The potential risks of long term exposure to low concentrations of antiretrovirals in treated and untreated water sources in Gauteng, South Africa

by

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DECLARATION

I, Ntsieni Rahab Ramalwa, declare that this work was not copied or repeated from any other studies either from national or international publications. Procedures were carried out in accordance with ethical rules as prescribed by Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Signature: 

Date: 30 November 2020

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SUMMARY

Access to safe and affordable drinking water and sanitation is highlighted in the Sustainable Development Goal Target 6.1 while Target 6.3 addresses the release of hazardous chemicals into water sources. Pharmaceuticals and personal care products in treated drinking water have been receiving growing attention from environmental and health organizations worldwide because they are more frequently being detected in water sources. The fact that pharmaceuticals are manufactured with the intention to cause biological effects continue raising concerns about the impact of unintentional exposure to pharmaceuticals on human health. Despite the relatively fast growing numbers of studies on the prevalence and potential risk associated with pharmaceuticals in potable water, few studies that have addressed the potential human health risks associated with ingestion of low doses antiretrovirals (ARVs) through drinking water. The aim of the study was to assess the potential risks posed by long-term exposure to trace levels of ARVs in treated and untreated water sources in South Africa (SA), more specifically the Gauteng Province. A review of national and international literature was conducted to determine the extent and risks posed by ARV contamination in water sources globally. From the review it was evident that there is paucity of data on pharmaceuticals in water sources worldwide, including Africa. Where such data was available, pharmaceuticals targeted and detected in each investigation were country-dependent and linked to the most commonly used drugs or antivirals in the region, e.g. oseltamivir in Japan, with only a few reviews reporting on the presence and fate of ARVs in environmental samples. From a review of global human immunodeficiency virus (HIV) epidemic it was evident that SA uses more ARVs per capita compared to any other country fighting the HIV/acquired immunodeficiency syndrome (AIDS) epidemic with 71% (5 million) of adults living with HIV on combination antiretroviral therapy (cART). From 2003 to 2019 the drugs used in the first-line regimen for adults were the most used for the management of HIV with tenofovir disoproxil fumarate, lamivudine (3TC), emtricitabine and efavirenz (EFV) used more widely from 2010-2019. A newly approved ARV, dolutegravir, was included in the first-line regimen from 2020. A systematic review, conducted to establish which ARVs have been detected in water sources in SA, revealed that all ARVs that have been used historically in the first-line (stavudine, 3TC, EFV, nevirapine) and in second-line (didanosine, ritonavir boosted lopinavir, zidovudine [AZT]) regimens have been detected

in one or more water sources, including treated drinking water, surface water and wastewater influent and effluent. To establish whether the low concentrations of ARVs in drinking water posed a possible health risk to individual ingesting polluted drinking water, a risk assessment was conducted. The method comprised of five general steps: a) selection of ARVs to be assessed; b) derivation of acceptable daily intake; c) derivation of predicted no effect concentrations; d) Exposure assessment - determination of environmental concentrations; and e) risk calculation. The risk quotient values needed for the risk assessment were sourced from studies that utilised acceptable daily intake values derived from dose-response model studies. The present study showed that from the current levels of AZT, 3TC and abacavir (ABC) detected in drinking water sources in SA, the possible human health risk was insignificant, although harmful to aquatic species. The predicted no effect concentrations were not available for the other ARVs present in the water sources in SA. Overall, this study showed that selected ARVs, namely EFV, in water were harmful to aquatic species, while the current levels of AZT, 3TC and ABC detected in drinking water sources in SA posed an insignificant human health risk. The study has therefore provided new data on the potential human health risk posed by exposure to low levels of ARVs in treated water sources in SA.

Key words: emerging contaminants, antiretrovirals, potable water, risk assessment, wastewater, pharmaceuticals

PRESENTATIONS

National presentations

1. **Ramalwa N,** De Jager C, Taylor MB. The potential risks of long term exposure to low concentrations of antiretrovirals in treated and untreated water sources in Gauteng, South Africa [Oral presentation]. Progress Report, University of Pretoria 19 October 2018: Pretoria.

2. **Ramalwa N**, De Jager C, Taylor M. Can long term exposure to low concentrations of antiretrovirals in treated and untreated water sources lead to development of resistance? [Oral Presentation]. The 7th Annual DAIDD Clinic, 17-21 December 2018: Stellenbosch, South Africa.

3. **Ramalwa N,** De Jager C, Taylor M. The potential risks of long term exposure to low concentrations of antiretrovirals in treated and untreated water sources in Gauteng, South Africa [Poster]. The 10th Annual MMED Clinic, 31 May - 7 June 2019: African Institute for Mathematical Sciences: Muizenberg, South Africa.

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ABBREVIATIONS AND ACRONYMS

Abbreviation	Full Name
AAF	Analgesics and anti-inflammatories
ABC	Abacavir
ABC-CBX	ABC–carboxylate
ABT	Albuvirtide
ACV	Acyclovir
ADI	Acceptable daily intake
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
ATV	Atazanavir
AZT/ZDV	Zidovudine
BIC	Bictegravir
CAB	Cabotegravir
cART	Combination antiretroviral therapy
COBI	Cobicistat
CSIR	Council for Scientific and Industrial Research
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DEET	diethyltoluamide
DEWAT	Decentralised Wastewater Treatment System
DLV	Delavirdine
DNA	Deoxyribonucleic acid
DOR	Doravine
DRV	Darunavir
DTG	Dolutegravir
DWAF	Department of Water Affairs and Forestry
DWEL	Drinking-water equivalent level
EC	Emerging contaminant

EC50	Half maximal effective concentration
EDC	Endocrine disrupting compound
EFV	Efavirenz
ENF(T-20)	Enfuvirtide
ETR	Etravirine
ETV	Entecavir
ETV	Entravirine
EVG	Elvitegravir
FDC	Fixed-dose combination
FPV	Fosamprenavir
FTC	Emtricitabine
FTC-CBX	Emtricitabine carboxylate
FTR	Fostemsavir
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HIC	High income countries
HIV	Human immunodeficiency virus
IBA	Ibalizumab - uiyk
IDV	Indinavir
INSTI	Integrase strand transfer inhibitor
L	Litre
LMIC	Low-to-middle income countries
LOEL	Lowest observed effect level
LPV	Lopinavir
LPVr	Ritronavir-boosted lopinavir
MBR	Membrane bioreactors
MBR	Membrane bioreactors
MEC	Measured environmental concentration
MERLIN-Expo	Modelling exposure to chemicals for risk assessment : a comprehensive library of multimedia and physiological based pharmacokinetic models for integration, prediction and uncertainty and sensitivity analysis

MOE	Margins of exposure
MSM	Men who have sex with men
MTD	Minimum therapeutic dose
MVC	Maraviroc
µg	Microgram
NDoH	National Department of Health
NDP	National Development Plan
NFV	Nelfinavir
ng	Nanogram
NHC	National Health Council
NHMR	National Health and Medical Research Council
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
NSAID	Non-steroidal anti-inflammatory drugs
NSP	National Strategic Plan
NVP	Nevirapine
OC	Oseltamivir carboxylate
PBPK	Physiological based pharmacokinetic
PBT	Persistent bio-accumulative and toxic substance
PEC	Predicted environmental concentration
PI	Protease inhibitor
PMTCT	Prevention of mother-to-child transmission
PNEC	Predicted no effect concentration
POD	Point of departure
POP	Persistent organic pollutant
PPCPs	Pharmaceuticals and personal care products
PrEP	Pre-exposure prophylaxis
RAL	Raltegravir
RNA	Ribonucleic acid
RPV	Rilpivirine
RQ	Risk quotient

RT	Reverse transcriptase
RTI	Reverse transcriptase inhibitor
RTV	Ritonavir
SA	South Africa
SADC	Southern African Development Community
SANAC	South African National AIDS Council
SANS241	South African National Standard 241
SDG	Sustainable Development Goal
SQV	Saquinavir
TAF	Tenofovir alafenamide fumarate
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TPV	Tipranavir
UF	Uncertainty factor
UK	United Kingdom
UN	United Nations
UNAIDS	United Nations Programme on HIV/AIDS
USA	United States of America
WHO	World Health Organization
WTP	Water treatment plant
WWTP	Wastewater treatment plant
3TC	Lamivudine

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CHAPTER 1

OVERVIEW

1.1GENERAL INTRODUCTION

Access to safe drinking water and sanitation as a basic human right was recognised by the United Nations (UN) General Assembly, Resolution 64/292, in 2010.[1] The human rights to water and sanitation are further embedded in Sustainable Development Goal (SDG) 6 which is to ensure access to safe water and sanitation for all people by 2030.[2] Target 6.1 of SDG 6 specifically addresses access to safe and affordable drinking water while Target 6.3 addresses the improvement of water quality and includes reducing pollution and the dumping and release of hazardous chemicals as well as increasing recycling and safe reuse.[3] Access to safe, readily available water is important for public health as exposure to contaminated water and the lack of sanitation is linked to the transmission of preventable health risks.[4] Linked to SDG 6 is SDG 3 which is to ensure healthy lives and promote well-being for all.[3] Target 3.3 of SDG 3 calls for the end of epidemics such as acquired immunodeficiency syndrome (AIDS) and preventing, amongst others, waterborne diseases, while Target 3.9 addresses the reduction of deaths and illness from hazardous chemicals and air, water, and soil pollution and contamination.[5] South Africa (SA) is one of the 193 member states that committed to the SDGs being attained by the year 2030 as outlined in the National Development Plan (NDP).[6] A recent newspaper report highlighted the contamination of South African water sources with pharmaceuticals, including antiretrovirals (ARVs), and indicated that in addition to being a potential health risk this could jeopardise SA attaining the SDG 6 goals.[7] The potential health risks posed by pharmaceuticals, specifically ARVs in SA's water sources therefore warrants further investigation.

1.1.1 Emerging contaminants in water and wastewater

In the literature the term contaminant is often used interchangeably with the term pollutant, but not all contaminants are pollutants.[8] A "*pollutant*" is a contaminant that

gets introduced into the natural environment, beyond acceptable limits, and can cause undesired effects to the inhabitants and/or resident communities.[9] A "*contaminant*" is defined as any physical, chemical, biological or radiological substance or matter that is present in the water.[10] Contamination refers to the presence of such substances where they should not be and/or at concentrations above background levels.[8] Contaminants can be divided into three categories: emerging contaminants (ECs), contaminants of emerging concern (CEC) and re-emerging contaminants with some authors using the terms ECs and CECs interchangeably.[11]

a) *Emerging contaminants* are chemical substances or compounds characterised by an apparent threat to the environment and/or human health with a lack of published data on environmental and/or human impact.[12-13] An EC may also refer to contaminants identified from an unknown source, a new exposure to human population or a novel recognition approach or technology.[12-13] These compounds or substances are not commonly monitored in the environment although they have the potential to enter the environment and cause known or unknown and/or suspected adverse ecological and/or human health effects.[14-15] It is possible that the release of such contaminants into the environment has been going on for decades, but may have been undetected until recently, owing to advances in analytical techniques and instrumentation. In other instances, new sources of ECs can occur as a result of the use of new chemicals or changes in the use and disposal of existing chemicals.[14-15]

b) *Contaminants of emerging concern* are contaminants that have been known to exist in the environment for a while but for which concerns have only been raised more recently. These contaminants are also referred to as "truly new" ECs, new compounds or molecules that were not previously known or that just recently appeared in the scientific publications.[16] The CECs, therefore, remain a moving target as new chemical compounds are continuously being produced and scientific techniques continuously improve, therefore improving the knowledge of current and past contaminants.[16]

c) *Re-emerging contaminants* are well-described, well-recognised contaminants that present with new problems.[16] These are contaminants that are already regulated,

which regain the "re-emerging" status as new information regarding their environmental and human health risks become available.[16]

Increasing numbers of ECs, including their metabolites, have been found in European aquatic bodies.[11] These ECs are further categorised into more than 30 classes related to their source.[11] Some of these classes include pesticides, disinfection by-products, pharmaceuticals and personal care products (PPCPs), industrial chemicals, endocrine disrupting compounds (EDCs), artificial sweeteners and food additives, nanomaterials, sunscreens, flame retardants, siloxanes, benzotriazoles and benzothiazoles.[11] There are different types of ECs that have widely varying physical and chemical properties: a) organic substances which can be subdivided in persistent bio-accumulative and toxic substances (PBTs) such as persistent organic pollutants (POPs), and more polar substances like pesticides, pharmaceuticals, industrial chemicals and b) Inorganic compounds (trace metals) and particulate contaminants such as nano-particles and microplastics.[17-18] In general, these ECs are derived from, but not limited to, pharmaceuticals, personal care products and EDCs.[19]

The detection of ECs and their transformation products in the various environmental compartments is critical in obtaining an understanding of where and how they occur and their destiny.[11] To date, obtaining such information remains challenging for the following reasons:

i) there are many currently known potential ECs, e.g. more than 1 036 detected in Europe alone,
ii) their relevance changes over time due to changes in production, use and disposal,
iii) new information on their occurrence, fate and hazardousness becoming available.[11]

The current worldwide high-tech methods for sampling and analysing ECs differ amongst monitoring laboratories. Laboratories are typically dedicated to certain EC classes and certainly do not cover the full range of ECs of potential concern. Moreover, for several known highly hazardous ECs that are regularly monitored, they often occur at very low concentrations that at times are insufficient to allow proper risk assessment from such exposures.[17-18] In addition, there are certain ECs such as hormones, pyrethroids and some

organophosphorus pesticides[20] that do have an effect on the aquatic environment at extremely low concentrations, i.e. at concentrations bordering on the detection limit.

Emerging contaminants are currently not included in the national and/or international routine monitoring programs and for this reason, their fate, behaviour and eco-toxicological effects are often not well understood and documented.[21] These ECs can be released from point pollution sources such as wastewater treatment plants (WWTPs) or diverse sources through deposition from the atmosphere or crop and animal production [14-15] and human faecal contamination of water bodies due to not having proper sewage systems in place,[22] as it is in many informal settlements.

1.1.1.1 Pharmaceuticals in water sources

Amongst the types of ECs, pharmaceuticals are the most concerning environmental contaminants as they are biologically active and are usually lipophilic and often have low biodegradability.[23] Pharmaceuticals are artificial or natural chemicals that are present in prescription medicines, over-the-counter therapeutic drugs and veterinary drugs.[24] They contain active ingredients that promote pharmacological effects and are significantly beneficial to society.[24] Pharmaceutical substances are used broadly in human and veterinary medicine and can enter the aquatic environment following manufacture.[25] The ongoing use of pharmaceuticals globally, in human and veterinary medical practices, aquaculture and agricultural products has led to the continual release of a wide range of pharmaceutical chemicals into the environment.[25]

Emerging contaminants, including pharmaceuticals, can enter the environment through many routes including human or animal excreta, wastewater overflow, treated sewage sludge, industrial and medical waste from health-care and veterinary facilities, landfill leachate and bio-solids.[12] (Figure 1.1) The majority of human pharmaceutical compounds enter aquatic systems after ingestion and subsequent excretion in the form of the non-metabolised parent compounds or as metabolites via the sewage treatment network.[25]

Various researchers have shown that a wide range of pharmaceuticals end up deposited into the environment as a result of inadequate wastewater treatment[26-28] and from improper disposal of expired and/or unused pharmaceutical stock.[29-31] Personal care

products are also present in wastewater and effluents and are a possible source of these products in treated drinking water.[27,32-33] Although wastewater treatment processes are not designed to remove PPCPs, they do so to varying degrees.[24]

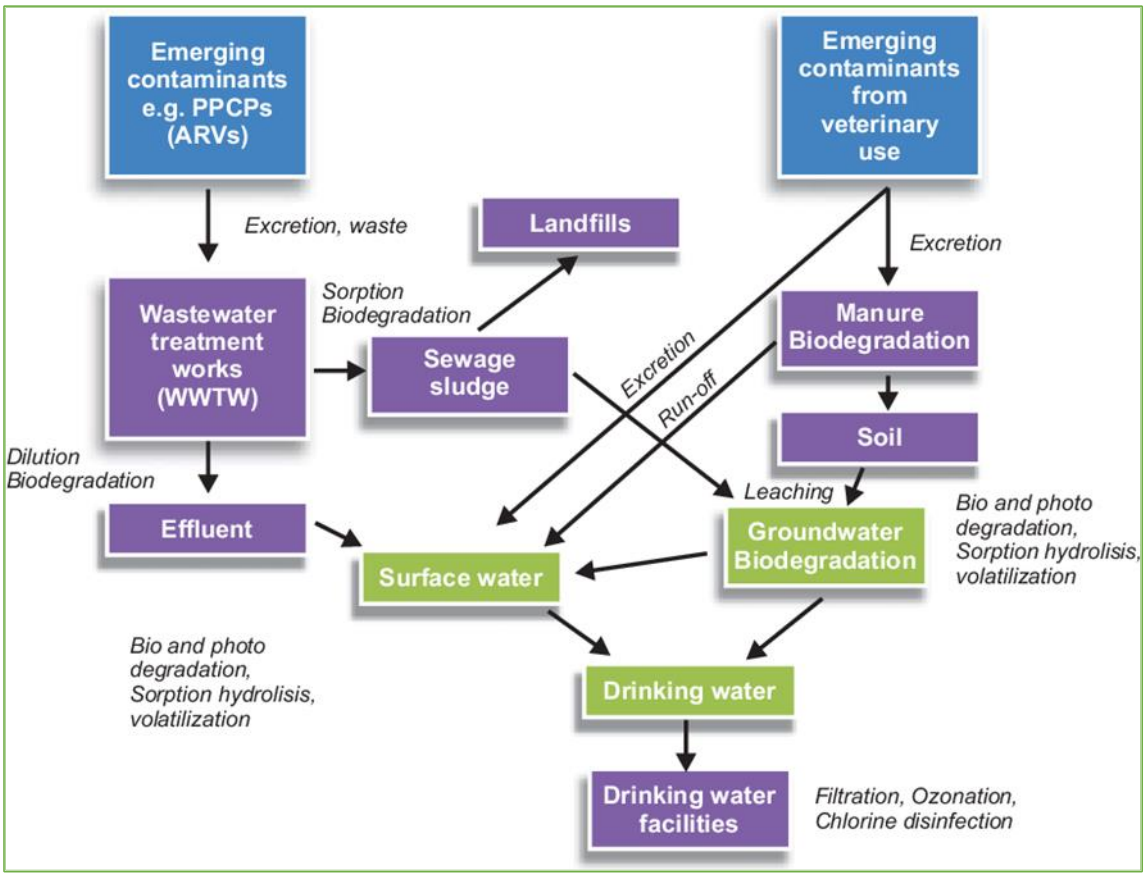


Figure 1.1: Pathways of emerging contaminants in the environment (The Water Wheel, Council for Scientific and Industrial Research (CSIR).[34] (*reproduced with permission from the author*)

Pharmaceutical and personal care products have been detected in water sources worldwide.[32] The presence of pharmaceuticals in the environment has been demonstrated for more than 30 years, with studies in the United States of America (USA) in the 1970s that reported the presence of heart medications, pain relievers and birth control medications in wastewater.[32] Traces of pharmaceuticals, typically at levels in the nanograms (ng) to low micrograms (µg) per litre (L) range, have been reported in the water sources, including surface water, wastewater, groundwater and, to some extent, treated drinking water.[24,35] Regular monitoring programs exist in many parts of the world, i.e. United Kingdom (UK), USA, Canada and Australia, for regulated chemical and microbiological parameters and these do not include PPCPs and therefore there is a need to integrate this emerging concern into future monitoring programmes.[24]

Pharmaceutical pollution in the environment is a global problem that affects both high-income countries (HIC) and low-to-middle income countries (LMIC). Several studies and surveys have reported the presence of pharmaceuticals in effluents from WWTPs and have identified these effluents as the main pathway of pharmaceuticals and their metabolites into rivers, lakes, reservoirs and groundwater aquifers that are used for drinking water supply.[36-41] Pharmaceuticals in ready-to-drink water have recently received growing attention from environmental and health agencies worldwide and have become one of the classes of ECs due to their frequent detection in the water environment.[24,42]

The presence of pharmaceuticals in water, even at very low concentrations, has raised concerns among stakeholders such as drinking-water regulators, governments, water suppliers and the public, regarding the potential risks to human health from exposure to traces of pharmaceuticals through drinking water.[24] Advances in analytical technology have been a key factor driving their increased detection.[24] Potable water sources are often contaminated by human and veterinary pharmaceuticals.[43-45] Incomplete removal by conventional WWTPs technologies, e.g., flocculation, sedimentation, and chlorination, has been observed[42] and consequently, pharmaceuticals have been detected in treated tap water in several HICs.[46]

According to the World Health Organization (WHO), appropriate regulations governing pharmaceutical disposal practices at point sources of hazards, widespread take-back programmes, guidance and enhanced consumer education is needed to support efforts for the proper disposal of unwanted and excess medicine. These regulations will reduce the impact of pharmaceuticals entering the environment, including water sources.[24] As most pharmaceuticals enter the water cycle through wastewater discharges or from poorly controlled manufacturing or production facilities that are primarily associated with generic medicines, the discharge of untreated or poorly treated wastewater to water bodies used as drinking water sources should be strongly discouraged.[24]

In HICs, effluents from pharmaceuticals producing factories have to meet strict guidelines,[47] but this becomes a concern in LMICs where a possibility of unmonitored release of contaminated effluents into surface water bodies exists. In LMICs, most WWTPs have not yet been upgraded or evaluated for the capacity to eliminate

pharmaceuticals, including ARVs.[47] This implies that there is still a global denial that there is a direct deposition of pharmaceuticals into surface water through disposal of improperly treated wastewater effluents, which could have detrimental health effects on humans.[47] Many of the ARVs pass unmetabolised or partially metabolised through the body and would therefore be excreted as such. Therefore in areas with poor or no sanitation, run-off would take the excreta straight into surface water.[47]

Regulations governing pharmaceuticals disposal practices at point sources of hazards, widespread take-back programmes, guidance and enhanced consumer education is needed to support efforts for the proper disposal of unwanted and excess medicine in order to reduce the environmental impact of pharmaceuticals entering the environment.[24] The Australian National Health and Medical Research Council (NHMRC) indicates that pharmaceutical disposal methods depend on the chemical composition of the material which must be checked with the manufacturer.[48] The components must be classified according to the known toxicity of the pharmaceutical involved and the degree of contamination. If in doubt on the disposal method, the pharmacist needs to be consulted.[48] Pharmaceutical waste can be disposed of as clinical waste if incinerated. Such waste should not be discharged into sewerage systems, although in some states and territories within Australia, discharge of small quantities of pharmaceutical waste is permitted. Where incineration is not possible, the relevant state or territory authorities should be consulted, including the sewerage authority, before developing a disposal policy.[48]

Furthermore, the NHMRC states that pharmaceutical waste, including any waste that may arise from pharmaceuticals that have passed their recommended shelf life, pharmaceuticals discarded due to off-specification batches or contaminated packaging, pharmaceuticals returned by patients or discarded by the public, pharmaceuticals that are no longer required by the institution and waste generated during the manufacture and administration of pharmaceuticals, should be disposed of appropriately.[48] Furthermore, it states that excess stock of pharmaceuticals, either in use or expired, may be returned to a relevant authority or collection centre for appropriate disposal or distribution.[48]

In the year 2008 a pilot study that was conducted in SA, on a random sample of 200 adults, found that 62.5% of the respondents threw unwanted medicine in the bin, 17% flushed it down the toilet, 6.5% poured it down the sink, 2.5% of respondents returned

medicines to the pharmacy for disposal and 2% buried it in the garden.[49] Another South African study also reported that the number of people returning medicines to the pharmacy for disposal was at 6% which is lower than other disposal methods as reported above.[49,50] The majority of people still discard unwanted medicines in the garbage and sewerage systems.[49,51] Similarly, the 1996 South African national drug policy states that the Department of Health (DoH), in cooperation with the private sector and in consultation with the state medical depots, will ensure that appropriate methods are applied for the removal and disposal of expired and returned stock, medical supplies and medical waste.[52] This phenomenon of medicine re-use following return from patients has also been recommended in literature as they considered most of them to still be in good condition.[53] This however, poses a lot of questions and uncertainties regarding the guarantee that the medicine has been subjected to safe storage conditions that did not compromise its safety and efficacy. Furthermore, the 1996 South African NDP states that the South African government will ensure through legislation that the removal and/or disposal of drugs, medical supplies and medical waste takes place in such a manner that is neither harmful nor dangerous to the community or environment.[52] Authorised inspectors will carry out regular inspections to ensure that the disposal of unwanted items takes place according to prescribed guidelines, which will carry a penalty for breach,[52] although adherence to such a policy remain questionable and with many gaps.

