



Presence, dynamics, and risk assessment of antiretrovirals in South African waters

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DECLARATION

Contributions of the research team.

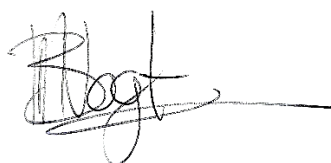
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Prof S Horn	Promotor • Provided academic supervision, assisted with sample preparation, reference material verification, and reviewed the manuscript for structure, clarity, and academic rigour.
Prof H Bouwman	Co-promotor • Offered expert guidance on research direction and methodology, reviewed the manuscript for scientific accuracy and coherence.
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Disclosure statement

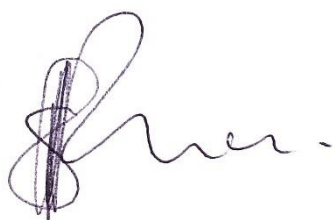
This work conforms with the North-West University SENATE RULES ON ACADEMIC INTEGRITY, Section 5; NWU Senate Rules on the Responsible and Ethical Use of Artificial Intelligence, approved on 29 May 2025.

The following is a statement from the promotor that confirms each individual's role in the study:

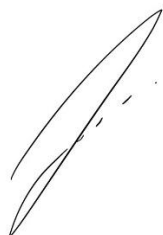
I declare that I have approved the article and that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of Bianca's thesis.



Student signature



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Co-promotor signature

DEDICATION

In loving memory of my father, whose quiet strength and unwavering support continue to guide me even in his absence.

And to my son, the light of my life, may this work be a legacy of perseverance and love.

PREFACE

The thesis has been compiled in accordance with the specifications outlined in the Manual for Higher Degrees Studies (2020) of the North-West University (NWU). The Harvard reference style has been consistently applied for all cited sources.

The outline of the thesis is as follows:

Chapter 1:

This chapter presents a general introduction to the study. It includes the thesis outline and concludes with the problem statement, research aims, objectives, and hypotheses.

Chapter 2:

This chapter provides a comprehensive literature review of antiretroviral drugs in South African waters. It discusses their physicochemical properties, occurrence, behaviour, and the analytical methods employed by various researchers. The chapter also identifies key concerns, knowledge gaps, and anticipated trends.

Chapter 3:

Building on this foundational understanding, Chapter 3 investigates the spatial and temporal trends of antiretroviral drug contamination. It elaborates on the sources and environmental pathways of these substances and explores certain contributing factors such as environmental conditions and usage patterns that influence distribution.

Chapter 4:

This chapter stresses the serious environmental risks posed based on the findings in Chapter 2. Emphasis is placed on the bioaccumulation potential and toxicity of selected compounds. Mechanisms of action and observed ecotoxicological effects are considered. It concludes with exploring combined effects and makes use of a prioritisation tool to rank drugs accordingly.

Chapter 5:

This chapter outlines a comprehensive evaluation of potential human health hazards associated with antiretroviral drugs detected in South African water sources. It employs two distinct hazard assessment approaches to assess ingestion-based exposure hazards across several sub-

populations. The chapter concludes with a quantification of cumulative hazard potential from multiple drug mixtures, offering insight into regulatory thresholds.

Chapter 6:

The chapter presents a specific multi-criteria decision analysis model designed to prioritise antiretroviral drugs in aquatic environments by integrating environmental occurrence, toxicity, and exposure parameters. The model combines weighted indicators to generate a final priority score, enabling systematic evaluation of compounds based on both ecological and human health relevance.

Chapter 7:

The final chapter synthesises the findings presented throughout the thesis. It revisits the central concepts and research undertaken in each chapter, addressing the overarching hypothesis, objectives, and aims. It reflects on the broader implications of the research and contributions to the field.

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ABSTRACT

Environmental contamination of antiretroviral drugs (ARVDs) is an emerging concern in South Africa, because of the high number of individuals on antiretroviral therapy (ART) programs as well as lack of pharmaceutical effluent guidelines. The current study offers among the first national assessments of ARVDs in South African waters, revealing contamination in drinking water (0.1 ng/L–27 µg/L), groundwater (0.3–13 ng/L), surface water (0.1 ng/L–102 µg/L), and wastewater (0.3 ng/L–814 µg/L). The study applied a multidisciplinary approach combining literature review, site-specific monitoring, spatial and temporal analysis, ecological and human health hazard assessments, and hybrid mixture toxicity modelling, while accounting for analytical, regulatory, and exposure-related uncertainties. By integrating multiple exposure and hazard indicators into a weighted multi-criteria model, the study enabled evidence-based ranking of selected ARVD contaminants. Disparities in ARVD detection and removal highlight the insufficiency of existing wastewater treatment technologies. Spatial analysis identified contamination hotspots near wastewater treatment plant discharges, particularly in the Hennops, Klip, and Jukskei Rivers. Site-specific pollution patterns were primarily independent of seasonal variation, although efavirenz and ritonavir exhibited seasonal peaks. Hazard assessments demonstrated moderate to high ecological risks for both individual ARVDs and mixtures, with lopinavir, efavirenz, and zidovudine identified as critical pollutants. A mixture toxicity model combining concentration and response addition confirmed additive and independent toxic effects in wastewater and surface water. Several ARVDs exceeded non-carcinogenic hazard thresholds, posing significant toxic potential to vulnerable populations such as children and pregnant females. The weighted multi-criteria model identified abacavir, lopinavir, efavirenz, emtricitabine, and nevirapine as the highest-priority ARVDs due to their combined high environmental occurrence, toxicity, and potential for human and ecological risk. The findings emphasise the urgent need for improved treatment technologies, targeted monitoring of priority ARVDs, and adaptive regulatory frameworks that integrate scientific uncertainty and cumulative exposure risks. This study supports the implementation of eco-pharmacovigilance strategies, proactive policy interventions and a more health-protective regulatory landscape in the face of emerging pharmaceutical contaminants.

Key terms: antiretroviral drugs; wastewater; environmental risk assessment; emerging contaminants; non-carcinogenic risks.

LIST OF ABBREVIATIONS

3TC	Lamivudine
°C	Degrees Celsius
ug/L	Microgram per litre
µg/kg/day	Microgram per kilogram per day
µL	Microlitre
µM	Micromolar
d	Day
g/d	Gram per day
g/mol	Grams per mole
h	Hour
L	Litre
L/d	Litre per day
L/kg	Liters per kilogram
L/min	Litres per minute
mg/kg/day	Milligrams per kilogram per day
mg/d	Milligram per day
mg/L	Milligrams per litre
mg/mL	Milligrams per millilitre
ML/d	Megalitres per day
mL/d	Millilitre per day
mL/g	Millilitre per gram
mM	Millimolar
ng/L	Nanogram per litre
ng/kg/day	Nanogram per kilogram per day
Psi	Pounds per square inch
V	Volts
ABC	Abacavir
ADD	Average daily dose
ADI	Acceptable daily intake
ADR	Adverse drug reactions
AF	Assessment factor
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of Variance
AOP	Advanced oxidation process
API	Active pharmaceutical ingredient
ARVD	Antiretroviral drug
ART	Antiretroviral treatment
ATC	Anatomical therapeutic chemical
ATV	Atazanavir
BCF	Bioconcentration factor
BOD	Biological oxygen demand
C₂H₃N	Acetonitrile
CA	Concentration addition
CA-RA	Concentration addition-response addition
CaCl₂·2H₂O	Calcium chloride dihydrate
CAGR	Compound annual growth rate
CAS	Chemical abstract service number
CDI	Chronic daily intake
CEC	Contaminants of emerging concern

CH₃COOH	Acetic acid
ChV	Chronic value (the geometric mean of the NOEC and the LOEC)
CLD	Compact letter display
CLDH	Calcined layered double hydroxide
COP	Chemical oxygen demand
COVID-19	Coronavirus disease of 2019
CSF	Cancer slope factor
d4T	Stavudine
DAD	Diode array detector
DDC	Zalcitabine
DDD	Defined daily doses
ddH₂O	Double-distilled water
ddl	Didanosine
DEA	Department of Environmental Affairs (South Africa)
DEWATS	Decentralised wastewater treatment systems
DID	Defined daily doses per 1000 inhabitants per day
DNA	Deoxyribonucleic acid
DoH	Department of Health (South Africa)
DT₅₀	50% degradation time
DTG	Dolutegravir
DRV	Darunavir
DWAF	Department of Water Affairs and Forestry (South Africa)
DWS	Department of Water and Sanitation (South Africa)
DWTP	Drinking water treatment plant
EC	Emerging chemical
EC₅₀	Effective concentration
ECHA	European Chemicals Agency
ECOSAR	Ecological Structure-Activity Relationships Program
EFI	Entry and fusion inhibitors
EFV	Efavirenz
EMEA	European Medicines Agency
EP PP	Environmentally persistent pharmaceutical products
ERA	Environmental risk assessments
ETR	Entravine
EU	European Union
FD	Frequency of detection
FDA	United States Food and Drug Administration
FDC	Fixed-dose combinations
FIA	Flow injector analysis
FLZ-d4	Deuterated fluconazole
FPV	Fosamprenavir
FTC	Emtricitabine
GAC	Granular activated carbon
GC-TOFMS	Gas Chromatography-Time of Flight Mass Spectrometry
GPS	Global Positioning System
H₂O₂	Hydrogen peroxide
HA	Hazard assessment
HCl	Hydrochloric acid
HCOOH	Formic acid
HHHA	Human health hazard assessment
HIV	Human immunodeficiency virus
HLB	Hydrophilic-Lipophilic Balance
HLB-L	Hydrophilic-lipophilic balance (low)
HF-LPME	Hollow Fibre Liquid Phase Microextraction

HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
HRSC	Human Sciences Research Council
HT	High-Throughput
HQ	Hazard quotient
IC₅₀	Inhibitory concentration 50%
IDV	Indinavir
IDW	Inverse distance weighting
II	Intergrase inhibitor
IS	Internal standard
ISO	International Organization for Standardization
KEGG	Kyoto Encyclopaedia of Genes and Genomes
KCl	Potassium chloride
km	Kilometre
K_{ow}	Octanol–water partition coefficient
K_{oc}	Organic carbon–water partition coefficient
LC/MS	Liquid Chromatography/Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LC-TOF-MS	Liquid Chromatography-Time-of-Flight Mass Spectrometry
LC-HRMS	Liquid Chromatography-High-Resolution Mass Spectrometry
LC-PDA-MS	Liquid Chromatography Coupled with Photodiode Array and Mass Spectrometry
LOAEL	Low observable adverse effect level
LOD	Limited of detection
LOEC	Lowest observed effect
Log D_{ow}	Log octanol–water distribution coefficient
Log K_{oc}	Log organic-carbon partition coefficient
LOQ	Limited of quantification
LPV	Lopinavir
LVI-UHPLC-MS/MS	Large Volume Injection Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry
[M+H]⁺	Protonated molecular ion; the mass of the molecule (M) plus one proton (H ⁺)
MASE-MIP	Membrane-Assisted Solvent Extraction and Molecularly Imprinted Polymer
Max	Maximum
MEC	Measured environmental concentration
MgSO₄·7H₂O	Magnesium sulfate heptahydrate
MIP	Molecularly imprinted polymer
Min	Minimum
MS	Mass Spectrometry
MVC	Maraviroc
n	Number
NaHCO₃	Sodium bicarbonate
NFV	Nelfinavir
NM	Newlands Mashu
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOEC	No observed effect concentration
NOEL	No observable adverse effect level
NRTI	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
NWA	National Water Act
NWU	North-West University
NWU-HREC	North-West University Health Research Ethics Committee

OECD	Organization for Economic Co-operation and Development
p	Probability value (used in statistical significance testing)
PD	Pharmacodynamic
PDA	Photodiode Array Detector
PEC	Predicted effect concentration
pH	Potential of hydrogen
P_{index}	Prioritisation index
PI	Protease inhibitor
PK	Pharmacokinetic
pKa	Acid dissociation constant
PLWH	People living with HIV
POD	Point of departure
PNEC	Predicted no effect concentration
QC	Quality control
QC/QA	Quality control/quality assessment
QTY	Quantity
QTOF	Quadrupole-Time-of-Flight
QSAR	Quantitative Structure-Activity Relationship
r	Boosted with ritonavir
R²	Coefficient of determination (R-squared); proportion of the variance
RA	Response addition
RAL	Raltegravir
RfD	Reference dose
RM	Repeated measures
RPV	Rilpivirine
RTI	Reverse transcriptase inhibitor
RTV	Ritonavir
RSD	Relative Standard Deviation
RQ	Risk quotient
SA	South Africa
SANS	South African National Standard
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SDB-L	Styrene-Divinylbenzene
STDEV	Standard deviation
SE	Soxhlet Extraction
SPE	Solid-Phase Extraction
SQV	Saquinavir
TD	Therapeutic dose
TDF	Tenofovir
TDS	Total dissolved solids
TD-GC×GC-	Two-Dimensional Gas Chromatography-Time-of-Flight Mass
TOFMS	Spectrometry
TEE	FDC comprising of tenofovir disoproxil fumarate, emtricitabine, and efavirenz
TOFMS	Time-of-Flight Mass Spectrometry
UA-Dμ-SPE-	Ultrasound-Assisted Dispersive Micro-Solid Phase Extraction Using
AC@PVP@Alginat	Activated Carbon Coated with Polyvinylpyrrolidone (PVP) and Alginate
UE	Ultrasonic Extraction
UF	Uncertainty factor
UHPLC-MS/MS	Ultra-High-Pressure Liquid Chromatography Tandem Mass Spectrometry
UNAIDS	Joint United Nations Programme on HIV/AIDS
UPLC-MS/MS	Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry

U.S.	United States
USA	United States of America
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultraviolet
WHO	World Health Organisation
WSA	Water Services Act
WWTP	Wastewater treatment plant
ZDV	Zidovudine

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CHAPTER 1 INTRODUCTION

1.1 Background

South Africa has the highest human immunodeficiency virus (HIV) incidence in the world, with a projected 12.7% (7.8 million) of the population living with HIV in 2022 (95% confidence interval: 7.2–8.4) (HSRC, 2023). Findings estimate that in 2022, 90% of adults knew their status, 91% of those diagnosed were on antiretroviral treatment (ART), and 94% of those on ART were virally suppressed (HSRC, 2023). Approximately 7.1 million people living with HIV (PLWH) in South Africa were receiving treatment for the associated acquired immune deficiency syndrome (AIDS) in 2022 (UNAIDS, 2017). More recent numbers are difficult to find. The extensive prescription of antiretroviral drugs (ARVDs) to combat the HIV/AIDS pandemic has inadvertently introduced a new environmental conundrum. By treating and trying to end this pandemic as an epidemiological and global health phenomenon, South Africa's freshwaters have become contaminated with ARVDs (Horn *et al.*, 2022).

Antiretroviral drugs have been detected in South Africa's freshwaters, as a result of the sheer number of ARVDs prescribed annually, its partial metabolism within the human body, its inadequate removal by wastewater treatment plants (WWTPs) and persistence in receiving waters due to resistance to breakdown and chronic release (Horn *et al.*, 2022; Swanepoel *et al.*, 2015). Consequently, ARVDs have emerged as contaminants of concern, prompting growing awareness due to their potential adverse effects on ecosystems and human health (Gani *et al.*, 2021; Ncube *et al.*, 2018; Ngumba *et al.*, 2016; Robson *et al.*, 2017).

Emerging chemical (EC) contaminants or contaminants of emerging concern (CECs), such as ARVDs, are substances not yet categorised as priority pollutants for systematic monitoring despite their continual release, documented toxicity, and environmental detection (Geissen *et al.*, 2015; Vogt *et al.*, 2020). In South Africa, ARVDs and their metabolites have been detected in surface water, groundwater, drinking water and wastewater, which suggests that ARVDs are an environmental concern (Abafe *et al.* 2018; Horn *et al.*, 2022; Mlunguza *et al.*, 2020; Mhuka *et al.*, 2020; Rimayi *et al.*, 2018; Schoeman *et al.*, 2015; Swanepoel *et al.*, 2015; Wood *et al.*, 2015; Xolo and Mahlambi, 2024). At the reported concentrations, both the public and non-target organisms are exposed to low chronic ARVDs (Ncube *et al.*, 2018).

Despite accumulating evidence, data on ARVD environmental fate, transport, and ecological impacts, specifically in developing countries such as South Africa, remain limited (Minguez *et al.*, 2016; Ncube *et al.*, 2018; Ngumba *et al.*, 2015; Nibamureke *et al.*, 2019; Robson *et al.*, 2017; Russo *et al.*, 2018). This lack of data emphasises a critical gap in understanding the risks posed by ARVDs to both ecosystems and non-target organisms.

The risk of adverse drug reactions (ADRs) derives from the safety profiles of multifaceted ARVDs and the effect of the disease on the immune systems (Mehta, 2011). Numerous ADRs related to ART have been documented ranging from mild to severe, and acute to chronic contingent on the environment (Masenyetse *et al.*, 2015). Concerns arise not only for patients who have developed certain severe or life-threatening reactions towards ART, or other pharmaceutical therapy, or those displaying ADRs, but also for the non-target organisms exposed via the environment.

As with many developing countries, information regarding the presence of ARVDs in South African environmental and drinking water is limited to a handful of studies, with no publications prior to 2015 known (Abafe *et al.*, 2018; Mhuka *et al.*, 2020; Mosekiemang *et al.*, 2019; Mtolo *et al.*, 2019; Rimayi *et al.*, 2018; Schoeman *et al.*, 2015, 2017; Swanepoel *et al.*, 2015; Wood *et al.*, 2015). Studies have also been limited to certain regions within the country, generally without considering temporal influences. Similar patterns have been observed elsewhere in Africa, for example Nantaba *et al.* (2024) reported concerning spatial trends and ecotoxic risks of pharmaceuticals in Lake Victoria sediments and surface water, emphasising the limited scope and uneven distribution of pharmaceutical monitoring across East Africa. These studies collectively highlight insufficient evidence regarding the ecological risks posed to non-target organisms by ARVDs. There is also a significant research gap in the availability of chronic studies regarding ARVDs.

Granted, the use of chronic toxicity tests with numerous species would be ideal for conducting ecological risk assessments for every ARVD, but it is not pragmatic to expect such an experimental endeavour due to time and financial constraints. The assessment of intrinsic ecotoxicological properties of ARVDs should therefore be accompanied by the usage of standardised acute tests in conjunction with protective assessment factors.

In the absence of chronic data, it can be advantageous to compare the predicted no effect concentrations (PNECs) calculated for compounds that have both acute and chronic data to reveal potential compounds of concern (Rodríguez-Gil *et al.*, 2018). This could help to identify established compounds for chronic testing when only acute data are readily accessible as well as

to ascertain those types of ARVDs for which acute toxicity data are appropriate substitutes for chronic toxicity data. In addition to limiting the number of organisms being tested, it is an important factor in the ethical framework.

South Africa currently lacks pollutant guidelines or regulations for pharmaceutical and personal care compounds. No national monitoring programs or threshold limits for ARVD contamination in aquatic ecosystems exist, leaving a regulatory void (Schoeman *et al.*, 2017). Some of the first steps in closing this gap are to: conduct comprehensive literature research; review existing environmental chemistry and fate data; evaluate toxicological data for acceptability; and establish background concentrations. Such efforts will help in addressing this environmental challenge while complementing South Africa's commitment to combatting HIV/AIDS.

This limited research on the presence of ARVDs in environmental and drinking water also affords no adequate indication of the priority ARVD class and/or individual compounds present in water bodies for future screening. A need for prioritisation and ranking methods to screen such target ARVDs for future research and risk assessment therefore exists. The current study will help to assess known background concentrations and potential human and environmental risks of parent compounds in waters. This will inform the development and recommendations for future regulatory purposes. The present study also proposes a simple prioritisation approach based on toxicity information and the number of prescriptions, accounting for metabolism and WWTP removal that can be applied to unmeasured and new compounds to account for ethical and financial constraints.

Chemical risk assessments ordinarily include two aspects: ecological- and human health. Humans are exposed to the same potential toxicants as any other biota, but the exposure scenarios differ, hence differentiated human and environmental risk assessments (Khan, 2017). These assessments help evaluate the probability that the environment may be influenced by exposure to ARVDs. Risk assessment methods based on ecological or toxicological evaluation criteria have been used to assess some ARVDs before, indicating individual or combined risk (Gani *et al.*, 2021; Ngumba *et al.*, 2016).

To correctly assess the significance of these compounds and formulate adequate pollutant control strategies within South Africa, it is a necessity to ceaselessly gather monitoring data and evaluate the hazards and risks posed by ARVDs (and other chemicals occurring in mixtures) to human health and the environment.

1.2 Problem statement

Given that ARVDs have been quantified in South African waters, continuous reports of ecotoxicity concerns, that ARVDs are continuously produced and prescribed, and that the HIV/AIDS epidemic will persist for the foreseeable future, new research questions exist that should be answered. This study therefore aims to: discuss the occurrence and dynamics of various ARVD drugs from studies conducted in South Africa waters; to discuss dynamics and perspectives of studying ARVD residues in the environment; identify pathways; to investigate spatial concentration profiles and produce distribution maps; to investigate seasonal variations, environmental factors, and trends over a specific time frame; to provide an in-depth overview of the environmental risks associated with ARVDs via risk quotients and hazard assessments; and to prioritise ARVDs and estimate priority scores for further research.

To the best of my knowledge, this overview is the first to gather all available data on ARVD environmental occurrence in South Africa and will be the most inclusive risk and hazard assessment to date. Currently, no data has been published regarding human hazard assessments of ARVDs in consumable waters from South Africa, nor has data been published on priority ARVDs that should receive regulatory attention and intervention.

The current study therefore, provides a first comprehensive assessment on the status of contamination; priority drug(s)/classes; long-term dynamic changes in response to specific environmental situations; and environmental risks incurred by pharmaceutical contaminants in freshwater ecosystems, which will greatly contribute to this particular scientific field. Ideally, this research will promote cost-effective and ethically sound methods that can be adopted across broader scientific investigations. This is important given that the greatest HIV/AIDS burden and consequence is at mainly developing countries.

