK and PATIL PA (2007) Genotoxic evaluation of morphine, buprenorphine, pentazocine, and noscapine by micronucleus and comet assay in albino mice. Indian Journal of Pharmacology **39** 265–268.

PYCKE BFG, ROLL IB, BROWNAWELL BJ, KINNEY CA, FURLONG ET, KOLPIN DW and HALDEN RU (2014) Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States. Environmental Science and Tehnology **48** 7881–7890.

RA J-S, LEE S-H, LEE J, KIM HY, LIM BJ, KIM SH and KIM SD (2011) Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters. Journal of Environmental Monitoring **13** 101–109. http://doi.org/10.1039/C0EM00204F.

RÄTTYÄ J, TURKKA J, PAKARINEN AJ, KNIP M, KOTILA MA, LUKKARINEN O, MYLLYLÄ VV and ISOJÄRVI JIT (2001) Reproductive effects of valproate, carbamazepine, and oxcarbazepine in men with epilepsy. Neurology **56 (1)** 31–36. https://doi.org/10.1212/WNL.56.1.31.

RAUT SA and ANGUS RA (2010) Triclosan has endocrine-disrupting effects in male Western mosquitofish, Gambusia affinis. Environmental Toxicology and Chemistry **29 (6)** 1287–1291. https://doi.org/10.1002/etc.150.

REILLY TJ, SMALLING KL, ORLANDO JL and KUIVILA KM (2012) Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States. Chemosphere **89** 228–234.

RICHARDS SM and COLE SE (2006) A toxicity and hazard assessment of fourteen pharmaceuticals to Xenopus laevis larvae. Ecotoxicology **15 (8)** 647–656. https://doi.org/10.1007/s10646-006-0102-4.

RIMAYI C, ODUSANYA D, WEISS JM, DE BOER J and CHIMUKA L (2018) Seasonal variation of chloro-s-triazines in the Hartbeespoort Dam catchment, South Africa. Science of the Total Environment **613–614** 472–482.

RIZZO L, MANAIA C, SCHWARTZ T, DAGOT C, PLOY MC, MICHAEL I and FATTA-KASSINOS D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of the Total Environment **447** 345–360.

ROBINS JC, MARSIT CJ, PADBURY JF and SHARMA SS (2011) Endocrine disruptors, environmental oxygen, epigenetics and pregnancy. Frontiers in Bioscience: Elite Edition **3** 690–700.

ROUTLEDGE EJ and SUMPTER JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry **15 (3)** 241–248.

SÁNCHEZ RODRÍGUEZ A, RODRIGO SANZ M and BETANCORT RODRÍGUEZ JR (2015) Occurrence of eight UV filters in beaches of Gran Canaria (Canary Islands). An approach to environmental risk assessment. Chemosphere **131** 85–90. https://doi.org/10.1016/j.chemosphere.2015.02.054.

SARAVANAN M, HUR J-H, ARUL N and RAMESH M (2014) Toxicological effects of clofibric acid and diclofenac on plasma thyroid hormones of an Indian major carp, Cirrhinus mrigala during short and long-term exposures. Environmental Toxicology and Pharmacology **38** 948–958.

SCHLUMPF M, COTTON B, CONSCIENCE M, HALLER V, STEINMANN B and LICHTENSTEIGER W (2001) In vitro and in vivo estrogenicity of UV screens. Environmental Health Perspectives **109** 239–244.

SCHOEMAN C, DLAMINI, M and OKONKWO, OJ (2017) The impact of a wastewater treatment works in southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. Emerging Contamination **3** 95–106.

SCHOEMAN C, MASHIANE M, DLAMINI M and OKONKWO OJ (2015) Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. Journal of Chromatography and Separation Techniques **6 (4)** 1000272–1000272.

SCHULTZ MM, PAINTER MM, BARTELL SE, LOGUE A, FURLONG ET, WERNER SL and SCHOENFUSS HL (2011) Selective uptake and biological consequences of environmentally relevant antidepressant pharmaceutical exposures on male fathead minnows Aquatic Toxicology **104** 38–47. http://doi.org/10.1016/j.aquatox.2011.03.011.

SEHONOVA P, PLHALOVA L, BLAHOVA J, BERANKOVA P, DOUBKOVA V, PROKES M, TICHY F and VECEREK V (2016) The effect of tramadol hydrochloride on early life stages of fish. Environmental Toxicology and Pharmacology **44** 151–157. Hyyp://doi.org/10.1016/j.etap.2016.05.006.