1.1.2 Removal of emerging contaminants through wastewater and drinking water treatment processes

Wastewater refers to liquid waste discharged from various sources namely domestic residences, commercial properties, health care facilities, industries, agriculture, etc.[54] About 99% of wastewater is water and only 1% solid wastes. Across the globe, water demands for various uses, namely household, commercial, industrial and agricultural purposes are increasing significantly.[54] Wastewater, therefore, has to undergo treatment processes to be re-used and this phenomenon has increased popularity as a means of preserving scarce freshwater resources and has led to widespread and growing applications for recycled wastewater, including irrigation of food crops, non-food crops, green spaces, recovering dry land, fire systems, industrial cooling or industrial processing, sanitation and as sources of drinking water.[55]

Several studies have reported the presence of pharmaceuticals in effluents from wastewater treatment facilities[36,39-41,56-57] and identified these effluents as the major drivers of pharmaceuticals and their metabolites into receiving water sources such as rivers, lakes, reservoirs and groundwater aquifers that are used for drinking water supply.[33,57-59] It is this presence of trace concentrations of pharmaceuticals in the water cycle, typically in the range of ng to low µg/L that has to date raised concerns regarding the efficacy of drinking water and wastewater treatment processes in removing pharmaceuticals during water purification processes.[24]

Wastewater re-use also helps to decrease the impact on the environment of disposal of sewage or industrial effluent. In addition to wastewater re-use, there is also the re-use of greywater. Greywater is defined as "untreated household wastewater which has not come into contact with toilet waste (faeces and/or urine)", and includes used water from bathtubs, showers, bathroom washbasins, and water from clothes washing machines and laundry tubs, etc. Although greywater does not include wastewater, pathogens (lower levels compared to level from wastewater) may still be present from different sources, e.g. babies' nappies or diapers, and also improper dumping of unused/unwanted pharmaceuticals.[55]

Wastewater treatment plants which are also known as sewage treatment plants or water pollution control plants, remove most contaminants from wastewater before it is released to local water channels.[60] At the plants, wastewater undergoes physical and biological processes for purification. Wastewater purification employs five major processes, i.e. preliminary treatment, primary treatment, secondary treatment, disinfection and sludge treatment. Primary and secondary treatments remove about 85% to 95% of pollutants per load/mass treated from the wastewater before the wastewater is disinfected and discharged into local waterways. Sludge, the by-product of the treatment process, is digested for stabilisation and is then dewatered for easier handling. The resulting material, known as bio-solids, is then applied to land to improve vegetation or processed further as compost or fertilizers,[60] which may lead to human exposure to contaminants within bio-solids as they are brought back to the environment/land.

Pharmaceuticals and personal care product removal during wastewater purification is dependent on their physical and chemical properties. Wastewater treatment plants that

have biological treatment such as activated sludge processes or bio-filtration have been shown to remove PPCPs at varying rates, ranging from less than 20% to more than 90%.[24] Efficiencies have been shown to vary depending on the operational configuration of the treatment plant. Factors influencing chemical removal include sludge age, activated sludge tank temperature and hydraulic retention time. Advanced processes that include reverse osmosis, ozonation and advanced oxidation technologies can result in higher removal of PPCPs.[24]

Water purification for drinking purposes can be conducted using either advanced water treatment processes or conventional processes. Advanced water treatment processes, like ozonation, membrane treatment and advanced oxidation, usually achieve higher removal rates (up to 100%) for pharmaceuticals in water, compared with conventional processes such as treatment with coagulation, filtration and chlorination which removes up to 50% of these compounds. For example, a bench-scale study showed that advanced oxidation processes can achieve up to 100% removal for the anti-inflammatory diclofenac sold under trade name Voltaren.[58]

Traditional drinking water treatment processes such as coagulation do not remove many of the PPCPs. Free chlorine can remove approximately 50% of PPCPs, chloramines are less effective.[24] Advanced drinking water purification processes (ozonation, oxidation, activated carbon and membranes) result in removal rates of over 90% of PPCPs.[24] Literature indicates that concentrations of PPCPs in drinking water are usually more than 1000-fold below the minimum therapeutic dose, i.e., the lowest clinically active dose.[24] However, for drinking water sources that are contaminated with pharmaceuticals, advanced treatment may be the option that can assist in optimising removal of pharmaceuticals during water treatment process.[24]

1.2 ANTIRETROVIRALS

Since the first antiviral drug, idoxuridine, was approved in 1963, 90 antiviral drugs from 13 functional groups have been approved for the treatment of nine infectious diseases.[61] These antivirals are used for the treatment of a broad spectrum of viral diseases including influenza, herpes simplex, varicella-zoster, human papillomavirus, hepatitis B and C and human cytomegalovirus infections.[61] Antiretrovirals are the drugs that are used to treat

retrovirus infections specifically human immunodeficiency virus (HIV)/AIDS.[62] As hepatitis B virus (HBV) uses the enzyme reverse transcriptase (RT) for replication, selected ARV drugs are also used for the treatment of hepatitis B infection.[63] Antiretroviral therapy (ART) for HIV is a lifetime treatment.[64] Owing to the chronic and lifelong use of ARVs, they can be viewed as *pseudo-persistent contaminants* in the environment because of their continuous use and release into the environment.[65] There are more than 25 ARV drugs (Figure 1.2) which are used in combinations of three or more drugs, referred to as combination antiretroviral therapy (cART).[66]

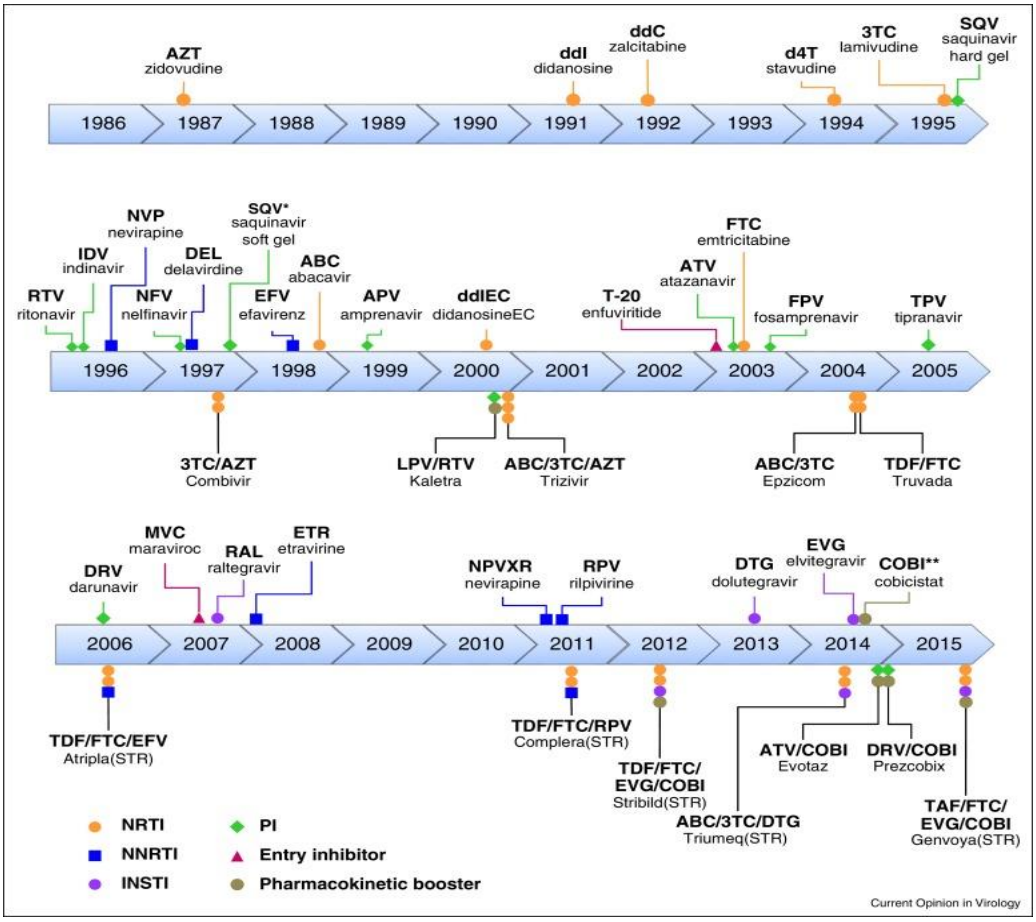


Figure 1.2: Antiretroviral drugs in six mechanistic classes.[66] (*reproduced with permission from publisher [Appendix C]*)

Based on their mechanism of action, ARVs are grouped into six major classes[61] (Figure 1.3). The classes and the associated drugs are:

a) *Reverse transcriptase inhibitors (RTIs)*

The RTIs inhibits the activity of RT enzyme, a viral deoxyribonucleic acid (DNA) polymerase that is required for replication of HIV and HBV.[63] There are two distinct types of RT inhibitors, the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).[67]

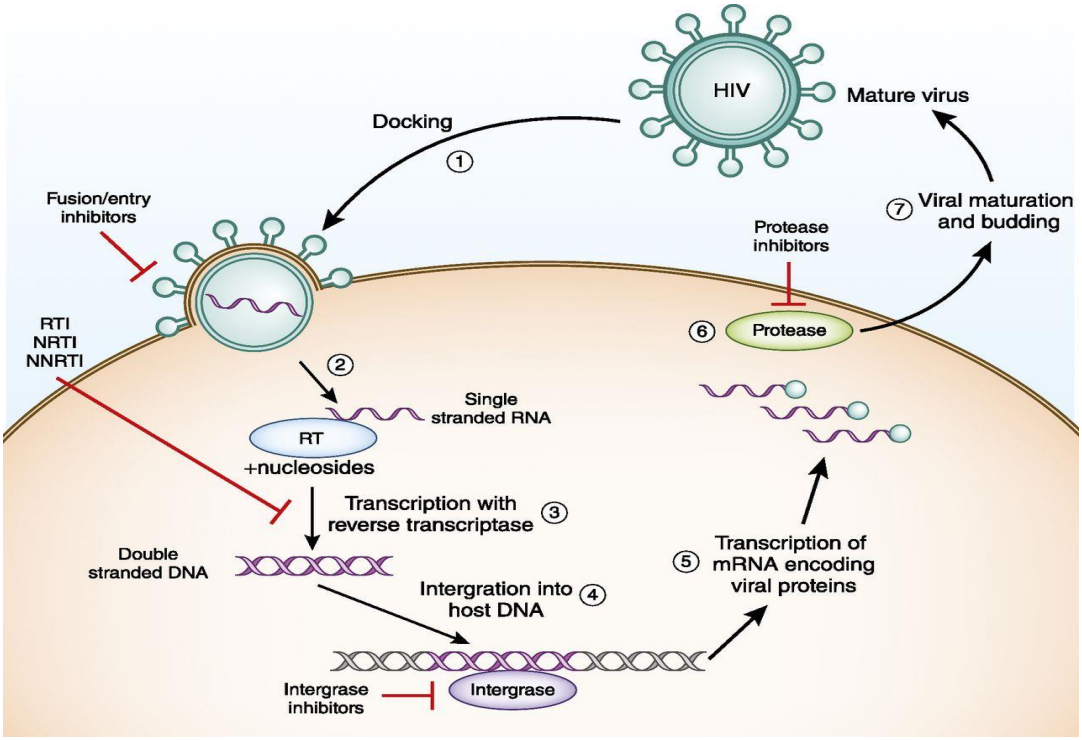


Figure 1.3: HIV-1 life cycle and classes of antiretroviral agents that interfere with these specific steps. The seven steps of HIV replication are depicted: 1) attachment and entry, 2) uncoating, 3) reverse transcription, 4) integration, 5) transcription, 6) assembly, 7) virus maturation and budding. Classes of antiretroviral drugs are shown as red lines near the life cycle step that they inhibit. NNRTI, non-nucleoside reverse transcription inhibitor; NRTI, nucleoside reverse transcription inhibitor; RT, reverse transcription; RTI, reverse transcription inhibitor.[68] (*reproduced with permission from publisher [Appendix C])*

i) Nucleoside/nucleotide reverse transcriptase inhibitors

The NRTIs competitively inhibit the HIV RT and are the backbone of cART, usually given as a combination of two NRTIs with a drug from one of the other classes.[67] The drugs in this class and their most common side effect are listed in Table 1.1

Table 1.1: Drugs in the nucleoside/nucleotide reverse transcriptase inhibitor class

Drug	Trade Name	Abbreviation	Major and most common side effects[69]
Nucleoside reverse transcriptase inhibitors			
Abacavir	Ziagen	ABC	Mitochondrial toxicity & myopathy (affects muscle contractility) Lactic acidosis & Hepatic steatosis
Didanosine	Videx	ddI	
Emtricitabine	Emtriva	FTC	
Lamivudine	Epivir	3TC	
Stavudine	Zerit	d4T	
Zidovudine	Retrovir	AZT/ZDV	
Zalcitabine*	Hivid	ddC	
Nucleotide reverse transcriptase inhibitors			
Tenofovir Disoproxil Fumarate	Viread	TDF	Decreased bone density & acute renal failure
Tenofovir alafenamide	Vemlidy	TAF	

*Discontinued

ii) Non-nucleoside reverse transcriptase inhibitors

The NNRTIs bind non-competitively to HIV-1's RT and prevents viral RNA conversion to DNA. Resistance to this drug class can develop from a single mutation.[70] Importantly, NNRTIs act specifically against HIV-1, whereas HIV-2, due to its structural properties, is unsurprisingly resistant to all NNRTIs.[71] The drugs in this class and their most common side effects are listed in Table 1.2.

Table 1.2: Drugs in the non-nucleoside reverse transcriptase inhibitor class

Drug	Trade Name	Abbreviation	Major and most common side effects[69]
Delavirdine	Rescriptor	DLV	Gastrointestinal intolerance Liver toxicity Skin rashes Stevens-Johnson Syndrome Teratogenic
Doravine	Pifeltro	DOR	
Efavirenz	Sustiva	EFV	
Entravirine	Intelence	ETR (TMC 125)	
Nevirapine	Viramune	NVP	
Rilpivirine	Edurant	RPV (TMC278)	
Elsufavirine	In development for use in LMICs		

b) *Protease inhibitors* (PI)

The PIs inhibit the viral protease which is the enzyme required for the cleavage of polyproteins to form the viral capsid and nucleocapsid and prevent the budding of mature virions from the infected cell.[72] Due to the risk of unwanted side effects at higher doses, PIs are often used as a component in a combination of different antiretroviral drugs.[72] The drugs in this class and their most common side effects are listed in Table 1.3.

Table 1.3: Drugs in the protease inhibitor class

Drug	**Trade Name**	**Abbreviation**	**Most common side effects[69]**
Atazanavir	Reyataz	ATV	Redistribution of body fat Hyperglycemia High cholesterol & triglyceride levels Gastrointestinal intolerance (nausea & diarrhoea)
Cobicistat	Tybost	COBI	
Darunavir	Prezista	DRV	
Fosamprenavir	Lexica, Telzir	FPV	
Indinavir	Crixivan	IDV	
Lopinavir/Ritonavir	Kaletra	LPV/r	
Nelfinavir	Viracept	NFV	
Ritonavir	Norvir	RTV	
Saquinavir	Invirase	SQV	
Tipranavir	Aptivus	TPV	

c) *Entry inhibitors*

Entry inhibitors act by preventing a cell-free virus from attaching to the receptors of a body cell.[47] Under this class, there are two sub-classes: the *entry inhibitors (CCR5 antagonists* and *gp120 attachment inhibitors)* and the *fusion inhibitors.*[73] The drugs in this class and their most common side effects are listed in Table 1.4a & b.

Table 1.4a: Drugs in the entry inhibitor class

Drug	Trade Name	Abbreviation	Most common side effects
CCR5 antagonists			
Maraviroc	Selzentry/Celsentri	MVC	
gp 120 attachment inhibitor			
Fostemsavir*		FTR	
Monoclonal antibody against CD4 receptor			
Ibalizumab**	Trogarzo	IBA	

***** Completed Phase 3

****** Newly approved

Table 1.4b: Drugs in the fusion inhibitor class

Drug	Trade Name	Abbreviation	Most common side effects[69]
Enfuvirtide	Fuzeon	ENF (T-20)	Local injection site reaction, Upper respiratory infections, cough, Postural hypotension, Joint & Muscle pain, Hepatotoxicity, Myocardial ischemia, Infarction
Albuvirtide	Aikening	ABT	

*Newly approved

d) *Integrase strand transfer inhibitors (INSTIs)*

Integrase strand transfer inhibitors act by preventing the insertion of the viral DNA into the DNA of the host cell. They target the HIV integrase enzyme.[47] The drugs in this class and their most common side effects are listed in Table 1.5

Table 1.5: Drugs in the integrase strand transfer inhibitor class

Drug	Trade Name	Abbreviation	Most common side effects[69]
Elvitegravir	Viteka	EVG	Nausea, Diarrhoea, Headache, Fever Weight gain
Raltegravir	Isentress	RAL	
Dolutegravir	Tivicay	DTG	
Cabotegravir*		CAB	
Bictegravir**		BIC	

* In Phase 3 of development

** Used in fixed-dose combination drugs

Limited research has been carried out globally to determine the presence and fate of pharmaceuticals (including ARVs) and personal care products and their degradation products. From the few studies undertaken, the focus has only been on a selected group of these products. Antiretrovirals are an emerging class of pharmaceuticals and their studies conducted to date are limited. Literature shows that there are currently far fewer data for Africa, Asia and South America compared to the Europe and North America.[74]

1.3 RELEVANCE OF THE STUDY

Despite the relatively fast-growing numbers of studies on ecological and/or environmental risk associated with pharmaceuticals in water, to date there are only a few studies that have addressed the potential human health risks associated with the ingestion of low doses pharmaceuticals through treated drinking water. As there are levels of ARVs in the environment including drinking water, it remains important to share findings on the investigation of the potential health impacts. Moreover, in the South African context, the surface water is mainly used as source water for water purification facilities as well as by higher socio-economic communities, mainly in urban areas, for recreational purposes while river and dam water is used by lower socio-economic communities, mainly in rural areas, for domestic and recreational purposes.[75] However, most studies on the presence of ARVs in African waters are conducted in urban areas. Also, there is a high population of HIV infected people residing in rural areas where there are no proper sanitation systems.[47]

People in most rural areas depend on untreated river water as the only source of drinking water which is also shared with their animals that are used as food sources. Due to droughts and lack of rainfalls especially in winter, the water level in the rivers become too low which could result in pre-concentration of pollutants in such rivers and those pollutants include ARVs.[47] The rural populations are therefore exposed to ARVs through untreated drinking water, bearing in mind some of the practices in rural areas which includes the use of the nearest bush for excretion of the body wastes which is usually washed off by rain to small rivers if not eaten by animals (that are also food sources).[47]

Additionally, as safe drinking water is a necessity for all human and other organisms, there is a growing interest in seeking to relieve the pressure of water scarcity. One of the options considered is reclaimed water (also referred to as recycled water). Although this option may be seen as a possible solution, attention about its technologies and potential risks is growing in the meantime. Most plants established WWTPs processes cannot ensure to remove all/certain contaminants completely from origin water sources and these may further aggravate water quality challenges.[76]

1.4 PROBLEM STATEMENT

Several studies have been conducted in Southern Africa and they have identified pharmaceuticals, including ARVs in drinking water[33-34,59,77-78] in low concentrations as these compounds would have naturally undergone metabolism and, where applicable, wastewater and drinking-water treatment processes[25]. The fact that pharmaceuticals are manufactured with the intention to cause biological effects has raised concerns about the impacts of unintentional pharmaceutical exposure on human health. There are few comprehensive, systematic studies on the occurrence of pharmaceuticals in drinking water. Limited data on the occurrence of pharmaceuticals in drinking water are a challenge in assessing potential human health risks from exposure to trace concentrations of pharmaceuticals in drinking water.[24]

We have therefore identified a gap in the comprehensive understanding of the presence of ARVs in drinking water. We therefore seek to provide a compressive insight into the extent and effects of unintended exposure ARVs through drinking water.

1.5 HYPOTHESIS

The study hypothesises that there are health effects that can be associated with long-term exposure to low levels of ARVs in water that has undergone wastewater and drinking water treatment processes in SA.

1.6 STUDY AIM

Through modelling, this study aims to assess the possible clinical risks associated with long-term exposure to low levels of ARVs in treated and untreated water sources in SA.

1.7 SPECIFIC OBJECTIVES

The specific objectives of this study are:

a) To review national and international literature to determine the extent and risks posed by ARV contamination in water sources worldwide.
b) To establish which ARVs, or derivatives thereof, are most commonly used in the public and private sector in SA, with special reference to Gauteng Province.
c) To establish which ARVs, and quantities thereof, have been detected in water sources in SA, with special reference to Gauteng Province.
d) To determine the potential health risks posed by ARVs in water to vulnerable individuals and communities through modelling using pharmacokinetic as well as compartmental models.

1.8 ETHICS APPROVAL

This study received ethics clearance from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria in February 2018, amended in June 2018 and was allocated the Ethics reference number: 13/2018. (Appendix B).

CHAPTER 2

THE EXTENT AND POTENTIAL RISKS POSED BY ANTI-RETROVIRAL DRUGS IN WATER SOURCES GLOBALLY: A REVIEW

2.1 GENERAL INTRODUCTION

Since the end of the 19th century, quality standards on drinking water supply have focused mainly on microbial risk,[55] nonetheless there is an emerging concern about potentially harmful chemicals, including small amounts of ECs, in water sources.[79] Due to the increase in pollutants in aquatic environments, this contamination threatens surface water resources, which has become a serious concern worldwide.[80-82] Literature indicates that ECs, including pharmaceuticals, occur globally in the environment, in both HICs and LMICs.[46] Contamination of water sources with pharmaceuticals result largely from their worldwide and continual usage by humans through ingestion and excretion, and overuse in domestic animals, as well as inappropriate disposal of expired or unwanted drugs.[83] These pharmaceuticals range from antivirals, analgesics, antibiotics, contraceptives, lipid regulators, β blockers, detergents, perfumes, dental products, etc. They are essential for the wellbeing of humans but unfortunately they might have detrimental effects on humans and aquatic life if they find their way into the water systems.[84] Although direct adverse effects of these contaminants have been scientifically established in aquatic biota, their effects on humans are still speculative.[85] Recently, research reports on environmental monitoring of pharmaceuticals in LMICs have been emerging.[33,59,78,86]

Currently most countries are facing a shift in their disease burden from one that is dominated by acute diseases towards one dominated by chronic diseases.[87] This change has profound implications for the supply and use of pharmaceuticals.[87] In addition, the global drug consumption has increased rapidly in recent years with the active ingredients of these drugs ending up in water sources.[87] The most abundant pharmaceuticals in wastewaters in any region are those that are consumed the most.[88] The detection of these pharmaceuticals in the environment therefore varies not only between countries, but also between different regions within the same country.[89] Detectable pharmaceuticals in one country or region may not appear in other countries or regions where they are not highly

prescribed.[89] This chapter aims to determine the extent and potential risks posed by selected ARV drugs in water sources worldwide, excluding SA.