Release and exposure management of possible future viral epidemic such as Covid-19 requiring intensive pharmaceutical treatment regimens will also benefit from this work. Through the implementation of cost-effective and ethically grounded ARVD surveillance, potential risks within aquatic ecosystems can be identified with greater precision. Importantly, these methods remain accessible to African nations irrespective of resource constraints, fostering broader regional collaboration. This could ultimately lead to new publications, attract future grant funding, and establish research programs dedicated to ARVD monitoring and mitigation strategies for aquatic ecosystems.

1.3 Research question/hypothesis

1.3.1 Main hypothesis:

In South Africa, ARVDs in freshwaters pose an increasing ecological and public health risk, especially in regions with intensive antiretroviral treatment rollout and inadequate environmental monitoring.

1.3.1.1 Sub-hypotheses:

- Current wastewater treatment technologies in South Africa are insufficient for removing ARVDs to environmentally safe levels.
- Absence of national monitoring guidelines results in inconsistent detection and reporting of ARVDs across provinces.
- ARVD concentrations in river water will be higher at effluent-receiving and downstream sites compared with upstream sites.
- Environmental factors such as temporal variation affect ARVD concentrations in South African waters.
- ARVD concentrations vary significantly across different sites and river systems, showing spatial heterogeneity.
- Seasonal variations in ARVD concentrations are influenced by hydrological processes.
- ARVDs in South African freshwaters pose environmental risks to aquatic ecosystems.
- ARVD mixtures exhibit combined ecotoxicological effects as predicted through a hybrid concentration addition-response addition (CA-RA) model.
- ARVDs in South African freshwaters pose a measurable non-carcinogenic health hazard to different sub-populations.
- Certain sub-populations are at greater toxic-potential from exposure to ARVDs in water sources.
- Mixtures of ARVDs may pose cumulative, unregulated non-carcinogenic hazards.
- ARVDs of greatest concern can be prioritised using a weighted multi-criteria model incorporating both human health hazard and ecological risk indicators.

1.3.2 Aims

Aim 1: To review and contribute to critical, region-specific data on the occurrence and environmental behaviour of ARVDs in South African water bodies, thereby filling existing gaps in global ARVD pollution research from underrepresented contexts.

Aim 2: To investigate the seasonal trends of selected ARVDs in four urban-influenced rivers in South Africa. To utilise geospatial analytical techniques to identify and map ARVD contamination hotspots across four river systems (Jukskei, Hennops, Klip and Crocodile rivers).

Aim 3: To assess the ecotoxicological risks posed by ARVDs in South African freshwater systems. To identify the most hazardous ARVDs and develop a prioritisation index to guide future risk mitigation efforts.

Aim 4: To conduct a human health hazard assessment for selected ARVDs in various environmental water sources, including surface water, wastewater, and drinking water.

Aim 5: To provide a rudimentary priority control list to identify and rank ARVDs of greatest concern for guided monitoring and regulatory intervention.

1.3.3 Objectives:

Objective of aim 1:

- To compile and critically review available data on the concentrations of ARVDs detected in various South African water matrices (wastewater, surface water, groundwater, drinking water).
- To identify and discuss the challenges and perspectives of studying ARVD residues in the environment.
- To establish baseline data, identify recurrent drug classes, occurrence, dynamics, and coverage in published articles.
- To review the adequacy of current water treatment technologies and removal efficiencies concerning ARVDs in a developing country context.
- To provide information that can aid in the development of national risk assessments, regulatory guidelines, and water treatment strategies in South Africa and similar regions.
- To identify priority ARVDs for future ecological risk assessment and mitigation efforts based on their environmental persistence, ecotoxicological potential, and frequency of detection.

Objective of aim 2:

- To quantify the concentrations of selected ARVDs in surface water samples from multiple sites along the Hennops, Jukskei, Klip, and Crocodile rivers.
- To assess potential spatial trends in ARVD contamination and to identify pollution hotspots linked to urban or wastewater inputs.
- To investigate the seasonal variation of ARVD concentrations and composition profiles by analysing data collected during different months (March, April, May, August, and October).
- To apply inverse distance weighting methods to create spatial concentration profiles and distribution maps of ARVDs across various sample sites and temporal intervals.

Objective of aim 3:

- To estimate the potential environmental risk associated with individual ARVDs using the risk quotient (RQ) approach based on measured environmental concentrations (MEC) and predicted no-effect concentrations (PNEC).
- To assess the toxicity of individual ARVDs on primary producers (algae), herbivorous consumers (crustaceans), and first-level carnivorous consumers (fish) using available acute and chronic toxicity data.
- To investigate the combined ecotoxicological effects of ARVD mixtures on aquatic ecosystems by evaluating the combined RQ values for various drug classes using a hybrid CA-RA model.
- To calculate the prioritisation index for each ARVD, considering both the highest RQ values and the frequency of exceedance of PNEC values at sampling sites.
- To rank the ARVDs according to their potential environmental risks, using the prioritisation index to highlight those that pose the greatest threat to aquatic ecosystems.

Objective of aim 4:

- To determine the acceptable daily dose for the ARVDs in question by applying uncertainty factors to account for variability in human sensitivity, data gaps, and extrapolation from animal studies to humans.
- To estimate the acceptable daily intake and chronic daily intake of ARVDs for different population groups, including vulnerable populations such as children and pregnant women, through ingestion of contaminated water.
- To conduct a human health hazard assessment.
- To calculate the hazard quotient (HQ) for each ARVD by two different approaches to assess potential non-carcinogenic health risks.
- To compare the outcomes of both hazard assessment approaches.
- To calculate the total effective non-carcinogenic hazard posed by the chemical mixture of ARVDs.
- To evaluate the cumulative health hazards by considering the additive effects of multiple ARVDs in the water and their potential interactions.
- To calculate the proportion of exposure scenarios where the estimated hazard exceeds acceptable safe limits.

Objectives of aim 5:

- To develop and apply an integrated multi-criteria model to prioritise ARVDs in aquatic environments based on combined ecological risk and human health hazard indicators.
- Develop and implement a weighted, normalised multi-criteria model that integrates multiple exposure and hazard indicators to derive a final priority score for each compound.
- To identify ARVDs of greatest threat based on their frequency of detection, environmental concentrations, toxicity profiles, and cumulative mixture effects.

1.4 References

- Abafe, O.A., Späth, J., Fick, J., Jansson, S., Stark, A., Pietruschka, B., and Martincigh, B.S. 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, 200:660–670.
- Dong, Z., Senn, D.B., Moran, R.E. and Shine, J.P. 2013. Prioritizing environmental risk of prescription pharmaceuticals. *Regulatory Toxicology and Pharmacology*, 65(1):60–67.
- Ferrer, I., Zweigenbaum, J.A. and Thurman, E.M. 2010. Analysis of 70 Environmental Protection Agency priority pharmaceuticals in water by EPA Method 1694. *Journal of Chromatography A*, 121(36):5674–5686.
- Gani, K.M., Hlongwa, N., Abunama, T., Kumari, S. and Bux, F. 2021. Emerging contaminants in South African water environment-A critical review of their occurrence, sources and ecotoxicological risks. *Chemosphere*, 269:128737.
- Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., Van der Ploeg, M., ... Ritsema, C.J. 2015. Emerging pollutants in the environment: a challenge for water resource management. *International Soil and Water Conservation Research*, 3(1):57–65.
- Horn, S., Vogt, T., Gerber, E., Vogt, B., Bouwman, H. and Pieters, R. 2022. HIV-antiretrovirals in river water from Gauteng, South Africa: Mixed messages of wastewater inflows as source. *Science of The Total Environment*, 806:150346.
- HSRC (Human Sciences Research Council). 2023. *Sixth South African National HIV Prevalence, Incidence, and Behaviour Survey (SABSSM VI) – Summary Sheet 2023*. [Online]. <https://sahivsoc.org/Files/SABSSMVI-SUMMARY-SHEET-2023.pdf> Date of access: 31 Mar. 2025.
- Khan, F. 2017. *Methods in chemical process safety*. Volume 1. London: Academic Press.
- Masenyetse, L.J., Manda, S.O., and Mwambi, H.G. 2015. An assessment of adverse drug reactions among HIV positive patients receiving antiretroviral treatment in South Africa. *AIDS Research and Therapy*, 12(1):6.

Mehta, U.C. 2011. Pharmacovigilance: the devastating consequences of not thinking about adverse drug reactions: the burden of adverse drug reactions (ADRs) on patient care has been found to be high globally and is particularly high in South Africa. *Continuing Medical Education*, 29(6).

Mhuka, V., Dube, S., and Nindi, M.M. 2020. Occurrence of pharmaceutical and personal care products (PPCPs) in wastewater and receiving waters in South Africa using LC-Orbitrap™ MS. *Emerging Contaminants*, 6:250–258.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., and Halm-Lemeille, M.P. 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. *Environmental Science and Pollution Research*, 23:4992–5001.

Mlunguza, N.Y., Ncube, S., Mahlambi, P.N., Chimuka, L., and Madikizela, L.M. 2020. Determination of selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre liquid phase microextraction and liquid chromatography-tandem mass spectrometry. *Journal of Hazardous Materials*, 382:121067.

Mosekiemang, T.T., Stander, M.A., and de Villiers, A. 2019. Simultaneous quantification of commonly prescribed antiretroviral drugs and their selected metabolites in aqueous environmental samples by direct injection and solid phase extraction liquid chromatography - tandem mass spectrometry. *Chemosphere*, 220:983–992.

Mtolo, S.P., Mahlambi, P.N., and Madikizela, L.M. 2019. Synthesis and application of a molecularly imprinted polymer in selective solid-phase extraction of efavirenz from water. *Water Science and Technology*, 79(2):356–365.

Nantaba, F., Wasswa, J., Kylin, H., Bouwman, H., Palm, W.U. and Kümmerer, K. 2024. Spatial trends and ecotoxic risk assessment of selected pharmaceuticals in sediments from Lake Victoria, Uganda, East Africa. *Science of The Total Environment*, 906:167348.

Ncube, S., Madikizela, L.M., Chimuka, L., and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. *Water Research*, 145:231–247.

Ngumba, E., Gachanja, A., and Tuhkanen, T. 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of the Total Environment*, 539:206–213.

Nibamureke, U.M.C., Barnhoorn, I.E.J., and Wagenaar, G.M. 2019. Nevirapine in African surface waters induces liver histopathology in *Oreochromis mossambicus*: A laboratory exposure study. *African Journal of Aquatic Science*, 44(1):77–88.

Omotola, E.O., Genthe, B., Ndlela, L. and Olatunji, O.S. 2021. Environmental Risk Characterization of an Antiretroviral (ARV) Lamivudine in Ecosystems. *International Journal of Environmental Research and Public Health*, 18(16):8358.

Rimayi, C., Odusanya, D., Weiss, J.M.J.M., de Boer, J., Chimuka, L. 2018. Contaminants of Emerging Concern in the Hartbeespoort Dam Catchment and the uMngeni River Estuary 2016 Pollution Incident, South Africa. *Science of the Total Environment*, 627:1008–1017.

Robson, L., Barnhoorn, I.E.J., and Wagenaar, G.M. 2017. The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56:225–232.

Rodríguez-Gil, J.L., Cáceres, N., Dafouz, R. and Valcárcel, Y. 2018. Caffeine and paraxanthine in aquatic systems: Global exposure distributions and probabilistic risk assessment. *Science of the Total Environment*, 612:1058–1071.

Russo, D., Siciliano, A., Guida, M., Andreozzi, R., Reis, N.M., Puma, G.L., and Marotta, R. 2018. Removal of antiretroviral drugs stavudine and zidovudine in water under UV254 and UV254/H₂O₂ processes: Quantum yields, kinetics and ecotoxicology assessment. *Journal of Hazardous Materials*, 349:195–204.

Schoeman, C., Mashiane, M., Dlamini, M., and Olomkwo, O.J. 2015. Quantification of selected antiretroviral drugs in wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography and Separation Techniques*, 6:272.

Schoeman, C., Dlamini, M., and Okonkwo, O.J. 2017. The impact of a wastewater treatment works in southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. *Emerging Contaminants*, 3:95–106.

Swanepoel, C., Bouwman, H., Pieters, R., and Bezuidenhout, C. 2015. *Presence, concentration, and potential implications of HIV-antiretrovirals in selected water resources in South Africa*. Water Research Commission. Report No2144/1/44 [ISBN 978-1-4312-0637-7].

Simbayi, L., Zuma, K., Zungu, N., Moyo, S., Marinda, E., Jooste, S., ... Mhlabane, N. 2019. *South African national HIV prevalence, incidence, behaviour and communication survey, 2017: towards achieving the UNAIDS 90-90-90 targets*. Cape Town: HSRC Press.

UNAIDS. Joint United Nations Programme on HIV/AIDS. 2017. *UNAIDS data 2017*. Geneva, Switzerland: UNAIDS. [Online].
http://www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf Date of access: 30 Jul. 2025.

Wood, T.P., Duvenage, C.S.J., Rohwer, E. 2015. The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution*, 199:235–243.

Xolo, T. and Mahlabi, P. 2024. Molecularly imprinted polymers as solid-phase and dispersive solid-phase extraction sorbents in the extraction of antiretroviral drugs in water: adsorption, selectivity and reusability studies. *Journal of Analytical Science and Technology*, 15(1):11.

CHAPTER 2 ANTIRETROVIRAL DRUGS IN SOUTH AFRICAN WATERS: A SYSTEMATIC AND CRITICAL REVIEW OF ENVIRONMENTAL DISTRIBUTION AND OCCURRENCE

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Abstract

The extensive use of antiretroviral drugs (ARVDs) as part of South Africa's comprehensive human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) treatment programme has drawn increasing concern regarding their presence in the aquatic environments. This review critically examines the occurrence, distribution, and implications of ARVD residues in South African waters, encompassing surface-, drinking-, ground-, and wastewater. A systematic analysis of 24 peer-reviewed studies published between 2015 and 2024 identified 19 ARVDs across various water systems, with nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) being the most frequently quantified drug classes. Ten ARVDs have been detected in drinking water (0.1 ng/L–26.9 µg/L), six in groundwater (0.3–13 ng/L), 13 in surface water (0.12 ng/L–102 µg/L), and 15 in wastewater (0.3 ng/L–814 µg/L). Nevirapine (NVP) and efavirenz (EFV) were the most frequently detected, with abacavir (ABC) exhibiting the highest reported concentrations (814 µg/L in influent). Quantitative analyses confirmed considerable variability in ARVD concentrations across water sources, likely influenced by drug solubility, prescription patterns, and insufficient removal efficiencies of wastewater treatment plants (WWTPs). However, background concentrations of ARVDs at certain upstream sites of WWTP discharge points were so high that it appeared as if the WWTPs had no influence on the ARVD concentrations at respective downstream sites. This review emphasises the inadequate removal capacity of current treatment technologies and the absence of standardised national monitoring frameworks, necessitating urgent research to address the gap. Disparities in analytical sensitivity, precision and reporting practices also hinder comparability across studies. This review advocates for improved monitoring, wastewater treatment technologies, and additional research on the environmental fate and presence of ARVDs in developing

countries. The findings contribute to understanding pharmaceutical pollution dynamics in high HIV burden settings and offer important implications for public health, water quality, and environmental management in South Africa.

Keywords: antiretroviral drugs; South Africa; wastewater treatment plants; pharmaceutical contaminants; AIDS.

2.1 A review of antiretroviral drugs in South Africa

Pharmacologically active substances present in aquatic environments are mostly derived from prescription and over-the-counter medications used for treatment of various human illnesses. Advances in analytical instruments and techniques make it possible to detect trace levels of various pharmaceuticals and their metabolites in wastewater, rivers, lakes, and groundwater all over the globe (Arumugam *et al.*, 2025; Nantaba *et al.*, 2020). One example of a pharmaceutical class of compounds detected in environmental water is ARVDs. These drugs are used in the treatment of people who contracted human immunodeficiency virus (HIV) and subsequent acquired immunodeficiency syndrome (AIDS) (Table 2-1) (DoH, 2019; Nel *et al.*, 2023). Numerous studies have detected ARVDs in aquatic environments around the world (Table 2-2).

The mobility, persistence, and potential bioaccumulation of these drugs in water systems are influenced by their unique physiochemical properties, including partition coefficients, solubility, and degradation rates. Detailed characteristics of key ARVDs are presented in Table 2-3. The Southern African region represents the documented core of the global HIV pandemic with South Africa as its epicentre, worldwide accounting for 55% of the 38 million people living with HIV (PLWH), 43% of all new HIV infections, and 43% of all AIDS-related deaths (Ekholuenetale *et al.*, 2022, UNAIDS, 2025).

Antiretroviral treatment (ART) has significantly decreased HIV-related morbidity and mortality in many African countries (Trickey *et al.*, 2024). In South Africa, an estimated 7.1 million adults and children are currently receiving ART in 2022 (UNAIDS, 2025). The success of South Africa's ART program (recognised as the largest ART programme in the world) also has a dimension of potential unintended consequences of these drugs entering aquatic ecosystems, with unknown effects on biodiversity and water quality.

The ART generally consists of a combination of ARVDs consisting of two nucleoside analogue reverse transcriptase inhibitors (NRTIs), with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitors (PIs) or three NRTIs (Yeni *et al.*, 2002). To keep up to

date of the World Health Organisation's (WHO) changing guidelines, South Africa's ART services have dramatically expanded in recent years (Table 2-2). Since 2016, all people with a positive diagnosis have been eligible for treatment, irrespective of their clinical progression stage (previously only people with Clinical Stage 3 infection received ART).

Consequently, the number of people eligible for treatment more than doubled from 3.39 million in 2015 to 7.7 million in 2018 (UNAIDS, 2020). Based on the known value of 7.7 million PLWH and the UNAIDS 90-90-90 goal (UNAIDS, 2020), 6.93 million people were expected to be on prolonged ART in 2020. This unique scale not only emphasises the success in addressing HIV/AIDS but also creates an unparalleled pharmaceutical load in its aquatic systems.

Despite South Africa's battle with HIV/AIDS since the 1990s, research into the presence of ARVDs in the environment is scarce, with no known publications prior to 2015 (Table 2-4). Information on ARVDs in South African environmental and drinking water remains sparse, as it is in many developing countries. These studies report data only in a few regions within the country.

Unlike some developed countries, South Africa lacks a national monitoring program for pharmaceuticals in water. To date, no national monitoring programme for ARVDs (or any other pharmaceutical) in South Africa's natural environment has been established. Although limited in scope, existing studies offer valuable insights into the presence and distribution of specific drug classes and compounds in waters.

Table 2-1: Drug classes of antiretrovirals prescribed in South African for combating human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS).

Class	ARVD	Trade name ^b	Abbrevia- tion	CAS #	2005 ^b	2008 ^c	2010 ^d	2014 ^e	2015 ^f	2017 ^g	2019 ^h	2020 ⁱ	2023 ^j
Nucleoside /nucleotide reverse transcriptase inhibitors (NRTIs)	Abacavir	Ziagen®	ABC	136470-78-5	X	X	-	X	X	X	X	X	X
	Didanosine	Videx EC®	ddl	69655-05-6	X	X	X	X	X	X	-	-	-
	Emtricitabine	Emtriva®	FTC	143491-57-0	-	X	X	X	X	X	X	X	-
	Lamivudine	Epivir®	3TC	134978-17-4	X	X	X	X	X	X	X	X	X
	Stavudine	Zerit®	d4T	3056-17-5	X	X	X	X	X	X	-	-	-
	Tenofovir	Viread®	TDF	206184-49-8	X	X	X	X	X	X	X	X	X
	Zalcitabine	Hivid®	DDC	7481-89-2	X	-	-	-	-	-	-	-	-
	Zidovudine	Retrovir®	ZDV	30516-87-1	X	X	X	X	X	X	X	X	X
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Efavirenz	Sustiva®	EFV	154598-52-4	X	X	X	X	X	X	X	X	X
	Entravine	Intelence®	ETR	269055-15-4	-	-	-	X	X	X	-	X	-
	Nevirapine	Viramune®	NVP	129618-40-2	X	X	X	X	X	X	X	X	X
	Rilpivirime	Edurant®	RPV	500287-72-9	-	-	-	X	-	X	-	X	-
Protease inhibitors	Atazanavir	Reyataz®	ATV	198904-31-3	X	X	X	X	X	X	X	X	X
	Darunavir	Prezista®	DRV	206361-99-1	-	-	-	X	-	X	X	X	X
	Fosamprenavir	Lexiva®	FPV	226700-79-4	-	X	-	-	-	-	-	-	-
	Indinavir	Crixivan®	IDV	150378-17-9	X	X	-	X	-	-	-	-	-
	Lopinavir	Kaletra®	LVP	192725-17-0	X	X	X	X	X	X	X	X	X
	Nelfinavir	Viracept®	NFV	159989-64-7	X	X	-	-	-	-	-	-	-
	Ritonavir	Norvir®	RTV	155213-67-5	X	X	X	X	X	X	X	X	X
	Saquinavir	Invirase®	SQV	127779-20-8	X	X	-	X	-	X	-	-	-
Integrase inhibitors	Raltegravir	Isentress®	RAL	518048-05-0	-	-	-	X	X	X	X	X	X
	Dolutegravir	Trivacy®	DTG	1051375-16-6	-	-	-	-	-	X	X	X	X
Entry and fusion inhibitors	Maraviroc	Selzentry®	MVC	376348-65-1	-	-	-	X	-	X	-	-	-

a Information obtained from <https://pubchem.ncbi.nlm.nih.gov/> and <https://www.drugbank.ca/drugs/> and <https://www.rxlist.com/script/main/hp.asp>; b SAHIVCA, 2005; c Maartens *et al.*, 2008; d DoH, 2010; e Meintjies, 2014; f

MIC, 2015; g Meintjies, 2017; h DoH, 2019; i Nel *et al.*, 2020; j Nel *et al.*, 2023.

Table 2-2: Occurrence of antiretroviral drugs (ARVDs) in wastewater from different parts of the world.