SHENG GP, YU HQ and LI XY (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnology Advances **28 (6)** 882–894. https://doi.org/10.1016/j.biotechadv.2010.08.001.

SIBEKO PA, NAICKER D, MDLULI PS and MADIKIZELA LM (2019) Naproxen, ibuprofen, and diclofenac residues in river water, sediments and Eichhornia crassipes of Mbokodweni river in South Africa: An initial screening. Environmental Forensics **20 (2)** 129–138. http://doi.org/10.1080/15275922.2019.1597780.

SIMON A, MALETZ SX, HENNER H, SCHÄFFER A and MAES HM (2014) Effects of multiwalled carbon nanotubes and triclocarban on several eukaryotic cell lines: Elucidating cytotoxicity, endocrine disruption, and reactive oxygen species generation. Nanoscale Research Letters **9** 396.

SLABBERT JL, VENTER EA, MOLETSANE M, VAN WYK JH, BLAISE C and ANECK-HAHN NH (2007) *An investigation of the estrogenic activity in water from selected drinking water treatment processes.* WRC Report No.1532/1/07. Water Research Commission, Pretoria, South Africa.

SNYMAN H VAN NIEKERK AM and RAJASAKRAN N (2006) Sustainable wastewater treatment – what has gone wrong and how do we get back on track? In: *Water Institute of Southern Africa Conference*, Durban, South Africa, May 2006. https://www.dwa.gov.za/events/MunicipalIndaba/Sanitation/05SustainableWasteWaterTreatment.pdf.

SOHONI P and SUMPTER JP (1998) Several environmental oestrogens are also anti-androgens. Journal of Endocrinology **158** 327–339. http://doi.org/10.1677/joe.0.1580327.

SOTO AM and SONNENSCHEIN C (2010) Environmental causes of cancer: Endocrine disruptors as carcinogens. Nat. Rev. Endocrinol. **6 (7)** 363–370. https://doi.org/10.1038/nrendo.2010.87.

STATISTICS SOUTH AFRICA (STATSSA) (2011) Formal census 2011. Population statistics. http://www.statssa.gov.za/; community survey for 2016. http://cs2016.statssa.gov.za/.

STATISTICS SOUTH AFRICA (STATSSA) (2017) Statistical release P0302. Mid-year population estimates for 2017. http://www.statssa.gov.za/publications/P0302/P03022017.pdf.

STATISTICS SOUTH AFRICA (STATSSA) (2019) Mid-year population estimates. Statistical release, P0302. http://www.statssa.gov.za/publications/P0302/P03022019.pdf.

SUBEDI B and KANNAN K (2014) Mass loading and removal of select illicit drugs in two wastewater treatment plants in New York State and estimation of illicit drug usage in communities through wastewater analysis. Environmental Science and Technology **48** 6661–6670. http://doi.org/10.1021/es501709a.

SUN Q, WANG Y, LI Y, ASHFAQ M, DAI L, XIE X and YU C-P (2017) Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environmental Policy and Law **225** 542–549.

SVALHEIM S, TAUBØLL E, LUEF G, LOSSIUS A, RAUCHENZAUNER M, SANDVAND F, BERTELSEN M, MØRKRID L and GJERSTAD L (2009) Differential effects of levetiracetam, carbamazepine, and lamotrigine on reproductive endocrine function in adults. Epilepsy and Behaviour **16 (2)** 281–287. https://doi.org/10.1016/j.yebeh.2009.07.033.

SWART JC, POOL EJ and VAN WYK JH (2011) The implementation of a battery of in vivo and in vitro bioassays to assess river water for estrogenic endocrine disrupting chemicals. Ecotoxicology and Environmental Safety **74 (1)** 138–143. https://doi.org/10.1016/j.ecoenv.2010.09.006.

SWART N and POOL EJ (2007) Rapid detection of selected steroid hormones from sewage effluents using an ELISA in the Kuils river water catchment area, South Africa. Journal of Immunoassay and Immunochemistry **28 (4)** 395–408. https://doi.org/10.1080/15321810701603799.

SWARTZ CD, GENTHE B, CHAMIER J, PETRIK LF, TIJANI JO, ADELEYE A, COOMANS CJ, OHLIN A, FALK D and MENGE JG (2018) *Emerging contaminants in wastewater treated for direct potable reuse: The human health risk priorities in South Africa. Volume I: A concise research report.* WRC Report No. K5/2369. Water Research Commission, Pretoria, South Africa.