2.1.1 Pharmaceuticals in water sources: a global picture

The occurrence of pharmaceuticals and their metabolites or transformed products in the aquatic environment has been investigated worldwide in several countries including Austria, Brazil, Canada, Croatia, China, UK , Germany, Greece, Italy, Spain, Switzerland, Taiwan, The Netherlands and the USA.[46] This has resulted in an increasing number of reports on the occurrence of PPCPs in environmental samples such as wastewater, seawater, river water, sediments and sludge.[84]

a) *Africa:* In most LMICs in Africa, the waste disposal system is mainly through landfill and some of the disposed waste is not easily degradable by environmental processes such as biodegradation or photodegradation.[74] Moreover, landfill leachate may contaminate groundwater which constitutes a major water supply for a large proportion of the population in arid regions of Africa.[74] African cities are densely populated with large usage of products containing these compounds, but lack adequate wastewater treatment facilities. Hence, untreated effluents are directly discharged to surface waters and soil.[90-91] Due to the fact that non-steroidal anti-inflammatory drugs (NSAIDs) are widely available over the counter and do not require any medical prescription, allowing for self-medication, this group of pharmaceuticals is widely detected in the environment.[92] The NSAIDs not surprising, are the most common drugs in the African aqueous environment and have been detected in selected African wastewater and surface water as follows, e.g. naproxen (59.3 ng/L),[93] ibuprofen (17.6 ng/L)[93] and diclofenac (222.7 ng/L).[94] In addition to ARVs, antibiotics such as ciprofloxacin (1.2 ng/L),[94] ampicillin (200 ng/L)[95] and trimethoprim (120 ng/L),[96] antimalarial drugs (sulfadoxine 50 ng/L)[59] and antiepileptic drug compounds (carbamazepine 2.7 ng/L) [97] amongst others, have been detected in a variety of water sources across Africa.

b) *Europe:* More than 3000 pharmaceutical compounds are commercially available in the European continent.[35,98] Pharmaceuticals and their metabolites have been detected in variable amounts in the European water sources.[88] Although lower concentrations were detected in Europe compared to Africa, diclofenac,

naproxen, ibuprofen, paracetamol and ketoprofen are the most common analgesics and anti-inflammatories (AAFs) in aquatic environments on both continents.[99] The concentrations of paracetamol, naproxen and ketoprofen were ~215 times, ~171 times and ~40 times lower than those reported in studies conducted in Africa, respectively.[99]

Codeine was more frequently reported in European studies than those in Africa.[100-105] This correlates with the reported codeine consumption globally, by country, in 2015.[106] In this report, France, UK and Spain were in the top eleven countries where codeine was the most frequently reported EC in the aquatic environment compared to other countries globally.[106] Another study showed that the use of codeine increased between 2006 and 2015 by 42% in France.[107] The consumption of codeine in Europe can be explained by the analgesic preferences of population in the countries and by the role of national guidelines, prescription policies and the marketing strategies of pharmaceutical companies.[107-108] Moreover, the environmental occurrence of codeine can also be linked to its low biodegradability.[99] Additionally, venlafaxine (used in treating depression and anxiety) has not only the highest concentration recorded in the anti-depressants therapeutic group but is also more frequently reported in European aquatic environments than in African water sources.[99] This is linked to an increasing consumption trend in Europe[109] as mental health disorders, in general, are the most common cause of disability. Depression alone causes 13.7% of all years lived with disability and ranks as the third most common condition after ischemic heart disease and stroke.[110]

c) *North and South America:* Although there are more studies in the North America compared to sparse data from the South America, pharmaceuticals have been detected in water sources from both continents.[46] The detection of pharmaceuticals in the treated wastewater was first reported in Kansas City, USA in 1976.[111] Thereafter, several studies were conducted in different environmental compartments. In one of these studies a total of 93 pharmaceuticals namely 27 antibiotics; 15 antidepressants; 9 antihypertensives; 7 analgesics; 7 anticonvulsants; 6 antilipidemics; 3 contraceptives; 3 stimulants; and 2 each of antihistamines, blood thinners, disinfectants, antacids, antitussives, anti-anxiety,

anti-inflammatory, and diuretic agents, were detected from surface waters (including rivers, lakes, oceans, and aquifers).[112] Also, in another study conducted in North America, the occurrence and distribution of 17 pharmaceuticals in surface and groundwater sources from Mexico City were determined.[113] The following pharmaceuticals were detected in surface water: ibuprofen (15-49 ng/L), diclofenac (28-32 ng/L), naproxen (52-186 ng/L), gemfibrozil (9-10 ng/L) and ketoprofen (21-42 ng/L).[113] The concentrations of detected pharmaceuticals were higher in surface water than in groundwater, where all were undetectable except diclofenac (1ng/L).[113] For the South American continent, a study was conducted at the Piracicaba River in the State of Sao Paul in Brazil where the following hormones were detected; estriol (90 ng/L), estrone (28 ng/L), progesterone (26 ng/L), 17β-estradiol (137 ng/L), and 17α-ethinylestradiol (194 ng/L). This contamination was linked to the inflow of sewage containing these hormones into the Piracicaba River.[114]

d) *Australia (Oceania):* Data exists demonstrating the presence of numerous pharmaceuticals in effluents, river systems, marine sediments and sewage sludge in Australia as well as New Zealand.[115] From a national survey of ECs in Australian rivers, which was conducted quarterly at 73 river sites across Australia for one year, ECs were detected in 92% of samples. Amongst other ECs detected were pharmaceuticals, namely: salicylic acid (1530 ng/L), paracetamol (7150 ng/L), carbamazepine (682 ng/L), and caffeine (3770 ng/L).[116] To determine the risk posed by the detected ECs to the aquatic environment, hazard quotients were calculated by dividing the maximum concentration detected for each compound by the predicted no-effect concentrations. Three of the 42 monitored compounds, namely; two pharmaceuticals (carbamazepine and sulfamethoxazole) and a herbicide (simazine) had a hazard quotient >1, suggesting that they may be causing adverse effects at the most polluted site.[116]

e) *Asia:* Emerging contaminants have also been detected in surface water sources in Asia. In lake Dongting in China, 12 pharmaceuticals were identified at concentrations ranging from 2 to 81 ng/L.[117] The contamination levels were relatively low on a global scale and the most abundantly detected compound was caffeine followed by diclofenac, diethyltoluamide (DEET), mefenamic acid,

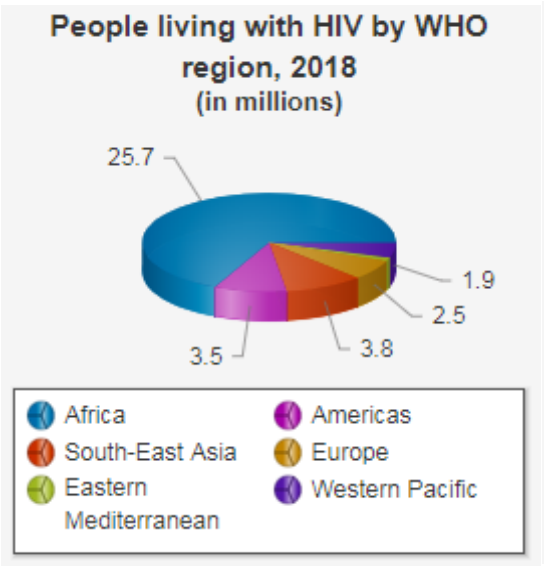
fluoxetine, ibuprofen, and carbamazepine.[117] Similarly, 15 pharmaceuticals were detected in surface waters (streams, ponds and lakes) of India by various researchers using various detecting methodologies. The quantities ranged from undetectable to 14 mg/L.[118] A study conducted in Japan demonstrated that the active metabolite of the drug Oseltamivir, Oseltamivir carboxylate (OC) is neither degraded nor removed by WWTPs.[119] It is therefore assumed that OC can be present in the aquatic environments,[120] especially those continents with high usage due to annual influenza pandemics (Asian, European and American regions). Japan is the top per-capita-consumer of Oseltamivir [120] and in a study conducted in Japan,[119] OC was present in Japanese waterways at clearly detectable levels. The levels increase at the peak of the influenza season. The study findings also suggested that the OC levels are higher closer to major WWTPs and further downstream in a river system.[120]

Although several studies have looked at pharmaceuticals in the aquatic environment globally, only a few pharmaceutical review articles report partially on the presence and fate of ARVs in environmental samples.[121-124] When one considers the use of ARVs and their occurrence in water sources globally, the global HIV and HBV epidemics need to be interrogated with regards to drug usage, particularly in places where the prevalence and ARV usage for those epidemics are high.

2.1.2 Global HIV epidemic

In 2018, the number of people living with HIV globally was estimated to be 37.9 million, with 23.3 million people on cART.[125] This reflects the continued transmission of HIV despite reductions in incidence, as well as the benefits of the expanded access to ARVs which have helped to reduce the number of people dying from HIV-related causes. Sub-Saharan Africa remains the most severely affected with 25.7 million people living with HIV, which is 70% of the global HIV population and accounts for more than two-thirds of the people living with HIV worldwide (Figure 2.1).[126] The most HIV prevalent countries within Sub-Saharan Africa are the Southern African Development Community (SADC) namely; Botswana, Lesotho, Malawi, Mozambique, Namibia, SA, Swaziland, Zambia and Zimbabwe.[127]

A



B

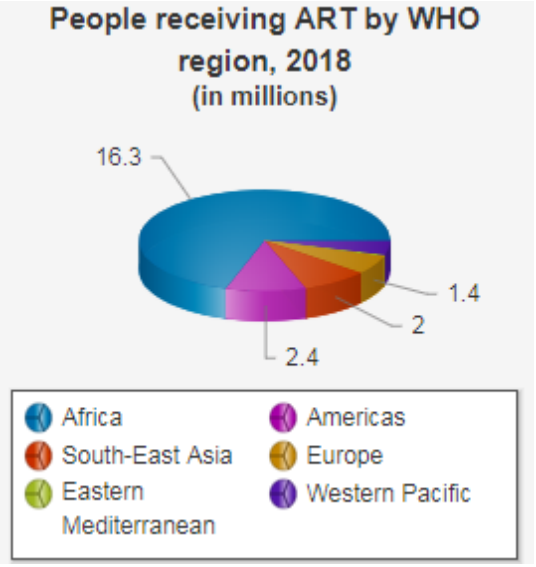


Figure 2.1: People living with HIV (A) and those receiving treatment (B) (in millions), by WHO region, 2018.[125] (reproduced as per WHO Policy on Open Access: https://www.who.int/about/who-we-are/publishing-policies/open-access)

Therefore looking at the burden of HIV and the ARV drug usage across the globe, it can be expected that more ARVs in the environment are detected on the African continent compared to the rest of the world.[47] In addition, the United Nations Programme on HIV/AIDS (UNAIDS) has set the 90–90–90 ambitious HIV treatment target that aims to diagnose 90% of all HIV-positive persons, provide ART for 90% of those diagnosed, and achieve viral suppression for 90% of those on ART by the year 2020.[128] This will have a great impact on what is detected in water sources globally as it will affect the ARV usage worldwide, particularly in those countries who have adopted the strategy.

Coupled with the HIV epidemic is the tuberculosis (TB) epidemic as TB is a main opportunistic infection amongst individuals living with HIV in Africa.[129] It is impossible to talk about one and omit the other. This means that the same concerns one has with other ARVs contaminating the environment would also be relevant when it comes to TB treatment drugs, mainly, isoniazid, rifampin, ethambutol and pyrazinamide, only considering adult dosing and excluding multidrug resistant cases as they are a small proportion. Tuberculosis is the most common presenting illness and cause of death among people with HIV [129] and people living with HIV are 20 to 30 times more likely to develop active TB disease than people without HIV.[130] It was estimated that 10 million people developed TB disease in the year 2017 (5.8 million men, 3.2 million women and 1 million are children). Cases were estimated in all countries globally and across all age groups,

but overall 90% were adults (aged ≥15 years), 9% were people living with HIV (72% in Africa) and two-thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and SA (3%).[131]

2.1.3 Global HBV epidemic

The WHO estimates that in 2015, 257 million people were living with chronic hepatitis B infection defined as hepatitis B surface antigen (HBsAg) positive for longer than six months, globally[132] (Figure 2.2). In 2015, hepatitis B resulted in an estimated 887 000 deaths, mostly from cirrhosis and hepatocellular carcinoma (HCC), the primary liver cancer.[129] As of 2016, 27 million people (10.5% of all people estimated to be living with hepatitis B) were aware of their infection, while 4.5 million (16.7%) of the individuals diagnosed were on treatment.[129]

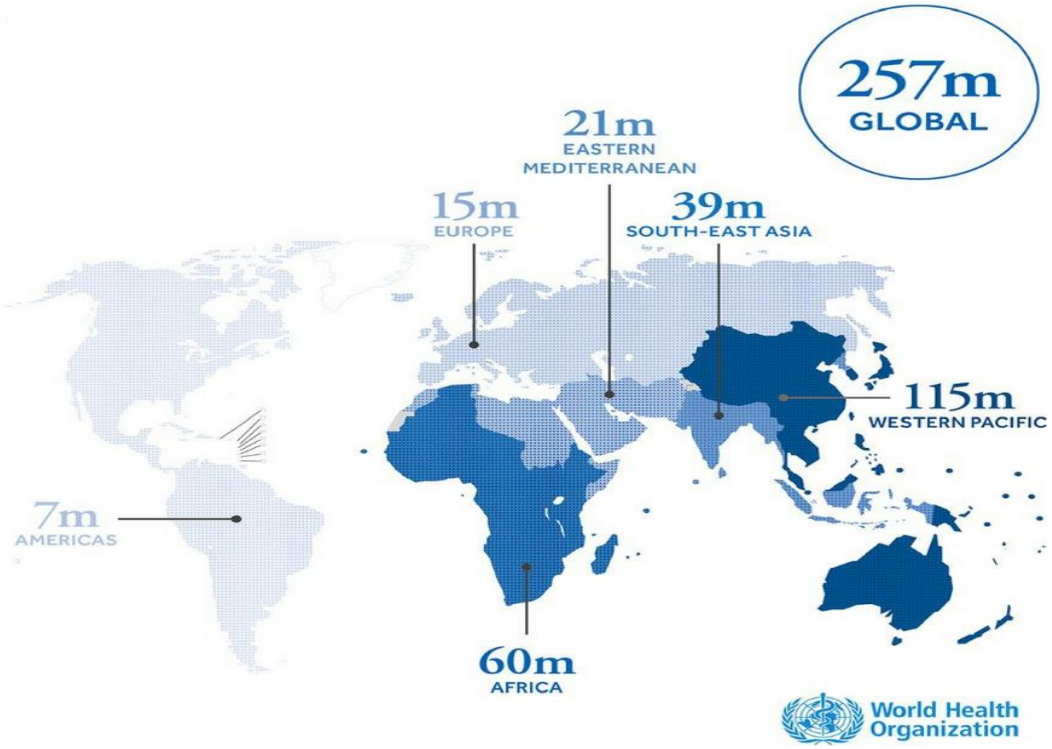


Figure 2.2: Prevalence of chronic hepatitis B worldwide, 2017.[133-134]

Hepatitis B prevalence is highest in the Western Pacific and the African regions, where 6.2% and 6.1% of the adult population, respectively, are living with hepatitis B.[132] The whole African continent is considered to have a high HBV endemicity.[135] Hepatitis B virus infection is hyper endemic, with > 8% of HBsAg chronic carriers in the general population, in some Sub-Saharan countries such as Nigeria, Namibia, Gabon, Cameroon

and Burkina Faso.[136] Of note, the epidemiology in Africa is characterised by a much higher HBsAg prevalence in rural than in urban areas.[137-138] In many regions, treatment is governed by international guidelines. Long-term suppression of HBV DNA is an achievable endpoint for most patients.[135] Treatment for HBV has been restricted to interferon, pegylated interferon or five nucleoside analogues: 3TC, adefovir, telbivudine, entecavir and TDF. Maintenance therapy is required for most people, as low rates of cure occur. Also, it is important to note that 3TC (or its 'equivalent', FTC) and TDF are used as first-line drugs for the treatment of HIV/AIDS as well.[139]

The incidence of HCC and cirrhosis is low in persons younger than 35 years of age, but rises in mid and later life.[140] In Africa however, a higher incidence of HCC has been reported in young male adults.[140] The WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 hours, and that the birth dose is followed by two or three subsequent doses. The vaccine is effective in 95% of infants and children but protection may fail in infants born to highly viraemic mothers. By 2012, 183 countries vaccinated infants against hepatitis B as part of primary vaccination schedules.[141] Most of the burden of disease from HBV infection comes from infections acquired before the age of 5 years. Prevention of HBV infection, therefore, focuses on children <5 years of age. In 2015, the estimated global prevalence of HBV infection in this age group was about 1.3%, which shows a decline compared to 4.7% recorded in the pre-vaccination era. This low incidence of chronic HBV infection in children under 5 years of age can be attributed to the widespread use of hepatitis B vaccine. This fall in the incidence of chronic HBV infections among children means that in the long term, the global hepatitis B epidemic will decline.[142]

Hepatitis B virus co-infection with HIV is common and the rates for HBV co-infection in HIV-positive people are given at a range between 5% and 30%, depending on the geographic region.[139] This co-infections commonly occur because of their endemicity in the same regions and their shared routes of transmission.[143-144] Sub-Saharan Africa has the largest burden of HIV infections in the world and is also an HBV endemic area [145] and it can therefore be expected that this situation contributes to the high usage of ARVs in these regions. Given this data, and considering the usage of ARV, it can then be expected that higher levels of environmental contamination with ARVs occurs in areas with high prevalences of HIV and HBV.

2.2 ANTIRETROVIRALS IN WATER SOURCES

The consumption of pharmaceuticals around the world is diversified, and, therefore, the types and concentrations of pharmaceuticals in surface water differ from one region to another largely driven by the disease burden.[87] Between 1963 and 2016, 90 antiviral drugs were formally approved to treat nine human infectious diseases.[61] There is an exponential increase in antiviral use and the occurrence of antiviral drugs in the environment is considered an emerging concern[124] and currently, half of all antiviral agents are ARVs.[124] The African continent is the hardest hit by the HIV pandemic hence this region uses more ARVs compared to the rest of the world.[47] The presence of antiviral drugs has been investigated in various aqueous environments globally.[84,124,146-149] Although only a few studies have been conducted on the occurrence and fate of antiviral drugs in the environment worldwide, the majority of the publications are on the drug oseltamivir and its metabolite, OC.[124]

2.2.1 Antiretrovirals in water sources globally, excluding Africa

Water sources, namely WWTP influent and effluent, surface water (rivers and streams) and drinking water (treated water and groundwater), have been analysed for ARVs in many regions globally. As expected, ARV usage is higher in the African region than the rest of the world, hence data on the detection of ARVs in environmental samples, compared to other antivirals, e.g. acyclovir (ACV),[150] is scanty for regions outside Africa. From a review of the literature, 13 studies report on the detection of ARVs in global non-African water sources (Table 2.1). For some of these studies the selection of ARVs to be tested was based on the consumption in a particular country at that time, e.g. Germany[145], France[151] and Finland[152] or as markers of cancer in urban sewage (ATV used for both chemotherapy or cART),[153] while other studies focussed on the development and validation of analytical methods[149,151] and/or the elimination efficiency of wastewater treatment processes.[154-155]

From data presented in Table 2.1 it is evident that the concentrations of ARVs detected in various water sources ranged from non-quantifiable (nq) to 564 ng/L. The concentrations also varied across water sources, with the highest concentration detected from WWTP effluents (564 ng/L), and the lowest concentrations in the drinking and

surface water (~3 ng/L). However, when reviewing such data it is important to keep in mind that the timing of the water sampling is crucial and impacts its detection rate as ARVs have variable half-lives, e.g. the half-life for ABC, RTV, 3TC and SQV were reported to be <5 days under biochemical conditions in surface water and wastewater.[151]

In the studies reported in Table 2.1, NVP was the most investigated ARV in water sources with the poorest removal in WWTPs activated sludge which was attributed to its photostability and poor biodegradability.[149] Of concern was the detection of NVP in river water in Germany[145] and groundwater in the USA[158] as individuals could unknowingly be exposed to low concentrations. The high detection frequency for NVP in the environment could be due to its wide use for the treatment of HIV and for the prevention of mother to child transmission (PMTCT).[78] In addition, poor removal efficiency for this drug during the sewage treatment process could also lead to frequent detection in surface water, [156] and NVP was also found to be resistant to degradation at relevant chlorination levels.[156] This might partially explain its ubiquitous presence in water sources. Zidovudine on the other hand, had the highest measured concentration among the ARVs in both influent and effluent wastewater, although the concentrations measured in Finland were much lower than what has been reported in other countries.[152] The presence of AZT in stream water in Germany[155] highlights the potential exposure of individuals to low concentrations of ARVs through polluted water sources.

Table 2.1: Antiretrovirals detected in water sources worldwide, excluding Africa.

Sample/ Source	ARV detected quantity ng/L		ARV tested for but not detected	Country	Continent
WWTP effluent	NVP AZT	7 - 32 98 - 564	ABC 3TC d4T	Germany	Europe[149]
Surface water	NVP d4T AZT	6 - 17 2 - 3 18 - 170	ABC 3TC		
Hospital effluent	RTV	108		Switzerland	Europe[154]
WWTP effluent	RTV	90		Switzerland	Europe[155]
Landfill leachates	ABC 3TC	185 355		USA	North America[157]
WWTP effluent	ABC IND 3TC NVP RTV SQV AZT	31 - 33 1.5 6.5 - 44 3 - 7.7 53 - 155 0.2 154 - 191	NFV	France	Europe[151]
WWTP effluent	ATV	<LOQ		Norway	Europe[153]
WWTP effluent			3TC	Belgium	Europe[158]
Drinking water	FTC# 3TC#	80 84	ABC AZT	Germany	Europe[159]
Surface water	AZT	22 - 30	ABC FTC 3TC		
WWTP effluent	AZT FTC 3TC	170* 170* 140*	ABC		
Lake water	3TC	12		Finland	Europe[152]
WWTP effluent	3TC AZT NVP	20 - 22 22 - 37 8 - 10			
Groundwater	3TC NVP	23 25		USA	North America[160]
Drinking water	DRV	3.4		Poland	Europe[161]
WWTP effluent	ATV	<LOQ		Greece	Europe[162]
Drinking water	3TC	28	ABC NVP	USA	North America[163]
WWTP effluent	FTC FTC-CBX ABC-CBX	51 330 86		Germany	Europe[164]

WWTP= Wastewater Treatment Plant, <LOQ = below limit of quantification, *Concentrations ranged from below limit of quantification to given value, #Concentrations for transformation products. 3TC = lamivudine, d4T = stavudine, ABC = abacavir, ABC-CBX = ABC–carboxylate, AZT = zidovudine, RTV = ritonavir, DRV = darunavir, SQV= saquinavir, FTC = emtricitabine, FTC-CBX = emtricitabine carboxylate, ATV = atazanavir, NFV = nelfinavir, IND = indinavir.