ARVD	Influent (ng/L)			Effluent (ng/L)			Removal (%)	Country	Reference
	Min	Max	Mean	Min	Max	Mean			
ABC			82			<LOQ(0.5)	>99	Germany	Prasse <i>et al.</i> 2010
			225			<LOQ(0.5)	>99	Germany	Prasse <i>et al.</i> 2010
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n/a	Germany	Boulard <i>et al.</i> 2018
			n/a	31	33		n/a	France	Aminot <i>et al.</i> , 2015
			<LOQ(20)–110			<LOQ(20)	>99	Germany	Funke <i>et al.</i> , 2016
ATZ						<LOQ(n/a)	n/a	Norway	Ferrando-Climent <i>et al.</i> , 2016
						<LOQ(n/a)	n/a	Greece	Ibáñez <i>et al.</i> 2017
						100–110	>83	Kenya	K'oreje <i>et al.</i> 2016
EFV			26–1020						
FTC	n.d.	n.d.	n.d.	<LOQ(5)	130	51	n/a	Germany	Boulard <i>et al.</i> 2018
IDV				n.d.	1.5		n/a	France	Aminot <i>et al.</i> , 2015
3TC	n/a	n/a	n/a	<LOQ(20)	58	21	n/a	Germany	Boulard <i>et al.</i> 2018
			210–720			<LOQ(100)	>76	Germany	Prasse <i>et al.</i> 2010
			30 300–60 680			19 900–31 070	>24	Kenya	K'oreje <i>et al.</i> 2016
				<LOQ(10)	5 430	3985	n/a	Kenya	Ngumba <i>et al.</i> , 2016a
			37–55		22		n/a	Finland	Ngumba <i>et al.</i> , 2016b
			270				n/a	France	Aminot <i>et al.</i> , 2015
			52–370			<LOQ(20)–48	n/a	Germany	Funke <i>et al.</i> , 2016
			n.d.			n.d.	n/a	Belgium	Vergeynst <i>et al.</i> 2015
MVC				n.d.	n.d.		n/a	France	Aminot <i>et al.</i> , 2015
NFV				n.d.	n.d.		n/a	France	Aminot <i>et al.</i> , 2015
NVP			33–3 300			1030–2 110	>11	Kenya	K'oreje <i>et al.</i> 2016
			5–22			7.2–32	n/a	Germany	Prasse <i>et al.</i> 2010
				<LOQ(12)	4 860	1357	n/a	Kenya	Ngumba <i>et al.</i> , 2016a
			13–19	45 879			n/a	Finland	Ngumba <i>et al.</i> , 2016b

RTV			110			90	25	Switzerland	Margot <i>et al.</i> 2013
SQV				n.d.	0.2		n/a	France	Aminot <i>et al.</i> , 2015
d4T			11.6–22.8			<LOQ(2.5)	>78	Germany	Prasse <i>et al.</i> 2010
	n.d.	<LOQ(13)		n.d.	<LOQ(13)		n/a	China	Peng <i>et al.</i> , 2014
ZDV			310–380			98–564	68	Germany	Prasse <i>et al.</i> 2010
			310–13 270			90–110	>99	Kenya	K'oreje <i>et al.</i> 2016
				<LOQ(122)	7 680	513	n/a	Kenya	Ngumba <i>et al.</i> , 2016a
			46–62		37		n/a	Finland	Ngumba <i>et al.</i> , 2016b
				154	191		n/a	France	Aminot <i>et al.</i> , 2015
			<LOQ(20)–390			<LOQ(20)–150	n/a	Germany	Funke <i>et al.</i> , 2016

3TC = lamivudine; ABC = abacavir; ATV = atazanavir; d4T = stavudine; EFV = efavirenz; FTC = emtricitabine; IDV = indinavir; MVC = maraviroc; n/a = not available; n.d. = not detected; NFV = nelfinavir;

RTV = ritonavir; SQV = saquinavir; ZDV = zidovudine. Values of limit of quantifications (LOQs) are given in brackets.

The sources of ARVDs in the environment are primarily municipal wastewater discharges from wastewater treatment plants (WWTPs), which include both household and hospital wastewater (Kümmerer, 2009). Various studies have confirmed the detection of ARVDs in waste, surface, groundwater, and drinking water (Table 2-4; Table 2-5) and have subsequently called for national regulating bodies to establish such consecutive monitoring and/or investigative practices (Horn *et al.*, 2022; Khulu *et al.*, 2022; Mhuka *et al.*, 2020; Mlunguza *et al.*, 2019; Vogt *et al.*, 2020; Wooding *et al.*, 2017).

Although the concentrations of ARVDs in drinking water samples in South Africa are low compared with other water sources (Table 2-5), it is disquieting that ARVDs are even present in drinking water (Swanepoel *et al.*, 2015). It is also unknown how daily exposure to potable water containing ARVD residues will affect PLWH and those who are on prolonged ART. The main explanations for the limited data are the analytical complexities in quantifying ARVDs, the ultra-trace level (ng/L) that antivirals are anticipated to be in drinking water, and the absence of distinct regulatory limits (Dévier *et al.*, 2013).

This chapter aims to address the critical gap in understanding ARVD concentrations, their variability, and their implications for water systems in a developing country context thereby enhancing the global understanding of ARVD distribution and effects in an African context.

Specifically, it seeks to:

- (1) Critically review, assess, and discuss the presence of ARVDs in South African freshwater systems.
- (2) Identify and discuss the challenges and perspectives of studying ARVD residues in the environment.
- (3) Evaluate the efficacy of current wastewater treatment technologies in removing ARVDs.
- (4) Assess the methodological thoroughness and internal validity of the studies included.
- (5) To contribute critical data from a developing country, complementing findings from regions with different ecological and pharmaceutical profiles.

Table 2-3: Physiochemical properties of antiretrovirals studied^a.

Drug class	Physicochemical properties							
	Abbreviation	Molecular formula	Molecular Weight (g/mol)	Water solubility (mg/mL)	pKa (base, acid) _{b,c}	Log K _{OW}	K _{OC}	Henry's Law constant (atm-cu m/mol at 25 °C)
<i>Nucleoside/nucleotide reverse transcriptase inhibitors</i>	ABC	C ₁₄ H ₁₈ N ₆ O	286.33	77	5.77, 15.41	1.18	N/A	1.9×10 ⁻¹⁶
	FTC	C ₈ H ₁₀ FN ₃ O ₃ S	247.25	112	2.65	-0.43	55	1.08×10 ⁻¹⁷
	3TC	C ₈ H ₁₁ N ₃ O ₃ S	229.26	70	4.30, 14.29	-1.44	N/A	1.9×10 ⁻²²
	ZDV	C ₁₀ H ₁₃ N ₅ O ₄	267.24	20.1	9.72	0.05	N/A	2.0×10 ⁻²²
	ddl	C ₁₀ H ₁₂ N ₄ O ₃	236.23	15.8	9.13	-1.24	N/A	2.4×10 ⁻²⁰
	TDF	C ₉ H ₁₄ N ₅ O ₄ P	287.21	13.4	4.13, 18.59	1.42	N/A	5.0×10 ⁻¹⁰
	d4T	C ₁₀ H ₁₂ N ₂ O ₄	224.21	83	-3.00, 9.95	-0.72	10	2.3×10 ⁻¹⁵
	DDC	C ₉ H ₁₃ N ₃ O ₃	211.22	76.4	14.67, 0.18	-1.3	NA	1.2×10 ⁻¹⁶
<i>Non-nucleoside reverse transcriptase inhibitors</i>	EFV	C ₁₄ H ₉ ClF ₃ NO ₂	315.68	8.55 ×10 ⁻³	-1.50, 12.52	4.69	17 × 10 ³	7×10 ⁻⁹
	NVP	C ₁₅ H ₁₄ N ₄ O	266.3	0.705	2.80	2.5	N/A	3.3×10 ⁻¹⁷
<i>Protease inhibitors</i>	IDV	C ₃₆ H ₄₇ N ₅ O ₄	613.79	0.015	7.37, 13.19	2.86	N/A	1.3×10 ⁻¹¹
	SQV	C ₃₈ H ₅₀ N ₆ O ₅	670.84	0.002	8.31, 5.11	2.5	N/A	9.9×10 ⁻³⁵
	RTV	C ₃₇ H ₄₈ N ₆ O ₅ S ₂	720.95	1.1×10 ⁻⁴	2.84, 13.68	6.27	N/A	2.7×10 ⁻³³
	DRV	C ₂₇ H ₃₇ N ₃ O ₇ S	547.66	8.7×10 ⁻³	2.38, 13.59	1.88	N/A	1.39×10 ⁻¹¹
	ATV	C ₃₈ H ₅₂ N ₆ O ₇	704.86	4–5	4.42, 11.92	2.88	3.8×10 ⁷	3.6×10 ⁻³²
	NFV	C ₃₂ H ₄₅ N ₃ O ₄ S	567.78	1.91×10 ⁻³	8.18, 9.32	8.98	N/A	1.38×10 ⁻¹¹
	LPV	C ₃₇ H ₄₈ N ₄ O ₅	628.81	7.70×10 ⁻³	-1.50, 13.39	5.94	1.0×10 ⁶	4.3×10 ⁻²⁸
<i>Integrase inhibitors</i> <i>Entry and fusion inhibitors</i>	RAL	C ₂₀ H ₂₁ FN ₆ O ₅	444.4	53.9	-1.50, 5.62	0.4	95	9.1×10 ⁻²³
	MVC	C ₂₉ H ₄₁ F ₂ N ₅ O	513.67	6.57×10 ⁻³	10.13, 7.02	5.8	N/A	9.49×10 ⁻¹⁵

a All information obtained from <https://www.ncbi.nlm.nih.gov/pccompound/>, <https://www.drugbank.ca/drugs/>, <https://comptox.epa.gov/dashboard/dsstoxdb/>, and <https://www.rxlist.com/script/main/hp.asp> accessed June 2020;

b The pKa values are as predicted in the DrugBank website (<https://go.drugbank.com/>).

2.2 Literature on ARVD concentrations in South Africa's environment

2.2.1 Review method

The present study was approved by the NWU Ethics Committee (NWU-00002-23-A1). The study conducted a comprehensive review of literature on ARVDs in South African water sources, guided by stringent inclusion criteria to ensure the reliability and relevance of findings. The search strategy incorporated a combination of keywords and phrases such as “antiretrovirals,” “pharmaceuticals,” “water sources,” “wastewater,” “surface water,” “antiretroviral therapy,” “emerging contaminants,” “South Africa,” and “ecotoxicology.” The literature search was conducted using the North-West University (NWU) library's database system, which aggregates content from ScienceDirect, Scopus, PubMed, and Wiley Online Library, among others. Boolean operators (AND/OR) were used to refine results.

Studies were included if they met the following criteria:

- (1) Published in English between 2015 and 2024.
- (2) Peer-reviewed or published in reputable journals.
- (3) Sampling conducted within South African borders.
- (4) Limited to freshwater environmental matrices (excluding marine or estuarine systems).
- (5) Based on original research (excluding reviews or editorials).
- (6) Focused on ARVDs prescribed for treatment in South Africa.

The review assessed the disparities in methodologies, and the interpretation of results. Table 2-5 summarises the maximum ARVD concentrations reported in South African waters across the included studies (also see Annexures A to Annexure E). Although dolutegravir, etravirine, fosamprenavir, and rilpivirine are listed in the ARVD therapy guidelines (Table 2-2), these ARVDs have yet to be investigated or reported in South African waters.

2.2.2 Quantitative data analysis

A quantitative approach was employed to analyse ARVD concentrations in South African waters. Descriptive statistics characterised central tendency and data distribution. Frequency distributions and percentages were presented graphically and tabulated. Normality was evaluated using the D'Agostino and Pearson omnibus test to guide the application of parametric (analysis of variance (ANOVA)) or non-parametric (Kruskal-Wallis) statistical analyses. Post-hoc tests (Tukey or Dunn's) identified pairwise differences between groups.

Data processing and visualisation were conducted using Microsoft Excel v. 2502 and GraphPad Prism v. 10.4.1, with statistical significance set at $p < 0.05$.

For the present study, only analyte concentrations reported as greater than the limit of quantification (LOQ) were included in the calculations. Values below the LOQ were excluded to ensure that all data used in the analysis met the minimum threshold for quantifiable accuracy and reliability. This approach was chosen to avoid potential inaccuracies associated with estimating concentrations below the LOQ and to ensure the robustness of the statistical analyses. While this may lead to some underrepresentation of trace-level contaminants, the focus remained on reliably quantifiable data for comparative and risk assessment purposes.

2.3 The presence of antiretrovirals in South African waters

A total of 24 studies met the literature review criteria out of the initial 54 studies identified during the search. This number illustrates the limited but focused body of work addressing ARVD contamination in South African water sources. Nineteen out of the 23 prescribed ARVDs mentioned in (Table 2-1) were reported. The maximum of each successfully quantified ARVD ($> \text{LOQ}$) at a specific sampling point was included from 579 water samples in total in all the publications.

Specifically, 29 drinking-, 242 surface-, 14 groundwater-, 144 WWTP influent, and 150 WWTP effluent water samples. The most prevalent drug class based on the number of analytes quantified in the various samples was NRTIs at 43% ($n = 25$), followed by PIs at 33% ($n = 19$), NNRTIs at 17% ($n = 10$), integrase inhibitors (II) at 3% ($n = 2$), and entry and fusion inhibitors (EFI) at 3% ($n = 2$) (Figure 2-1). The prevalence of NRTIs comes as no surprise as these drugs form the backbone of ART and are generally used in combination with NNRTIs and PIs to effectively suppress HIV replication. The data (Figure 2-1 a) reflect the basic ART regimen.

Two drug classes, II and EFI, were only quantified in wastewater samples (Figure 2-1 b), possibly due to the manner in which II and EFI are used and eliminated from the body, the methods used in analysis, and/or the WWTP processes involved. For example, the renal clearance of unchanged raltegravir (RAL) is only a minor pathway of elimination (9% of the total dose) (Drugbank, 2023). Maraviroc (MVC) also has a higher clearance in faeces (approximately 76% recovery) (PubChem, 2023) than in urine; these ARVDs are not readily detected in surface or groundwater even though they are eliminated from the body.

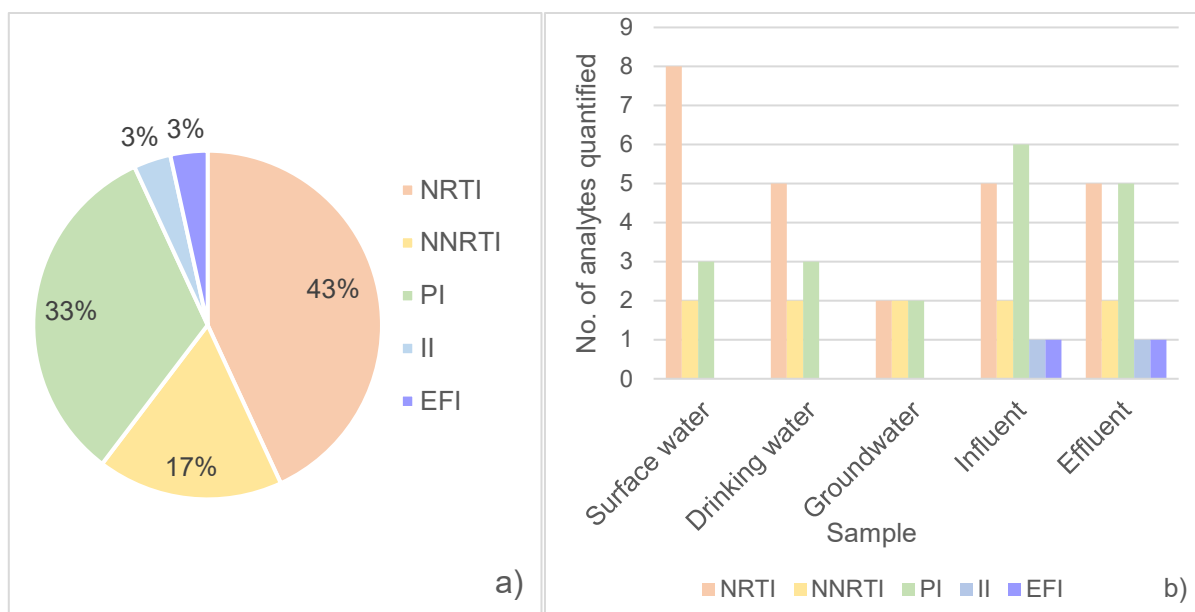


Figure 2-1: a) Percentage of antiretroviral drug classes; b) number of analytes detected in water samples from South Africa. (EFI = entry/fusion inhibitor; II = integrase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor).

The most frequently documented ARVD in any water sample was nevirapine (NVP) ($n = 130$), followed by efavirenz (EFV) ($n = 119$), zidovudine (ZDV) ($n = 58$), and LVP ($n = 50$) (Table 2-4). The least frequently documented ARVDs were saquinavir (SQV), zalcitabine (DDC), and nelfinavir (NFV) ($n < 3$). Apart from NFV, the data from all drugs were considered to have a high degree of dispersion based on the coefficient of variation results (66–688%).

Normality testing (D'Agostino-Pearson) indicated non-normal data distribution ($p < 0.05$) warranting non-parametric analysis. The Kruskal-Wallis test revealed significant differences between ARVD groups ($p < 0.0001$), and Dunn's post-hoc analysis confirmed pairwise group-level differences ($p < 0.05$).

Although only two NNRT analytes has been quantified in South African waters, this drug class accounted for the majority of ARVD detections. Efavirenz (14.5 ± 25.8 ug/L) also had the third highest mean concentrations after FTC (18 ± 36.4) in all water samples. Despite being the most recurrent drug, NVP had a seemingly low mean concentration in all samples (7 ± 25.1 ug/L).

Seldom quantified drugs such as darunavir (DRV), lamivudine (3TC), and tenofovir (TDF) had comparatively high mean concentrations in all water samples (7.8–12 ug/L). An NRTI, abacavir (ABC), was found to have the greatest mean analyte concentration of any sample

(184±254 ug/L) with a remarkably large standard deviation suggesting substantial variability across sampling points. These variations in mean concentrations and detection frequencies (e.g., NVP vs. ABC) might reflect the influence of the human metabolism, ART prescription patterns, environmental factors, and wastewater processes. Such variation highlights the requisite for drug-specific environmental behaviour studies (Table 2-4; Table 2-5).

The high detection of NNRTIs and NRTIs could be due to the great number of prescribed ARVDs in fixed-dose combinations (FDC) which includes TDF + 3TC/FTC + EFV/NVP as a first-line regimen, TDF + 3TC/FTC + dolutegravir (DTG) and ZDV/TDF + 3TC/FTC + lopinavir/ritonavir (LPV/r) or atazanavir/ritonavir (ATV/r), or DTG as a second-line regimen (2019 ART Guideline). The high detection for NVP in the environment, according to Madikizela *et al.* (2017) and Schoeman *et al.* (2015), could be due to its weak removal efficiency during treatment processes and/or its wide use to treat HIV.

The WHO (2019) and the South African Department of Health (DoH) have recommended EFV as the preferred NNRTI to NVP since 2014 (Wood and Fox, 2013). Nevirapine is no longer recommended as a first-line ART regimen in South Africa due to its potential for severe liver toxicity (SAHIVCS, 2023). Therefore, EFV (600 mg once daily) is more commonly used as part of the first-line treatment regimen rather than NVP (200 mg once daily). These changes in ART regimen and discrepancies in prescribed dosages could explain why NVP had high detection frequencies, but a comparatively low mean concentration, whereas EFV had both high detections frequencies and a high mean concentration.

Urinary excretion rates of EFV (14–34%) are higher than those of NVP (< 3% of the initial dose), which would lead to higher concentrations of EFV entering the WWTP (Schoeman *et al.*, 2017). The collective data support this statement, given that mean EFV concentrations (23.6±37.5 ug/L) entering WWTPs were higher than those of NVP (0.7±0.8 ug/L).

Emtricitabine was quantified at high concentrations, especially in wastewater samples. Potentially due to the drug being regularly prescribed at 200mg (FDC tablet) in the NNRTI-based (TDF + 3TC/FTC + EFV/NVP), Integrase strand transfer inhibitor (InSTI) -based (TDF + 3TC/FTC + DTG) and/or PI-based (ZDV/TDF + 3TC/FTC + LPV/r or ATV/r or DTG) regimens (DoH, 2019).

From Table 2-3, FTC was noted to have the highest water solubility of all investigated analytes (112 mg/mL). Due to FTC's high water solubility properties, it is more likely to be present in wastewater samples at significant concentrations. Especially since FTC is prescribed to a large number of PLWH and 86% is excreted via urine.

Lopinavir had the third highest mean concentration of all samples (14.47 ± 16.1 ug/L). Lopinavir/ritonavir (LPV/r) is often prescribed as a boosted PI at large dosage, for example, 400/100mg 12-hourly or 800/200mg daily. Boosting PIs involves the addition of low-dose RTV, which inhibits PI metabolism. This in turn boosts PI plasma concentration and prolongs the half-life (Meintjies, 2014). Interestingly, the data roughly reflects this prescription dosage (LPV/r), as both LPV and RTV were regularly detected, but LPV had mean concentrations roughly six times greater than RTV.

Abacavir is one of the most potent NRTIs used to treat HIV (Nannou *et al.*, 2020). Following oral administration, it is extensively metabolised (83% bioavailability) meaning that a large percentage of the administered dose undergoes metabolic transformation in the body before being excreted. Subsequently, ABC metabolites are expected to dominate in waste and surface water, with minimal presence from the parent compound. It is therefore striking that such exceptionally high concentrations of ABC were observed (278–814 $\mu\text{g/L}$) (Table 2-5).

In South Africa, data on ABC's occurrence in water systems remain limited, with only four authors reporting on it (Abafe *et al.*, 2018; Ngwenya and Mahlambi, 2023; Späth *et al.*, 2021; Xolo and Mahlambi, 2024). Some international information regarding ABC is available in Germany (Boulard *et al.*, 2018; Funke *et al.*, 2016; Prasse *et al.*, 2010), South Korea (McCurry *et al.*, 2014) and France (Aminot *et al.*, 2015) (Table 2-2).