SZYMONIK A, LACH J and MALIŃSKA K (2017) Fate and removal of pharmaceuticals and illegal drugs present in drinking water and wastewater. Ecological Chemistry and Engineering S **24 (1)** 65–85.

TANAKA H, YAKOU Y, TAKAHASHI A, HIGASHITANI T and TOMORI K (2000) Comparison between estrogenicities estimated from DNA recombinant yeast assay and from chemical analyses of endocrine disruptors during sewage treatment. In: *First World Water Congress of IWA*, Paris, France.

TAUBØLL E, GREGORASZCZUK EL, TWORZYDØ A, WÓJTOWICZ AK and ROPSTAD E (2006) Comparison of reproductive effects of levetiracetam and valproate studied in prepubertal porcine ovarian follicular cells. Epilepsia **47 (9)** 1580–1583. https://doi.org/10.1111/j.1528-1167.2006.00668.x.

THAI PK, LAI FY, BRUNO R, VAN DYKEN E, HALL W, O'BRIEN J, PRICHARD J and MUELLER JF (2016) Refining the excretion factors of methadone and codeine for wastewater analysis – combining data from pharmacokinetic and wastewater studies. Environment International **94** 307–314. http://doi.org/10.1016/j.envint.2016.05.033.

TRAN NH, REINHARD M and GIN KY-H (2018) Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions – a review. Water Research **133** 182–207.

TRUTER JC, VAN WYK JH and NEWMAN BK (2015) In vitro screening for endocrine disruptive activity in selected South African harbours and river mouths. African Journal of Marine Science **37 (4)** 567–574.

TRUTER JC, VAN WYK JH, OBERHOLSTER PJ, BOTHA AM and DE KLERK AR (2016) An in vitro and in vivo assessment of endocrine disruptive activity in a major South African river. Water, Air, and Soil Pollution **227**. http://doi.org/10.1007/s11270-016-2748-8.

UNITED NATIONS OFFICE ON DRUGS AND CRIME (UNODC) (2016) *World Drug Report*. United Nations, New York, NY, USA.

UNITED STATES DIGITAL SERVICE (USDS), 2017. *International Narcotics Control Strategy Report: Volume I: Drug and Chemical Control I*. USDS, Washington, DC, USA.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (1997) *Special report on environmental endocrine disruption: An effects assessment and analysis*. USEPA, Washington, DC, USA. http://www.epa.gov/raf/publications/pdfs/ENDOCRINE.PDF.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (2005) *Prevention, pesticides and toxic substances.* USEPA, Washington DC, USA. http://envirocancer.cornell.edu/turf/pdf/mancozeb_fs.pdf.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (2018) *2018 edition of the drinking water standards and health advisories.* USEPA, Washington DC, USA.

VALENTINE JL, KEARNS GL, SPARKS C, LETZIG LG, VALENTINE CR, SHAPPELL SA, NERI DF and DEJOHN CA (1995) GC-MS determination of amphetamine and methamphetamine in human urine for 12 hours following oral administration of dextro-methamphetamine: Lack of evidence supporting the established forensic guidelines for methamphetamine confirmation. Journal of Analytical Toxicology **19** 581–590.

VAN NUIJS ALN, PECCEU B, THEUNIS L, DUBOIS N, CHARLIER C, JORENS PG, BERVOETS L, BLUST R, MEULEMANS H, NEELS H and COVACI A (2009) Can cocaine use be evaluated through analysis of wastewater ? A nation-wide approach conducted in Belgium. Addiction **104** 734–741. http://doi.org/10.1111/j.1360-0443.2009.02523.x.

VAN WYK JH, ARCHER E, BABALOLA OO, TRUTER JC, VAN RENSBURG EJ and DABROWSKI J (2014) *Pesticides as endocrine disruptors in South Africa: Laboratory and field studies.* WRC Report No. 1932/1/14. Water Research Commission, Pretoria, South Africa.

VAN ZIJL MC, ANECK-HAHN NH, SWART P, HAYWARD S, GENTHE B and DE JAGER C (2017) Estrogenic activity, chemical levels and health risk assessment of municipal distribution point water from Pretoria and Cape Town, South Africa. Chemosphere **186** 305–313. https://doi.org/10.1016/j.chemosphere.2017.07.130.