In studies where both influent and effluent water from WWTPs were tested, ARVs were also detected in WWTP effluent, even though they are detected in lower concentrations.[145,155] In an early investigation in Germany, 3TC (720 ng/L), AZT (380 ng/L), d4T (11.6 ng/L) and ABC (220 ng/L) were detected in grab influent wastewater samples from one WWTP, with 3TC, d4T and ABC undetected in the corresponding effluent. In contrast AZT was still detected (98 ng/L) in the effluent of the WWTP. A similar pattern was noted in a second WWTP where 24-hour composite samples were collected.[145] The removal efficiencies for ABC, 3TC and d4T ranged from 87 to > 99%. In contrast AZT and NVP were detected in both the influent and effluent from both WWTPs, with a removal efficacy of 0 - 68% for AZT and 0% for NVP.[145] Although the early German studies reported high removal efficiencies for ABC and 3TC from wastewater,[149] a more recent study in Germany showed that even though the parent compound ABC was below the level of detection in wastewater effluent, the main metabolite, ABC–carboxylate (ABC-CBX), was detected at a mean concentration of 86 ng/L in effluents.[164] Investigations in France however showed ABC (31-33 ng/L) and 3TC (6.5-44 ng/L) in wastewater effluent during two samplings,[151] while 3TC was detected, albeit at low concentrations (20-22 ng/L), in wastewater effluent in Finland.[152-153] Although studies report high removal efficiencies of 3TC from wastewater,[159,165-166] the carboxy metabolite of 3TC mostly exhibited negative removal rates, where it was found at concentrations of 25 ng/L in the influents compared to 220 ng/L in the effluents in Germany.[159] Emtricitabine, which is metabolised to a small extent in the human body (10–30%), was detected in influents of municipal WWTPs at concentrations up to 980 ng/L,[159] but with a removal efficiency of 74% concentrations in the effluents which were much lower.[159] In a different study, FTC and its metabolites, FTC-carboxylate (FTC-CBX) and FTC-S-oxide were investigated in wastewater effluents and were found to have negative removal rates resulting in concentrations of up to <330 ng/L.[164] This highlights that selected ARVs, or their metabolites, are persistent enough to by-pass most wastewater treatment processes.[47] The persistence of ARVs is further highlighted by the presence of 3TC (28 ng/L) in drinking water in the USA.[159]

The PIs were only investigated in water sources in Europe. Ritonavir was detected in 54% of the hospital effluent samples analysed, with a removal rate of 78% in a pilot-scale membrane bioreactors (MBR) installed and operated for one year at a Swiss hospital,[154] while in another study also conducted in Switzerland, RTV concentrations up to 110 ng/L

were measured in WWTP effluents. The removal efficiency when applying conventional WWTP treatment was <25%, while ozonation and powdered activated carbon on ultrafiltration membrane surfaces increased removal efficiency by 8% and 56% respectively.[155] Approximately 20% of another PI, IND, which is considered as a 'heavy' ARV and not recommended for initial therapy because of pill burden and the risk of nephrolithiasis,[167] is excreted unchanged in the urine.[168] In France, IND was detected in very low concentrations in WWTP effluents (1.5 ng/L).[151] The same study also reported the detection of RTV (53 -155 ng/L) and a very low quantity of another PI, SQV (0.2 ng/L), from WWTP effluents.[148] Darunavir is the most recent protease PI used as a component of HAART in combination with the pharmacokinetic booster RTV.[169] It is 94% excreted via urine and it was also detected in drinking water in Poland.[161] However, there is not enough data available to interpret its presence in drinking water or WWTPs. Atazanavir was also detected during non-target screenings of WWTPs in Norway[150] and Athens, Greece.[158] It has been suggested that the hydraulic residence time for ATV, which is usually only a few hours for wastewaters in the activated sludge system, accounts for the accumulation of ATV in the effluent of decentralised wastewater treatment systems (DEWATS) and other WWTPs.[165] However, the degradation kinetics and breakdown products of ATV should be explored to understand its fate and removal in WWTPs.[165]

The current literature also shows that ARVs have been detected from landfill leachates.[157] This problem usually arises from municipal solid waste disposal. Waste disposal is a global concern, especially in LMICs, and as urbanisation continues to advance, the management of solid waste becomes a public health and environmental concern in urban areas.[170] Landfills are commonly the final repository for heterogeneous mixtures of municipal solid and liquid waste composed of discarded materials from residential, commercial and industrial sources.[157] Studies characterising the composition of CECs in landfill leachate indicate that the landfills can be sources of CECs.[172-177] To provide the first national-scale assessment of CECs in landfill leachate across the USA, fresh leachate samples from 19 landfills in 16 states were collected and analysed for 202 CECs.[157] The analysed CECs included 100 prescription pharmaceuticals, 33 industrial chemicals, 30 household chemicals, 19 non-prescription pharmaceuticals, 16 steroid hormones, and 4 plant/animal sterols.[157] Together 129 of 202 CECs analysed were detected in one or more leachate samples collected in this study. Amongst other CECs detected were 62 prescription pharmaceuticals, of which the ARVs ABC (185 ng/L) and 3TC (355 ng/L), were detected.[157]

2.2.2 Antiretrovirals detected in water sources on the African continent, excluding SA.

From the literature survey, it was evident that there was a paucity of data on ARVs in water sources in Africa (excluding SA),[99,171] with only three publications (one published in 2012 and two in 2016), all reporting on ARVs in water sources in Kenya (Table 2.2).

Table 2.2: Antiretrovirals detected in water sources in African countries, excluding SA

Sample/Source	ARV detected quantity ng/L		ARV tested not detected	Country
Surface water	3TC NVP AZT	3 150‼ 33 440‼ 18 300‼	EFV‼	Kenya[56]
Surface water	3TC NVP AZT EFV	300 -161 000* 330 – 5 620 nd – 17 410 nd - 560		Kenya[172]
Groundwater	NVP AZT	20 – 1 600** 20 - 30	3TC EFV	
WWTP influents / effluents	3TC NVP AZT EFV	50 913 / 26 947*** 2 076 / 1 723 15 167 / 97 753 / 107		
Surface water	3TC NVP AZT	5 428 4 859 7684		Kenya[173]
WWTP effluents	3TC NVP AZT	3 985 1 357 513		

WWTP= Wastewater Treatment Plant,
3TC = lamivudine, NVP = nevirapine, AZT = zidovudine, EFV = Efavirenz.
‼ indicatively identified (results suggestive of presence)
* concentration range from 14 sampling points from three rivers
** concentration range between three shallow water wells
*** average concentrations from three separate WWTPS monitored

From Table 2.2 it is evident that in the studies in Kenya only selected RTIs were targeted for analysis. The selected ARVs were those in the first-line cART regimen used in Kenya[174] at the time the water samples were taken (2012-2014). From the data it is evident that the water sources in the Nairobi region are significantly contaminated with ARVs, which can be ascribed to the high HIV/AIDS prevalence and consequent consumption of ARVs (4.4 tons) in the area[56] In addition, the limited performance of available wastewater

treatment processes against pharmaceuticals, particularly ARVs, results in the release of contaminated effluents to surface water. The release of untreated or partially treated wastewater, as well as limited dilution of the effluents due to drought effects (low rainfall and high evapotranspiration), may be another contributing factor.[159] Comparing the data from Kenya to data from other continents (Table 2.1), the ARV levels in water sources are significantly higher.[47]

Of the NRTIs, 3TC was detected at high concentrations in all the water sources tested, but not at all in the groundwater. The detection of 3TC in surface water in 2012[56] was also the first report of this ARV being detected in surface water. A subsequence study reported 3TC concentrations ranging from 300 ng/L to 161 000 ng/L in surface river water[179] which is not surprising as the removal efficiency during wastewater treatment was low, ranging from 24-59%.[179] This differs from the elimination efficiency of 93% reported in Germany, where 3TC was not detected in wastewater effluent.[145] Zidovudine was the most frequently detected NRTI, but at concentrations lower than that recorded for 3TC, i.e. <LOD – 17 410 ng/L in river water. The concentration of AZT in wastewater effluents from the different studies was low (513 ng/L[164]; 97 ng/L[179]) as the removal efficacy from wastewater was shown to be 99%.[179]

When reviewing the occurrence and fate of NNRTIs in the Kenyan water sources, NVP was present in all the water sources tested, but at concentrations lower than reported for the NRTIs. The levels, however, were very similar to those reported elsewhere.[158,165] After ingestion, NVP is either excreted unchanged (2.7%) or metabolised into several hydroxylated metabolites, which may be further glucuronidated before excretion.[175] In one of the Kenyan studies, the removal efficacy of NVP in wastewater treatment was shown to be lower (11-49%).[179] This phenomenon is likely as a result of the deconjugation of the hydroxylated metabolites of NVP, its recalcitrance and the lack of binding of the NVP to the primary settling tank sludge. Efavirenz was only detected at very low concentrations in river water (not detected - 560 ng/L) and in the influent (460-1020 ng/L) and effluent (100-110 ng/L) of WWTPs.[179] This equates to a removal efficacy 83-92% by wastewater treatment processes,[179] which is important when one considers the potential neurologic and teratogenic side effects of EFV.[174]

2.3 POTENTIAL RISKS RELATING TO ANTIRETROVIRALS IN WATER SOURCES

Pharmaceutical compounds were not thought to pose a significant risk to human health through drinking water and from the consumption of fish,[176] however, a study conducted in Spain, which evaluated the potential toxicity of urban wastewater effluents contaminated with ECs found that PPCPs do contribute to water toxicity.[177] The major concern about the toxic implications of pharmaceuticals is that some of them were designed specifically to maximise their biological activity at low doses and to target certain metabolic, enzymatic, or cell-signalling mechanisms.[74] This mode of action concept can be applied to all aquatic biota, which is unintentionally exposed to pharmaceuticals in their natural environment, thus raising the risk of ecotoxicological effects.[178] Although PPCPs are detected in the freshwater environment at relatively low concentrations, many of them and their metabolites are biologically active and can impact non-target aquatic organisms.[74] Many of the pharmaceutical drug targets are evolutionally conserved between species.[115] This means that analogous proteins to those that human drugs target may also be present in other vertebrate and invertebrate animals and plants.[115] Moreover, while not all pharmaceuticals and their metabolites are persistent, their continuous use and release to the environment means many are considered "pseudo-persistent".[179] Pseudopersistent pharmaceuticals are suggested to have greater potential for environmental persistence than other organic contaminants because their source continually replenishes even when acted on by environmental processes such as biodegradation, photodegradation and particulate sorption. Hence, pharmaceuticals that may degrade would eventually and effectively behave as persistent compounds because of their constant release into the environment.[179]

Several studies have assessed health risks associated with exposure to pharmaceuticals through drinking water. Human health risk assessments of pharmaceuticals in drinking water have been conducted in the UK, Australia and the USA.[24] In the assessments conducted from the above-mentioned countries, the approaches of acceptable daily intake (ADI) or minimum therapeutic dose (MTD) were adopted as the point of departure (POD) in the studies to assess potential risks to human health through exposure to pharmaceuticals in drinking water. Margins of exposure (MOEs) were derived by comparing measured or modelled exposure levels in drinking water with a reference

exposure concentration, which was usually the ADI or MTD or sometimes a drinking-water equivalent level (DWEL). A judgement of safety was then based on the magnitude of this MOE for each pharmaceutical of interest. From the assessments conducted above in three countries, the results indicated that appreciable adverse health impacts to humans are very unlikely from exposure to the trace concentrations of pharmaceuticals that could potentially be found in drinking-water. Available data have shown that for those substances that have been detected, the concentrations are more than 1000 - fold less than the MTD, which is the lowest clinically active dosage.[24] These findings are in line with other studies over the past decade that also supported the conclusion that apparent risks to health arising from trace levels of pharmaceuticals in drinking water are extremely unlikely.[23,180-184] Given the low likelihood of human health risks, it is therefore not recommended to implement routine monitoring programmes that are resource intensive and detract from other drinking-water concerns that are more important and more acute like the threat of waterborne pathogens. However, where specific circumstances indicate a potential for elevated concentrations, screening values and targeted investigative monitoring could be considered. The latter is particularly true for a country like SA, where a great possibility of doubling the ARV usage through test and treat policy exists.[24]

Regarding the ecotoxicity of ARVs in non-human species, data is sparsely available.[47,175] According to available data for freshwaters, ABC has been found to be harmful to green algae with the half maximal effective concentration (EC50) value of 57 mg/L, which is of concern since green algae are the main primary producers in aquatic ecosystems.[185] In a Kenyan study where the environmental risk was evaluated by calculating the risk quotients (RQs) for algae, daphnia and fish, AZT and NVP were shown to have potential ecotoxicological effects on the acquatic organisms, with algae being the most affected.[173] Efavirenz has also been proven to be hazardous in the environment, as it is persistent and toxic to aquatic life. *Oreochromis mossambicus* fish exposed to EFV for 96 hours at a concentration of 20.6 ng/L triggered liver damage, as well as higher total fish deaths compared to the control sample.[186]

Although there are data on the potential side effects of therapeutic doses of ARVs in humans (Tables 1.1-1.5), there are no data on the effect of long-term or prolonged exposure to low doses of ARVs in water sources, either through ingestion or transdermally.[175] A serious concern relating to the presence of pharmaceuticals,

specifically ARVs and antibiotics, in water sources is the potential creation of resistant strains of the targeted microorganisms in the body through the unintentional exposure to these compounds in contaminated water sources.[47] Moreover, maximally-suppressive cART, which reduces the likelihood and effects of viral mutations, is the best tool to minimise the occurrence of resistance. However, suboptimal regimens, interrupted regimens and poor adherence to regimens are major factors determining the development of resistance, as the therapeutic concentrations in the body drops, but may still exert selective pressure.[33] It may therefore be possible that resistance can be promoted by low concentrations of ARVs in drinking water, by means of maintaining low concentrations of ARVs in HIV-positive treatment-naïve patients.[33] At present, it is not yet known if residue concentrations may result in the development of drug resistant HIV strains.[33] The possibility of resistance development from ARV residues in drinking water may be low or negligible (the dilution and attenuation from discharge to eventual uptake may be large enough) but urgent investigation is needed to disprove this possibility.[32]

2.4 DISCUSSION

There is paucity of data on ARVs in water sources worldwide, excluding Africa.[99] There are also limited studies on ARVs in African (excluding SA), but the reported studies revealed that African water sources are investigated more intensely, particularly targeting ARVs.[175] This highlights the greater awareness of the current HIV situation and the use of ARVs on the continent with the highest burden of HIV disease.[175] Not only was the detection frequency of ARVs higher in Kenyan water bodies, the concentrations were generally higher than those detected in Europe and North America, which could be ascribed to higher ARV usage. The presence of these compounds in the Kenyan water sources cannot be solely attributed to WWTP discharge but also to inadequate sanitation, the use of pitlatrines, open defecation practices as well as malfunctioning WWTPs, as untreated human waste is often discharged untreated into water systems.

The risks associated with ARVs in water sources may vary across regions and will be country dependent. Antiretrovirals in the aquatic environment exhibits discrepancies which makes it difficult to observe a global pattern.[175] This discrepancies are mainly due to differences in consumption and prescription rates, different treatment technologies and environmental and geographical conditions,[175] disease burden and country specific public

health priorities. To some countries, HIV is the biggest problem hence they may target ARVs, while in other countries influenza for example, could be the main battle hence they target antiviral Oseltamivir and its metabolite, OC. Considering the number of ARVs available globally, which differ according to region, there is a need for point specific prediction data on risk potential.[47]

Lastly, assessing the potential health risk to exposed individuals is fraught with uncertainties. Gauging the environmental risk from a pollutant, in this case ARVs, by making use of the RQ, which is the ratio of the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC), is problematic because calculating this ratio can be challenging due to lack of information regarding the effects of the particular pollutant and difficulties calculating the PNEC. Researchers who have previously used RQ to assess the low levels of pollutants on the health of the ecosystem found variable results.[32] Also, PEC and PNEC are point specific and depend on specific dosage, they also depend on prescription and consumption data as prescription data is often biased due to the assumption that all prescribed medicines were consumed.[47] What is also not addressed in the literature and what would be very difficult to evaluate is the potential exposure of the HIV-negative individuals, who may or may not have underlying conditions, e.g. kidney failure, to low levels of ARVs with nephrotoxicity or pregnant women to potentially teratogenic ARVs. In addition, potential drug interactions need to be considered in HIV-infected and uninfected individuals on medication for other conditions[187-188] who are exposed to low levels of ARVs through water sources, and these interactions maybe difficult to determine or quantify. Antiretroviral drugs are also known to affect the gut microbiome and the resultant intestinal dysbiosis affects the immune homeostasis of HIV-infected individuals,[189-190] an effect which may be difficult to determine or ascribe to expose to low levels or a mixture of ARVs in water sources.

2.5 CONCLUSION

Detection of ARVs in water sources is a global public health concern. The potential risks associated with human exposure to ARVs through drinking water is a concern, particularly in areas that practice indirect water reuse and where sewage effluents get released to surface waters that in turn are used as a source of drinking water or for irrigation purposes.[182] It is also important to realise that at any given point in time, the aquatic environment can be contaminated by multiple pharmaceuticals and ARVs from

different classes, which increases the overall threat via the cocktail effect of which the risk might be higher than anticipated.[191] Future studies should focus on individual drugs, synergistic and/or antagonistic effects as well as possible mixture effect over an extended period of time.[182]

CHAPTER 3

HISTORICAL, CURRENT AND FUTURE ANTIRETROVIRAL USE IN SOUTH AFRICA

3.1 INTRODUCTION

Human immunodeficiency virus was discovered in the year 1983.[192] To date, there is no cure for HIV/AIDS, however, ART is available for the treatment and management of people living with HIV (PLWH) using ARV drugs.[193] Antiretroviral therapy is a lifetime treatment.[64] South Africa is scheduled to achieve the global health community goal of ending the public health threats due to HIV/AIDS by 2030.[194-195] Addressing this global health catastrophe includes providing optimal prevention strategies and treatment regimens for individual persons living with or at risk of HIV.[194] The scale up of ART has been one of the major public health success stories within SA, with the greatest gains made in the world's worst affected regions of East and Southern Africa.[196] An estimated 23.3 of the 37.9 million people living with HIV globally were reported to be on ART by the end of June 2019, which is more than three times as many as in 2010.[197] The global ARV usage is expected to rise, particularly for those countries, like SA, who have adopted the WHO test and treat strategy. While SA is on track to meet its testing and treatment targets, the scale up of the treatment programme in the country and the concentration and possible accumulation of HIV ARVs in the environment could be creating new health challenges such as environmental contamination.[198] Therefore, there is a need to review literature on the country's HIV/AIDS situation as well as the ARV usage within the country to enable proper projections on the quantities of anti-HIV drugs that could potentially end up in the environment.

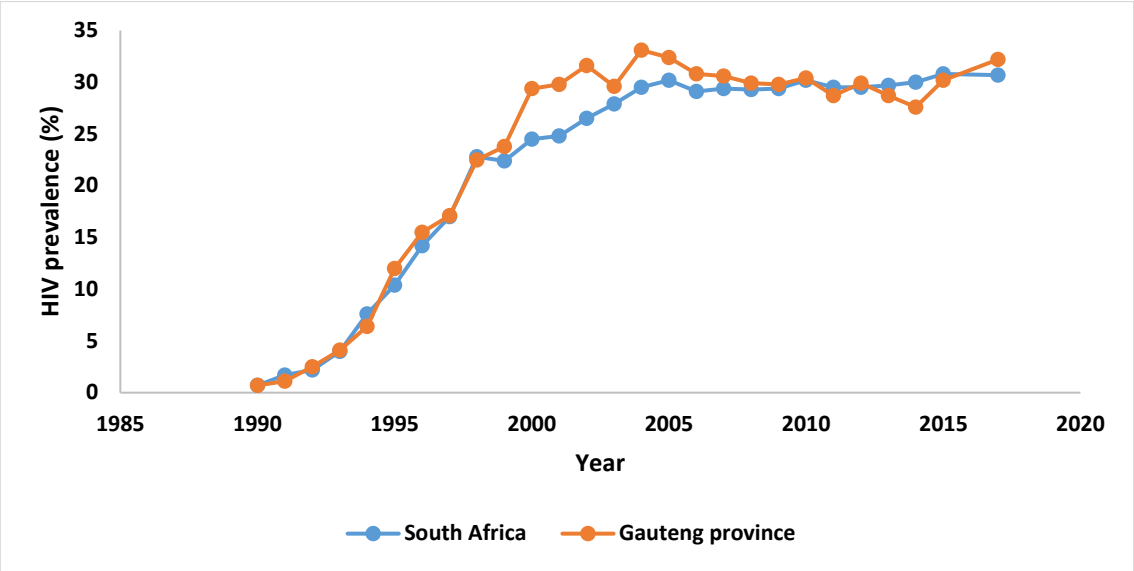
3.2 THE HIV EPIDEMIC IN SOUTH AFRICA

The first official case of AIDS in SA was reported in 1982 from a South African homosexual man who contracted the virus while in California, USA.[199] Later that year, 250 random blood samples were taken from homosexual men living in Johannesburg, SA of which a staggering 12.8% were infected with the virus.[199] This was followed by very

little attention being paid to the epidemic over the next decade.[200] Since the first cases of AIDS in 1982, the HIV epidemic in SA has evolved through four phases:

Phase 1: 1982 – 1987- an initial **concentrated epidemic** phase
Phase 2: 1988- 1994 - the initiation of the **generalised HIV epidemic**
Phase 3: 1995 – 2000 - the **rapid spread** of HIV
Phase 4: the post-2000 **AIDS mortality phase** where deaths due to AIDS became evident and increased rapidly.[201]

Although the first cases of HIV/AIDS were identified in men who have sex with men (MSM), and the HIV serotype B, a serotype dominant in the US and Western Europe continued to spread among MSM, HIV subtype C, the dominant subtype in Africa and Asia, started spreading in the general population in SA around the year 1988.[201] Until 1988, HIV in SA was largely restricted to the homosexual community and haemophiliacs who had received blood transfusions.[201-202] However, from that point onwards, heterosexual transmission became the dominant mode of HIV transmission. After an initially slow introduction of HIV into the heterosexual community, the numbers of individuals with HIV infection grew exponentially from 1990 to 1994. The HIV prevalence in pregnant women increased from 0.8% to 7.6% during this period.[203-204]. This contributed to a significant rise in the number of perinatal infections and in the subsequent five years, an even more rapid spread occurred throughout the country, particularly in certain areas of rural KwaZulu-Natal (KZN).[201] South Africa has been implementing the national antenatal sentinel HIV prevalence survey since 1990, with the 2017 antenatal sentinel survey being the 27th such survey conducted in SA.[205] Between 1990 and 2015, the survey primarily focused on estimating HIV prevalence trend over time, using anonymous unlinked testing of blood samples collected from pregnant women attending routine antenatal care (ANC) across the country. The survey has shown that HIV prevalence increased dramatically from 7.6% in 1994 to 24.5% in 2000 (Figure 3.1), with wide variations between provinces and local communities.[205] HIV prevalence in young pregnant women (20–24 years) in a rural KZN community increased from 21.1% in 1995, 39.3% in 1998 and 50.8% in 2001.[204] The national HIV seroprevalence in pregnant women increased from 24.8% in 2001 to a peak of 30.2% in 2005 and thereafter decreased to 29.1% in 2006.[205] (Figure 3.1).



Note: the prevalence reported in 2015 and 2017 is for both first and follow-up ante-natal clinic visit attendees.