Concentrations reported by Späth *et al.* (2021) aligned with Prasse *et al.* (2010), but were lower compared with those published by Abafe *et al.* (2018). Abacavir was the most prevalent ARVD, exceeding NVP and EFV concentrations in local studies by Ngwenya and Mahlambi (2023) and Xolo and Mahlambi (2024). High concentrations of ABC ranging from 278 to 814 $\mu\text{g/L}$ were observed in influents of WWTPs in KwaZulu-Natal.

It should be noted that NNRTIs and PIs are usually very soluble. Based on their solubility (Table 2-3), these ARVDs are expected to readily dissolve and move with water as it travels into the environment. They are also expected to be transported downstream and through soil, which increases their probability of reaching groundwater (Savjani *et al.*, 2012). It is therefore

not surprising that these drug classes were found in drinking- and groundwater samples (Figure 2-1 b).

Table 2-4: Summary of all 19 antiretroviral drugs (ARVDs) successfully quantified in all South African waters. Drug class, and detection frequency, and concentration ranges are provided.

ARDV	Class	No. of detections ^a	No. of studies	Concentration		
				Mean±SD (µg/L)	Max (µg/L)	Min (ng/L)
ABC	NRTI	24	5	184±253.7	814	0.1
ATV	PI	10	3	1.04±1.35	3.5	64
ddI	NRTI	5	2	0.02±0.03	0.05	0.4
DRV	PI	8	2	11.31±14.88	43	69
EFV	NNRTI	119	16	14.5±25.8	140.4	0.3
FTC	NRTI	30	5	18.06±36.44	172	1
IDV	PI	6	1	0.21±0.22	0.59	25
3TC	NRTI	31	9	11.95±32.11	150	0.15
LVP	PI	50	5	14.47±16.05	63.69	1.3
NFV	PI	3	1	0.001±0.0	0.001	0.8
NVP	NNRTI	130	16	7.02±25.08	207.7	0.1
MVC	II	4	1	0.13±0.13	0.32	39
RAL	EFI	7	2	4.55±5.96	17	61
RTV	PI	41	4	2.3±1.53	5.14	0.02
SQV	PI	2	2	0.09±0.13	0.18	1.3
d4T	NRTI	8	2	0.16±0.29	0.78	0.3
TDF	NRTI	14	4	7.83±15.59	48.4	0.6
DDC	NRTI	3	1	0.04±0.03	0.07	8
ZDV	NRTI	58	7	3.2±11.09	66.98	0.3

a = based on the maximum concentration reported in published literature at a specific sampling point (LOQ > 0.005 µg/L); EFI = entry and fusion inhibitors ; II = integrase inhibitor ; max = maximum; min = minimum; NNRTI = Non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; SD = standard deviation.

Table 2-5: Metrix of antiretroviral compounds quantified in various South Africa waters.
Concentrations are in (µg/L).

ARVD	Max	Min	Average	STDEV	SUM	n	FD (%)
Surface water							
ABC	102	0.000	35.01	39.6	280	8	3.3
ddl	0.054	0.054	0.05	0.002	0.1	2	0.8
EFV	21.7	0.000	2.6	4.7	105	40	17
FTC	17.9	0.001	4.1	6.1	49.3	12	5
3TC	0.95	0.000	0.2	0.24	2.8	14	6
LPV	63.7	0.001	18.7	19	467.8	25	11
NFV	0.001	0.001	0.001	0.0	0.001	1	0.4
NVP	52.3	0.001	2.7	9.3	183	66	28
RTV	5.1	0.02	3.23	1.4	71	22	9
d4T	0.78	0.001	0.26	0.34	1.3	5	2
TDF	0.24	0.001	0.14	0.1	0.7	6	3
DDC	0.07	0.03	0.05	0.03	0.1	2	1
ZDV	2.74	0.000	0.74	0.67	26.6	36	15
Drinking water							
ABC	0.001	0.001	0.001	0.0	0.001	1	3
ddl	0.003	0.000	0.001	0.001	0.004	3	10
EFV	24.6	2.33	11.06	11.9	33.17	3	10
LVP	26.85	9.04	17.95	12.6	35.89	2	7
NFV	0.001	0.001	0.001	0.0	0.001	1	3
NVP	0.46	0.000	0.088	0.16	0.79	9	31
RTV	1.95	1.95	1.95	0.0	1.95	1	3
d4T	0.001	0.001	0.001	0.0	0.001	1	3
DDC	0.008	0.008	0.008	0.0	0.008	1	3
ZDV	1.18	0.002	0.42	0.4	2.92	7	24
Groundwater							
EFV	0.005	0.005	0.005	0.0	0.005	1	7
NFV	0.001	0.001	0.001	0.0	0.001	1	7
NVP	0.013	0.000	0.003	0.0	0.028	8	57
SQV	0.001	0.001	0.001	0.0	0.001	1	7
d4T	0.001	0.000	0.001	0.0	0.001	2	14
TDF	0.002	0.002	0.002	0.0	0.002	1	7
Influent							
ABC	814	0.29	337.8	309	3715	11	8
ATV	3.5	0.06	1.29	1.6	5.2	4	3
DRV	43	0.07	15.25	20.1	61	4	3
EFV	140.4	0.01	20.2	32	849.2	42	30
FTC	172	0.3	46.1	59	372	8	6
IDV	0.59	0.26	0.35	0.2	1.4	3	2
3TC	86	0.009	11.95	27	119.5	9	6
LVP	24.4	1.2	7.6	8	83.2	10	7
NVP	261	0.007	29.83	65.3	716	23	16
MVC	0.32	0.08	0.14	0.1	0.57	3	2

RAL	17	0.06	5	7.1	25	4	3
RTV	3.2	0.06	1.3	1	10.5	8	6
SQV	0.18	0.18	0.18	0.0	0.36	1	0.7
TDF	48.4	0.1	13.6	21.3	81.42	5	4
ZDV	53	1.23	21.5	24.6	129	5	4
Effluent							
ABC	178	0.57	85.5	57	684	8	6
ATV	3.5	0.08	0.87	1.3	5.2	6	4
DRV	17	0.13	7.4	8.5	29.5	4	3
EFV	103	0.52	19.8	27	733	37	27
FTC	41.7	0.11	12.4	18.6	124	10	7
IDV	0.04	0.03	0.04	0.009	0.1	3	2
3TC	150	0.01	31.3	53	251	8	6
LVP	38.5	0.13	10.7	13.5	139	13	9
NVP	207.7	0.000	24.6	52	762	31	23
MVC	0.04	0.04	0.04	0.0	0.04	1	0.7
RAL	4.1	0.09	2.56	2.2	7.7	3	2
RTV	1.95	0.053	1.2	0.6	15	10	7
TDF	25	0.002	12.5	18	25	2	1
ZDV	67	0.000	8	21	80	10	7

3TC = lamivudine; ABC = abacavir; ARVD = antiretroviral drug; ATV = atazanavir; d4T = stavudine; DDC = zalcitabine; ddl = didanosine; DRV = darunavir; EFV = efavirenz; FD = frequency of detection; FTC = emtricitabine; IDV = indinavir; LPV = lopinavir; max = maximum; min = minimum; MVC = maraviroc; n = number of samples; NFV = nelfinavir; NVP = nevirapine; RAL = raltegravir; RTV = ritonavir; std = standard; stdev = standard deviation; SQV = saquinavir; TDF = tenofovir; ZDV = zidovudine.

2.4 Diverse analytical approaches to ARVD detection and quantification

An overview of the analytical methods employed in various South African studies for the detection and quantification of ARVDs in environmental water matrices is presented in Table 2-6. Different parameters (for e.g. extraction and detection methods, analytes quantified, recovery, and matrix types) are listed that provide insight into the sensitivity, precision, and applicability of different methodologies across diverse water sources used by the authors. Although not all studies provide complete method validation parameters. The lack of standardised reporting somewhat hinders a direct comparison of method performance.

There are some clear discrepancies amongst the different studies when comparing the sensitivity across methods used. ARVDs may be present in trace concentrations (Vogt *et al.*, 2020). Therefore, methods with low limits of detection (LODs) and LOQs are more capable of reliably measuring these compounds and are critical for environmental monitoring. Across the studies (Table 2-6), LODs ranged from as low as 0.000002 µg/L (Wood *et al.*, 2015) to as high as 28 µg/L (Vogt *et al.*, 2020), reflecting considerable variation in method performance. Despite being fast and having a high degree of resolution, LC-related techniques are costly

and not easily accessible in the majority of African countries (Madikizela *et al.*, 2020). Consequently, the majority of African countries are deficient in the advanced scientific apparatus necessary for the trace analysis of emerging pollutants such as ARVDs. This limitation poses challenges for routine analyses in smaller WWTPs located in rural and less developed towns (Madikizela *et al.*, 2017; Madikizela *et al.*, 2020).

Techniques such as LC-MS/MS where the authors extracted ARVDs using solid-phase extraction (SPE) cartridges provided some of the highest sensitivity (Abafe *et al.*, 2018; Rimayi *et al.*, 2018). These techniques were the most frequently used (Abafe *et al.*, 2018; Akawa *et al.*, 2021; Mtolo *et al.*, 2019; Olabode, 2021; Sigonya *et al.*, 2022; Vogt *et al.*, 2020; Xolo and Mahlambi, 2024).

Wood *et al.* (2015) demonstrated the value of combining advanced ultra-high pressure liquid-chromatography tandem-mass-spectrometry (UHPLC-MS/MS) technology with optimised extraction methods using SPE, achieving detection limits nearly 1 000 times lower than studies like Akawa *et al.* (2021) or Mtolo *et al.* (2019). Although advanced, the sensitivity of the system used by Holton *et al.* (2022) was lower than that of Wood *et al.* (2015), perhaps due to differences in pre-treatment or optimisation of detection parameters.

Some methods incorporated high-resolution chromatographic techniques (UHPLC) with advanced mass spectrometry (Orbitrap HRMS) (Mhuka *et al.*, 2020a) to improve detection sensitivity and specificity. Xolo and Malahambi (2024) combined the benefits of mass spectrometry with photodiode array detection for enhanced selectivity and quantification. These findings underline the importance of modern instrumentation and optimised extraction methods in achieving reliable detection of ARVDs in diverse water matrices.

Regarding the precision assessment, the percentage relative standard deviation (RSD, or % coefficient of variation) represents the variation of replicate measurements expressed as a percentage of the mean (Mani *et al.*, 2012). A low %RSD ($\leq 10\%$) is generally acceptable for highly precise methods, particularly in regulated fields like pharmaceutical or environmental analysis. A higher %RSD ($> 20\%$) might indicate variability in the method, perhaps due to sample matrix heterogeneity, instrument limitations, or operator error (Boleda *et al.*, 2013; Piri-Moghadam *et al.*, 2016).

Reported %RSD values for ARVD analyses in literature vary, depending on matrix complexity and compound characteristics. Detailed comparisons of %RSD values across studies in this review, as well as recovery (%R) data and discussion of matrix effects, are provided in the supplementary material (Annexure D).

Regarding recovery values (%R), Abafe *et al.* (2018) and Horn *et al.* (2022) reported high values ranging from 24–139% and 55–196% respectively. This might indicate interference, leading to an overestimation of analytes. Overestimation increases the difficulty of comparing ARVDs concentrations across different studies and/or matrices.

Compared with other studies, Späth *et al.* (2021) reported comparatively low recoveries for certain analytes such as SQV and etravirine (0–14%). These compounds were therefore excluded from the present study. This emphasises the importance of improved analytical techniques. The majority of the remaining studies had moderate recovery values (80–120%) that reflect efficient extraction and reliable quantification. Some authors also reported low %R values ($\leq 81\%$) (Khulu *et al.*, 2022; Mtolo *et al.*, 2019; Vogt *et al.*, 2020; Wood *et al.*, 2015).

Comparisons drawn from Table 2-6 highlight how advancements in chromatographic techniques and extraction methodologies directly influence detection capabilities since the ability to detect ARVDs at sub-nanogram levels ensures that even trace contamination can be identified. While many studies quantified well-known ARVDs (EFV, NVP, FTC), there is limited exploration of newer or less common ARVDs. Expanding the analyte scope would improve monitoring comprehensiveness.

Table 2-6: Overview of the analytical methods used for quantifying antiretroviral drugs in South African waters.

Reference	LOD range (µg/L)	LOQ range (µg/L)	Detection Method	Extraction Method	ARVD analytes successfully quantified	% RSD	%R	Matrix	Province(s) in South Africa
Abafe <i>et al.</i> , 2018	0.002–0.02	0.012–0.065	LC-MS/MS	SPE–Oasis HLB cartridge	3TC, NVP, ZDV, ABC, EFV, LPV, SQV, NFV, ATV, DRV, IDV, MVC, RAL, RTV		24–139	Wastewater	KwaZulu-Natal
Akawa <i>et al.</i> , 2021	0.20–0.23	0.67–0.75	HPLC equipped with diode array detector analysis	UA–Dµ–SPE – AC@PVP@alginat	ZDV, NVP	≤ 6.8	91–99	Wastewater, surface water	Gauteng,
Holton <i>et al.</i> , 2022			LC-MS/MS	SPE–Oasis HLB cartridge	3TC, FTC	0.005–141.4		Wastewater, surface water	Western Cape
Horn <i>et al.</i> , 2022	0.01–0.58	0.05–1.94	UHPLC/MS QTOF	SPE–HLB-L disk	EFV, ZDV, LPV, NVP, RTV	5.4–32.3	55–196	Wastewater, surface water	Gauteng
Khulu <i>et al.</i> , 2022	0.12–0.24	0.39–0.81	LC-QTOF/MS	MASE-MIP	NVP	3	41	Surface water	Gauteng, KwaZulu-Natal,
Mhuka <i>et al.</i> , 2020a	0.003–8.41	0.01–30.7	LC coupled Orbitrap™ HRMS	SPE–Oasis HLB cartridge	ATV, EFV, 3TC, NVP, RTV			Wastewater, surface water	Gauteng
Mhuka <i>et al.</i> , 2020b	0.003–8.41	0.01–30.7	LC-MS/MS	SPE–Oasis HLB cartridge	ATV, EFV, 3TC, NVP, RTV			Wastewater, surface water	Gauteng
Mlunguza <i>et al.</i> , 2020	0.009–0.16	0.033–0.53	Ultra HPLC-HRMS	HF-LPME	TDF, EFV, FTC		92–111	Wastewater, surface water	KwaZulu-Natal, North West
Mosekiema ng <i>et al.</i> , 2019	0.128–16.34	0.425–37.5	LC-MS/MS	SPE–SDB-L cartridges	EFV, FTC, 3TC, NVP,	≤ 10	68–113	Wastewater, surface water	Western Cape
Mtolo <i>et al.</i> , 2019	0.41	1.39	LC-PDA system	SPE cartridge, MIP	EFV,	0.27	81	Wastewater, surface water	KwaZulu-Natal,
Netshithothole <i>et al.</i> , 2024	1.2–1.8	4.1–6.2	HPLC	SPE–Oasis HLB cartridge	EFV	0.25–3.9		Wastewater	Eastern Cape
Ngwenya and	0.68–0.77	2.1–2.4	UE and SE followed by LC-	SPE–Oasis HLB cartridge	ABC, EFV, NVP	0.73–11.2	61–108	Wastewater, surface water	KwaZulu-Natal,

Mahlambi, 2023			PDA						
Olabode, 2021	0.068–0.76	0.186–2.3	HPLC	Phenomenex SPE cartridges	3TC, NVP, ZDV	0.18–0.5	99.8–102.3	Wastewater	Western Cape
Rimayi <i>et al.</i> , 2018	0.010–0.0001 25	0.0001 3–0.0007	LC-MS/MS	SPE–Oasis HLB cartridge	EFV, FTC, NVP, 3TC			Surface water, groundwater	North West, Gauteng, KwaZulu-Natal,
Robson <i>et al.</i> , 2017			LC-MS/MS		EFV,			Surface water	Limpopo
Schoeman <i>et al.</i> , 2015	0.0018–0.0078	0.006–0.026	GC-TOFMS	SPE cartridge	EFV, NVP	3.5–10.2	106–109	Wastewater	Gauteng
Schoeman <i>et al.</i> , 2017	0.0018–0.0078	0.006–0.026	HT TOFMS	SPE–Bond Elute sodium sulphate cartridges	EFV, NVP	3.5–10.2	106–109	Wastewater	Gauteng
Sigonya <i>et al.</i> , 2022	0.075–0.48	0.23–1.45	HPLC equipped with PDA (HPLC-PDA)	SPE–Bond Elut Plexa cartridges	EFV, FTC, TDF	0.56–4.7	62–104	Wastewater, surface water	KwaZulu-Natal,
Späth <i>et al.</i> , 2021		0.002–1	UHPLC	SPE–Oasis HLB cartridge	ABC, ATV, DRV, 3TC, NVP, RAL	4.0–16	90–110	Wastewater	KwaZulu-Natal
Swanepoel <i>et al.</i> , 2015		0.02–1.0	HPLC-QTOF/MS	SPE–DEX	D4t, 3TC, ddl, NVP, ZDV, ABC, TFV, EFV, LPV, SQV, NFV	7.0–29	88–108	Surface water, drinking water, wastewater, groundwater	Free State, Eastern Cape, North West, Gauteng, KwaZulu-Natal
Vogt <i>et al.</i> , 2020	28–1161	93–3871	UHPLC/MS QTOF	SPE–Oasis HLB cartridge	NVP, ZDV, EFV, LPV, NFV, RTV		55–70	Surface water, drinking water, wastewater, groundwater	Gauteng

Wood <i>et al.</i> , 2015	0.0000 02– 0.014	0.0000 4– 0.018	UHPLC-MS/MS	SPE–Oasis HLB cartridge	ZDV, NVP, LPV, TDF, ddl, d4T, DDC, 3TC		57–74	Wastewater, surface water	Free State, North West, Gauteng, Limpopo, Western Cape, Northern Cape
Wooding <i>et al.</i> , 2017	0.044	0.148	UPLC-MS/MS & GC x GC-TOFMS	TD-GC×GC- TOFMS and LVI-UHPLC- MS/MS	NVP, EFV	12	112	Surface water	Gauteng, Limpopo
Xolo and Mahlambi, 2024	0.70– 0.91	1.87– 2.51	Optimised SPE, UE and SE followed by LC-PDA method	SPE–Oasis HLB cartridge	ABC, EFV, NVP	1.37– 9.9	92–98	Wastewater, surface water	KwaZulu-Natal,

GC-TOFMS = Gas Chromatography-Time of Flight Mass Spectrometry; HLB = Hydrophilic-Lipophilic Balance; HF-LPME = Hollow Fibre Liquid Phase Microextraction; HPLC = High-Performance Liquid Chromatography; HRMS = High Resolution Mass Spectrometry; HT = High Throughput; LC/MS = Liquid Chromatography/Mass Spectrometry; LC-PDA-MS = Liquid Chromatography Coupled with Photodiode Array and Mass Spectrometry; LVI-UHPLC-MS/MS = Large Volume Injection Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry; MASE-MIP = Membrane Assisted Solvent Extraction and Molecularly Imprinted Polymer; MS = Mass Spectrometry; PDA = Photodiode Array Detector; QTOF = Quadrupole-Time-Of Flight; RSD = Relative Standard Deviation; SDB-L = Styrene-Divinylbenzene; SE = Soxhlet Extraction; SPE = Solid-Phase Extraction; TD-GC×GC-TOFMS = Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry; TOFMS = Time-of-Flight Mass Spectrometry; UA-Dμ-SPE –AC@PVP@alginat = Ultrasound-Assisted Dispersive Micro-Solid Phase Extraction Using Activated Carbon Coated With Polyvinylpyrrolidone (PVP) and Alginate UE = ultrasonic extraction; UHPLC-MS/MS = Ultra-High Pressure Liquid Chromatography Tandem Mass Spectrometry; UPLC-MS/MS = Ultra-Performance Liquid Chromatograph Tandem Mass Spectrometer

2.4.1 Occurrence in wastewater

Wastewater treatment plants play a critical role in managing pharmaceutical contaminants (Nantaba *et al.*, 2020), including ARVDs, which have been increasingly detected in wastewater influent and effluent due to population growth and expanded ART programs. It can therefore be assumed that a region with a high population density, with increased numbers of PLWH, will have a greater risk of ARVD contamination of water. Gani *et al.* (2020), concurred that ARVD consumption might not be uniform in all regions of South Africa. Antiretroviral drugs were reported across five of South Africa's nine provinces in wastewaters (see Table 2-6, Table 2-7, and Figure 7-1 in Annexure E).

Regions with high population densities, such as the provinces of Gauteng (831 people/km²), KwaZulu-Natal (132 people/km²), and the Western Cape (57 people/km²) (albeit with high local variability due to urbanisation), are particularly at risk of ARVD contamination (Table 2-7). Influent samples were predominantly collected from these provinces, with limited collected in Limpopo (46 people/km²) and Eastern Cape (43 people/km²) (Table 2-7). Moreover, these provinces experienced meaningful population growth with increases ranging from 22% to 28% between 2011 and 2022. The most recurrent ARVDs detected in influent were EFV (n = 42), NVP (n = 23), and LVP (n = 10) (Table 2-5).

Table 2-7: Provincial population distribution in South Africa according to the 2022 census (Stats SA, 2022) as well as the total number of antiretroviral drug studies reported in wastewater per province.

Province	Population	% Increase (2011–2022)	Area (km ²)	Density (people/km ²)	Number of ARVD studies
Western Cape	7 433 019	27.7	129 462	57	3
Eastern Cape	7 230 204	10.2	168 966	43	1
Northern Cape	1 355 946	18.3	372 889	4	-
Free State	2 964 412	8	129 825	23	-
KwaZulu-Natal	12 423 907	21	94 361	132	9
North West	3 804 548	8.4	104 882	36	-
Gauteng	15 099 422	23	18 178	831	8
Mpumalanga	5 143 324	27.3	76 495	67	-
Limpopo	6 572 720	21.6	125 755	52	1
South Africa	62 027 503	19.8	1 221 037	51	22

- represents no current reports of ARVD in wastewater from that province.