VELASCO-SANTAMARÍA YM, KORSGAARD B, MADSEN SS and BJERREGAARD P (2011) Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish (Danio rerio). Aquatic Toxicology **105 (1–2)** 107–118. https://doi.org/10.1016/j.aquatox.2011.05.018.

VELDHOEN N, SKIRROW RC, BROWN LLY, VAN AGGELEN G and HELBING CC (2014) Effects of acute exposure to the non-steroidal anti-inflammatory drug ibuprofen on the developing North American bullfrog (Rana catesbeiana) tadpole. Environmental Science and Technology **48** 10439–10447.

VELDHOEN N, SKIRROW RC, OSACHOFF H, WIGMORE H, CLAPSON DJ, GUNDERSON MP, VAN AGGELEN G and HELBING CC (2006) The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development. Aquatic Toxicology **80** 217–227.

VILLENEUVE DL, CRUMP D, GARCIA-REYERO N, HECKER M, HUTCHINSON TH, LALONE CA, LANDESMANN B, LETTIERI T, MUNN S, NEPELSKA M, OTTINGER MA, VERGAUWEN L and WHELAN M (2014) Adverse Outcome Pathway (AOP) development I: strategies and principles. Toxicological Sciences **142** 312–320. https://doi.org/10.1093/toxsci/kfu199.

VILLENEUVE DL, COADY K, ESCHER BI, MIHAICH E, MURPHY CA, SCHLEKAT T and GARCIA-REYERO N (2019) High-throughput screening and environmental risk assessment: State of the science and emerging applications. Environmental Toxicology and Chemistry **38 (1)** 12–26.

VOUTSA D, HARTMANN P, SCHAFFNER C and GIGER W (2006) Benzotriazoles, alkyphenols, and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environmental Science and Pollution Research **13** 333–341.

VUONG C, VAN UUM SHM, O'DELL LE, LUTFY K and FRIEDMAN T (2010) The effects of opioids and opioid analogs on animal and human endocrine systems. Endocrine Reviews **31** 98–132. http://doi.org/10.1210/er.2009-0009.

WAGENAAR G, BOTHA T and BARNHOORN I (2012) Sperm motility and testicular histology as reproductive indicators in Clarias gariepinus from an eutrophic impoundment, South Africa. Journal of Applied Ichthyology **28 (6)** 990–997. https://doi.org/10.1111/jai.12065.

WATANABE T, SHIOZAWA T, TAKAHASHI Y, TAKAHASHI T, TERAO Y, NUKAYA H, TAKAMURA T, SAWANISHI H, OHE T, HIRAYAMA T and WAKABAYASHI K (2002) Mutagenicity of two 2-phenylbenzotriazole derivatives, 2-[2-(acetylamino)-4-(diethylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and 2-[2-(acetylamino)-4-(diallylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and their detection in river water in Japan. Mutagenesis **17 (4)** 293–299.

WEICH L, PERKEL C, VAN ZYL N, NAIDOO L, NOWBATH H, FLEGAR S, RAMJEE H, HITZEROTH V and KRAMER L (2017) South African guidelines for the management of opioid use disorders (Part 1). Professional Nursing Today **21** 24–31.

WEICH L, PERKEL C, VAN ZYL N, RATAEMANE ST and NAIDOO L (2008) Medical management of opioid dependence in South Africa. South African Medical Journal **98** 280–283.

WERNERSSON A-S, CARERE M, MAGGI C, TUSIL P, SOLDAN P, JAMES A, SANCHEZ W, DULIO V, BROEG K, REIFFERSCHEID G, BUCHINGER S, MAAS H, VAN DER GRINTEN E, O'TOOLE S, AUSILI A, MANFRA L, MARZIALI L, POLESELLO S, LACCHETTI I, MANCINI L, LILJA K, LINDEROTH M, LUNDEBERG T, FJÄLLBORG B, PORSBRING T, LARSSON DJ, BENGTSSON-PALME J, FÖRLIN L, KIENLE C, KUNZ P, VERMEIRSSEN E, WERNER I, ROBINSON CD, LYONS B, KATSIADAKI I, WHALLEY C, DEN HAAN K, MESSIAEN M, CLAYTON H, LETTIERI T, CARVALHO RN, GAWLIK BM, HOLLERT H, DI PAOLO C, BRACK W, KAMMANN U and KASE R (2015) The European technical report on aquatic effect-based monitoring tools under the water framework directive. Environmental Science Europe **27 7**. http://foi.org/10.1186/s12302-015-0039-4.