Figure 3.1: The HIV epidemic curve among antenatal women, in South Africa and Gauteng province, 1990–2017.[205]

There is a noticeable upward trend in HIV prevalence amongst pregnant women in SA, particularly before the national rollout of ARVs in 2004.[205] A similar trend was observed for cases in Gauteng province (Figure 3.1). The 2019 HIV/AIDS and ART statistics for

7.5 million people living with HIV

19% adult HIV prevalence

200,000 new infections

72,000 AIDS related deaths

71% adults living with HIV on ARTs

(= 5 083 800 adults in SA)

(= 1 166 680 adults in Gauteng*)

47% children living with HIV on ARTs

(= 159 800 children in SA)

https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/south-africa

*https://www.spotlightnsp.co.za/2019/08/05/graphs-that-tell-the-story-of-hiv-in-south-africas-provinces/

Fact Box 1: The 2019 HIV/AIDS and ART statistics for SA

3.3 ANTIRETROVIRAL THERAPY IN SOUTH AFRICA

A literature review was conducted for the purpose of addressing the question: which ARVs were and are mostly used in SA? Like most countries in Sub-Saharan Africa, SA bases its health guidelines on WHO recommendations, which are based on the best available scientific evidence, especially the HIV and AIDS guidelines.[206] The first WHO HIV treatment guidelines were released in 2002, and updated in 2006, 2010 and 2013.[206]

a) Antiretroviral therapy in SA: period prior to 2004

Prior to 2004, when the first national ART programme was launched in SA, access to ARVs in SA was faced with many challenges.[207] In most LMICs, the private sector has a long history of ART provision through donor-funded projects.[208] Although there were no specified ART guidelines during this period, since 1999, SA used NVP in the PMTCT projects, which started in Western Cape. Thereafter, in 2001, the National Department of Health (NDoH) introduced two pilot sites in each province. To date, the roll out of PMTCT has expanded dramatically.[209] In addition to PMTCT projects, demonstration projects were also conducted in the Western Cape Province in 2001 and 2002, in Khayelitsha and Gugulethu respectively, wherein ART was provided to HIV infected individuals with advanced disease through government health services.[210-214] Care was first offered as part of the primary-care HIV intervention for children in 2002, with follow-up for children in this analysis extending to 3 years.[215] Children were defined as patients under the age of 14 years starting ART. Individuals were considered eligible for ART if they had a WHO stage IV illness or a CD4 count less than 200 cells/µl.[207] The adult regimens used throughout comprised of two NRTIs and one NNRTI. Initially, the NRTI backbone in Khayelitsha comprised of AZT and 3TC, but was later changed to d4T and 3TC in line with the national programme. Paediatric regimens varied, with NNRTIs and PIs being variously used with the NRTI backbone[215] (Table 3.1). In addition, Combivir, a combination of two NRTIs, AZT and 3TC, was introduced in SA in 2000 and was widely used. Each Combivir tablet contained 300 mg of AZT and 150 mg of lamivudine and was taken every twelve hours with or without food. It was the first fixed-dose combination (FDC) therapy made available for HIV infected individuals.[216]

Table 3.1: Recommended ARV regimens for adults and children, 2003 guidelines.[207]

	Adults	**Children**	
		6 months – 3 years	**>3 years old and >10 kg**
First-line: a	d4T+3TC+EFV	d4T+3TC+LPV/r	d4T+3TC+EFV
First-line: b	d4T+3TC+NVP		
Second-line	AZT+ddI+LPV/r	AZT+ddI+NVP	AZT+ddI+LPV/r

First-line **a** = first-line treatment option, all patients were commenced on d4T, 3TC and EFV; **b** = first-line treatment option where EFV is replaced by NVP. 3TC = lamivudine, d4T = stavudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, NVP = nevirapine, AZT = zidovudine, ddI = didanosine.

b) Antiretroviral therapy in SA: 2004 – 2009

On 1 April 2004, ARV distribution began at several service points across SA.[200] The first South African national ARV treatment guidelines were released in 2004, aligned with the 2003 operational plan for comprehensive HIV and AIDS care, management and treatment.[217] By September 2005, 17 months after rollout began, 85 000 people were enrolled on ART in the public health sector. By then, 199 public healthcare facilities (just over 5%) were providing ARVs for the treatment of HIV.[200] In 2006, the 2007-2011 National Strategic Plan (NSP) was signed off by government. The NSP ambitiously committed provision of ARVs to all eligible individuals. Tenofovir disoproxil fumarate was licensed in 2007, along with a FDC pill that combined TDF with 3TC (Truvada). By the end of 2007, an estimated 424 009 patients were receiving ARVs.[199-200]

c) Antiretroviral therapy in SA: 2010 – 2019

The NDoH revised the 2004 ARV guidelines in 2010, expanding treatment to all children under 1 year, all pregnant women regardless of CD4 count and all TB-HIV co-infected patients with a CD4 count less than 350 cells/µl, a CD4 count <200 cells/µl (for everyone else who is HIV positive). The ART regimens for both first- and second-line therapy were also changed to make treatment safer and more tolerable, i.e, d4T used in the first line treatment option was replaced with ABC and TDF in children and adults, respectively[218], although there were people still on d4T, regardless of this recommendation from NDoH (Tables 3.2a. & 3.2b). Another objective in the 2010 ART guidelines was the expansion of the use of FDCs, e.g. FTC and TDF (Truvada) and other co-packaged formulations.[218] In the following year, in a further boost for the treatment programme, the South African

National AIDS Council (SANAC) endorsed the National Health Council (NHC) policy to initiate treatment for all those who test positive with a CD4 count of 350 or less.[219]

Table 3.2a: Recommended ARV regimens for adults and children, 2010 ART guidelines.[218]

	Adults	**Children**	
		6 months – 3 years	**>3 years old and >10 kg**
First-line: i	TDF+3TC/FTC+EFV/NVP	d4T/ABC+3TC+ LPV/r	d4T/ABC+3TC+EFV
First-line: ii	d4T+3TC+EFV/NVP		
First-line: iii	AZT+3TC+EFV/NVP		
Second-line: i	TDF+3TC/FTC+LPV/r		
Second line: ii	AZT+3TC+LPV/r	AZT+ddI+NVP	AZT+ddI+LPV/r

First-line i = all new patients needing treatment; ii = currently on d4T regimen and no side effects; iii = contraindication to TDF. Second-line i = failing d4T or AZT-based first-line regimen; ii = failing TDF-based first-line regimen. TDF = tenofovir, d4T = stavudine, 3TC = lamivudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, NVP = nevirapine, AZT = zidovudine, ddI = didanosine, FTC = emtricitabine.

Table 3.2b: Recommended ARV regimens for pregnant women and infants, 2010 ART guidelines.[218]

	Pregnant women	**Infants**
First-line:	TDF+3TC/FTC+NVP	NVP
TDF contraindication	AZT+3TC+NVP	
Not eligible as yet (CD4 >350)	AZT from 14 weeks sdNVP+AZT in labour TDF+FTC sd stat after delivery	

sd = single dose, TDF = tenofovir, 3TC = lamivudine, NVP = nevirapine, AZT = zidovudine, FTC = emtricitabine.

The numbers enrolled for ART in SA kept rising with consecutive, revised guidelines and NSPs. The 2012 -2016 NSP was launched and it included the marginalised groups, which meant expanded inclusivity.[220] Late in 2012, the NDoH announced that Atripla, a FDC which contains a combination of three ARVs: EFV (600 mg), FTC (200 mg), and TDF (300 mg), would be used in the first-line treatment of HIV-positive patients from 1 April 2013.[221] With the introduction of Atripla, all new patients, pregnant women and breastfeeding mothers were offered this FDC, meaning that patients would have to take

one tablet once a day instead of three or more pills multiple times a day.[221] In the same year, 2013, WHO published its first consolidated ARV treatment guidelines and SA followed suite and published its first consolidated guidelines in 2015 (Table 3.3). Of importance was the phasing out of d4T and ddI regimens. Children on d4T- and ddI-based regimens were to be changed to ABC-based regimens. All adults and adolescents on d4T containing regimens were to be changed to TDF-based regimens so that no patients were on d4T. [217]

Table 3.3a: National consolidated guidelines the management of HIV in children, adolescents and adults (including pregnant and breastfeeding women), 2015.[222]

	Late adolescents (>15 years) & adults	**Infants, children & early adolescents**	**Adolescents (10-15 years)**
First-line: i	TDF + 3TC (or FTC) + EFV*	ABC + 3TC + LPV/r (>3 years & < 10 kg)	ABC + 3TC + EFV (<15 years or < 40 kg)
First-line: ii	TDF + FTC (or 3TC) + NVP (or LPV/r) (contraindication to EFV)	ABC + 3TC + EFV (3-10 years & > 10 kg)	TDF + 3TC/FTC + EFV (≥15 years or ≥ 40 kg)
First-line: iii	ABC + 3TC + EFV (or NVP) (contraindication to TDF)		
Second-line: i	AZT + 3TC + LPV/r (failed TDF-based regimen) AZT + TDF + 3TC + LPV/r (if HBV co-infected)	Under consultation (failed PI-based regimen)	AZT + 3TC + LPV/r
Second line: ii	- + 3TC (or FTC) + LPV/r (failed d4T or AZT-based regimen)	AZT + 3TC + LPV/r AZT + ABC + LPV/r (failed NNRTI-based regimen)	AZT + ABC + LPV/r

TDF = tenofovir, d4T = stavudine, 3TC = lamivudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, NVP = nevirapine, AZT = zidovudine, FTC = emtricitabine, ABC = abacavir, HBV = hepatitis B virus, * = offered as FDC, NNRTI = non-nucleoside reverse transcription inhibitor.

Table 3.3b: National consolidated guidelines for the prevention of mother-to-child transmission, 2015.[222]

	Pregnant & breastfeeding women	**Unbooked labour or delivery (with no ART)**	**Prophylaxis in infants**
First-line: i	TDF+3TC/FTC+EFV	sdNVP, sdTruvada + AZT	NVP , AZT
First-line: ii		sdNVP, sdTruvada (for caesarean section)	

sd = single dose. TDF = tenofovir, 3TC = lamivudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, NVP = nevirapine, NVP = nevirapine, AZT = zidovudine, FTC = emtricitabine, Truvada = TDF + FTC,

The 2013 WHO guidelines recommended treatment initiation for adults and adolescents > 10 years old whose CD4 count falls below 500 cells/μl and universal treatment for persons with active TB disease; HBV co-infection with severe chronic liver disease; pregnant and breastfeeding women with HIV and those who are HIV positive in a serodiscordant partnership.[223] The South African NDoH adopted the 2015 WHO HIV treatment guidelines.[224] The only WHO recommendation not adopted in the new South African ART guidelines was the recommendation to initiate serodiscordant couples regardless of CD4 count; which initiated a lot of discussion around the issue.[206] South Africa has made huge improvements in getting people to test for HIV in recent years. In addition to having the world's largest ART programme, SA has undergone further expansion with the implementation of 'test and treat' guidelines in 2016 [225] and is the first country in Sub-Saharan Africa to fully approve pre-exposure prophylaxis (PrEP), which is also being made available to people at high risk of infection.[226] South Africa is making good progress towards the UNAIDS 90-90-90 targets, particularly with regards to testing and viral suppression. However, progress towards the second UNAIDS target (90% of HIV-diagnosed individuals on ART) is generally poor (Figure 3.2).[227]

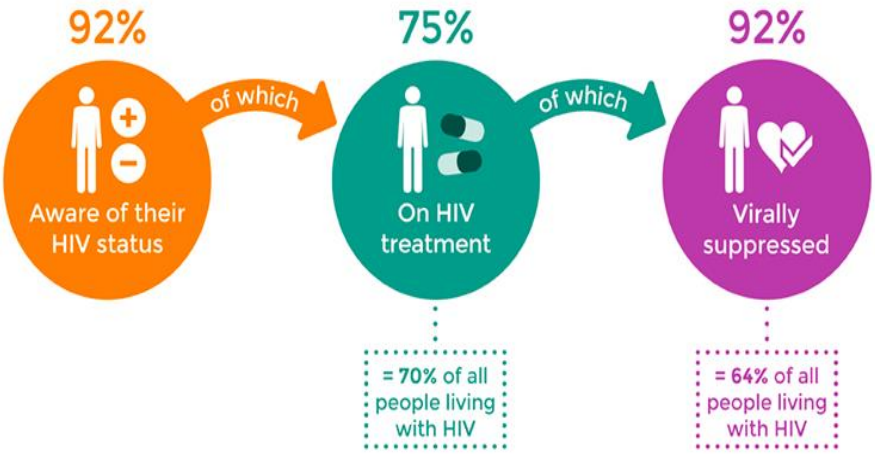


Figure 3.2: Progress towards 90-90-90 targets for South Africa (all ages).[225,227]

In 2019, 7 500 000 people were living with HIV, 92% (6 900 000) of people living with HIV were aware of their status, of which 75% (5 175 000) of those who knew their status were on treatment, were on treatment.[225,227] Of those diagnosed and on treatment, 92% (4 761 000) were virally suppressed. This on the other hand equates to 70% of all people living with HIV in SA on treatment and 64% virally suppressed (if one considers all people living with HIV as the denominator).[227]

d) Antiretroviral therapy in SA: 2020 onwards

In November 2019, DTG-based regimens were introduced to the South African ARV programme. The Minister of Health launched the FDC referred to as "TLD" (TDF 300 mg + 3TC 300 mg + DTG 50 mg). Currently, SA is using the 2019 HIV clinical guidelines that have been revised in 2020 to include a new formulation of the FDC for all eligible adults, adolescents and children over the age of 10 years and weighing 35 kg or more (Table 3.4).[228] From 2020 onwards, we can therefore expect DTG to start dominating amongst the most used ARVs in SA as the country has recently moved to DTG-based regimens for first-line treatment.

With the release of consecutive guidelines, the scale-up of ART in SA has continued as new ARVs have become available with improved efficacy, safety and robustness. With regards to HIV treatment, at any given time the majority of patients are on first-line treatment. From mid-2016, the number of people on ART increased to 3.4 million, with about145 000 of them on second-line treatment and >700 on third-line treatment.[229] The number of those on treatment has further increased to over 4.7 million in 2018.[227] A standard dose of typical ART medication contains active compounds in the range of 50-600 mg of the active compounds per patient per day (Table 3.5).[228]

Table 3.4: Regimens used up to 2019 and those currently in use in South Africa.

	Up to December 2019 regimens[222]	**Current Regimens**[228]
Adult first–line		
	TDF + FTC + EFV	TDF + 3TC +DTG
Contraindications to EFV	TDF +FTC+ NVP	N/A
Contraindications for TDF	AZT + 3TC + EFV ABC +3TC + EFV	ABC + 3TC +DTG AZT + 3TC +DTG
Contraindication to DTG	N/A	TDF + FTC +EFV
Adult second–line		
Failing on d4T or AZT with EFV regimen	TDF + FTC + LPV/r	TDF + 3TC +DTG
Failing on TDF/FTC with EFV regimen	AZT +3TC+ LPV/r	AZT + 3TC +TDG
Contraindication on LPV/r	Replace LPV/r with ATV/r	Replace LPV/r with ATV/r
Failing on d4T or AZT with DTG	N/A	AZT + 3TC +LPV/r
Adult third-line		
Only after drug resistance test and recommendation by the committee	Guided by drug resistance	Guided by drug resistance
Paediatric first-line		
Neonates : birth-<4 weeks (2.5-3kg)	AZT+ 3TC +NVP	AZT+3TC+NVP
Infants and children: (<3 years, <10 kg) (3-10 years ,>10 kg), (≥4 weeks 3-20 kg)	ABC +3TC +LPV/r ABC +3TC +EFV	ABC +3TC +LPV/r
Children (<10 years, 25-35 kg)	ABC +3TC +EFV	ABC +3TC +DTG
Adolescents (≥10 years, ≥35 kg)	TDF +FTC +EFV	TDF +3TC +DTG/EFV
Paediatric second-line		
Failing on EFV-based regimen <20 kg ≥20 kg	LPV with at least 1 active NRTI	LPV-based regimen DTG-based regimen
Failing on EFV-based regimen <20 kg ≥20 kg	Drug resistance test	Drug resistance test DTG-based regimen

TDF = tenofovir, FTC = emtricitabine, EFV = efavirenz, 3TC = lamivudine, DTG = dolutegravir, NVP = nevirapine, AZT = zidovudine, ABC = abacavir, LPV/r = lopinavir/ritonavir, d4T = stavudine, NRTI = nucleoside reverse transcription inhibitor.

Table 3.5: Annual consumption, per adult, of commonly used first line ARV drugs in South Africa

Drug class	Drug Name	Daily dosage per adult (mg)[a]	Annual consumption per person[b]	% of drug excreted[c]	Annual amount entering the environment[d]
NRTIs	d4T	60	**21 900 mg**	Not yet elucidated in humans	
	3TC	300	**109 500 mg**	#70% unchanged #5% metabolites	76 650 mg 5 475 mg
	AZT	600	**219 000 mg**	#29% unchanged #45% metabolites	63 510 mg 98 550mgg
	FTC	200	**73 000 mg**	#73% unchanged #13% metabolites * 14% metabolites	53 290 mg 9 490 mg 10 222 mg
	ABC	600	**219 000 mg**	#1.2% unchanged #81% metabolites *16% metabolites	2 628 mg 177 380 mg 35.040 mg
NtRTI	TDF	300	**109 500 mg**	#80% unchanged	87 600 mg
NNRTIs	NVP	200	**146 000 mg**	#<3% unchanged	<4 380 mg
	EFV	600	**219 000 mg**	*61% unchanged *33% unchanged, <1% metabolites	133 590 mg 72 270 mg <2 190 mg
INSTI	DTG	50	**18 250 mg**	#<1% unchanged #25% metabolites *53% unchanged	183 mg 4 563 mg 9 673 mg
PIs	LPV/r	800/200	**292 000/73 000 mg**	#2.2% unchanged #7.8% metabolites *19% unchanged *61% metabolites	6 424/1 606 mg 23 360/ 56 940 mg 55 480/13 870 mg 178 120/44 530 mg
	RTV	200	**73 000 mg**	#3.5% unchanged #7.5% metabolites *33% unchanged *53% metabolites	2 555 mg 5 475 mg 24 090 mg 38 690 mg
	ATV/r	300/100	**109 500 / 36 500 mg**	#7% unchanged #6% metabolites *20% unchanged *59% metabolites	7 665/2 555 mg 6 570/2 190 mg 21 900/7 300 mg 64 605/21 535 mg

NVP = Nevirapine, d4T = stavudine, 3TC = lamivudine, AZT = zidovudine, EFV = Efavirenz, FTC = emtricitabine, ABC = abacavir, TDF = tenofovir, DTG = dolutegravir, LPV = lopinavir, RTV = ritonavir, ATV = atazanavir, [a]National Deparment of Health. 2015. South African Antiretroviral Treatment guidelines, 2010, 2015, 2019 (Adults), [c] Proportion of drugs excreted, based on urine and faecal excretions, www.drugbank.ca/drugs/DB00339, [d]Amount entering the environment is % of annual consumed compound excreted by a single adult patient, # Urine excretion, *Faecal excretion. NRTI = nucleoside reverse transcriptase inhibitor, NtRTI = nucleotide reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor, INSTI = integrase strand transfer inhibitor , PI = protease inhibitor.

If the number of patients on ARV treatment is considered, it is reasonable to expect that, despite the loss of some analytes due to their metabolism and transformation, ARVs could potentially contribute significantly to the overall pharmaceutical load in the environment and water sources. In 2010, Schoeman and colleagues estimated that a daily dose of ARV combination therapy equated to a total of 542 944 kg of ARVs ingested per year for 1.5 million people on ARVs, with 162 833 kg reaching aquatic systems each year.[78] In 2019, 1 166 680 adults were on ART in Gauteng province.[230] Assuming that in 2019, all adults were on the first-line therapy of TDF+FTC+EFV (Table 3.4), a single adult would consume 0.402 kg ARVs annually, which would equate to a massive 469 005 kg of ARVs ingested annually in Gauteng province only. From Table 3.5 it is evident that a large percentage of ARVs are excreted in urine or faeces as unchanged drug or as a metabolites. An individual on first-line therapy would therefore excrete 0.235 kg unchanged ARVs or their metabolite in faeces or urine annually. If all the excreted ARVs passed through the formal sewage system, approximately 274 169 kg ARVs or their metabolites could reach the Gauteng waterways each year through surface runoff and wastewater treatment plant effluents if not removed by wastewater or water treatment processes.

3.4 DISCUSSION

To date, SA ranks amongst the worst afflicted countries by HIV/AIDS pandemic.[201] South Africa uses more ARVs per capita, compared to any other country fighting HIV/AIDS.[231] Treatment and guideline policies for HIV/AIDS have been driven by WHO recommendations, with SA having its first operational plan for HIV care in 2003 with updates as new recommendations from WHO became available, i.e. when the eligibility CD4 count threshold changed in 2010, 2013 and 2015. Also, the change from the 2003-2004 to the 2010 HIV treatment guidelines which included replacement of d4T with TDF and ABC for adults and children first-line treatment options, respectively. Most recently, SA amended its 2019 HIV treatment guidelines to include DTG-based regimens. Replacing EFV with DTG and the change from d4T to TDF have been the biggest shifts within the country's ART programme. This translates to the fact that the usage of the two drugs, d4T and EFV, will diminish. From 2020 going forward, the use of new drugs like DTG and other future drugs, may increase. It is important to note that although the number of people accessing ARVs from private health care is lower than those accessing public

health care, even before government dropped threshold for initiating ARVs, private health care users did not have to wait until they met eligibility criteria. Antiretroviral drugs that are reserved as second-line regimens in public sector are often used as first-line treatment option in private sector, where users may have access to tertiary care and alternative drugs with greater tolerability and fewer side effects.[208] However, from 2016, SA has embarked on the universal test and treat for all patients living with HIV, making everyone who is HIV positive eligible for treatment regardless of their CD4 count. This will in turn increase the number of patients on ARV treatment.

From the review it is evident that some ARVs, e.g. AZT, 3TC, NVP, d4T, EFV have been in use since the first guidelines were implemented in 2003 (Table 3.1), with the use of ABC, TDF, FTC and LPV/r increasing after the introduction of the ART 2010 guidelines.[218] This list includes some drugs that have been phased out, i.e. d4T, and some scheduled to be phased out, namely EFV and NVP. The fixed-dose combination TLD (TDF, 3TC and DTG) is set to replace what was previously used as fixed-dose combination TEE (TDF, FTC, EFV). Although NVP is to be phased out, it is still in use as side effects are already being reported for DTG, i.e. weight gain in women.[232] Not included in these lists are less commonly used ARVs, e.g. RAL, ATV/r, DRV/r which can be used as a third drug for occupational post-exposure prophylaxis[233] or ARVs used in salvage therapy or specialised treatment regimens.

Evidence is available in the literature indicating that after ingestion ARVs are only partially transformed and are excreted from the human body in urine and faeces in their original form and/or as metabolites (www.drugbank.ca/drugs/DB00339). As ARV treatment requires continual lifelong use, excreted metabolites and parent compounds can enter the environment continuously. The drugs that are recommended for use in the first-line treatment option can be expected to be in larger quantities in the environment compared to those reserved for second-line or third-line treatment options. This poses a potential public health concern because the public might be unintentionally exposed to low concentrations of ARVs or their metabolites through the use of polluted water sources.

3.5 CONCLUSION

While SA's ARV strategy is successfully dealing with the pandemic new problems, i.e. the accumulation of ARVs in the environment, have been identified which could pose health risk to human and aquatic life. It is clear that several hundred kilograms of ARVs could reach the aquatic systems of SA every year. As ART is a lifelong treatment, with more and more people becoming initiated on treatment, the load of ARV drugs entering the environment and waterways will increase.[33] The main issue of concern relates to the continual exposure of HIV-infected and -uninfected individuals to low concentrations of ARVs through the consumption of polluted treated and untreated drinking water. It is therefore important to determine which ARVs have been detected in South African water sources.

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CHAPTER 4

ANTIRETROVIRAL DRUGS DETECTED IN SOUTH AFRICAN WATER SOURCES

4.1 INTRODUCTION

Hundreds of tons of ARVs are annually dispensed and consumed in SA. Awareness about the detection of ARVs in aquatic environments has grown in recent years. Many studies have confirmed the presence of ARVs in various water sources at trace levels in the range of ng-µg/L.[234] Reviews to date indicate that more than 30 different ARVs have been widely detected in treated drinking waters worldwide.[234] The detection of these compounds in drinking water is largely due to their presence in source water and the inability of treatment processes to reduce pharmaceuticals totally or to below detection limits.[234] Antiretrovirals in water are increasingly reported in SA although available data is still limited. In general, research on the occurrence and fate of CECs in Africa is limited, however, there has been a significant increase in the number of publications from 2011 to date. From the studies conducted in Africa focusing on CECs in aquatic environments, 59% of such studies emerged from SA.[171] The aim of this systematic review was to identify and summarise the published studies on ARVs and quantities thereof that have been detected in water sources of SA.

4.2 METHODS

4.2.1 Search Strategy

A systematic review was conducted to address the question: which ARVs have been detected in the aquatic environments of SA, with special reference to Gauteng province? A literature search was conducted from MEDLINE/PubMed database and from Grey literature databases: University of Pretoria library: http://www.library.up.ac.za/. Literature identifying articles published between 1 July 2015 and 31 July 2020 were searched. Any combination of the following key words was used: (Antiretroviral OR Antiviral) AND (Water OR Aqueous OR wastewater) AND ("South Africa"). Citation searching or backward searching was also conducted, wherein reference lists of the identified relevant literature were hand-searched for any publications referenced that are eligible for inclusion.[235] Verbal leads on researcher names were also followed up.

4.2.2 Inclusion Criteria

Accessible full-text articles written in English, original studies and reviews were considered. An inclusion protocol was developed according to the reporting guidelines established by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) in 2009. [236]

4.2.3 Exclusion Criteria

Studies were excluded if they were: not written in English, duplicates, conducted in other areas besides SA or if the article indicated detection of other pharmaceuticals not ARVs. All papers identified by the search were initially screened for relevance using the title and abstract followed by the use of eligibility and inclusion criteria on the published studies or reports. Records considered eligible were therefore included in the qualitative synthesis if the records indicates that ARVs were detected in SA from the aqueous environment. Studies conducted outside SA or making references to other countries were excluded.