The highest documented concentration overall was ABC at 814 µg/L, followed by NVP at 261 µg/L in WWTP influent from KwaZulu-Natal (Ngwenya and Mahlambi, 2023) (Table 2-5). Emtricitabine also had high reported concentrations (172 µg/L) in WWTP influent in the Western Cape (Mosekiemang *et al.*, 2019). Similar to influent, effluent samples were collected from the same provinces, namely KwaZulu-Natal, Gauteng, and the Western Cape. The most frequently documented ARVDs in effluent samples were EFV (n = 37), NVP (n = 31), and LVP (n = 13). The highest effluent concentration documented was 207 µg/L for NVP in the Khayelitsha informal settlement, Western Cape (Olabode, 2021). Followed by ABC (178 µg/L) at the Umbilop WWTP in KwaZulu-Natal, (Xolo and Mahlambi, 2024).

Wastewater analysis yielded complementary results with similar ARVDs noted as the most recurrent and highest detected ARVDs in both effluent and influent. Nearly all ARVDs detected in influent were also found in effluent, although most at reduced concentrations. This supports the notion that current WWTPs do not achieve complete removal of these pharmaceuticals (Nantaba *et al.*, 2021). Upon comparing the data for influent and effluent samples (Figure 2-2 a–b), a similar mean pollutant profile was observed. A broad concentration range was evident for several drugs in both datasets (< 1 to > 100 µg/L). Drugs such as ABC, DRV, 3TC, and TDF, had high mean concentrations in wastewater but generally much lower frequencies of detection.

Nevirapine is a prevalent drug in wastewater. However, NVP had comparatively low mean concentrations for its high frequency of detection (FD) in influent (Table 2-5). Efavirenz revealed both high mean concentrations and FD. Notably, two ARVDs had higher mean effluent concentrations than influent (3TC, LVP), indicating incomplete removal during treatment or some other process-related factor. From the data, we can deduce that the drug class dominance based on prevalence in wastewater samples was NNRTI > PI > NRTI > II > EFI.

Abacavir's low log K_{ow} contributes to its predominance in the aqueous phase (Table 2-3) (Xolo and Mahlambi, 2024). Ngwenya and Mahlambi (2023) reported greater removal efficiencies (75–91%) in all WWTPs studied, with lower ABC concentrations observed in effluents. However, comparative studies of the Umbilo and Darvil WWTPs (KwaZulu-Natal) revealed conflicting influent concentrations. Xolo and Mahlambi (2024) documented 61.2 µg/L and 168 µg/L respectively, while Ngwenya and Mahlambi (2023) reported substantially higher concentrations (814 µg/L and 278 µg/L). Mean effluent levels (93 ± 30 µg/L) were three to four orders of magnitude higher than those recorded in France (0.03 µg/L) (Aminot *et al.*, 2015),

Germany, and South Korea ($< 0.01 \mu\text{g/L}$) (Boulard *et al.*, 2018; McCurry *et al.*, 2014), indicating far greater environmental discharge in South Africa. These discrepancies give emphasis to local differences in wastewater management and point to higher ABC removal efficiencies in WWTPs compared to decentralised wastewater treatment systems (DEWATS). Additional comparative studies are necessary to refine intersystem performance assessments.

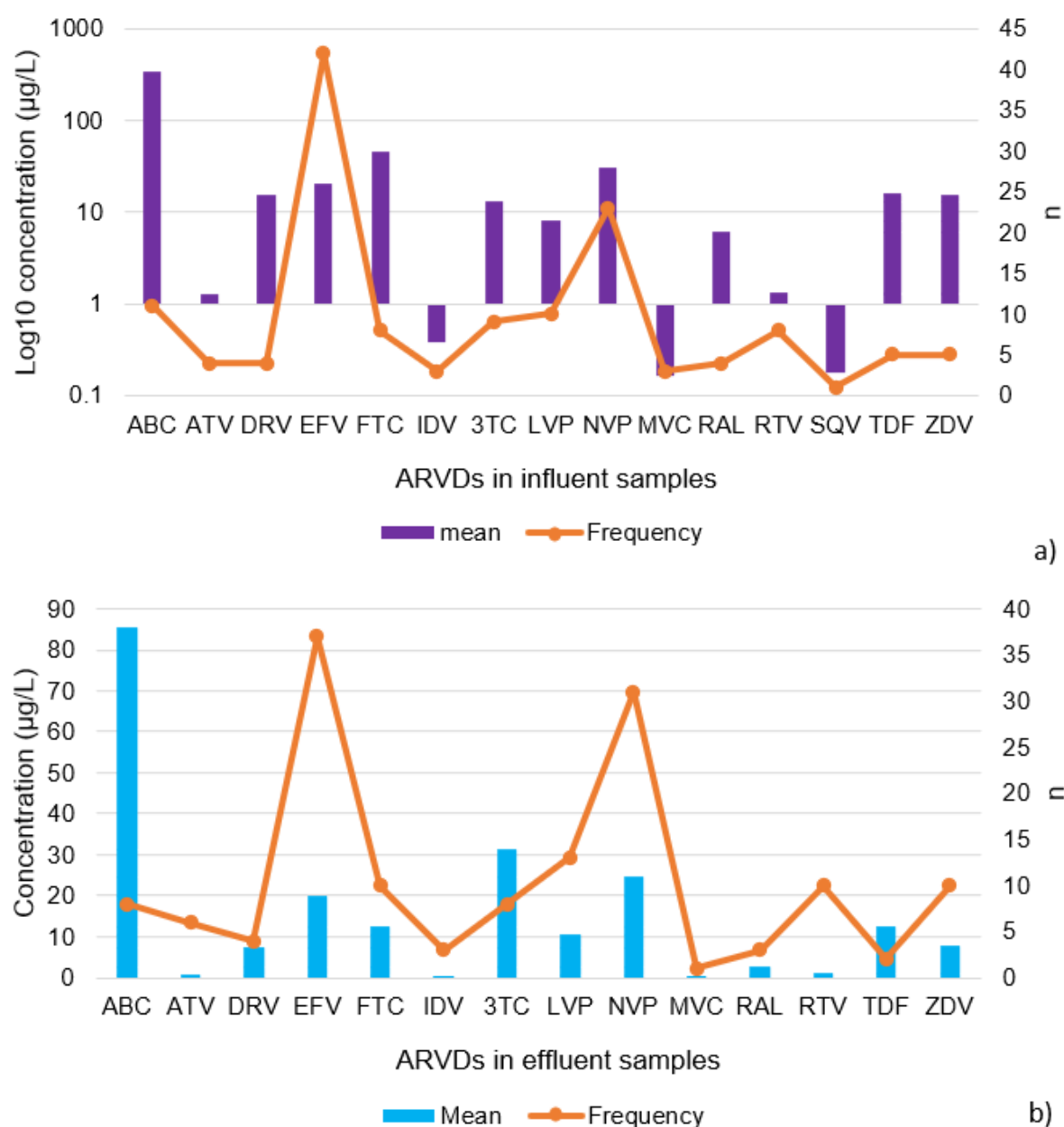


Figure 2-2: Mean influent (a) and effluent (b) water concentrations and frequency of detection for the various antiretroviral drugs quantified in South African waters.

The Kruskal-Wallis test revealed significant differences between ARVDs measured in influent ($p < 0.0001$). Dunn's multiple comparison tests further identified significant differences between ABC, indinavir (IDV), NVP, and MVC (Figure 2-3).

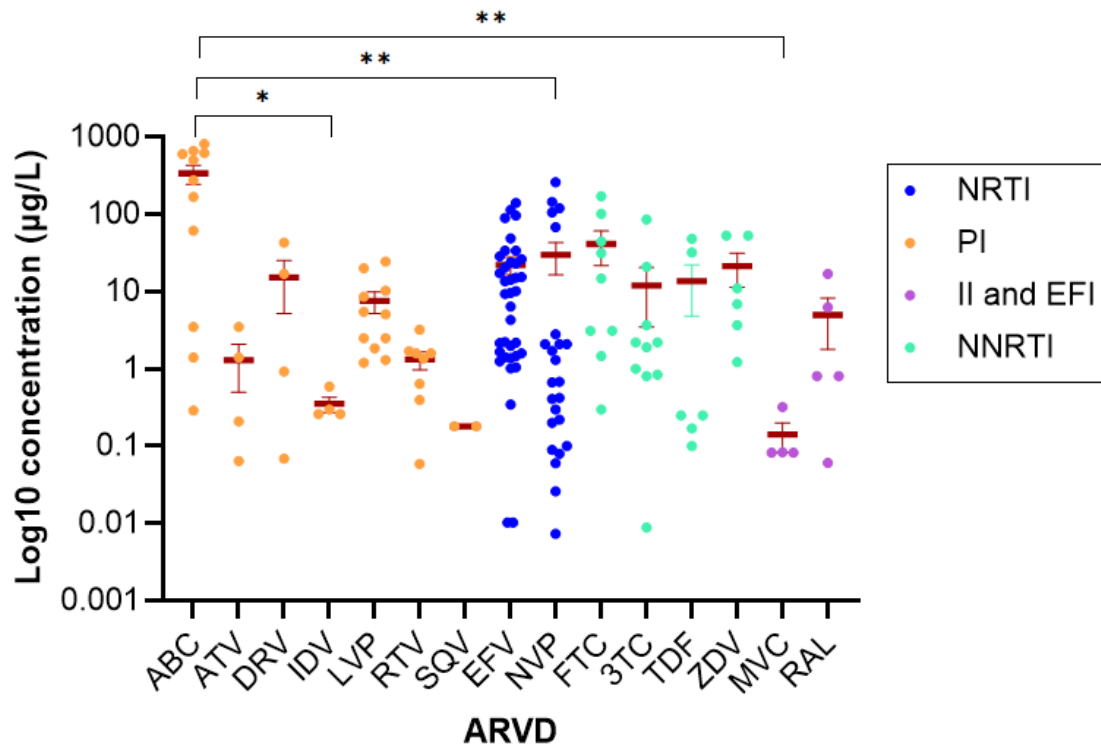


Figure 2-3: Statistical differences between antiretroviral drug mean concentrations detected in South African influent water. Error bars represent mean and standard error of the mean. Post-hoc test results are denoted by significance levels (* = $p < 0.05$; ** = $p < 0.01$). EFI = entry/fusion inhibitor; II = integrase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.

The Kruskal-Wallis test revealed significant differences between ARVDs measured in effluent ($p < 0.0001$). Dunn's multiple comparison test further identified significant differences between ABC, ATV, IDV, NVP, ZDV, as well as between EFV, IDV, and NVP (Figure 2-4).

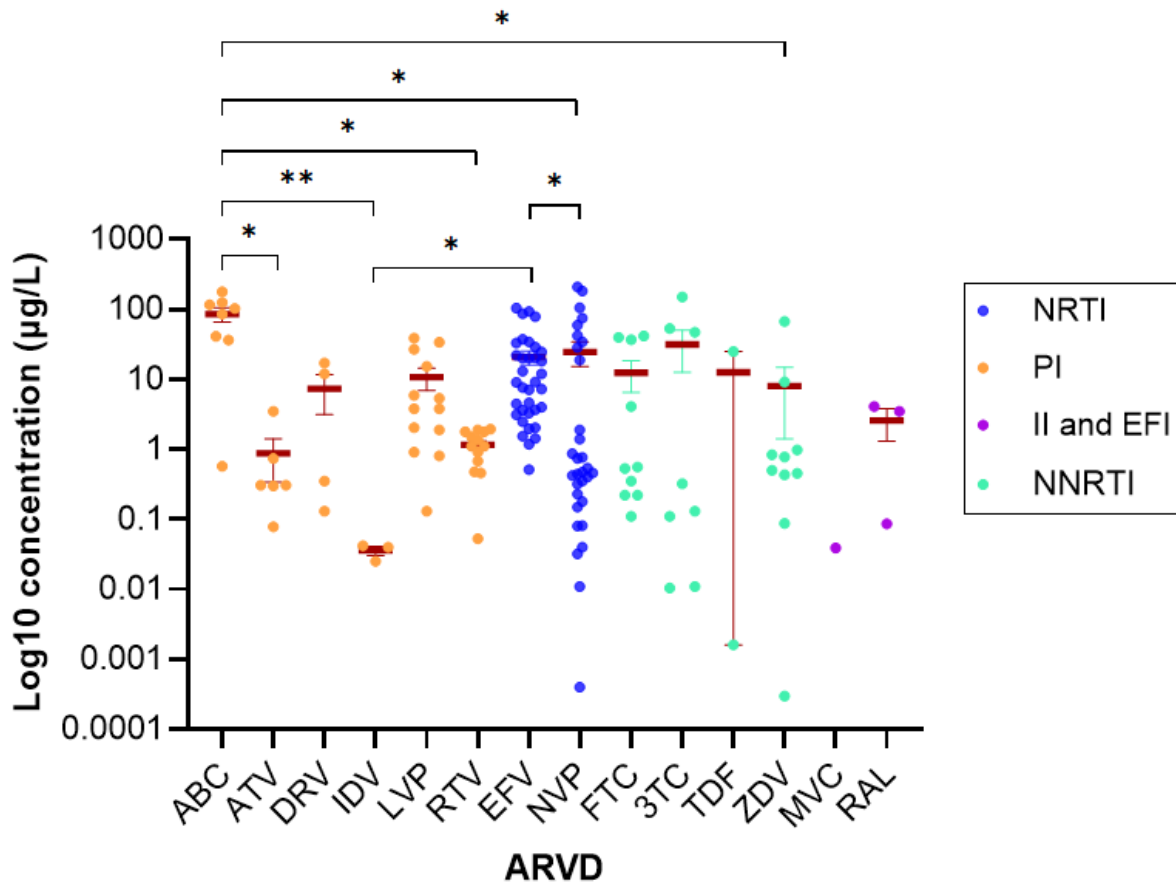


Figure 2-4: Statistical differences between ARVD mean concentration detected in South African effluent water. Error bars represent mean and standard error of mean. Post-hoc test results are denoted by significance levels (* = $p < 0.05$; ** = $p < 0.01$). EFI = entry/fusion inhibitor; II = integrase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.

Insufficient sanitation infrastructures across most African countries have the potential to augment ARVD concentrations in surface water. Ncube *et al.* (2018), projected increased ARVD concentrations in water systems in developing countries because countries have extensive ART programmes and limited pharmaceutical removal protocols. Five out of seven WWTPs investigated by Horn *et al.* (2022) revealed higher concentrations of ARVDs downstream than at the corresponding upstream sites, suggesting that WWTPs are potentially representing point sources of ARVD pollution. The background concentrations of ARVDs at certain upstream sites were already so high that it appeared as if the WWTPs had no influence on the ARVD concentrations at the respective downstream sites.

For example, Netshithothole *et al.* (2024) noted that during their sampling period the identified WWTPs were not operational, and their sewage was bypassing all the treatment stages, flowing untreated into the nearby Buffalo River, Eastern Cape. Such infrastructure failures can potentially lead to extreme ARVD pollution in receiving waters. The corresponding increase in WWTP capacity with an increase in population density represents another level of risk, as WWTPs become overburdened, resulting in the discharge of low-quality effluent into the natural environment. The direct disposal of urine and faecal matter contaminated with ARVD residues may subsequently enter groundwater or accrue in rivers via runoff (Ncube *et al.*, 2018).

Disparities in ARVDs quantified in wastewater could also be due to differences in wastewater treatment practices. Decentralised wastewater treatment systems (DEWATS) and traditional WWTPs have different attributes and processes. DEWATS are smaller, have a lower capacity, are typically located close to wastewater sources, and are designed to treat small communities on site (Arora *et al.*, 2016).

Whilst traditional plants are large-scale facilities, they have a higher capacity, are located further away from wastewater sources, and are designed to treat large communities from a wide area. DEWATS may use different treatment technologies, which may subsequently affect their ability to remove ARVDs from wastewater. Analytical inconsistencies arising from sampling approaches and diurnal flow patterns may also influence reported removal rates, as the diurnal incoming load is dispersed over multiple days in the effluent owing to the residence time distribution of wastewater (Baalbaki *et al.* 2017).

In summary, ARVD concentrations in South African wastewaters were considerably higher, often several orders of magnitude, than those found in other countries with more advanced treatment systems (Aminot *et al.*, 2016; Boulard *et al.*, 2018; Funke *et al.*, 2016; Ngumba *et al.*, 2016b; Prasse *et al.*, 2010). For example, a study by K'oreje *et al.* (2016) in Kenya reported a maximum EFV influent concentration of 1.02 µg/L and a maximum effluent concentration of only 0.11 µg/L. This is in strong contrast to the concentrations reported in South Africa (Mtolo *et al.*, 2019; Xolo and Mahlambi, 2024).

These higher concentrations could be due to a variety of reasons, such as disparities in population density, usage of ARVDs, and wastewater treatment practices, to name a few. Discrepancies in analytical performance, such as the slope of the calibration curve (sensitivity), as well as the LOD and LOQ must be considered, as they can influence the

reported concentrations and the comparability between studies. Overall, the study underlines the requirement for enhanced wastewater management and pharmaceutical disposal, especially in areas with high population density, to mitigate the potential risk of ARVD contamination in the environment.

2.4.2 Occurrence in surface water

Thirteen ARVDs have been quantified in surface water samples from eight provinces: Gauteng, Eastern Cape, KwaZulu-Natal, Limpopo, North West, Free State, Northern Cape, and Western Cape (Table 2-6). The current study could find no literature to date reporting ARVD levels in environmental water from the Mpumalanga province. The most frequently documented ARVDs were NVP ($n = 66$), EFV ($n = 40$), and ZDV ($n = 36$). The highest documented concentration was ABC at 102 $\mu\text{g/L}$ (Ngwenya and Mahlambi, 2023). Abacavir is also the ARVD with the greatest mean concentration in surface water ($35 \pm 39.6 \mu\text{g/L}$).

The Kruskal-Wallis test revealed statistically significant differences in surface water concentrations among 13 ARVDs ($H = 82.5$, $p < 0.0001$; $n = 242$), with median concentrations varying significantly ($p < 0.05$). Dunn's post-hoc comparisons identified multiple pairwise distinctions, notably between 3TC vs. LPV, NVP vs. RTV, and LPV vs. NVP ($p < 0.0001$), indicating a distinct separation in their median values.

Other drugs showed moderate ($0.001 \leq p < 0.01$) or lower significance ($0.01 \leq p < 0.05$). Greater mean rank differences (e.g., 3TC vs. LPV: -128, LPV vs. TDF: 133) implied more substantial differences in detection between those particular ARVDs, while smaller mean rank differences suggest closer concentrations that are still statistically significant.

The results suggest that, while certain ARVD groups present distinct differences, others share similar characteristics, indicating that their median values were comparable (also see Figure 2-5). Table 2-8 revealed a recurring trend of specific groups, such as LPV, NVP and 3TC, constantly demonstrating distinct characteristics when compared with other ARVDs. Drugs such as NVP and FTC also showed notable trends, reinforcing certain separations across the dataset. These results emphasise the variability of ARVDs quantified, such as differential input quantities, environmental persistence, prevalence, or stability.

Table 2-8: Summary of significant pairwise comparisons from Dunn's Test performed on surface water samples from South Africa.

ARVD comparison	Mean rank difference	Adjusted P value	Significance
EFV vs. LPV	-70.8	0.0043	**
3TC vs. LPV	-128	<0.0001	****
3TC vs. RTV	-106.4	0.0005	***
LPV vs. NVP	104.8	<0.0001	****
LPV vs. d4T	129.9	0.0091	**
LPV vs. TDF	133	0.0063	**
LPV vs. ZDV	82.8	0.0003	***
NVP vs. RTV	-83.2	<0.0001	****

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

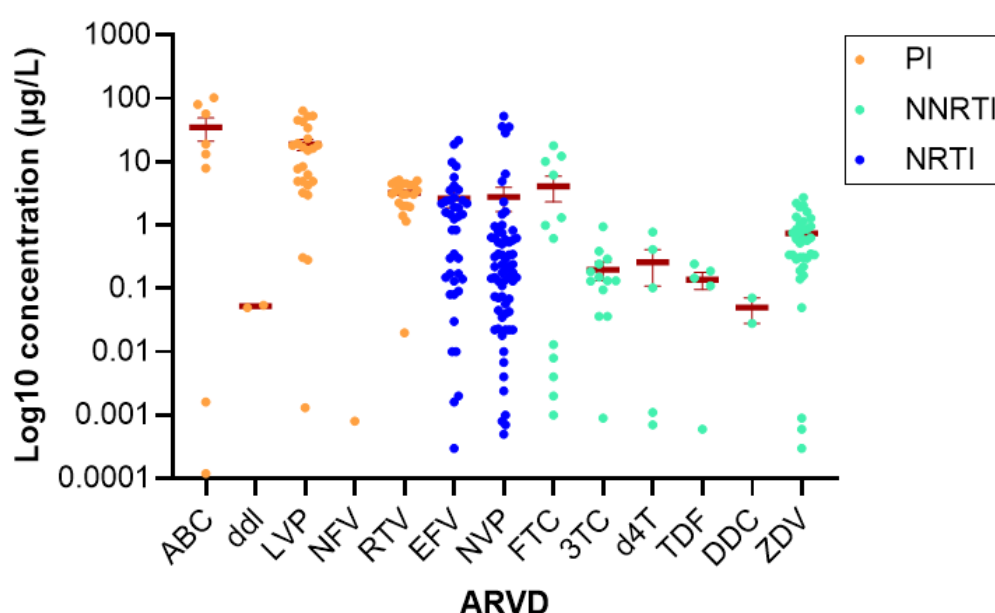


Figure 2-5: Graphical representation of the mean surface water concentrations detection for the various ARVDs quantified in South African waters. Error bars represent mean and standard error of mean. EFI = entry/fusion inhibitor; II = integrase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.