WESTLUND P and YARGEAU V (2017) Investigation of the presence and endocrine activities of pesticides found in wastewater effluent using yeast-based bioassays. Science of the Total Environment **607–608** 744–751. http://doi.org/10.1016/j.scitotenv.2017.07.032.

WORLD HEALTH ORGANISATION (WHO) (2012) State of the science of endocrine disrupting chemicals. http://apps.who.int/iris/bitstream/10665/78102/1/WHO_HSE_PHE_IHE_2013.1_eng.pdf.

WORLD HEALTH ORGANISATION (WHO) (2011) *Guidelines for drinking-water quality*, 4th ed., WHO, Geneva, Switzerland.

WORLD HEALTH ORGANISATION (WHO) (2019) *Progress on household drinking water, sanitation and hygiene 2000–2017. Special focus on inequalities.* United Nations, New York, NY, USA; WHO, Geneva, Switzerland.

WILKINSON J, HOODA PS, BARKER J, BARTON S and SWINDEN J (2017) Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. Environmental Pollution **231** 954–970.

WOOD TP, DUVENAGE CSJ and ROHWER E (2015) The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. Environmental Pollution **199** 235–243. https://doi.org/10.1016/j.envpol.2015.01.030.

WOODING M, ROHWER ER and NAUDE Y (2017) Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: A disposable sorptive sampler with comprehensive gas chromatography – time-of-flight mass spectrometry and large volume injection with ultra-high-performance liquid chromatography – tandem mass spectrometry. Journal of Chromatography A **1496** 122–132.

WRITER JH, BARBER LB, RYAN JN and BRADLEY PM (2011) Biodegradation and attenuation of steroidal hormones and alkylphenols by stream biofilms and sediments. Environmental Science and Technology **45 (10)** 4370–4376. https://doi.org/10.1021/es2000134.

WU L-H, ZHANG X-M, WANG F, GAO C-J, CHEN D, PALUMBO JR, GUO Y and ZENG EY (2018) Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Science of the Total Environment **615** 87–98.

WU QY, HU HY, ZHAO X and SUN YX (2009) Effect of chlorination on the estrogenic/antiestrogenic activities of biologically treated wastewater. Environmental Science and Technology **43** 4940–4945. http://doi.org/10.1021/es8034329.

XIAO S, LV X, ZENG Y, JIN T, LUO L, ZHANG B, ZHANG G, WANG Y, FENG L, ZHU Y and TANG F (2017) Mutagenicity and estrogenicity of raw water and drinking water in an industrialized city in the Yangtze River Delta. Chemosphere **185** 647–655. http://doi.org/10.1016/j.chemosphere.2017.07.066.

XU Z, DU P, LI K, GAO T, WANG Z, FU X and LI X (2017) Tracing methamphetamine and amphetamine sources in wastewater and receiving waters via concentration and enantiomeric profiling. Science of the Total Environment **601–602** 159–166. http://doi.org/10.1016/j.scitotenv.2017.05.045.

YUEH M-F, LI T, EVANS RM, HAMMOCK B and TUKEY RH (2012) Triclocarban mediates induction of xenobiotic metabolism through activation of the constitutive androstane receptor and the estrogen receptor alpha. PLoS ONE **7 (6)** e37705. https://doi.org/10.1371/journal.pone.0037705.

ZENG X, LIU Z, HE L, CAO S, SONG H, YU Z, SHENG G and FU J (2015) The occurrence and removal of organophosphate ester flame retardants/plasticizers in a municipal wastewater treatment plant in the Pearl River Delta, China. Journal of Environmental Science and Health, Part A **50 (12)** 1291–1297, http://doi.org/10.1080/10934529.2015.1055158.

ZHANG K, YUAN G, WERDICH AA and ZHAO Y (2020) Ibuprofen and diclofenac impair the cardiovascular development of zebrafish (Danio rerio) at low concentrations. Environmental Pollution **258** 113613.

ZHAO X, ZHANG X-F, XU L, LIU L-Y, SONG W-W, ZHU F-J, LI Y-F and MA W-L (2017) Occurrence and fate of benzotriazoles UV filters in a typical residential wastewater treatment plant in Harbin, China. Environmental Pollution **227** 215–222.