4.3 RESULTS AND DISCUSSION

The flow of articles through the review process is displayed in Figure 4.1. The literature search resulted in 192 articles of which 54 were excluded as they were identified to be duplicates. Therefore, 138 records were identified through database synthesis and screened on the basis of title and abstract. Of the 138, a further 104 were excluded as they were irrelevant with the full text records from 34 records were screened further. From these 34, a further 24 were excluded as they were either not conducted in SA (n=16), irrelevant (n=6) or the studies identified other antivirals other than ARVs (n=2). As a result, a final number of 10 original articles were found to be relevant and were included in the systematic review. There were no publications prior 2015. Relevant information such as author, publication date, sample/source, target ARV(s), amount detected in ng/L, year of publication were documented for each article.

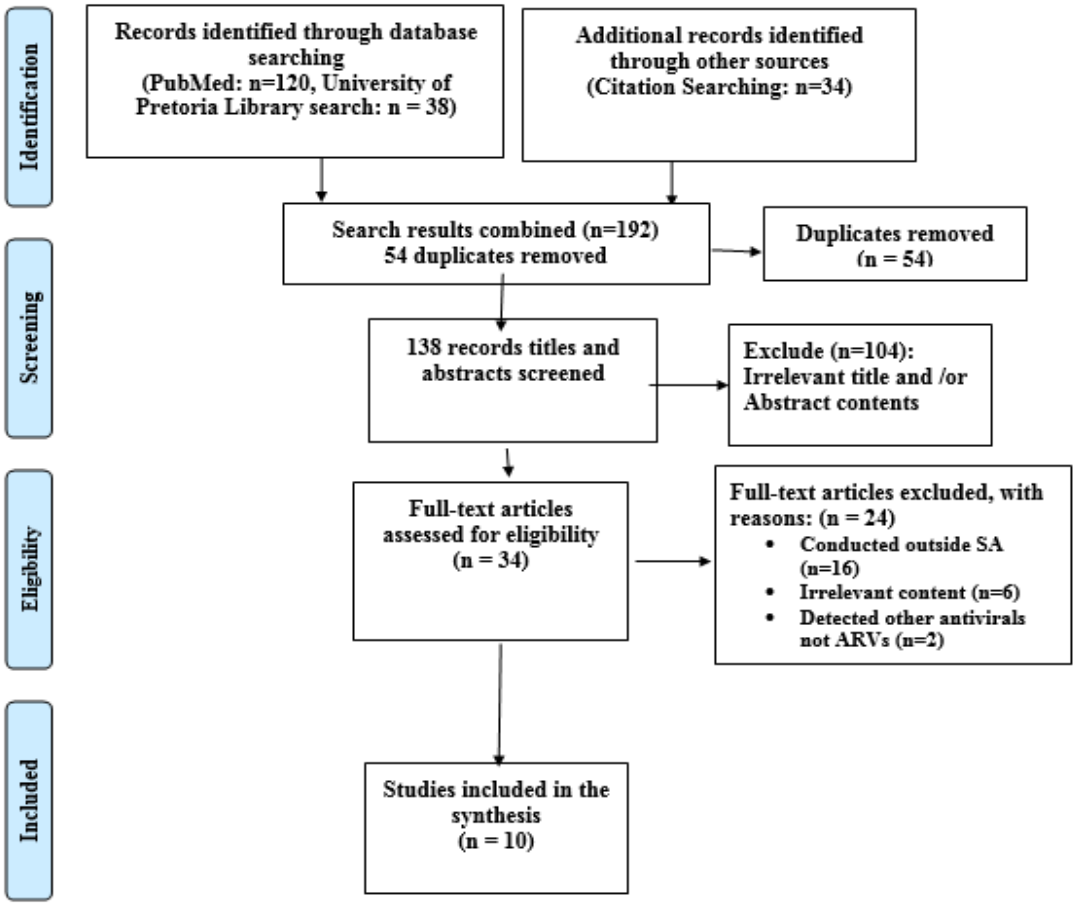

Figure 4.1: Flow diagram of the study and publication selection for the review process.[237]

The detection and analysis of ARVs in water sources is sparsely reported globally, including SA. The systematic review conducted resulted in ten studies on the occurrence of ARVs in water sources which were conducted in SA. Data indicating place where the water was sampled, source sampled, ARVs detected and quantities in ng/L, ARVs not detected though targeted, sample collection dates, author, and year of publication are presented in Table 4.1

From the results it is evident that the detection of ARVs from various water sources in SA is recent, as reported for the rest of the world. This indicates that the contamination of water sources with ARVs as a CEC is only recently getting attention. The earliest publications on ARVs in water sources across SA date from 2015, with the available dates of sample collection ranging from February 2011 to April 2018. The earliest record of ARVs in water in SA was the detection of AZT, NVP and LPV in surface water collected from the Hartbeespoort dam, North West province in February 2011 (Table 4.1a).[77]

Table 4.1: Antiretrovirals detected in South African water sources.

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
Surface water	TDF 3TC d4T AZT NVP	243 151-242 102-778 224-627 294-1480	ddC ddI ABC IDV RTV LPV EFV	Roodeplaat Dam System Gauteng	August 2013; July 2014	Wood et al, 2015
WWTP effluent	AZT	973	ddC TDF 3TC ddI d4T ABC NVP IDV RTV LPV EFV		July 2014	
Surface water	AZT	51.1	TDF 3TC ddI d4T ABC NVP IDV RTV LPV EFV	Vaal Dam Gauteng	February 2014	
Surface water	3TC AZT NVP	94.5-132 156-188 nq-177	ddC TDF ddI d4T ABC IDV RTV LPV EFV	Rietvlei Dam Gauteng	July 2014	
Surface Water	ddC TDF ddI	71.3 145-189 54.0	3TC d4T ABC AZT NVP IDV RTV LPV EFV	Orange River System Eastern/ Northern Cape	February 2014	
Surface Water	nd		ddC TDF 3TC ddI d4T ABC AZT NVP IDV RTV LPV EFV	Theewaters-kloof Dam Western Cape	February 2014	
Surface Water	nd			Inanda Dam KwaZulu Natal	February 2014	
Surface Water	nd			Renosterkop Dam Mpumalanga	February 2014	

Table 4.1*: continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
WWTP effluent	AZT LPV	452 130	ddC TDF 3TC ddI d4T ABC NVP IDV RTV EFV	East Rand Gauteng	February 2014	Wood et.al, 2015.[77]
Surface Water	NVP	143	ddC TDF 3TC ddI d4T ABC AZT IDV RTV LPV EFV	Limpopo	February 2014	
Surface Water	AZT NVP LPV	350 130 283	ddC TDF 3TC ddI d4T ABC IDV RTV EFV	Hartebees-poort Dam, North West	February 2011	
Surface Water	ddC AZT NVP LPV	54.1 139 137 305	TDF 3TC ddI d4T ABC IDV RTV LPV EFV		February 2014	
Tap Water	ddC AZT	8.4 72.7	TDF 3TC ddI d4T ABC NVP IDV RTV LPV EFV		February 2014	

WWTP = Wastewater Treatment Plant, 3TC = lamivudine, NVP = nevirapine, AZT = zidovudine, ABC = abacavir, d4T = stavudine, TDF = tenofovir, NFV = nelfinavir, LPV = lopinavir, ddI = didanosine, SQV= saquinavir.

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
WWTP effluent	AZT TDF	0.3 1.6	d4T 3TC ddI ABC EFV NFV LPV/r SQV NVP	Kutsong, Gauteng	January – March 2014	Swanepoel et al, 2015.[33]
WWTP effluent	NVP	0.4	d4T 3TC ddI ABC EFV NFV LPV/r SQV AZT TDF	Randfontein, Gauteng		
Surface water (River)	d4T NVP	1.1 4.0	3TC ddI ABC EFV NFV LPV/r SQV AZT TDF	South of Kutsong, Gauteng		
Surface water (River)	NVP AZT TDF EFV	0.5-6.8 0.3-0.9 0.6 0.8	d4T 3TC ddI ABC NFV LPV/r SQV	North West	January – March 2014	
Surface water (River)	NVP AZT	0.8 0.6	d4T 3TC ddI ABC NFV LPV/r SQV TDF EFV	KwaZulu-Natal	January – March 2014	
Surface water (River)	d4T ABC	1.1 1.6	NVP AZT 3TC ddI NFV LPV/r SQV TDF EFV	Eastern Cape	January – March 2014	

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
Surface water (River)	AZT ABC	0.9 1.2	d4T 3TC ddI EFV NFV LPV/r SQV NVP TDF	Northern Cape	January – March 2014	Swanepoel et al, 2015.[33]
Surface water (River)	3TC	0.9	d4T ddI ABC EFV NFV LPV/r SQV NVP AZT TDF	Gariep Dam, Free State		
Tap water (treated)	NVP AZT ABC ddI NFV	0.5-3.5 0.4-1.9 0.5 0.6 1.1	d4T 3TC EFV LPV/r SQV TDF	Gauteng		
Tap water (treated)	NVP ddI	0.3-1.0 0.4-3.3	d4T 3TC EFV ABC NFV LPV/r SQV TDF AZT	North West	January – March 2014	
Tap water (treated)	d4T	0.9	3TC NVP ddI ABC NFV LPV/r SQV TDF EFV AZT	Durban, KwaZulu-Natal	January – March 2014	

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
Groundwater	NVP	2.5	3TC d4T ddI ABC NFV LPV/r SQV TDF EFV AZT	Wildfontein, Gauteng	January – March 2014	Swanepoel et al, 2015.[33]
Groundwater	NVP d4T TDF NFV SQV	0.3-5.3 0.3-0.9 2.4 0.9 1.3	3TC ddI ABC LPV/r EFV AZT	North West	January – March 2014	
WWTP influent	NVP EFV	2100 17400		Gauteng	~ 2014	Schoeman et.al, 2015.[78]
WWTP effluent (treated)	NVP EFV	350 7100				

WWTP = Wastewater Treatment Plant, 3TC = lamivudine, NVP = nevirapine, AZT = zidovudine, ABC = abacavir, d4T = stavudine, TDF = tenofovir, NFV = nelfinavir, LPV = lopinavir, ddI = didanosine, SQV= saquinavir, ddC = zalcitabine, IND = indinavir, < LOD = Concentrations ranged from < limit of detection to given value.

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
Surface water (dam)	ABC EFV FTC 3TC LPV NVP RTV	<LOQ 174 361 21 204 379 489	ddI IND d4T TDF AZT	Roodeplaat dam system, Gauteng	2013 - 2016	Wood et.al, 2017.
WWTP influent	NVP EFV	50-190 5500-14000		Southern Gauteng	May 2016 to June 2016	Schoeman et.al, 2017.[238]
WWTP effluent	NVP EFV	90-473 4000				
Wastewater sludge	NVP EFV	<3.4 17.7-43.6 (mg/kg)				
Surface water	NVP EFV	<44.4 -227 nd-148		Rietvlei Dam system Gauteng	February 2015 & March 2016	Wooding et.al, 2017.[239]
	NVP	<44.4	EFV	Albisini Dam Limpopo	August 2015	
Surface water	NVP EFV	6-71 2-303	3TC FTC TDF	Hartbeespoort Dam North West	November 2014 to September 2015	Rimayi et al, 2018.[240]
Groundwater	NVP EFV	8-13 2-5	3TC FTC TDF			
Surface water	NVP EFV FTC	nd-57 134-303 nd-13	3TC TDF	Jukskei River Gauteng	May 2015	
	NVP EFV 3TC FTC TDF	nd-81 nd-138 <0.15-0.6 <0.13-8.0 nd-0.3		uMngeni River estuary KwaZulu Natal	May 2016	

WWTP = Wastewater Treatment Plant, NVP = Nevirapine, EFV = Efavirenz, 3TC = lamivudine, FTC = emtricitabine, TDF = tenofovir, < LOQ = Concentrations ranged from < limit of quantification to a given value, nd = not detected

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
DEWATS influent	MVC AZT NVP RAL DRV ATV IND RTV LPV 3TC EFV	83 53000 2100 17000 43000 64 260 3200 2500 2200 34000	ABC SQV	eThekwini KwaZulu-Natal	August 2016	Abafe et.al, 2018.[165]
DEWATS effluent	AZT NVP RAL DRV ATV IND RTV LPV 3TC EFV	500 1900 3500 17000 78 25 1500 3800 130 34000	ABC MVC SQV			
WWTP influent	ABC MVC AZT NVP RAL DRV SQV ATV IND RTV LPV 3TC EFV	3500-14000 82-320 6900-11000 670-2800 61-810 69-920 <LOD-180 210-1400 300-590 1600 1200-1300 840-1900 24000-34000				
WWTP effluent	MVC AZT NVP RAL DRV ATV IND RTV LPV EFV	<LOD-39 87-430 540-1400 <LOD-86 130-150 300-740 40-42 460-910 1900-3800 20000-33000	ABC SQV 3TC			

WWTP = Wastewater Treatment Plant, ABC = abacavir, MVC = maraviroc, AZT = zidovudine, NVP = nevirapine, NVPM = 12-hydroxy-nevirapine, RAL = raltegravir, DRV = darunavir, SQV= saquinavir, ATV = atazanavir, IND = indinavir, RTV = ritonavir, LPV = lopinavir, 3TC = lamivudine, EFV = efavirenz, FTC = emtricitabine, FTC-CBX = emtricitabine carboxylate, AZTG = zidovudine glucoronide, EFVM = 8,14-dihydroxy-efavirenz, < LOD = Concentrations ranged from < limit of detection to given value.

Table 4.1: *continued*

Sample/ Source	ARV detected quantity ng/L		ARV tested not detected	Region(s) within SA	Sample collection dates	Authors and Publication Year
WWTP influent	3TC FTC NVPM NVP EFV EFVM	4-21 31-172 <LOD-1 <LOD-1 1-15 1.5-12.4	AZTG AZT RTV	Western Cape	April and July 2016, September 2016, April 2018	Mosekieman g et.al, 2019.[166]
WWTP effluent	3TC FTC NVPM NVP EFV EFVM	<LOD 5 <LOD <LOD-1 2-4 <LOD	AZTG AZT RTV			
Surface water			AZTG AZT 3TC FTC NVPM NVP EFV EFVM RTV (<LOD)			
WWTP influent	EFV 3TC NVP RTV	50.9-2169 <ILOD-1001 <ILOQ-26.3 4.08-393.9	ATV	Daspoort, Pretoria, Gauteng	December 2016 to March 2018	Mhuka et al 2020.[241]
WWTP effluent	ATV EFV 3TC NVP RTV	<ILOD-308.2 210-2042 <ILOD-323.4 <ILOQ-80.5 14.4-675.9				
Surface water (upstream WWTP)	EFV 3TC NVP RTV	116.7-345.3 <ILOD-8.9 <ILOQ-7.3 <ILOD-58.8		Apies River, Pretoria		
Surface water (downstream WWTP)	EFV 3TC NVP RTV	170.9-514.6 <ILOD-10.3 <LOQ-10.9 5.0-52.6				

WWTP = Wastewater Treatment Plant, MVC = maraviroc, AZT = zidovudine, NVP = nevirapine, NVPM = 12-hydroxy-nevirapine, RAL = raltegravir, DRV = darunavir, SQV= saquinavir, ATV = atazanavir, IND = indinavir, RTV = ritonavir, LPV = lopinavir, 3TC = lamivudine, EFV = efavirenz, FTC = emtricitabine, FTC-CBX = emtricitabine carboxylate, AZTG = zidovudine glucoronide, EFVM = 8,14-dihydroxy-efavirenz, <LOD = concentrations ranged from < limit of detection to given value. < LOQ = concentrations ranged from < instrument limit of quantification to given value, < ILOD = concentrations ranged from < instrument limit of detection to given value.

It is important to note that most of the data presented in these studies from across SA represent a snap shot of results from single grab samples taken at selected sites at specific time points. There are no longitudinal studies where ARVs in the water from the same site were analysed at daily, monthly or yearly time points. Data from selected studies (Table 4.1c) indicates that only two authors attempted to address seasonality as the sampling was conducted for all four seasons between November 2014 and September 2015.[235] However, sampling was only conducted once and no follow up was done.[240] In the other study, a once-off follow-up sampling was conducted in the same season of the following year.[239] The reported concentrations in Tables 4.1a-e may therefore not be a true reflection of the extent and severity of ARV pollution in SA water sources on a day-to-day basis.

The concentrations of ARVs in water sources across SA, are higher than those detected in HICs (Table 2.1 vs Tables 4.1a-e).[77] The presence of ARVs in water is not that common in other studies conducted outside Africa and this is mainly attributed to the high HIV burden experienced in a number of African countries, including SA.[77] Concentrations do not only vary between LMICs and HICs, the detection of pharmaceuticals in the environment has been reported to vary even within countries due to factors such as population demographics, pharmaceutical usage statistics, and sewage treatment methods per region or province.[74] From the results presented in Tables 4.1 a-e, the ARVs concentrations detected match the HIV prevalence within the country. The HIV prevalence recorded from the 2017 ante-natal sentinel HIV survey in SA varies by province, with Gauteng coming fifth after KZN (40%), Mpumalanga (37%), Eastern Cape (34%) and Free State (33%), while the lowest HIV prevalence was in Western Cape (16%).[205] These would mean that if the 90-90-90 targets were met, in terms of environmental HIV drug concentrations much larger loads of ARVs in the environment would be expected in those areas with high HIV prevalence, with the highest concentrations expected from KZN and the lowest expected from the Western Cape. Although concentrations in surface water were investigated in all provinces, most work has been conducted in Gauteng, North West and KZN provinces, with the highest concentrations recorded in Gauteng, i.e. NVP (1480 ng/L), d4T (778 ng/L), AZT (627 ng/L), RTV (489 ng/L), FTC (361 ng/L), EFV (345 ng/L), TDF (243 ng/L) and 3TC (242 ng/L). (Table 4.1) Within Gauteng, interestingly, the highest concentrations were detected in the Pretoria area, along the Roodeplaat dam system, the Apies and Pienaars rivers,

[77,241-242] compared to concentration detected along the Jukskei River where the maximum of EFV (303 ng/L), NVP (57 ng/L) and FTC (13 ng/L) were detected. Although ddC is not in use, as it was phased out over a decade ago in SA, this drug has been detected in the Orange river (54.1 ng/L) and from the North West, Hartebeespoort dam (71.3 ng/L) from the water samples collected in February 2014.[77] The highest concentrations of LPV were also obtained from the Hartebeespoort dam at 305 ng/L.[77] Studies on surface waters in the Western Cape however indicated that no ARVs have been detected to date.[77,166]

The majority of the studies conducted in SA on ARVs detected in water sources have been conducted in urban areas. Such studies are equally important in rural areas as some of these areas are recognised as water scarce regions wherein communities resort to the use of groundwater and surface water for drinking and/or irrigation purposes.[175] Results from the present study shows that groundwater analysis has only been conducted in Gauteng and North West. From Gauteng, NVP (2.5 ng/L) was detected while a range of ARVs were obtained from in NW: d4T (0.9 ng/L), TDF (2.4 ng/L), NFV (0.9ng/L), SQV (1.3 ng/L) and EFV (5 ng/L). Since ARVs were reported to be present in the groundwater, it can be expected that drinking water derived from groundwater sources may be contaminated too.[243] Analysis from drinking tap water shows that the maximum value for NVP (3.5 ng/L) was detected in tap water from Gauteng[33] and with higher concentrations of AZT (72.2 ng/L) recorded for tap water from a source in the North West.[77] Stavudine was detected in a tap water sampled between January and March 2014 in Durban at 0.9 ng/L, although in the 2010 ARV treatment guidelines, SA recommended that the country take steps to progressively reduce the use of d4T because of its well-recognized toxicity.[218,244]

In addition, although the general expectation is that the wastewater treatment processes reduce or eliminate contaminants before discharge of the effluent waters into the environment, shortcomings of the WWTPs regarding the removal of ECs and CECs has been highlighted.[245] Monitoring WWTPs to assess their potential impact when their effluents are released into the environment is crucial.[241] In a number of studies ARVs have been detected in WWTPs effluents (Tables 4.1a-e) throughout SA. However, the presence of ARVs in water sources across the country, as it is in the rest of the Sub-Saharan Africa, cannot be solely attributed to discharge from WWTPs, rather, additional considerations must be made that may not be relevant to similar European studies.[77] These

includes, inadequate sanitation in certain parts of the country, the use of pit latrines and malfunctioning WWTPs which all translate to exposing the environment to untreated human waste.[77] Archer and co-workers cited sampling protocols and possible illegal waste dumping as potential reasons for the higher concentrations observed in the river samples downstream of the WWTP effluent discharge point in Gauteng province.[245] Although some researchers showed higher concentrations of pharmaceuticals in rivers and dams adjacent to WWTPs,[240,242] in some studies concentrations of ARVs upstream to the WWTP had higher concentrations than the samples taken downstream of the WWTP.[241]

The occurrence of ARVs in WWTPs also depends on multiple factors including medical needs in the area, prescription practices, operation of WWTPs and the timing of sampling.[175] Where data is available on WWTPs influent and effluent measurements, the highest quantities for NVP, EFV and 3TC measured from WWTPs influents were detected in KZN (2800 ng/L, 34000 ng/L and 1900 ng/L) followed by Gauteng (2100 ng/L, 17400 ng/L and 1001ng/L) and the lowest measurements detected in Western Cape (1 ng/L, 15 ng/L and 21 ng/L). The highest WWTPs effluents were measured from KZN, NVP at 1400 ng/L, EFV at 33000 ng/L and RTV at 910 ng/L while the highest measured AZT from WWTPs effluent was measured in Gauteng at 973 ng/L.[77,165] The poor removal of NVP in WWTPs activated sludge has been reported in early German studies and it was attributed to its photostability and poor biodegradability.[149] Similar findings were reported in the Western Cape in SA, where low concentrations for both the parent drug and 12-OH-NVP were reported, and the possible occurrence of additional metabolites in their analysed samples were implied.[166] The removal efficiencies for WWTPs has been grouped into negative removal, where a compound showed increased concentrations from influent to effluent, low to moderate removal and high removal efficiency.[241] Nevirapine was reported to exhibit negative removal efficiency where the reported concentrations increased from influents of a maximum of 26.34 ng/L to a maximum effluent concentration of 80.53 ng/L.[241] Lamivudine was also detected in German wastewaters at a concentration up to 720 ng/L, but an almost complete removal during wastewater treatment was reported.[149] Similar removal efficiencies were reported in SA.[165-166] The inefficiency of chlorination in the removal of ARVs from WWTPs, as well the observed increases in concentration in effluent streams in some cases have been reported in SA before, particularly for NVP and EFV.[156,238] On the other hand, although ABC was

detected in WWTP influents in KZN, this compound was not detected in the WWTPs effluents.[165] Similar findings were recorded in the early German studies, where complete removal efficiencies (>99%) of ABC from WWTPs were recorded.[149] In addition, although it was targeted, ABC was not detected from groundwater and WWTP effluents across SA (Table 4.1). This could be linked to high removal efficiencies of these ARVs by WWTPs as reported in a study conducted in Europe.[149] Similarly, SQV was not detected from surface water, WWTP effluents and potable tap water (Table 4.1). Although data about its presence in WWTPs is scarce, this could be mainly due to its limited use within the country,[217-218,228] which may possibly lead to lower environmental concentrations that could be below detection limits.[245] Its half-life has also been reported to be less than five days under biochemical conditions in surface water and wastewater.[151] Various wastewater treatment methods were compared for the removal of ARVs it was observed that biological treatment was more effective compared to MBR treatment and advanced stage treatment by ultra-violet irradiation appears to be more effective compared to chlorination.[166] As the country envisions the use of DEWATS for high density areas located far from the traditional, centralised WWTPs, data on the removal efficiencies from DEWATS is crucial.[246] In the first published study that investigated the performance of DEWATS in SA, researchers found the effluent concentrations from DEWATS and conventional WWTPs to be similar which allows for new insights for planning waste water treatment and potential water re-use application in areas with limited access to conventional sewer systems.[165]

The present study shows that all ARVs that have been used historically in the first-line (d4T, 3TC, EFV, NVP) and in second-line (ddI, LPV/r, AZT) ART guidelines have been detected to date. Finally, ARVs such as DTG have been included in the 2020 ART first-line treatment option and these would need attention in future studies.