2.4.3 Occurrence in groundwater

According to Dévier *et al.* (2013), antiviral drugs may be present in groundwater, particularly those adjacent to municipal wastewater. This is due to antiviral drugs displaying relatively high biological stability, thereby being insufficiently removed during bank filtration and sand filtration. Groundwater performs a fundamental role in ensuring the functioning of ecosystems and the well-being of humans because it is often a source of drinking water. Groundwater has

become a vital source of potable water for the livelihood of 50% of the South African population living in small towns and villages (WRC, 2017). Groundwater is under increasing pressure due to pollution. Information about ARVDs in groundwater globally is very limited. However, the few available studies reveal the degree of pollution of the emerging concern.

Only six ARVDs have been quantified in South African groundwater: EFV, NFV, NVP, SQV, d4T, and TDF, ranging from 0.3–13 ng/L, from only two authors (Rimayi *et al.*, 2018; Swanepoel *et al.*, 2015) (Figure 2-6). The Kruskal-Wallis test revealed no statistically significant differences between the ARVDs ($p = 0.71$). Of the analytes recorded, NVP had the greatest concentration (13 ng/L) and was the most recurrently detected ARVD ($n = 8$), followed by EFV (5 ng/L) sampled from the Hartbeespoort Dam catchment area, North West province (Rimayi *et al.*, 2018).

Owing to the maximum concentration of NVP, the overall mean ARVD concentrations were higher in the Swanepoel *et al.* (2015) report than in the Rimayi *et al.* (2018) report, but still in the same order of magnitude. Swanepoel *et al.* (2015) reported on NNRTIs, and Rimayi *et al.* (2018) on PIs and NRTIs. Considering the absence of groundwater reports and the fact that the two authors reported on different ARVDs, an adequate comparison between the reports could not be drawn.

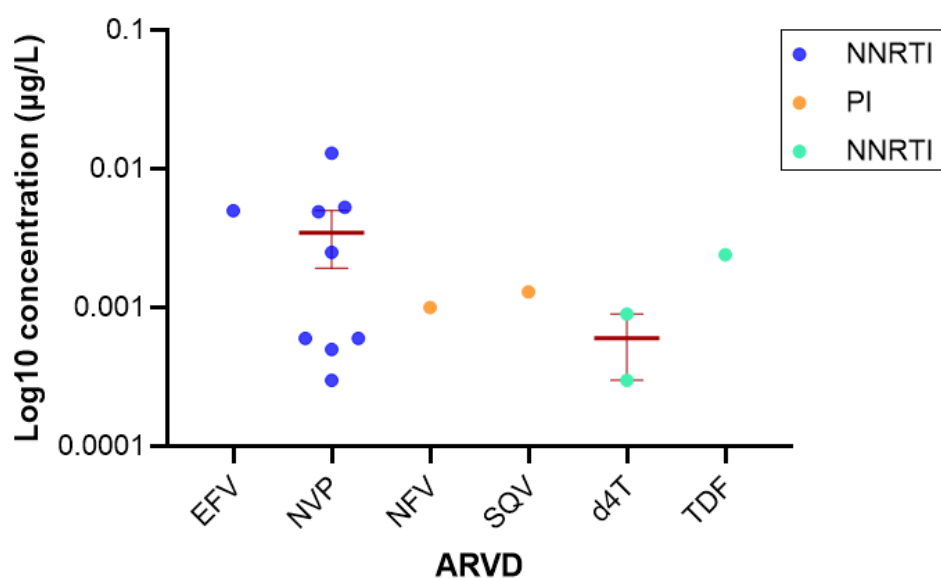


Figure 2-6: Concentrations of antiretroviral drugs in groundwater from South Africa. Error bars represent the mean and standard error of mean.

Nevirapine's high concentrations in groundwater may stem from high prescription volumes coupled with improper disposal at landfill sites where leaching through soil facilitates subsurface contamination. Another source could be due to poor sanitation and waste management practices, which allow for the leaching and contamination of surface water. Such bodies of water could act as a conduit for the transport of NVP into groundwater, thereby contaminating it. Agricultural application of stabilised sewage sludge also presents a pathway for drug residues and metabolites to enter soils and infiltrate groundwater, potentially contributing to accumulation within the food web (Kotzya *et al.*, 2009).

The maximum recorded value of NVP in groundwater from the United States of America (USA) was 28 ng/L (Fisher *et al.*, 2016) and 1600 ng/L from Kenya (K'oreje *et al.*, 2016). Some other global studies reported on ARVDs in groundwater: 30 ng/L ZDV in Kenya (K'oreje *et al.*, 2016); 3.9 ng/L FTC, and 1.8 ng/L 3TC in Germany (Boulard *et al.*, 2018). Kenya recorded groundwater ARVD concentrations an order of magnitude higher than South Africa. The USA also documented comparable concentrations. Numerous ARVDs have been shown to be present in water sources at concentrations far greater than their international counterparts (Table 2-2), yet information is limited, especially on groundwater. More studies on groundwater are needed for cross-national comparisons.

Tenofovir, d4T, NFV, EFV, and LPV had only been reported for South Africa. Given that groundwater is used as a source of drinking water, this exposure pathway may lead to adverse health effects for humans. NVP concentrations in South Africa have been documented to yield minor seasonal fluctuations (8–13 ng/L) with a relative standard deviation of only 2% for the four seasons sampled (Rimayi *et al.*, 2018). In the main, the sum of 11 emerging pollutant concentrations in groundwater was lower than in surface water concentrations in three of the four seasons (Rimayi *et al.*, 2018). Rimayi *et al.* (2018) were the only authors that reported on a seasonal variation of ARVDs in groundwater.

Zidovudine was detected in groundwater sampled from Kenya, possibly due to illicit use and direct spillage into water (K'oreje *et al.*, 2016), but ZDV has not been recorded in South African groundwater. FTC and 3TC have been recorded in Germany at maximum concentrations of 3.9 ng/L and 1.8 ng/L (Boulard *et al.*, 2018). As analytical technology for quantifying ARVDs at ultra-trace level (sub-ng/L) in South Africa improves, so does the potential for quantifying similar or even greater discoveries of compounds previously unquantifiable. Saquinavir displays high activity against HIV (Nannou *et al.*, 2020). Yet, finite information is available regarding its presence in water resources. In South Africa, SQV was detected in groundwater

from the North West at 1.3 ng/L (Swanepoel *et al.*, 2015), as well as in influent from the Phoenix WWTP in KwaZulu-Natal at 180 ng/L (Abafe *et al.*, 2018). Aminot *et al.* (2015), reported SQV levels in France's groundwater at a mere 0.2 ng/L (Aminot *et al.*, 2015).

There is a commonly held perception that groundwater is usually safeguarded against contamination by virtue of the presence of a soil layer characterised by low permeability, which serves to capture micropollutants through its sorption capacity (Rozman *et al.*, 2015). Nonetheless, this is not the case for all pollutants, as seen in the current review. The groundwater conduit theory (Klaus, 2018) stresses the vulnerability of groundwater in South Africa, where poor sanitation may allow untreated wastewater to leach into surface waters that eventually infiltrate groundwaters and contaminate them.

Overall, the theory emphasises the need for improved sanitation and wastewater management to safeguard the groundwater and ensure its safe use for human consumption. The groundwater conduit theory and its far-reaching implications for a number of major industries, such as the pharmaceutical industries, designate groundwater pollution as a prominent social, economic, and environmental law predicament.

2.4.4 Occurrence in drinking water

Perhaps the biggest concern is that 10 parent ARVD compounds from three different drug classes have been detected in drinking water in South Africa (Swanepoel *et al.*, 2015; Vogt *et al.*, 2020; Wood *et al.*, 2015). Study samples were mostly collected from the Gauteng and North West provinces, with only one sample collected in KwaZulu-Natal (Swanepoel *et al.*, 2015). The following maximum ARVD concentrations were found in drinking water: ABC (0.5 ng/L), d4T (0.9 ng/L), NFV (1.1 ng/L), ddl (3.3 ng/L), DDC (8.4 ng/L), NVP (460 ng/L), ZDV (1180 ng/L), and RTV (1950 ng/L).

Nevirapine was the most frequently recorded ARVD in drinking water ($n = 9$) (Table 2-5) (Figure 2-7). One-way ANOVA revealed significant differences among ARVDs ($p = 0.004$). Tukey's multiple comparison test at $p < 0.05$ further revealed significant differences between LPV, ddl, and NVP as indicated in Figure 2-7. The highest concentration of ARVDs documented in South African municipal tap water samples was LPV (26.9 $\mu\text{g/L}$) near the Crocodile River (west), followed by EFV at 24.6 $\mu\text{g/L}$ near Olifantsfontein, Gauteng province (Vogt *et al.*, 2020). Vogt *et al.* (2020) were also the only authors to document LPV, EFV, and RTV in drinking water samples, whilst Swanepoel *et al.* (2015) were the only authors to document ABC, ddl, NFV, and d4T in drinking water samples.

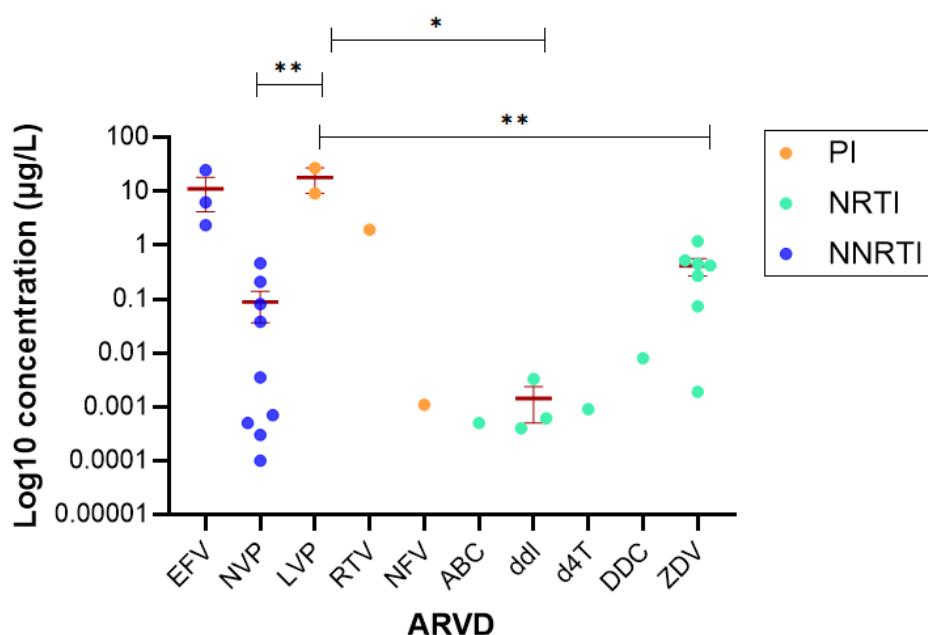


Figure 2-7: Concentrations of antiretroviral drugs documented in South African drinking water. Error bars represent the mean with standard error of mean. Post-hoc test results are denoted by significance levels (* = $p < 0.05$; ** = $p < 0.01$).

Given that the different authors quantified different ARVDs, direct comparisons between the compounds could not be drawn except for NVP and ZDV. Their mean concentrations were reviewed further. Vogt *et al.* (2020) documented mean NVP concentrations in drinking water samples that were roughly 200 times greater than those documented by Swanepoel *et al.* (2015). Vogt *et al.* (2020) also documented mean ZDV concentrations that were 300 times greater than Swanepoel *et al.* (2015) and roughly eight times greater than Wood *et al.* (2015).

The mean concentrations for all ARVDs detected in drinking water were reported as 4976 ng/L by Vogt *et al.* (2020), 41 ng/L by Wood *et al.* (2015), and 1.2 ng/L by Swanepoel *et al.* (2015), in descending order. Vogt *et al.* (2020) therefore reported far greater concentrations than their precedents. Overall, the mean values for drinking water revealed greater concentrations for the PI and NNRTI classes (Figure 2-7). Lopinavir, EFV, and RTV are practically insoluble (Table 2-3). Owing to the low solubility of LPV, EFV, and RTV and the amount reported in drinking water only being a fraction of the therapeutic dose, it is anticipated that the current risk of these ARVDs in waters would be negligible. The question then remains however, what happens to these drugs in other matrixes in the environment?

In a collaborative study by the United States (U.S.) Geological Survey (USGS) and the U.S. Environmental Protection Agency (USEPA), 3TC was identified in 25% of the source-treated water samples (Furlong *et al.*, 2017). Specifically, Furlong *et al.* (2017) documented 3TC at a concentration of 0.027 µg/L in drinking water samples from the U.S. of America. Similarly, Gieburtoiwics *et al.* (2017) reported DRV at a concentration of 0.003 µg/L in Polish drinking water. Funke *et al.* (2016) identified various ARVD metabolites in German drinking water samples: carboxy-abacavir (0.080 µg/L), carboxy-emtricitabine (0.041 µg/L), and carboxy-lamivudine (0.084 µg/L). To date, none of these ARVDs have been quantified in South African waters.

Both NFV and DDC have been detected in drinking water (Swanepoel *et al.*, 2015; Wood *et al.*, 2015) (Table 2-5) (Figure 2-7). To the best of our knowledge, this is also the only report of NFV and DDC in drinking water in the world. These ARVDs are no longer prescribed in ART regimens since 1996 (Meintjies *et al.*, 2017). Subsequently, no current information on NFV and DDC therapeutic doses are available. South African guidelines to combat HIV in the early 2000s did, however, include NFV (2250 mg/day) and DDC (2.25 mg/day) (Martin, 2005; Maartens *et al.*, 2008). The discontinuation of DDC and NFV could be why these ARVDs were scarcely quantifiable in environmental samples. DDC was only distributed for 15 years, yet occurred still quantifiable in water samples nine years after it had been discontinued (Wood *et al.*, 2015), raising questions about ARVD drug persistence.

As the ARVD concentrations in drinking water are knowingly lower than those used in related therapies, the short-term effects on the human body are not expected to be grave. Nevertheless, the long-term effects may become a serious problem. Explicit studies on chronic toxicity following continual long-term exposure to low concentrations of ARVDs are not yet accessible. According to Kostich and Lazorchak (2008), it is expected that certain high-risk groups of the population, such as children, the elderly, and people with impaired liver and kidney functions, may be susceptible to low-level exposure to pharmaceutical residues in drinking water and the environment.

Antivirals may be inadvertently consumed through drinking water, and even at a trace level, these may exert alterations to the homeostatic mechanisms in the human body (Simazaki *et al.*, 2015). Nannou *et al.* (2020) completed a worldwide review and argued that based on the data from France, South Africa, Germany, Japan, Poland, and USA, the removal efficiency of ARVDs during drinking water treatment is acceptable, seeing as the majority of occurring concentrations are below or near quantification limits (< LOQ–169 ng/L). However, drinking

water samples in the current review ranged between 0.0001 and 26.9 µg/L, with a combined average of 2.6 ± 6.6 µg/L for all ARVDs in South African waters. This review reports ARVDs in drinking water of a magnitude greater than that reported by Nannou *et al.* (2020). Therefore, it is urgent and necessary to conduct further ecotoxicological studies and determine the potential hazards of South Africans drinking water contaminated with ARVDs. Although advanced treatment technologies such as ozonation and activated carbon filtration (Deblonde *et al.*, 2011) used in countries like Germany, the U.S., and Poland have shown some potential in degrading ARVDs, studies have reported that residues may still persist in treated drinking water.

In South Africa, WWTPs and drinking water treatment plants (DWTPs) have distinct purposes, processes, and regulatory standards (Table 2-9). The WWTPs are regulated under the National Water Act and monitored by the Department of Water and Sanitation (DWS) for effluent quality. These WWTPs (as are many others in the world) are not optimised to remove emerging contaminants such as ARVDs. The DWTPs are regulated under South African National Standard (SANS 241) standards for drinking water quality (SABS, 2006) and similarly lack capacity to eliminate trace pharmaceuticals. Instead DWTPs rely on conventional processes designed to remove pathogens and turbidity thereby prioritising microbial and chemical safety for consumption.

In contrast to international treatment technologies, South African DWTPs rarely incorporate advanced treatment technologies. This discrepancy highlights a critical gap in the local infrastructure. Without the integration of advanced or targeted treatment technologies, there is a higher risk that ARVDs and other pharmaceutical residues (Nantaba *et al.*, 2020) may persist through the treatment process and re-enter to the drinking water supply. This is especially concerning in urban centres where water is reclaimed from polluted rivers.

Therefore, to align with international best practices and through common sense, the adoption and scaling of advanced treatment technologies in South Africa (such as ozonation, granular activated carbon, membrane filtration, or advanced oxidation processes) should be prioritised to improve public health protection and ensure compliance with emerging global standards for micropollutant removal.

Table 2-9: South African Drinking Water Treatment Plant (DWTP) and Wastewater Treatment Plant (WWTP) comparison (City of Tshwane Water Services³, 2022; DWAF 1996; 2002; 2004; Hansen, 2015; Laher, 2023; Swanepoel *et al.*, 2017¹; Van der Walt, 2001²).

Aspect	DWTP	WWTP
Main purpose	To produce safe, clean drinking water from surface water, groundwater, or dams.	To treat and purify sewage before discharging it back into the environment (usually rivers or dams).
Water source	Raw water from rivers, dams, boreholes.	Domestic, industrial, and stormwater sewage.
Treatment focus	Disinfection and removal of suspended solids, pathogens, and some chemical pollutants.	Biological and chemical degradation of organic matter, nutrients, and solids.
Typical treatment steps	Coagulation, flocculation, sedimentation, filtration (sand or dual-media), disinfection (chlorination).	Screening, grit removal, primary sedimentation, biological treatment, secondary sedimentation, disinfection.
Target contaminants	Microbes (e.g., <i>Escherichia coli</i>), turbidity, metals, nitrates, taste/odour compounds.	Organic matter, nutrients (N, P), pathogens, suspended solids.
Pharmaceutical removal	Limited – not specifically designed to remove ARVDs or other pharmaceuticals.	Some removal via biodegradation or sorption, but generally inefficient for ARVDs.
Advanced technologies used	Rarely used in SA: GAC, AOPs (ozone, UV/H ₂ O ₂) in limited cases.	Also rare, but some pilot studies use MBRs, ozonation, or activated carbon.
GAC	Sometimes used in advanced drinking water plants (for taste/odour or pesticides), but not widespread.	Not commonly used due to cost; limited use.
AOPs	Used in developed countries for micropollutant removal (e.g. U.S.); rarely applied in SA DWTPs.	Mostly research studies; effective but costly.
End product	Potable water meeting SANS 241 standards.	Treated effluent, discharged into rivers or used for irrigation.
Monitoring standards	SANS 241:2015 – Microbiological, chemical, and physical parameters.	NWA and General Authorisations for discharge quality (section 39 of NWA).
Regulatory body	DWS.	DWS, and local municipalities.
Examples in SA	Vaalkop DWTP ¹ , Rietvlei DWTP ² .	Daspoort WWTP ³ , Sunderland Ridge WWTP ³ , Olifantsfontein WWTP ³ .

AOPs = Advanced Oxidation Processes; ARVDs = Antiretroviral Drugs; BOD = Biological Oxygen Demand; COD = Chemical Oxygen Demand; DWS = Department of Water and Sanitation; DWTP = Drinking Water Treatment Plant; GAC = Granular Activated Carbon; H₂O₂ = hydrogen peroxide N = nitrogen; NWA = National Water Act; P = phosphorus; SA = South Africa; SANS = South African National Standard; UV = Ultraviolet (disinfection); WWTP = Wastewater Treatment Plant.

2.5 Causes for concern

One concern is that excessive amounts of these bioactive compounds (ARVDs), designed to target various biological systems, are entering aquatic systems without effective technology and infrastructure to remove and mitigate the consequences thereof. Although data on ecological impacts remain limited, Mlunguza *et al.* (2020) found trace levels of FTC, TDF, and EFV in wastewater, surface water, and in plant tissues of *Eichhornia crassipes*. The study revealed that ARVDs are taken up by plant roots, stems, and leaves, and are translocated to aerial tissue. The highest concentrations were found in plant roots, with tenofovir disoproxil, FTC, and EFV reaching concentrations of 8.7 µg/kg, 13.4 µg/kg, and 29.6 µg/kg, respectively. This proves that aquatic plants (such as hyacinths) can accumulate organic pollutants, including pharmaceuticals, in polluted water bodies.

The fate of these compounds in the environment is also uncertain. The persistence of some pharmaceuticals, including ARVDs, is influenced by the aerobic physicochemical properties of water (Aminot *et al.*, 2018). The half-lives of ABC, 3TC, RTV, and SQV are less than 5 days under biochemical conditions in surface water and wastewater, but NVP and ZDV are quite stable in surface water and wastewater (Aminot *et al.*, 2018).

Schoeman *et al.* (2017) confirmed the persistence of NVP under most wastewater treatment practices (< 22.5% removal). Ncube *et al.* (2018) argued that the removal efficiencies of EFV (83–92%), 3TC (24–59%), NVP (11–49%), and ZDV (99%), as documented by K'oreje *et al.* (2016), suggest that certain ARVD drugs are persistent enough to circumvent most wastewater treatment practises and persist in surface water.

One route of how pharmaceuticals find their way into river water is through improper disposal of unused and expired medicines. This often happens despite policies that allow patients to return expired and unused drugs to pharmacies for safe disposal (SA, 1965). In a study by Maharaj *et al.* (2020), 84% of participants indicated they disposed of their unused/expired ARVDs, 56% of these participants chose their method of disposal to be the bin (threw unused/expired ARVDs in the trash), with only 6% and 7% of participants returning unused ARVDs to a hospital or pharmacy, respectively.

Maharaj *et al.* (2020) also reported that 28% of participants indicated they had expired or unused ARVDs and 44% had other unused and expired medicines (antibiotics and anti-tuberculosis) at home. According to the latest census, up to 22% of households dump or leave their rubbish anywhere they find fit (Stats SA, 2022).

Another important concern in addressing ARVD pollution is the absence of accurate usage and prescription data. This includes data such as kilograms dispensed annually per province or catchment area which limits current assessment and intervention strategies. Based on this limited study by Maharaj *et al.* (2020) and the reported 6.93 million South Africans receiving ART in 2020 (UNAIDS, 2020), this could mean that several tonnes of prescribed ARVDs could be thrown into the bin annually. These drugs then end up in landfill sites subjected to various environmental conditions and could potentially leach into surrounding aquatic environments. According to Groundwork, an environmental and social justice organisation, approximately half of South Africa's 1 327 documented landfill sites are unlicensed, of which 58 are regarded as hazardous (Southern Africa Treads, 2018).

Contaminated sites, such as old factories or landfills, may produce contaminant plumes with high concentrations of pharmaceuticals (D'Alessio and Ray, 2016). The plume at Grindstedt landfill in Denmark, for example, discharges over 1000 kg/year of sulfonamides and barbiturates into the underlying groundwater (Balbarini *et al.*, 2018). Despite some degradation, landfill studies have revealed that pharmaceutical compounds pose a severe risk to groundwater and surface water when compared with more degradable compounds typically studied at contaminated sites. The following maximum concentrations of antivirals were found in raw landfill leachates: ABC 0.19 µg/L; 3TC 0.36 µg/L; acyclovir 12 µg/L; ganciclovir 1.1 µg/L; and oseltamivir 0.2 µg/L in the United States and China (Masoner *et al.*, 2014; Peng *et al.*, 2014; Masoner *et al.*, 2016; Yu *et al.* 2020).

Due to the ARVD treatment, PLWH experience immune-suppression, which makes them susceptible to opportunistic infections such as tuberculosis, receive additional pharmaceutical regimens. These pharmaceuticals could have the same disposal route as the ARVDs (Maharaj *et al.*, 2020). According to Balakrishna *et al.* (2017), concentrations of pharmaceuticals detected in hospital effluents are frequently higher than those detected in domestic WWTP effluent. Wastewater from hospitals and clinics bearing pharmaceuticals can be discharged into nearby rivers, leading to contamination. Regions with a high density of hospitals and HIV/AIDS clinics have a greater risk of severe ARVD contamination compared to those with a low density.

South Africa has a serious wastewater treatment problem given that large volumes of WWTP effluent that do not meet the required effluent quality standards flow into the country's rivers and other environmental media (Department of Environmental Affairs (DEA)), 2020). Based on the latest available report, 30% of WWTPs were in critical condition and required regulatory action, 19.5% were in poor condition and needed urgent action, and only 7% were reported to be in good condition. Nearly half of the WWTPs in South Africa are not functioning properly (Toxopeüs, 2019). These metrics may have changed since.

2.5.1 Mitigation of pharmaceutical pollution in catchments across South Africa

Using a risk-based approach, the SA DEA (2020) identified 18 river catchments across South Africa that are potentially at very high to high risk of contamination by environmentally persistent pharmaceutical products (EPPPs), which included ARVDs. These river catchments are the Berge, Breede, Bushmans, Crocodile River West, Fish, Gourits, Kei, Komati, Kromme, Limpopo, Mfolozi, Mkomazi, Olifants, Orange, Sundays, Swartkops, Tugela, and Vaal catchments. ARVDs with high concern in the DEA (2020) study were TDF, DDC, 3TC, NVP, LPV, and ddl. ARVDs of medium concern were dT4 and ZDV. Accordingly, these catchments need to be prioritised for management in terms of strategies for reducing the entry of pharmaceuticals in all environmental media.

The current review and the DEA study both identified certain drugs such as ddl, 3TC, NVP, and TDF as having a high environmental presence and potential for contamination (DEA, 2020). Many of the specific river catchments suggested to be prioritised in the DEA study, are regions identified in the current review as systems experiencing considerable ARVD contamination (Limpopo, Olifants, and the Crocodile River West catchments). Concurring with the findings in this review, the DEA study suggests that insufficient wastewater treatment facilities and inefficiencies in removing ARVDs are major contributors to the documented environmental contamination.

It is imperative that the authority's robust ART programme continue to be upheld, along with the safeguarding of the substantial financial investment made (R207 billion) (Hopkins *et al.*, 2018). To mitigate the risk of secondary health issues, close monitoring of the quantity and concentration of ARVDs in the physical environment must be conducted. Additionally, governmental funding for research examining the impact of ARVDs on human health in aquatic environments is of paramount importance.

Questions to which South Africa particularly does not know the answers are: What is the persistence duration of these drugs in aquatic environments? Is the waste management of these drugs efficient? Could medical waste enter domestic waste and end up in municipal landfill sites? What are the prospective human and environmental risk?

2.5.2 Future trends

Although certain ARVDs have yet to be quantified in South African waters, the continued scale-up of HIV treatment intensifies the possibility of their future detection. As of early 2025, an additional 1.1 million additional PLWH in South Africa are expected to receive ART under national programs, drastically increasing the potential for pharmaceutical residues to enter aquatic environments through wastewater pathways (UNAIDS, 2025b). This growing treatment coverage support concerns that analytes such as fosamprenavir (FPV), etravirine (ETR), RPV, and DTG will likely emerge in future water monitoring efforts.

Considering the high frequency of detection within the NRTI drug class, it is reasonable to anticipate that NRTIs, along with ETR and RPV, will also be detected in environmental monitoring efforts. Newer ARVDs will be detected, but changes in concentrations of existing and legacy ARVDs may be complicated by existing high backgrounds.

Programmatically, DTG has become central to South Africa's HIV treatment strategy following WHO endorsed transitions from EFV-based regimens (WHO, 2018; Vitoria *et al.*, 2018). South Africa has adopted DTG-based FDC (TDF 300 mg + 3TC 300 mg + DTG 50 mg) as first-line treatment due to its superior viral suppression, tolerability, and reduced resistance (DoH, 2019; Hamers *et al.*, 2018). Subsequently, DTG was the most procured pharmaceutical consumed in the public sector in South Africa for the year 2019 (DEA, 2020). DTG constituted 68% of the quantity of prioritised pharmaceuticals procured and continued to lead public procurement in January to June 2020. Caution remains for use during early pregnancy due to potential risks of neural tube defects (Alhassan *et al.*, 2020; Zash *et al.*, 2019). Suggesting the need for critical attention to the environmental risk posed by it in the environment (DEA, 2020).

Tenofovir was sixth on the list of priority pharmaceuticals awarded for public sector procurement, with procurement value nearly doubling over the same period as DTG. The transition from EFV to DTG is anticipated to reduce EFV concentrations in the environment while increasing levels of DTG, TDF, and 3TC.

Regions with a high density of WWTPs, hospitals, pharmaceutical industries, insufficient and ineffective waste treatment systems, informal settlements, and regions with increasing population density are expected to be at greater risk of accumulating ARVD residues based on the review findings.

2.6 Conclusions

Some of the most extensive research on the presence of ARVDs in the environment has been conducted in South Africa, highlighting their emergence as a considerable concern due to South Africa's extensive ART program. This review represents the first comprehensive assessment of ARVD contamination specific to South Africa's water sources. Ten ARVDs have been detected in drinking water (0.1 ng/L–26.9 µg/L), six in groundwater (0.3–13 ng/L), 13 in surface water (0.12 ng/L–102 µg/L), and 15 in wastewater (0.3 ng/L–814 µg/L). Discrepancies in the frequency of detection, removal efficiencies, and concentrations among the different drug classes and individual ARVDs were noted. The concentrations of ARVDs in the water sources of developing countries, such as South Africa, are expected to increase, seeing as the countries with high ART programmes do not have pharmaceutical removal or treatment guidelines for WWTP effluents. Current water treatment technologies are insufficient to completely remove these drugs, as evident by this review, necessitating urgent research and technological advancements to address the gap.

Information is limited for the Northern Cape, Mpumalanga, Eastern Cape, Western Cape, and the Free State Provinces, underscoring the need for targeted investigations. The current study emphasises the urgent need for risk assessments, national monitoring programs, and improved water treatment technologies to mitigate the presence of ARVDs in the environment. A national survey evaluating the state of ARVDs in various water resources should be prioritised, in conjunction with the establishment of regulatory limits set by governing bodies. Such measures are needed to address the growing ARVD contamination problem, to protect water resources, and to safeguard both ecosystems and public health.

2.7 Recommendations

- To increase comparability, standardised procedures for ARVD analysis across water matrices should be created, if possible, to harmonise monitoring methods. Standardised monitoring and reporting methods will improve international comparability of data, fostering global collaboration on ARVD management.

- Future studies should expand the analyte scope. New research should consider pharmacological advancements and trends in environmental pollution. Current research primarily focuses on older or more commonly prescribed ARVDs. Research into newer drugs such as rilpivirine, dolutegravir, and etravirine should be considered.
- Current ARVD research is limited by the complexity of analytical methods, variability in sensitivity and the financial burden of advanced technologies. The standardisation of method validation is recommended. Comprehensive reporting of validation metrics should be promoted, such as recovery percentage, RSD and matrix effects if possible.
- Advanced techniques such as matrix-matched calibration and isotope dilution should be considered in order to improve measurement accuracy.
- Another consideration is the analysis of ARVDs in different aquatic species, sediments, and soils to obtain more insights about their role and effects in the ecosystem. This will help to understand the broader ecological impact.
- The effect of mixtures should be investigated. There is currently insufficient information on the combined ecological and human health impacts of ARVD mixtures in aquatic environments. While individual drugs may exist within the predicted no-effect concentration, mixture effects due to similar ARVDs with the same mechanism of action against the virus may result in synergic or additive effects (Backhaus and Faust, 2012).
- It is recommended that absolute ARVD usage and prescription data be routinely assessed and reported. Specifically in kilograms per annum per province and catchment. This will help with accurate exposure modelling, prioritising monitoring efforts, and aligning water quality interventions with geographic treatment burdens.
- The monitoring scope should be expanded since there is a limited geographical scope. Investigations into ARVD contamination in underrepresented provinces, including Mpumalanga, Northern Cape, and Eastern Cape, should be conducted, to build a comprehensive understanding of contamination patterns. Thus far, there are no reports of any investigations into freshwater sources from Mpumalanga, Northern Cape, or Eastern Cape (Figure 7-1, Annexure E).
- The efficiency of different treatment systems (for e.g., DEWATS vs. traditional WWTPs) should be investigated further by removing ARVDs under local conditions.
- Suggested areas of focus include studying drugs such as EFV's material flow, removal efficiency across various WWTPs, and its impact on surface water and solid waste (e.g., sludge).
- Enhance wastewater treatment technologies to improve ARVD removal efficiency. These could include membrane bioreactors, activated carbon adsorption, or advanced

oxidation processes. Strategies could also include chlorination processes, supported by AI-based predictive models, such as response surface methodology, to enhance treatment efficiency by identifying optimal operating conditions per Kang *et al.*'s (2024) example.

- Standardised guidelines should be developed and enforced for ARVD removal in WWTPs, particularly in regions with extensive ART programs.
- WWTP capacities should be improved and increased. Current WWTP infrastructure should be expanded to accommodate the growing population density and to prevent overburdening existing facilities. Outdated WWTPs should be upgraded, and maintenance should be improved to handle increasing influent loads more effectively. By reducing ARVD concentrations in WWTP effluents, the risk of contamination in surface and groundwater will be mitigated, preserving ecosystems and aquatic biodiversity. By mitigating and minimising ARVD pollution, the potential risks associated with exposure will also be reduced.
- Efforts to improve water infrastructure in underserved regions, such as in Limpopo and the Eastern Cape, should be intensified in order to ensure safe, treated piped water access and to mitigate the risks of ARVS contamination from surface water.
- Given the documented presence of ARVDs in Hartbeespoort's water and findings by Mlunguza *et al.* (2020) that demonstrate the uptake of FTC, TDF, and EFV by *Eichhornia crassipes*, it is recommended that the uptake and accumulation of ARVDs in aquatic plants, particularly water hyacinths (*E. crassipes*), should be investigated further. Quantifying ARVD concentrations in plant roots, stems, leaves, and aerial tissues will help evaluate the ecological impact and inform potential phytoremediation strategies for managing ARVD contamination.
- Criteria for prioritising ARVDs for priority attention in South Africa should include physicochemical properties, analytical feasibility, consumption and sales, therapeutic dose, long-term use estimates, metabolism and excretion, degradability and persistence, predicted environmental concentration (PEC), and a relative risk approach (PEC/PNEC).
- Stakeholders should encourage pharmaceutical take-back programs to prevent ARVDs from entering wastewater through direct disposal. Communities should also be educated on proper pharmaceutical disposal practices to minimise environmental contamination.
- Interdepartmental collaboration between health, environmental, and water management sectors should be promoted to tackle ARVD pollution holistically.

2.8 References

- Abafe, O.A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., ... Martincigh, B.S. 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, 200:660–670.
- Alhassan, Y., Twimukye, A., Malaba, T., Orrell, C., Myer, L., Waitt, C., ... Taegtmeyer, M. 2020. Engendering health systems in response to national rollout of dolutegravir-based regimens among women of childbearing potential: a qualitative study with stakeholders in South Africa and Uganda. *BMC Health Services Research*, 20(1):1–12.
- Aminot, Y., Litrico, X., Chambolle, M., Arnaud, C., Pardon, P. and Budzinski, H. 2015. Development and application of a multi-residue method for the determination of 53 pharmaceuticals in water, sediment, and suspended solids using liquid chromatography-tandem mass spectrometry. *Analytical and bioanalytical chemistry*, 407(28): 8585–8604.
- Aminot, Y., Fuster, L., Pardon, P., Le Menach, K. and Budzinski, H. 2018. Suspended solids moderate the degradation and sorption of waste water-derived pharmaceuticals in estuarine waters. *Science of the Total Environment*, 612:39–48.
- Arora, J., Yadav, A. and Saroj, D. 2016. Potential of decentralised wastewater treatment systems applicable to India. *Current World Environment*, 11(2):338.
- Arumugam, A., Lee, K.E., Ng, P.Y., Shamsuddin, A.S., Zulkifli, A. and Goh, T.L. 2025. Pharmaceuticals as emerging pollutants: Implications for water resource management in Malaysia. *Emerging Contaminants*, 100470.
- Baalbaki, Z., Sultana, T., Metcalfe, C. and Yargeau, V. 2017. Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics. *Chemosphere*, 178:439–448.
- Backhaus, T. and Faust, M. 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environmental science and technology*, 46(5):2564–2573.

Balakrishna, K., Rath, A., Praveenkumarreddy, Y., Guruge, K.S. and Subedi, B. 2017. A review of the occurrence of pharmaceuticals and personal care products in Indian water bodies. *Ecotoxicology and environmental safety*, 137:113–120.

Balbarini, N., Rønde, V., Maurya, P., Fiandaca, G., Møller, I., Klint, K.E., ... Bjerg, P.L. 2018. Geophysics based contaminant mass discharge quantification downgradient of a landfill and a former pharmaceutical factory. *Water Resources Research*, 54:5436–5456.

BHIVA, British HIV Association. 2020. Letter from Roche Products Limited regarding Viracept (nelfinavir mesilate) 250mg film-coated tablets and Invirase (saquinavir) 200mg hard capsule. [Online]. <https://www.bhiva.org/Letter-from-Roche-Products> Date of access: 21 Oct. 2020.

Bingul, Z., Irdemez, S., Yildiz, Y.Ş. and Demircioglu, N. 2023. Organic and inorganic matter removal from tannery wastewater using the electrocoagulation process. *International Journal of Environmental Science and Technology*, 20(2):2171–2180.

Boleda, M.R., Galceran, M.T. and Ventura, F. 2013. Validation and uncertainty estimation of a multiresidue method for pharmaceuticals in surface and treated waters by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1286:146–158.

Boulard, L., Dierkes, G., Ternes, T. 2018. Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: benefits and limitations. *Journal of Chromatography* 1535:27–43.

City of Tshwane Water Services. 2022. Water Services Development Plan 2022-2026. Pretoria, South Africa: City of Tshwane. [Online]. https://ws.dws.gov.za/wsdp/Assessment/Documents/WSDP_WSA_54_2024.pdf Date of access: 2 Apr. 2025.

D'Alessio, M., and C. Ray. 2016. Pharmaceuticals and groundwater resources. In: A. Fares, ed. *Emerging Issues in Groundwater Resources*. Advances in Water Security. Cham: Springer.

DEA, Department of Environmental Affairs (South Africa). 2020. Inventory of Environmental Persistent Pharmaceutical Pollutants and Management Action Plans in South Africa.

[Online]. Development of Inventory of Environmental Persistent Pharmaceutical Pollutants and Management Plans in South Africa. MC_DEA_EPPP_01.

Deblonde, T., Cossu-Leguille, C., and Hartemann, P. 2011. Emerging pollutants in wastewater: A review of the literature. *International Journal of Hygiene and Environmental Health*, 214(6):442–448.

Dévier, M.H., Le Menach, K., Viglino, L., Di Gioia, L., Lachassagne, P. and Budzinski, H. 2013. Ultra-trace analysis of hormones, pharmaceutical substances, alkylphenols and phthalates in two French natural mineral waters. *Science of the Total Environment*, 443:621–632.

DoH, Department of Health Republic of South Africa. 2010. *Clinical guidelines for the management of HIV and AIDS in adults and adolescents*. [Online]. https://www.who.int/hiv/pub/guidelines/south_africa_art.pdf Date of access: 5 Nov. 2020.

DoH, Department of Health Republic of South Africa. 2019. *2019 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy, Adolescents, Children, Infants and Neonates*. <https://sahivsoc.org/Files/2019%20Abridged%20ART%20Guidelines%2010%20October%202019.pdf> Date of access: 5 Oct. 2020.

Dong, L., Hu, S. and Gao, J. 2020. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug discoveries and therapeutics*, 14(1):58–60.

Drugbank, 2023. Drugbank: Drugs. [Online]. <https://go.drugbank.com/> Date of access: Jan. 2023.

DWAF, Department of Water Affairs and Forestry (South Africa). 1996. *South African water quality guidelines*: Vol. 3 (Industrial use) and 4 (Commercial irrigation). 2nd ed. Pretoria.

DWAF, Department of Water Affairs and Forestry (South Africa). 2002. *An Illustrated Guide to Basic Sewage Purification Operations* (ISBN 0-620-29884-7).

DWAF, Department of Water Affairs and Forestry (South Africa). 2004. Revision of General Authorizations in terms of section 39 of the National Water Act 1998 (Act No. 36 of 1998)—engaging in a controlled activity. Government Gazette 26187, Pretoria.

Dziuban, E.J., Raizes, E. and Koumans, E.H. 2015. A farewell to didanosine: harm reduction and cost savings by eliminating use of didanosine. *International journal of STD and AIDS*, 26(12):903–906.

Ekholuenetale, M., Nzoputam, C.I. and Okonji, O.C. 2022. Association between socio-economic factors and HIV self-testing knowledge amongst South African women. *Southern African Journal of HIV Medicine*, 23(1).

Ferrando-Climent, L., Reid, M.J., Rodriguez-Mozaz, S., Barceló, D., and Thomas, K.V. 2016. Identification of markers of cancer in urban sewage through the use of a suspect screening approach. *Journal of Pharmaceutical and Biomedical Analysis*, 129:571–580.

Fisher, I.J., Phillips, P.J., Colella, K.M., Fisher, S.C., Tagliaferri, T., Foreman, W.T., and Furlong, E.T. 2016. The impact of onsite wastewater disposal systems on groundwater in areas inundated by hurricane Sandy in New York and New Jersey. *Marine Pollution Bulletin*, 107:509–517.

Funke, J., Prasse, C., and Ternes, T.A. 2016. Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. *Water Research*, 98:75–83.

Furlong, E.T., Batt, A.L., Glassmeyer, S.T., Noriega, M.C., Kolpin, D.W., Mash, H., and Schenck, K.M. 2017. Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States: pharmaceuticals. *Science of the Total Environment*, 579:629–1642.

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H.P., ... Thomaidis, N.S. 2020. Wide-scope target screening of > 2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. *Journal of Hazardous Materials*, 387:121712.

Gani, K.M., Hlongwa, N., Abunama, T., Kumari, S. and Bux, F. 2020. Emerging contaminants in South African water environment-A critical review of their occurrence, sources and ecotoxicological risks. *Chemosphere*, 128737.

Giebułtowicz, J., Nałecz-Jawecki, G. 2016. Occurrence of immunosuppressive drugs and their metabolites in the sewage-impacted Vistula and Utrata Rivers and in tap water from the Warsaw region (Poland). *Chemosphere*, 148:137–147.

Hamers, R.L., de Wit, T.F.R. and Holmes, C.B. 2018. HIV drug resistance in low-income and middle-income countries. *The Lancet HIV*, 5(10):588–96.

Hansen, K. 2015. Overview of wastewater treatment in South Africa. Association for Water and Rural Development (AWARD) Publication. <https://award.org.za/wp/wp-content/uploads/2020/07/AWARD-Tech-Report-42-Overview-of-waste-water-treatment-in-South-Africa-2015-v1.pdf> . Date of access: 2 April. 2025.

HIVInsite, 2020. The Center for HIV Information at the University of California San Francisco (UCSF). *Zalcitabine (Hivid)*. <http://hivinsite.ucsf.edu/InSite?page=ar-01-03> Date of access: 21 Oct. 2020.

Hopkins, K.L., Doherty, T. and Gray, G.E. 2018. Will the current National Strategic Plan enable South Africa to end AIDS, tuberculosis and sexually transmitted infections by 2022?. *Southern African Journal of HIV Medicine*, 19(1).

Horn, S., Vogt, B., Pieters, R. and Bouwman, H. 2020. Impact of Potential COVID-19 Treatment on South African Water Sources Already Threatened by Pharmaceutical Pollution. *Environmental Toxicology and Chemistry*, 39(7)1305–1306.

Horn, S., Vogt, T., Gerber, E., Vogt, B., Bouwman, H. and Pieters, R. 2022. HIV-antiretrovirals in river water from Gauteng, South Africa: Mixed messages of wastewater inflows as source. *Science of The Total Environment*, 806:150346.

Ibáñez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N.S. and Hernández, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. *Journal of Hazardous Materials*, 323:26–35.

Kang, Z., Duan, L. and Zahmatkesh, S. 2024. Optimizing removal of antiretroviral drugs from tertiary wastewater using chlorination and AI-based prediction with response surface methodology. *Science of The Total Environment*, 934:172931.