4.4 CONCLUSION

The presence of ARVs in water sources raises concerns not only to aquatic life and wild life, but also for humans who may unintentionally consume ARV-contaminated water through treated and potable tap water or in rural areas polluted surface or groundwater. The potential of transdermal exposure during domestic or recreational use of surface

water is unknown. Data is lacking on the possible effects of prolonged human exposure to low concentrations of ARVs as they are considered pseudo-persistent contaminants. In addition the lack of routine monitoring programmes for ARVs in water sources, the analytical difficulties in quantifying ARVs in water sources and the absence of defined regulatory limits are major limitations. The problem is further compounded by the scarcity of water and lack of proper sanitation, especially in some drought stricken regions. The situation is dire especially for communities that use untreated contaminated water sources for domestic and irrigation purposes. Additionally, to get a much clearer picture of the true burden of this problem there is a need to put emphasis on the continual development of new analytical methods capable of simultaneous detection of all or most therapeutic classes of ARVs, while also taking cognisance of continually changing ART guidelines. Now that there is clear evidence that ARVs are detected in water sources, including drinking water, it is important to assess the potential risks associated with such exposure on human health.

CHAPTER 5

ASSESSMENT OF THE POTENTIAL RISKS POSED BY ANTIRETROVIRAL DRUGS IN SOUTH AFRICAN WATER SOURCES

5.1 INTRODUCTION

Antiretrovirals have been detected in surface waters, groundwater and drinking water at low levels (ng-µg/L) and this has raised concerns on whether these levels may have a detrimental effect on human health and aquatic species.[247-248] Because of conservative shared drug targets in some physiological processes, many aquatic species have similar targets to those found in humans.[249-250] The question then arises as to whether or not long term exposure to low levels of ARVs in drinking water can elicit the same or similar side effects as those experienced by patients on long term ARV therapy, e.g. ocular disease in patients on cART,[251] mitochondrial toxicity in the new born due to maternal usage of AZT[247] and weight gain and obesity due to INSTIs.[252] There is still no clear evidence of the effect of trace amounts of ARVs through drinking water on human health. The absence of evidence however, does not mean that humans are safe from any adverse effects of repeated exposure to drug waste in water.[198] With regard to aquatic species, fish exposed to NVP in the aquatic environment developed slightly reduced growth rate was noted between 30 and 60 days of exposure.[253]

There is a paucity of data on the potential human health risks associated with exposure to ARVs through water sources worldwide and there are no reported studies on the risks posed by ARVs in drinking water in SA. The aim of this section of the study was to determine the potential health risks posed by ARVs in drinking water from various sources to vulnerable individuals and communities of SA, namely, PLWH on cART, PLWH not yet on treatment, HIV uninfected individuals with co-morbidities and HIV uninfected healthy adults. This study focussed on the adult population of SA, with special reference to Gauteng province. A human ARV exposure pathway diagram (Figure 5.1) was proposed to address the potential development of HIV drug resistant strains or other possible toxic effects.[254]

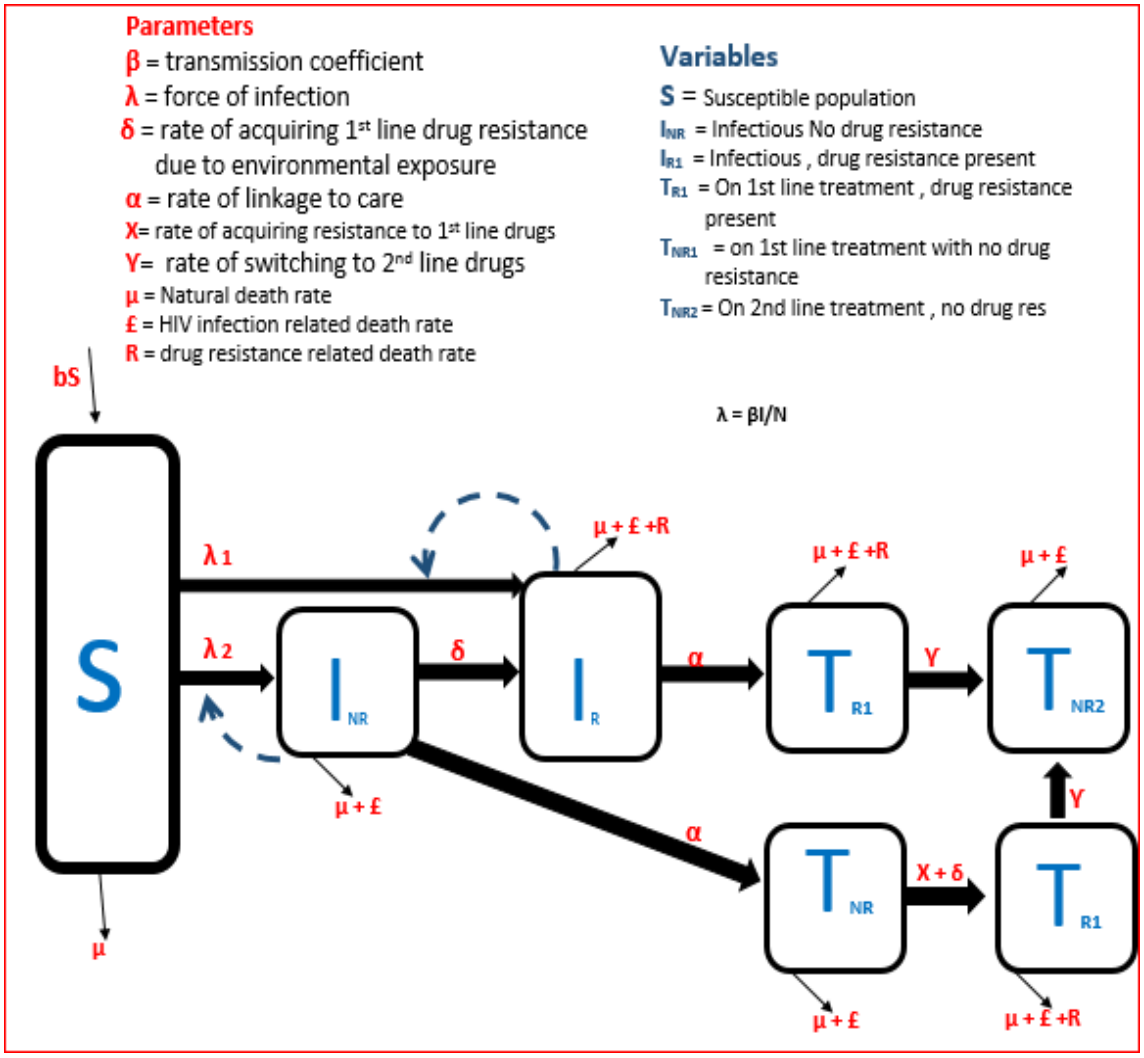

Figure 5.1: ARV exposure pathway using a model diagram. The exposure pathway diagram showing compartments, parameters and variables.

This diagram is a graphical depiction of how HIV positive people may be affected (should the detected drinking water values have any effect) by ingesting water contaminated with ARVs. From this diagram, susceptible individuals enter the population through birth (bS), at an annual constant rate as a function of how many uninfected (susceptible) individuals in the population (bS) at a given time. Individuals can become infected and then move from susceptible (S) into Infectious compartment as they experience a force of infection (λ) proportional to the prevalence of infectious individuals in the population. Once infected, individuals can develop resistance to first-line HIV treatment drugs without any drug resistant strain(s). Those that develop resistance to first-line drugs can move to second-line treatment drugs compartment whereas those that initially did not develop any drug resistance, may develop it later on due to other causes, i.e. non-adherence to treatment, drug interactions etc. This group will also move to second-line drug compartment as they develop resistance to first-line treatment. There is also a small proportion of susceptible individuals who will get infected with a resistance strain, who are therefore, also moved to second-line drug treatment compartment for better management of HIV. Similarly, individuals may be changed from first-line cART to other regimens due to effects other than drug resistance. The equation $\lambda = \beta I/N$ represents the values for λ_1 and λ_2 in the diagram, which is the product of transmission coefficient and infection rate divide by the population size.

5.2 MATERIALS AND METHODS

5.2.1 Overview of human risk assessment process

The evaluation of the potential risks of ARVs in water sources presented in this study comprised of five general steps: a) selection of ARVs to be assessed; b) derivation of ADIs; c) derivation of PNECs; d) Exposure assessment - determination of environmental concentrations; and e) risk calculation. The methods of risk assessment used in this analysis were based on the method described by Schwab et al. (2005)[247] for other ECs.

5.2.1.1 Selection of ARVs to be evaluated

Antiretrovirals were selected from the list of those detected in water sources across SA, which were used in the first-line treatment option and for which PNECs were available at the time of writing the report.

5.2.1.2 Derivation of ADIs

The ADI is an estimated daily chronic dose of a pharmaceutical that may not result in an adverse health effect in a population, including sensitive sub-populations.[247] To derive ADIs, POD estimate is needed, which is defined as, either the lowest daily therapeutic dose or the no observed effect level (NOEL) or the lowest observed effect level (LOEL), obtained from pre-clinical toxicology studies. The ADIs for each of the three ARVs selected for the human health risk assessment were therefore determined by dividing the POD by uncertainty factors (UFs) using methods established by the USEPA.[247,255] Uncertainty is due to the limited knowledge or lack of precise knowledge, either qualitative or quantitative about the factors affecting exposure and adequacy of model outputs for decision making.[256] Sources of uncertainty in modelled estimates (ADI and POD), were identified and appropriate UFs were selected based on five extrapolation uncertainties: LOEL to NOEL (UF1), duration of exposure (UF2), interspecies variability (UF3), intra-individual susceptibility (UF4) and data quality (UF5). Extrapolation uncertainties and considerations for selection of uncertainty factors are listed in Table 5.1.[247] This approach of incorporating UFs was applied in the equation to calculate ADIs to reduce the PODs to a dose where there was no doubt that no effect occurred (therapeutic or adverse effects). Incorporating UFs also enabled the integration of protection of sensitive individuals and sub-populations.[247] The ADIs for each ARV was estimated using the equation:

$$ADI = \frac{1000 \times POD}{UF1 \times UF2 \times UF3 \times UF4 \times UF5}$$

Where ADI is measured in µg/kg/day and POD is measured in mg/kg/day.

Table 5.1: Extrapolation uncertainties and considerations for selection of uncertainty factors as specified by Schwab et al. (2005).[247]

Extrapolation uncertainties	Considerations for uncertainty factor selection
UF1	10 recommended when NOAEL is not available. 3 recommended when LOAEL is a therapeutic response, operative only on disease state. 1 recommended when the LOEL is associated with a homeostatic response or an equivocal effect (i.e. the LOEL is a NOAEL).
UF2	10 recommended when no relevant chronic data available. 3 recommended when no chronic data are available but pharmacokinetic or pharmacodynamics analyses suggest little persistence of compound or effect. 1 recommended when no chronic data are available but pharmacokinetic or pharmacodynamics analyses suggest little persistence of compound and effect. 1 recommended when adequate chronic data are available.
UF3	10 recommended when no human data are available unless considerations below apply. 3 recommended when absorption, distribution, metabolism and excretion (ADME) data are similar for multiple species, including humans or non-human primates. 1 used when derivation is based on human data.
UF4	10 recommended if NOAEL is from a general adult population and/or animal study, with no multigenerational study of toxicity. 3 recommended when effect is therapeutic and there is little difference between the median and minimally effective dose. 3 recommended when using an adjusted LOEL, NOEL or therapeutic dose specific to a sensitive sub-population. 1recommended when sufficient post-marketing data indicate the absence of specific and particularly sensitive individuals or when using a LOEL or NOEL for a specifically identified sensitive human population based on a large post marketing study.
UF5	10, 3, 1 or a number smaller than 1 are recommended for a professional judgement on the quality of data available on compound

UF = uncertainty factor, NOAEL = no observed adverse effect level, NOEL = no observed effect level, LOAEL = lowest observed advert effect level, LOEL = lowest observed effect level.

5.2.1.3 Derivation of PNECs

For the estimation of PNECs, acceptable daily limits were combined with assumptions regarding potential exposure through drinking water. The PNECs for a scenario whereby human exposure to ARVs through water as a drinking water source only was used. The PNECs for both adults and children were estimated, with children used to reflect the worst-case scenarion.[176,247] The parameters used for deriving PNECs are those recommended by the USEPA for deriving ambient water quality criteria [257] as given in Table 5.2.

Table 5.2: Human exposure parameters relating to adult and child receptors for the derivation of PNECs.[247,257]

Parameter	**Units**	**Symbol**	**Receptor**	
			Adult	Child
Body weight	kg	BW	70	14
Water consumption	L/day	$IngR_{DW}$	2	1
Exposure frequency	days/year	EF	350	350
Exposure duration	years	ED	30	6
ADI averaging time	days	AT	10,950	2190

The PNECs for each ARV was estimated as follows:

$$\mathbf{PNEC_{dw}\ (ng/L)} = \frac{\mathbf{1000} \times \mathbf{ADI} \times \mathbf{BW} \times \mathbf{AT}}{\mathbf{IngR_{DW}} \times \mathbf{EF} \times \mathbf{ED}}$$

Where PNEC is in ng/L and ADI is in µg/kg/day, therefore the conversion factor of 1000 was used, BW is the adult body weight (kg/person); $IngR_{DW}$ is the adult drinking water ingestion rate (L/person/day); AT is the averaging time (days); EF is the exposure frequency (days/year); and ED is the exposure duration (years).[247]

5.2.1.4 Exposure assessment

Measured environmental concentrations (MECs) for selected ARVs in SA were available from data published in the scientific literature (Table 4.1 a-e).

5.2.1.5 Risk calculation

Risk quotients were calculated as an indicator for human health risk. The MEC of the target ARV was divided by the PNEC of the target ARV, to estimate the possible threat posed by a specific ARV.[258]

$$\mathbf{RQ} = \frac{\mathbf{MEC}}{\mathbf{PNEC}}$$

The RQ < 0.1 indicates insignificant risk, 0.1 – 1 indicates low risk, > 1 – 10 indicates moderate risk and > 10 indicates high risk.[153,258-259]

5.2.2 Ecotoxicological risk assessment

Data regarding ARVs with estimated PNECs for aquatic species was sourced from the literature and the calculation of the ecotoxicological risk was the same as for the human health risk assessment.

5.3 RESULTS

5.3.1 Potential human health risk assessment

5.3.1.1 Selection of ARVs to be evaluated and determination of environmental concentrations

Based on their use in the first line treatment option and also on the fact that their PNECs were available from literature, [176,183,247] six ARVs were selected for the assessment: AZT, ABC, 3TC (PNECs calculated for human health risks) [176] and TDF, FTC and EFV (PNECs calculated for ecotoxicological risks).[260-262] For each of the six ARVs selected, maximum concentrations detected in treated tap water, surface water and WWTP effluents were used in the risk calculations. In addition, these concentrations were multiplied by 1000 to estimate the RQs for situations where higher concentrations of ARVs may be present in the water sources (Table 5.3).

Table 5.3: Antiretrovirals included for selected water sources in Gauteng and other provinces in SA.

ARV ***Class***	**ARV use**	***Detected concentration** ng/L	**Detected value** (X 1000)	**Water source**	**Province**
AZT ***NRTI***	Adult first-line, Adult second-line & Paediatric first-line	72.7	7 270	Tap water	North West
		973	97 300	WWTP effluent	Gauteng
		627	62 700	Surface water	Gauteng
3TC ***NRTI***	Adult first-line, Adult second-line & Paediatric first-line	323.4	32 340	WWTP effluent	Gauteng
		242	24 200	Dam system	Gauteng
		130	13 000	DEWATS effluent	KZN
ABC ***NRTI***	Adult first-line & Paediatric first-line	1.6	160	River water	Eastern Cape
FTC ***NRTI***	Adult first-line & Adult second-line	361	361 000	Surface water	Gauteng
		5	5000	WWTP effluents	Western Cape
TDF ***NtRTI***	Adult first-line, Adult second-line & Paediatric first-line	243	243 000	Surface water	Gauteng
		1.6	160	WWTP effluents	Western Cape
EFV ***NNRTI***	Adult first-line, Adult second-line, Paediatric first-line & Paediatric second-line	514.6	514 600	Surface water	Gauteng
		33 000	33 000 000	WWTP effluents	KZN

*** =** Refer to Table 4.1 in chapter 4, NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleotide reverse transcriptase inhibitor, NtRTI = nucleotide reverse transcriptase inhibitor, ARV = antiretroviral, AZT = zidovudine, 3TC = lamivudine, ABC = abacavir, FTC = emtricitabine, EFV= efavirenz, TDF = Tenofovir, WWTP = wastewater treatment plant, DEWATS = decentralised water treatment sites, KZN = KwaZulu-Natal, ng/L = nanogram per litre.

5.3.1.2 Derivation of ADIs

The calculated ADIs for AZT, 3TC and ABC are given in Table 5.4

Table 5.4: Acceptable daily intake derivation for the selected antiretrovirals.

ARV	**POD** (mg/day)	**UF1**	**UF2**	**UF3**	**UF4**	**UF5**	**ADI** (µg/kg/day)
AZT	300	3	1	1	10	10	14.3
3TC	100	3	1	1	10	3	15.9
ABC	600	3	1	1	10	5	57.1

ARV = antiretroviral, AZT = zidovudine, 3TC = lamivudine, ABC = abacavir, POD = point of departure, UF = uncertainty factor, ADI = acceptable daily intake.

5.3.1.3 Derivation of PNECs

The estimated PNECs for AZT, 3TC, and ABC are presented in Table 5.5.

Table 5.5: Estimated human health PNECs for selected ARVs.[176]

ARV	**ADI** (µg/kg/day)	**PNEC**(ng/L) child	**PNEC**(ng/L) adult
AZT	14.3	208 780	520 997
3TC	15.9	232 140	579 290
ABC	57.1	833 660	2 080 343

ARV = antiretroviral, AZT = zidovudine, 3TC = lamivudine, ABC = abacavir, ADI = acceptable daily intake, PNEC = predicted no effect concentration, ng/L = nanogram per litre.

Due to the fact that children were assumed to drink more water on the body weight basis, compared to adults, the PNECs for children are always lower than those calculated for adults.[176,247]

5.3.1.4 Risk calculation

Risk quotients for the selected ARVs on different water sources in Gauteng and other provinces in SA are presented in Table 5.6.

From Table 5.6, it is evident that all RQs are considerably less than one, with ratios ranging from 0.00 – 0.01. This indicates that based upon currently available data, these compounds do not appear to pose an appreciable risk to human health from the consumption of drinking water containing trace levels of ARVs. And even if the detected concentrations were to increase a thousandfold, the RQs would still be < 1, except for AZT surpassing RQ of 0.1, indicating low risk to human health.

Table 5.6: Human health risk quotients for selected water sources in Gauteng and other provinces in SA.

ARV *Class*	**Detected concentration** ng/L	**RQ (child)**	**RQ (adult)**	**Detected concentration** (X 1000)	**RQ (child)**	**RQ (adult)**	**Water source**
AZT ***NRTI***	72.7	0.00	0.00	7 270	0.03	0.01	Tap water
	973	0.01	0.00	97 300	0.47	0.19	WWTP effluent
	627	0.00	0.00	62 700	0.30	0.12	Surface water
3TC ***NRTI***	323.4	0.00	0.00	32 340	0.10	0.06	WWTP effluent
	242	0.00	0.00	24 200	0.10	0.04	Dam system
	130	0.00	0.00	13 000	0.60	0.02	DEWATS effluent
ABC ***NRTI***	1.6	0.00	0.00	160	0.00	0.00	Surface water

ARV = antiretroviral, NRTI = nucleoside reverse transcriptase inhibitor, AZT = zidovudine, 3TC = lamivudine, ABC = abacavir, RQ = risk quotient, WWTP = wastewater treatment plant, DEWATS = decentralised water treatment sites, KZN = KwaZulu-Natal, ng/L = nanogram per litre.

5.3.2 Ecotoxicological effects

The estimated aquatic PNECs for TDF, FTC and EFV are presented in Table 5.7.

Table 5.7: Estimated aquatic PNECs for TDF, FTC and EFV.[260-262]

ARV	**PNEC**$(ng/L)_{fish}$
TDF	610 000
FTC	900 000
EFV	10

ARV = antiretroviral, TDF = Tenofovir, EFV = efavirenz, PNEC = predicted no effect concentration, ng/L = nanogram per litre.

From Table 5.7 it is evident that the aquatic PNEC for EFV is low (10 ng/L)

The calculated ecotoxicological RQs for FTC, TDF and EFV in surface and wastewater effluent are presented in Table 5.8.

Table 5.8: Ecotoxicological risk quotients for selected water sources in Gauteng and other provinces in SA.

ARV	Detected concentration (ng/L)	PNE_{fish} (ng/L)	RQ	Detected concentration (x 1000)	RQ
FTC	361 (surface)	900 000	0.00	361 000	**0.40**
NRTI	5 (effluent)	900 000	0.00	5000	0.01
TDF	243 (surface)	610 000	0.00	243 000	**0.39**
NtRTI	1.6 (effluent)	610 000	0.00	160	0.00
EFV	514.6 (surface)	10	**51.46**	514 600	**51 460**
NNRTI	33 000 (effluent)	10	**3 300**	33 000 000	**3 300 000**

NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleotide reverse transcriptase inhibitor, NtRTI = nucleotide reverse transcriptase inhibitor, ARV = antiretroviral, FTC = emtricitabine, TDF = Tenofovir, EFV= efavirenz, ng/L = nanogram per litre, RQ = risk quotient.

Higher RQs were observed on the ecotoxicological risks for EFV exposure for fish, indicating that EFV in water sources at measured concentrations were found to be hazardous to fish. If 1000-fold higher concentrations were detected, FTC and TDF would indicates lower risk (RQ > 0.1)

5.4 DISCUSSION AND CONCLUSION

South Africa, like the rest of Africa, uses more ARVs in the fight against HIV/AIDS and as expected, ARVs are increasingly being detected in water sources.[47] This has raised concerns regarding the potential human health effects of long-term exposure to trace levels of ARVs detected in drinking water sources. The present study shows that from the current levels of AZT, 3TC and ABC detected in drinking water sources in SA, the possible human risk is insignificant. The calculated RQs were at the range of 0.00 – 0.01. Even though concentrations were to increase to a thousandfold, the risk to humans is deemed negligible with RQs for AZT only rising to low risk levels (RQ > 0.1). This could be particularly true for SA, where there is a great possibility of doubling the ARV usage as the country expands on the HIV test and treat policy. In addition, for AZT, ABC and 3TC, in order for them to reach the LOEL, a 70 kg adult person would have to drink > 20 L/day or > 162 000 L/day or >1 million L/day to reach RQs > 1 for AZT, ABC and 3TC, respectively. For reference, drinking 10 litres of water in an hour can be fatal.[263] The results from this study are in agreement with those of Cunningham et al. (2009) who

showed that RQs calculated for all CECs including those of ARVs (AZT, 3TC, ABC), were below 1, ranging from 0.067 to 2.9 x 10^{-7}.[176] As early as 2004, Jones et al. postulated that the human health risk posed by trace levels of pharmaceuticals in water was negligable.[264] This is further supported by other researchers who reported on low quantities of pharmaceuticals in drinking and/or surface water sources.[23,176,180-181,264] This study also confirms previous findings that PNECs for children are always lower than those of adults as ADIs were based on the assumption about potential exposure through drinking water. Children's PNECs indicate a worst-case scenario as they are more conservative.[176,183,247]

Initially, for this study, the plan was to use pharmacokinetic and stochastic models to predict human health effects posed by ARVs in water sources. However, the benchmark dose, i.e. a dose associated with a specified measure or change of a biological effect, for ARVs as well as other specific parameters for ARV exposure to humans for such models could not be calculated or sourced in the available literature. However, for modelling exposure to chemicals for risk assessment the comprehensive library of multimedia and physiological based pharmacokinetic (PBPK) models for integration, prediction and uncertainty and sensitivity analysis (The MERLIN-Expo software) was explored. It is an environmental exposure assessment software tool, which models the exposure to chemicals for risk assessment. It is a comprehensive library within which the candidate explored the "Human Model". Unfortunately, there were no parameters for ARVs within this model, although other data for other pharmaceuticals was available and consequently this library was not used. The ADI values, however, could be sourced from dose-response model studies and applied to this investigation.[265] Even if the detected concentrations were associated with a human health risk, it would be difficult to attribute or associate trace levels of an ARV in water to a specific condition, e.g. metabolic disorders due to TDF, without data from large case control studies. Even though the potential of HIV drug resistance was identified as one of the potential effects from exposure to trace levels of ARVs in water sources, this would be difficult to prove or disprove as the dynamics of HIV drug resistance are complex with mutations induced that can affect a whole class of ARVs, thus compromising a regimen, INSTIs.[266] In addition, there are novel mechanisms of drug resistance, for which there are no diagnostic assays.

There are a number of uncertainties to be considered when determining the potential health risk through ingestion of contaminated water, particularly the ARV concentrations in drinking water.[247] The ARVs enter the environment mainly as a result of excretion following therapeutic use, and because ARVs are receptor mediated, levels entering water sources will often be diluted and are likely metabolised to well below levels that could result in any effect from water consumption.[247] In addition, photodegradation is an important factor regarding elimination process for many pharmaceuticals in surface water. Zhou et al. (2015) investigated photodegradation of antivirals including AZT and 3TC and found that AZT was easily transformed via direct photolysis whereas 3TC was mainly transformed via indirect photolysis. Nitrate enhanced their photodegradation in fresh water while in sea water, AZT photolysis was inhibited and that of 3TC enhanced.[267] This is an important point to consider for those ARVs that are not completely removed by wastewater treatment processes which use chlorination. Nevirapine has been detected widely in the environment in SA (Table 4.1 a-e) and other regions of the world (Table 2.1 and 2.2). However, Wood et al. (2016) showed that some of the NVP disinfection transformation products retained viral activity although not found to be more toxic than NVP.[156] This indicates that ARVs behave differently in the environment and this could partially explain the differences in concentrations detected in different water sources. Dolutegravir has only been used in SA since late 2019 in SA and therefore it has not been detected from water sources. Consequently even though the PNEC for aquatic species has been established (950 ng/L),[262] the RQ could not be calculated as the environmental concentration has not yet been established.

Another potential contributing factor is sampling error. The sampling methods used did not address the possibility of short-term variations in the ARV concentrations and the common approach was to apply *ad hoc* grab sampling within a treatment plant or at different positions of a river system and dams and at different seasons of the year. Sampling times and frequency of sampling are important in ARV analysis[47] and should be taken into consideration. Antiretrovirals are taken at specific prescribed times of the day for the rest of the person's life which may imply that there are certain times during the day that the ARVS are excreted and levels in water systems are very high.[47] In addition, sampling errors related to long sampling intervals and inadequate sampling modes as contributors to over-interpretation of pharmaceutical ecotoxicological data have been highlighted previously.[268] Predictive models should therefore incorporate these

sampling errors. Future studies should take passive sampling approaches, automated samplers and online sensors into consideration.[47]

Although not much has been done to date, ecotoxicological effects of ARVs in non-human species has been investigated.[47] Ecotoxicological data indicates that ARVs in water are harmful to aquatic species [186,253] and results from the present study showed that EFV concentrations of 514.6 ng/L and 33 000ng/L detected in surface water and WWTP effluents in SA, respectively, was hazardous to fish. Similar results were obtained previously in a study conducted in SA by Robson et al. (2017), where the exposure (96 hours) of fish to EFV at a concentration of 20.6 ng/L triggered liver damage, as well as higher total fish deaths compared to the control sample.[186] The ecotoxicological risk depends on the ARV detected or contaminating the water, e.g. EFV was found to be hazardous at low concentrations as low as 10.3 ng/L and from the PNECs calculated for aquatic risks, its PNEC was comparatively low.[269] In SA, Schoeman et al. (2015) and Abafe et al. (2018) detected high levels of EFV in Gauteng (71 00 ng/L)[78] and KZN (33 000 ng/L)[165] and this highlights the risks to aquatic species, that currently exists in those areas. Available data for ABC indicated an EC50 value of 57 mg/L to be harmful to green algae.[185] Emtricitabine exhibited negligible ecotoxicity risk for three trophic levels (algae, daphnia, and fish), since the median measurements for the RQs were 0.00.[173] Ecotoxicological effects are not limited to ARVs, Musee (2018) investigated adverse effects of triclosan (TCS) and triclocarban (TCC) on aquatic species in surface water and found that TCS can pose risks in wastewater and freshwater, whereas TCC poses risks to freshwater but none in wastewater and further indicated that both chemicals posed no risk to the terrestrial life, e.g. earthworms, in Gauteng, SA.[270]

Regardless of the absence of any proven human health risks associated with exposure to trace levels of AZT, 3TC and ABC in the present analysis, the safety of potable drinking water remains a major focus as it is a necessity for human survival as it is a direct route for micro pollutants to enter the human body. Further studies should investigate potential human health effects of chronic exposure to mixtures of different classes of PPCPs Current and outdated wastewater treatment technologies should be reassessed and repurposed for the removal of ARVs and other PPCPs.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

The human right to safe potable drinking water is embedded in the SDGs of the UN. In addition, the One Health concept recognises that the health of any community is interconnected to the health of animals and the environment. While biological pollutants such as bacteria, viruses and parasites in water sources are considered to pose a significant human health risk,[271] ECs and CECs, which include PPCPs, are being released into the environment continuously by many anthropogenic activities.[272] Pharmaceuticals, which are widely used in human and veterinary medicine, contain active ingredients that are beneficial to society. However, the occurrence of trace levels of pharmaceuticals and their metabolites in water sources, including drinking water sources, has become a public health concern. This study focused on a specific group of pharmaceuticals, i.e. ARVs, which are used for the treatment of HIV. Globally, SA has the greatest number of people on ARVs[231,272] and the intentional and unintentional release of ARVs into the environment is of considerable concern with regard to the possible human health and ecotoxicological effects. This concern is based on a dearth of knowledge regarding the effect of low concentrations of ARVs to human health through the consumption of ARV-contaminated water. The aim of this study was to assess the potential risks of long term exposure to low concentrations of ARVs in treated and untreated water sources in Gauteng, South Africa.

The first objective of the study was to review the national and international literature to determine the extent and risks posed by ARV contamination in water sources worldwide. This objective was achieved in two parts. In the first section, an overview of ECs and pharmaceuticals in water sources globally was conducted. From the review it was evident that the pharmaceuticals targeted and detected in each investigation were country-dependent and linked to the most commonly used drugs or antivirals in the region, e.g. oseltamivir in Japan.[119-120] The second part addressed the extent and potential risks posed by ARV contamination in water sources worldwide, excluding SA. Although several studies have looked at pharmaceuticals in the aquatic environment globally, only a few review articles reported on the presence and fate of ARVs in environmental samples.

However, the presence of ARVs in water sources was considered a public health concern and there is paucity of data on ARVs in water sources worldwide, excluding Africa. There are also limited studies on ARVs in Africa, but the reported studies, specifically in Kenya, showed that African water sources are investigated more intensely with regard to ARVs. This highlights the greater awareness of the current HIV situation and the use of ARVs on the continent with the highest burden of HIV disease. This review also highlighted that German studies indicated high removal efficiencies of ABC[149] and 3TC[159,165-166] through wastewater treatment processes. The potential risks associated with human exposure to ARVs through drinking water is an area of concern, particularly in regions that practice indirect water reuse and where sewage effluents get released to surface waters that in turn are used as a source of drinking water.[59] From the risk assessment studies conducted in other countries outside Africa, it was concluded that there was a low likelihood of human health risk associated with unintentional exposure to low levels of ARVs[176] and other pharmaceuticals[23,183,247] via drinking water contaminated with ECs.

The second objective was to establish which ARVs are most commonly used in the public and private sector in SA, with special reference to the Gauteng province. A literature review was conducted and NDoH guidelines were scrutinised to establish which ARVs are most commonly used in the public and private sector in SA. South Africa uses more ARVs per capita compared to any other country fighting HIV/AIDS.[231] Treatment and guideline policies for HIV/AIDS have been driven by WHO recommendations and at any given time, the drugs recommended for use in the first-line option are the ones mostly used.[229] From the review it is evident that some ARVs, e.g. AZT, 3TC, NVP, d4T, EFV have been in use since the first guidelines were implemented in 2003,[207] with the use of ABC, TDF, FTC and LPV/r increasing after the introduction of the ART 2010 guidelines.[218] From 2010, the discontinuation of d4T has been encouraged with the accelerated phasing out in people who have already initiated cART.[218] In addition EFV and NVP, currently in use in SA, are scheduled to be phased out.[273] The introduction of the INSTI, namely DTG, into the first-line therapy in late 2019 as a replacement for EFV was highlighted.[228] Up to 2019, TDF, 3TC, FTC and EFV were the drugs used in the first-line therapy for adults[218] and were used mostly for the management of HIV. Approximately 71% of adults living with HIV are on ART,[225-226] which equates to over 5 million adults on ART. From the review of the ARV guidelines it is evident that as more effective drugs are developed, cART modalities will be adapted and improved resulting

in different patterns in the ARV environmental profile. Monitoring changes in ART guidelines is therefore important for both WWTP operators and laboratory analysts so that wastewater treatment processes and analytical methodology can be adapted accordingly.

The third objective was to establish which ARVs, and quantities thereof, have been detected in water sources in SA, with special reference to Gauteng province. To achieve this objective, a systematic review was conducted to establish which ARVs and quantities thereof, have been detected in water sources in SA. A final number of 10 original articles were found to be relevant and were included in the systematic review. From the review, it was evident that all ARVs that have been used historically in the first-line, namely d4T, 3TC, EFV, NVP, and in second-line (ddI, LPV/r, AZT) ART guidelines have been detected in one or more water sources. Concentrations of ARVs in treated tap water ranged from below the detection limit of the assay to 72.7 ng/L – the latter being for AZT on potable water in the North West province. Concentrations of up to 53 000 ng/L were recorded for AZT and 33 000 ng/L for EFV in WWTP influent samples from KwaZulu-Natal. From the results it was also evident that the ARVs concentrations detected matched the HIV prevalence by province. The HIV prevalence in SA varies by province, with Gauteng coming fifth after KZN (40%), Mpumalanga (37%), Eastern Cape (34%) and Free State (33%), while the lowest HIV prevalence was in Western Cape (16%). These implies that if the 90-90-90 targets are met, in terms of environmental HIV drug concentrations, much higher viral loads of ARVs in the environment would be expected in those areas with high HIV prevalence, with the highest concentrations expected from KZN and the lowest expected from the Western Cape. Within the Gauteng province, the highest concentrations were detected in the Pretoria area, along the Roodeplaat dam system, the Apies and Pienaars rivers, compared to concentration detected along the Jukskei River in the Alexandra, Johannesburg area. Surprisingly, ARVs such as ddC and d4T were detected although ddC has already been phased out and d4T is not used widely. The newly introduced ARVs, e.g. DTG, that have been included in the 2020 ART first-line treatment option would need to be included in future environmental surveillance studies.

The fourth objective was to determine the potential health risks posed by ARVs in potable water to vulnerable individuals and communities by means of modelling using

pharmacokinetic as well as compartmental models. To answer this objective, a risk assessment was conducted to evaluate the potential risks of ARVs in water sources. The method comprised of five general steps: a) selection of ARVs to be assessed; b) derivation of ADIs; c) derivation of PNECs; d) exposure assessment - determination of environmental concentrations; and e) risk calculation. The values needed for this objective were sourced from studies that utilised ADIs derived from dose-response model studies. The present study showed that for the current levels of AZT, 3TC and ABC detected in drinking water sources in SA, the possible human health risk was insignificant with calculated RQs ranging from 0.00 – 0.01. Even if concentrations were to increase by a thousandfold, the risk to humans was still deemed low with RQs for AZT only rising to low risk levels (RQ > 0.1). Although currently no human health risks were identified, drug-dependant ecotoxicological risks were identified. Efavirenz was found to be highly toxic to fish. This highlights that ARVs in water sources need to be monitored closely, as although they currently pose a low risk, should concentrations increase the potential risk could escalate.

The null hypothesis of this study was that exposure to low levels of ARVs through ingestion of contaminated drinking water could adversely affect human health and the alternative hypothesis was that ARVs in water had no effect on human health. Based on the results in this study for AZT, 3TC and ABC, the null hypothesis was rejected as current evidence indicated that the human health effects were negligible.

There is a paucity of data on the potential human health risks associated with exposure to ARVs through water sources worldwide. As there are no reported studies on the potential human health risks posed by ARVs in drinking water in SA this is the first of such studies making use of the RQ method. This study is novel and makes a positive contribution to the growing body of knowledge on potential human health risks posed by ECs in water sources. Although results on human health presented in this study are for three ARVs (AZT, 3TC and ABC), it is suggested that exposure to trace levels of any other ARV would also result in negligible human health risk. As only snapshot grab samples were used in SA for the analysis of ARVs, future studies should consider passive sampling approaches, automated samplers and online sensors to further investigate potential human health effects of chronic exposure to mixtures of different classes of PPCPs. As the safety of potable drinking water remains crucial, current and outdated wastewater treatment

technologies should be reassessed and repurposed for the removal of ARVs and other ECs. The recommendation from this study is for monitoring and management of the pharmaceuticals entering the environment via different routes, e.g. informal dumping and non-functional WWTPs, as access to safe drinking water is a basic human right and is a necessity for human survival.

CHAPTER 7

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APPENDIX A

The potential risks of long term exposure to low concentrations of antiretrovirals in treated and untreated water sources in South Africa.

Ntsieni Ramalwa [1,2] **, Tiaan de Jager** [1] **, Maureen Taylor** [1,3]

[1]School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa.

[2]National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa

[3] Virology TAD, National Health Laboratory Service, Pretoria South Africa

BACKGROUND

- **Pharmaceuticals**
  - Are chemicals present in prescription medicines, over-the-counter therapeutic drugs and veterinary drugs
  - Contain active ingredients that promote pharmacological effects and are significantly beneficial to society.
- **Antivirals**
  - Pharmaceuticals used for treatment and prophylaxis of broad spectrum of viral infections: HIV/AIDS, influenza, herpes infections and hepatitis B & C
  - Widely used, not fully metabolised and shed in the urine and faeces
  - Enter the environment via wastewater or via disposal of unused/unwanted drugs
- **Antiretrovirals (ARVs)**
  - South Africa (SA) has an estimated 7.3 million people infected with HIV
  - Largest ARV treatment programme worldwide: test and treat, pre- and post- exposure prophylaxis, access to and use of ARVs increasing
  - ARVs detected and/or quantified in various water sources : untreated (surface) and treated (drinking) water sources : few studies worldwide and in SA

PROBLEM STATEMENT

- Pharmaceuticals are manufactured with the intention to cause biological effects
  - concern about potential impact on human health of unintentional exposure via contaminated water sources

OBJECTIVES

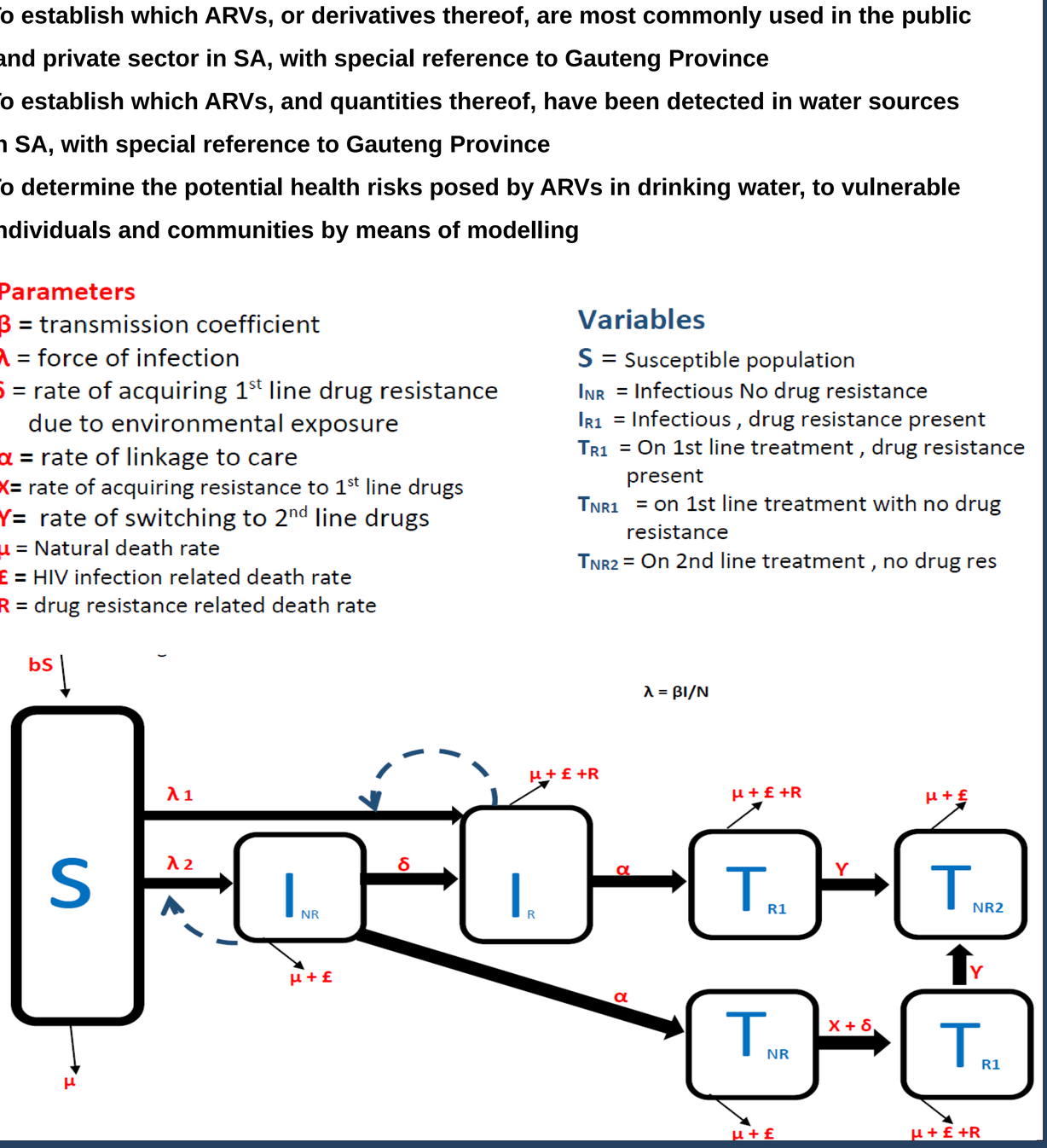
1. **To review national and international literature to determine the extent and risks posed by ARV contamination in water sources worldwide**
2. **To establish which ARVs, or derivatives thereof, are most commonly used in the public and private sector in SA, with special reference to Gauteng Province**
3. **To establish which ARVs, and quantities thereof, have been detected in water sources in SA, with special reference to Gauteng Province**
4. **To determine the potential health risks posed by ARVs in drinking water, to vulnerable individuals and communities by means of modelling**

Parameters

β = transmission coefficient
λ = force of infection
δ = rate of acquiring 1st line drug resistance due to environmental exposure
α = rate of linkage to care
X= rate of acquiring resistance to 1st line drugs
Y= rate of switching to 2nd line drugs
μ = Natural death rate
£ = HIV infection related death rate
R = drug resistance related death rate

Variables

S = Susceptible population
I_{NR} = Infectious No drug resistance
I_{R1} = Infectious , drug resistance present
T_{R1} = On 1st line treatment , drug resistance present
T_{NR1} = on 1st line treatment with no drug resistance
T_{NR2} = On 2nd line treatment , no drug res



METHODS

- The initial part of the study will be a systematic review of national and international literature to determine the extent and potential risks posed by ARV contamination in water sources globally
- The second part of the study will be an investigation to establish which ARVs and derivatives thereof, are commonly used in the public and private sector in SA, with special reference to Gauteng Province
- The third part of the study will be to establish which ARVs, and quantities thereof, have been detected in water sources in SA, through systematic literature review
- **The last and novel part of the study will focus on developing and applying health risk assessment models to project the potential risks posed by long term exposure to low levels of ARVs in water sources to different SA paediatric and adult populations, namely**
  - HIV infected individuals on treatment
  - HIV infected individuals not on treatment
  - **A STOCHASTIC MODEL** will be used : discreet time chain binomial model, as there are possible outcomes for a given set of parameters and the population for this study is large but sub-populations are small. Averages or proportions will be used at some point but eventually whole numbers will be used to represent individuals.
  - Possible ARV drug resistance or potential toxic effects are viewed as emergence of a new “pathogen” and that will have to be quantified (it is therefore a quantitative outcome) and individuals will represented as discreet.

POTENTIAL IMPACT OF STUDY

- New models to determine potential health risks posed by pharmaceuticals in water sources
- New data on possible health impact of ARV exposure through water sources
- Recommendations to water industry with regard to which ARVs to target for routine analysis (current usage and new drugs in pipeline)

ACKNOWLEDGEMENTS

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NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

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APPENDIX B

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
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Faculty of Health Sciences Research Ethics Committee

1/02/2018

Approval Certificate
New Application

Ethics Reference No: 13/2018

Title: The clinical relevance of antiretrovirals detected in treated and untreated water sources in Gauteng, South Africa

Dear Ntsieni Ramalwa

The **New Application** as supported by documents specified in your cover letter dated 24/01/2018 for your research received on the 24/01/2018, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 31/01/2018.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**13/2018**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Level 4-60*

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
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Faculty of Health Sciences Research Ethics Committee

28/06/2018

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No: 13/2018

Title: The potential risks of long term exposure to low concentrations of antiretrovirals in treated and untreated water sources in Gauteng, South Africa

Dear Ntsieni Ramalwa

The **Amendment** as described in your documents specified in your cover letter dated 8/05/2018 received on 8/05/2018 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 27/06/2018.

Please note the following about your ethics amendment:
- Please remember to use your protocol number (**13/2018**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committe may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

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We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

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Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

20 July 2020

Approval Certificate
Annual Renewal

Ethics Reference No.: 13/2018
Title: The potential risks of long-term exposure to low concentrations of antiretrovirals in treated and untreated water sources in Gauteng, South Africa

Dear Ms NR Ramalwa

The **Annual Renewal** as supported by documents received between 2020-06-19 and 2020-07-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2020-07-15.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2021-07-20.
- Please remember to use your protocol number (13/2018) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

APPENDIX C

Ntsieni Ramalwa

From: Chavon Walters <CWalters@csir.co.za>
Sent: 11 February 2021 12:25 PM
To: Ntsieni Ramalwa
Cc: Ntsieni
Subject: Re: Request for permission to use a figure from your article

Good day Ntsieni

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You may use this email as proof that permission was granted.

Best wishes
Chavon

CSIR
Touching lives through innovation

Chavon Walters
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Tel: +27 888-2625
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www.csir.co.za

Current status and prospects of HIV treatment

Author: Tomas Cihlar,Marshall Fordyce

Publication: Current Opinion in Virology

Publisher: Elsevier

Date: June 2016

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Publication Title	AMERICAN SOCIETY OF NEPHROLOGY. CLINICAL JOURNAL. ONLINE	Rightsholder	American Society of Nephrology
		Publication Type	e-Journal
Article Title	Clinical Pharmacology in HIV Therapy.	Start Page	435
		End Page	444
Author/Editor	American Society of Nephrology.	Issue	3
Date	01/01/2006	Volume	14
Language	English		
Country	United States of America		

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Instructor name	Professor Maureen Taylor	Expected presentation date	2021-02-19

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