Khulu, S., Ncube, S., Nuapia, Y., Madikizela, L.M., Tutu, H., Richards, H., Ndungu, K., Mavhunga, E. and Chimuka, L. 2022. Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer. *Chemosphere*, 286:131973.

Klaus, K. 2018. The Conduit Theory: Protecting Navigable Waters from Discharges to Tributary Groundwater. *Vt. L. Rev.*, 43:871.

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J. and Van Langenhove, H. 2012. From multi-residue screening to target analysis of pharmaceuticals in water: development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. *Science of the Total Environment*, 437:153–164.

K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K. 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere*, 149:238–244.

K'oreje, K.O., Okoth, M., Van Langenhove, H. and Demeestere, K. 2020. Occurrence and treatment of contaminants of emerging concern in the African aquatic environment: Literature review and a look ahead. *Journal of environmental management*, 254:09752.

Kostich, M.S. and Lazorchak, J.M. 2008 Risks to aquatic organisms posed by human pharmaceutical use. *Science of the Total Environment*, 389:329–339.

Kotyza, J., Soudek, P., Kafka, Z. and Vaněk, T. 2009. Léčiva—nový “environmentální polutant. *Chemické listy*, 103:540–547.

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

Laher, A. 2023. *Introduction to water treatment*. Rand Water Forum meeting. [PowerPoint]. <https://randwater.co.za/media/forums/presentations/Introduction%20to%20Water%20%20Treatment.pdf>. Date of access: 4 Apr. 2025.

Maartens, G., Venter, F., Meintjes, G., Cohen, K., Andrews, S., Conradie, F., ... Sanne, I. 2008. Southern African HIV clinicians society: guidelines for antiretroviral therapy in adults. *World Health Organisation site*.

Madikizela, L.M. and Chimuka, L. 2017. Simultaneous determination of naproxen, ibuprofen and diclofenac in wastewater using solid-phase extraction with high performance liquid chromatography. *Water Sa*, 43(2):264–274.

Madikizela, L.M., Ncube, S. and Chimuka, L. 2020. Analysis, occurrence and removal of pharmaceuticals in African water resources: A current status. *Journal of Environmental Management*, 253:109741.

Maharaj, P., Baijnath, S. and Naidoo, P. 2020. Knowledge and practices of HIV infected patients regarding medicine disposal among patients attending public ARV clinics in KwaZulu Natal, South Africa. *BMC Public Health*, 20(1):1–9.

Mani, D.R., Abbatiello, S.E. and Carr, S.A. 2012. Statistical characterization of multiple-reaction monitoring mass spectrometry (MRM-MS) assays for quantitative proteomics. *BMC Bioinformatics*, 13:1–18.

Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., De Alencastro, L.F., ... Barry, D.A. 2013. Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? *Science of the Total Environment*, 461:480–498.

Martin, D. 2005. Guidelines: antiretroviral therapy in adults. *SA Journal of HIV Medicine*, 16:18–31.

Masoner, J. R.; Kolpin, D. W.; Furlong, E. T.; Cozzarelli, I. M.; Gray, J. L. 2014. Contaminants of emerging concern in fresh leachate from landfills in the conterminous United States. *Environmental Science: Process and Impacts*, 16:2335–2354.

Masoner, J. R.; Kolpin, D. W.; Furlong, E. T.; Cozzarelli, I. M.; Gray, J. L. 2016. Landfill leachate as a mirror of today's disposable society: Pharmaceuticals and other contaminants of emerging concern in final leachate from landfills in the conterminous United States. *Environmental Toxicology and Chemistry*, 35:906–918.

McCurry, D.L., Bear, S.E., Bae, J., Sedlak, D.L., McCarty, P.L. and Mitch, W.A. 2014. Superior removal of disinfection byproduct precursors and pharmaceuticals from wastewater in a staged anaerobic fluidized membrane bioreactor compared to activated sludge. *Environmental Science and Technology Letters*, 1(11):459–464.

Meintjes, G., Black, J., Conradie, F., Cox, V., Dlamini, S., Fabian, J., ... Moorhouse, M. 2014. Adult antiretroviral therapy guidelines 2014: guideline. *Southern African Journal of HIV Medicine*, 15(4):121–143.

Meintjes, G., Moorhouse, M.A., Carmona, S., Davies, N., Dlamini, S., Van Vuuren, C., ... Nel, J. 2017. Adult antiretroviral therapy guidelines 2017. *Southern African Journal of HIV Medicine*, 18(1).

Mhuka, V., Dube, S. and Nindi, M.M. 2020. Occurrence of pharmaceutical and personal care products (PPCPs) in wastewater and receiving waters in South Africa using LC-OrbitrapTM MS. *Emerging Contaminants*, 6:250–258.

MIC, The Medicines Information Centre. 2015. South African antiretroviral treatment guidelines (ADULT) 2015. Clinical Pharmacology, University of Cape Town. [Online]. http://www.mic.uct.ac.za/sites/default/files/image_tool/images/51/MIC_Poster_Adult_2015_No%20Logo.pdf

Mlunguza, N.Y., Ncube, S., Mahlambi, P.N., Chimuka, L. and Madikizela, L.M. 2019. Adsorbents and removal strategies of non-steroidal anti-inflammatory drugs from contaminated water bodies. *Journal of Environmental Chemical Engineering*, 7(3):103142.

Mlunguza, N.Y., Ncube, S., Mahlambi, P.N., Chimuka, L. and Madikizela, L.M. 2020. Determination of selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre liquid phase microextraction and liquid chromatography-tandem mass spectrometry. *Journal of Hazardous Materials*, 382:121067.

Mosekiemang, T.T., Stander, M.A., and de Villiers, A. 2019. Simultaneous quantification of commonly prescribed antiretroviral drugs and their selected metabolites in aqueous environmental samples by direct injection and solid phase extraction liquid chromatography - tandem mass spectrometry. *Chemosphere*, 220:983–992.

- Mtolo, S.P., Mahlambi, P.N. and Madikizela, L.M. 2019. Synthesis and application of a molecularly imprinted polymer in selective solid-phase extraction of efavirenz from water. *Water Science and Technology*, 79(2):356–365.
- Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E. and Lambropoulou, D. 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. *Science of The Total Environment*, 699:134322.
- Nantaba, F., Wasswa, J., Kylin, H., Palm, W.U., Bouwman, H. and Kümmerer, K. 2020. Occurrence, distribution, and ecotoxicological risk assessment of selected pharmaceutical compounds in water from Lake Victoria, Uganda. *Chemosphere*, 239:124642.
- Nantaba, F., Palm, W.U., Wasswa, J., Bouwman, H., Kylin, H. and Kuemmerer, K. 2021. Temporal dynamics and ecotoxicological risk assessment of personal care products, phthalate ester plasticizers, and organophosphorus flame retardants in water from Lake Victoria, Uganda. *Chemosphere*, 262:127716.
- Ncube, S., Madikizela, L.M., Chimuka, L. and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. *Water Research*, 145:231–247.
- Nel, J., Dlamini, S., Meintjes, G., Burton, R., Black, J.M., Davies, N.E., ... Moosa, M.Y. 2020. Southern African HIV Clinicians Society guidelines for antiretroviral therapy in adults: 2020 update. *Southern African Journal of HIV Medicine*, 21(1):1–39.
- Nel, J., Wattrus, C., Osih, R. and Meintjes, G. 2023. 2023 Southern African HIV clinicians society adult antiretroviral therapy guidelines: What's new?. *Southern African Journal of HIV Medicine*, 24(1).
- Netshithothole, R., Managa, M., Botha, T.L. and Madikizel, L.M. 2024. Occurrence of selected pharmaceuticals in wastewater and sludge samples from wastewater treatment plants in Eastern Cape province of South Africa. *South African Journal of Chemistry*, 78:7–14.

- Ngumba, E., Gachanja, A. and Tuhkanen, T. 2016a. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of the Total Environment*, 539:206–213.
- Ngumba, E., Kosunen, P., Gachanja, A. and Tuhkanen, T. 2016b. A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards. *Analytical Methods*, 8(37):6720-6729.
- Ngumba, E., Gachanja, A., Nyirenda, J., Maldonado, J. and Tuhkanen, T. 2020. Occurrence of antibiotics and antiretroviral drugs in source-separated urine, groundwater, surface water and wastewater in the peri-urban area of Chunga in Lusaka, Zambia. *Water SA*, 46(2):278–284.
- Ngwenya, N. and Mahlambi, P. 2023. Methods optimization and application: Solid phase extraction, ultrasonic extraction and Soxhlet extraction for the determination of antiretroviral drugs in river water, wastewater, sludge, soil and sediment. *Journal of Pharmaceutical and Biomedical Analysis*, 230:115358.
- Olabode, G.S. 2021. *Spectroscopic and chromatographic analysis of Nevirapine, Lamivudine and Zidovudine anti-retroviral compounds in wastewater samples from selected wastewater treatment plants*. Cape Town: Cape Peninsula University of Technology (Thesis – PhD).
- Peng, X., Wang, C., Zhang, K., Wang, Z., Huang, Q., Yu, Y. and Ou, W. 2014. Profile and behavior of antiviral drugs in aquatic environments of the Pearl River Delta, China. *Science of the Total Environment*, 466:755–761.
- Piri-Moghadam, H., Ahmadi, F. and Pawliszyn, J. 2016. A critical review of solid phase microextraction for analysis of water samples. *Trends in Analytical Chemistry*, 85:133–143.
- Prasse, C., Schlüsener, M.P., Schulz, R. and Ternes, T.A. 2010. Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance?. *Environmental Science and Technology*, 44:1728–1735.

Prasse, C., Wagner, M., Schulz, R. and Ternes, T.A. 2011. Biotransformation of the antiviral drugs acyclovir and penciclovir in activated sludge treatment. *Environmental Science and Technology*, 45(7):2761–2769.

PubChem, 2023. National Centre for Biotechnology Information. PubChem Annotation Record for Maraviroc, Source: Hazardous Substances Data Bank (HSDB). [Online]. <https://pubchem.ncbi.nlm.nih.gov> Date of access: 3 Jan. 2023.

Rimayi, C., Odusanya, D., Weiss, J.M.J.M., de Boer, J., and Chimuka, L. 2018. Contaminants of Emerging Concern in the Hartbeespoort Dam Catchment and the uMngeni River Estuary 2016 *Pollution Incident, South Africa*, 627:1008–1017.

Robson, L., Barnhoorn, I.E.J., and Wagenaar, G.M. 2017. The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56:225–232.

Rozman, D., Hrkál, Z., Eckhardt, P., Novotná, E. and Boukalová, Z. 2015. Pharmaceuticals in groundwaters: a case study of the psychiatric hospital at Horní Beřkovice, Czech Republic. *Environmental Earth Sciences*, 73(7):3775–3784.

Russo, D., Siciliano, A., Guida, M., Andreozzi, R., Reis, N.M., Puma, G.L. and Marotta, R. 2018. Removal of antiretroviral drugs stavudine and zidovudine in water under UV254 and UV254/H₂O₂ processes: Quantum yields, kinetics and ecotoxicology assessment. *Journal of Hazardous Materials*, 349:195–204.

SA. South African Government. 1965. Medicines and Related Substances Control Act, No. 101 of 1965. Government Printers, Pretoria.

SABS, South African Bureau of Standards. 2006. *The South African Drinking Water Quality Standard, SANS 241: 2006 Edition 6.1*. Pretoria, South Africa: South African Bureau of Standards.

SAHIVCS (Southern African HIV Clinicians Society). 2005. Antiretroviral therapy in adults. Guidelines March 2005. [Online]. <https://sahivsoc.org/Files/2005%20Adult%20ART%20Guidelines.pdf> Date of access: 5 Nov. 2020.

SAHIVCS (Southern African HIV Clinicians Society). 2023. Non-nucleoside reverse transcriptase inhibitor class of antiretroviral drugs. [Online].

<https://sahivsoc.org/Guidelines/Module4> Date of access: 2 Jan. 2023.

SANS, South African National Standards. 2015. *SANS 241-1 Drinking water. Part 1: microbiological, physical, aesthetic and chemical determinants*. 2nd ed. Pretoria. South African Bureau of Standards (SABS).

Savjani, K.T., Gajjar, A.K. and Savjani, J.K. 2012. Drug solubility: importance and enhancement techniques. *International Scholarly Research Notices*, 2012.

Schoeman, C., Mashiane, M., Dlamini, M. and Okonkwo, O.J. 2015. Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography and Separation Techniques*, 6(4):1–10.

Schoeman, C., Dlamini, M. and Okonkwo, O.J. 2017. The impact of a Wastewater Treatment Works in Southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. *Emerging Contaminants*, 3(2):95–106.

Simazaki, D., Kubota, R., Suzuki, T., Akiba, M., Nishimura, T. and Kunikane, S. 2015. Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Research*, 76:187–200.

South Africa National Aids Council (SANAC). 2017. *Let our actions count: South Africa's national strategic plan for HIV, TB and STIs 2017–2022*. Pretoria: SANAC.

Southern Africa Treads. 2018. South Africa's landfill sites, a ticking time bomb. Published Groundwork. *Southern Africa Treads*: 23.

Späth, J., Arumugam, P., Lindberg, R.H., Abafe, O.A., Jansson, S., Fick, J. and Buckley, C.A. 2021. Biochar for the removal of detected micropollutants in South African domestic wastewater: a case study from a demonstration-scale decentralised wastewater treatment system in eThekweni. *Water SA*, 47(4):396–416.

Stats SA (Statistics South Africa). 2011a. Ekurhuleni: Tembisa. [Online].

http://www.statssa.gov.za/?page_id=4286&id=11262 Date of access: 17 Jun. 2021.

Stats SA (Statistics South Africa). 2011b. City of Johannesburg: Ivory Park. [Online]. http://www.statssa.gov.za/?page_id=4286&id=11297 Date of access: 17 Jun. 2021.

Stats SA (Statistics South Africa). 2022. Statistical Release P0301.4 Census 2022. [Online]. https://census.statssa.gov.za/assets/documents/2022/P03014_Census_2022_Statistical_Release.pdf Date of access: 17 Jun. 2023.

Swanepoel, C., Bouwman, H., Pieters, R. and Bezuidenhout, C. 2015. Presence, concentrations and potential implications of HIV-anti-retrovirals in selected water resources in South Africa. Water Research Commission. *WRC Report*, 2144(1):14.

Swanepoel, A., Du Preez, H.H. and Cloete, N. 2017. The occurrence and removal of algae (including cyanobacteria) and their related organic compounds from source water in Vaalkop Dam with conventional and advanced drinking water treatment processes. *Water SA*, 43(1):67–80.

Toxopeüs, M. 2019. *The state of sanitation and wastewater treatment services in SA – OPINION*. Politicsweb. [Online]. <https://www.politicsweb.co.za/opinion/the-state-of-sanitation-and-waste-water-treatment-> Date of access: 4 Nov. 2020.

Trickey, A., Glaubius, R., Pantazis, N., Zangerle, R., Wittkop, L., Vehreschild, J., ... Casabona, J. 2024. Estimation of improvements in mortality in spectrum among adults with HIV receiving antiretroviral therapy in high-income countries. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 95(1S):e89–e96.

UNAIDS (The Joint United Nations Programme on HIV/AIDS). 2020. AIDS info. *People living with HIV receiving ART - as of 30 June 2019*. [Online]. <http://aidsinfo.unaids.org/> Date of access: 4 Jun. 2020.

UNAIDS (The Joint United Nations Programme on HIV/AIDS). 2025a. *South Africa*. [Online]. <https://www.unaids.org/en/regionscountries/countries/southafrica> Date of access: 20 Jan. 2025.

UNAIDS (The Joint United Nations Programme on HIV/AIDS). 2025b. 1.1 million people started HIV treatment in South Africa in 2024 – the largest number ever in a single year. [Online].

https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2025/february/20250225_1-1-million-people-treatment-south-africa Date of access: 29 Jun. 2025.

Van der Walt, C.J., Taljaard, G.F., Zdyb, L. and Haarhof, J. 2001. Granular activated carbon for the treatment of eutrophic water at Rietvlei Dam. *Chemical Technology-Bedfordview*, (1):8–10.

Vergeynst, L., Haeck, A., De Wispelaere, P., Van Langenhove, H. and Demeestere, K. 2015. Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography–magnetic sector mass spectrometry: Method quality assessment and application in a Belgian case study. *Chemosphere*, 119:S2–S8.

Vitoria, M., Hill, A., Ford, N., Doherty, M., Clayden, P., Venter, F., ... Domanico, P.L. 2018. The transition to dolutegravir and other new antiretrovirals in low-income and middle-income countries: what are the issues? *Aids*, 32(12):1551-1561.

Vogt, B., Bouwman, H., Bezuidenhout, C., Horn, S., Vogt, T., Bothma, L., ... Potgieter, J. 2020. *Quantification, Fate and Hazard Assessment of HIV-ARVs in Water Resources*. Report no. 2594/1/19. Report to the Water Research Commission. Pretoria: Water Research Commission.

WHO (World Health Organization). 2010. *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach-2010 revision*. World Health Organization.

WHO (World Health Organization). 2013. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*. Geneva: World Health Organization. [Online].
https://iris.who.int/bitstream/handle/10665/85321/9789241505727_eng.pdf?sequence=1
Date of access: 3 Jan. 2022.

WHO (World Health Organization). 2018. *Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: interim guidelines: supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection (No. WHO/CDS/HIV/18.51)*.

WHO (World Health Organization). 2019. *Update of recommendations on first- and second-line antiretroviral regimens*. Geneva, Switzerland: World Health Organization; 2019 (WHO/CDS/HIV/19.15). Licence: CC BY-NC-SA 3.0 IGO.

WHO (World Health Organization). 2020a. WHO Director-General's opening remarks at the media briefing on COVID-19–11 March 2020. <https://www.who.int/dg/speeches/2020/11-march-2020> Date of access: 12 May 2024.

WHO (World Health Organization). 2020b. *Naming the Coronavirus Disease (COVID-19) and the Virus that Causes it*. [Online]. [https://www.who.int/news/item/28-02-2020-naming-the-coronavirus-disease-\(covid-19\)-and-the-virus-that-causes-it](https://www.who.int/news/item/28-02-2020-naming-the-coronavirus-disease-(covid-19)-and-the-virus-that-causes-it) Date of access: 12 May 2024.

WHO (World Health Organization). 2020c. World Health Organization. *Thailand joins the WHO "Solidarity Trial": global testing of effective treatments of COVID-19 across 8 countries – an aggressive effort to save lives from the pandemic (press release), 20 March 2020*. [Online]. <https://www.who.int/news/item/20-03-2020-thailand-joins-the-who-solidarity-trial> Date of access: 4 Jun. 2023.

Wood, R. and Fox, J. 2013. HIV/AIDS in South Africa. *The Lancet*, 382(9907):1634–1645.

Wood, T.P., Duvenage, C.S.J., and Rohwer, E. 2015. The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution*, 199:235–243.

Wood, T.P., Basson, A.E., Duvenage, C., and Rohwer, E.R. 2016. The chlorination behaviour and environmental fate of the antiretroviral drug nevirapine in south African surface water. *Water Res*, 104:349–360.

Wooding, M., Rohwer, E.R., and Naudé, Y. 2017. Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: a disposable sorptive sampler with comprehensive gas chromatography – time-of-flight mass spectrometry and large volume injection with ultra-high performance li. *Journal of Chromatography A*, 1496:122–132.

WRC, Water Research Commission. 2017. National Groundwater Strategy. (WRC Project No. 1074 TT 714/17).

Wu, F., Zhang, J., Xiao, A., Gu, X., Lee, W.L., Armas, F., ... Endo, N. 2020. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *Msystems*, 5(4):10–1128.

Xolo, T. and Mahlambi, P. 2024. Molecularly imprinted polymers as solid-phase and dispersive solid-phase extraction sorbents in the extraction of antiretroviral drugs in water: adsorption, selectivity and reusability studies. *Journal of Analytical Science and Technology*, 15(1):11.

Yeni, P.G., Hammer, S.M., Carpenter, C.C., Cooper, D.A., Fischl, M.A., Gatell, J.M., ... Montaner, J.S. 2002. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *Jama*, 288(2):222–235.

Yu, X., Sui, Q., Lyu, S., Zhao, W., Liu, J., Cai, Z., Yu, G. and Barcelo, D. 2020. Municipal Solid Waste Landfills: An Underestimated Source of Pharmaceutical and Personal Care Products in the Water Environment. *Environmental Science and Technology*, 54(16):9757–9768.

Zash, R., Holmes, L., Diseko, M., Jacobson, D.L., Brummel, S., Mayondi, G., Isaacson, A., Davey, S., Mabuta, J., Mmalane, M. and Gaolathe, T. 2019. Neural-tube defects and antiretroviral treatment regimens in Botswana. *New England Journal of Medicine*, 381(9):827–840.

Zulu, M.B. 2020. Comparative evaluation of the impacts of two wastewater treatment works on the water quality of Roodeplaat Dam in Tshwane, Gauteng. *UNISA Institutional Repository*. <https://uir.unisa.ac.za/handle/10500/27399> Date of access: 23 Aug. 2023.

CHAPTER 3 SEASONAL AND GEOGRAPHICAL DISPARITIES IN ANTIRETROVIRAL DRUG CONTAMINATION OF RIVER WATERS: SOUTH AFRICA

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Abstract

Antiretroviral drugs (ARVDs), cardinal to public health, are emerging as environmental contaminants in river systems. The present study explores the influence of seasonal changes and geographic location on ARVD concentrations in four major rivers (Hennops, Jukskei, Klip, Crocodile) in the Gauteng province, South Africa. It investigates the spatial and temporal distribution of five ARVDs (efavirenz, lopinavir, nevirapine, ritonavir, and zidovudine) over a five-month period. Using spatial interpolation and detailed water analyses, contamination hotspots were identified particularly in proximity to wastewater treatment plants (WWTPs). The samples were extracted by solid-phase extraction (SPE) and analysed by using liquid chromatography coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF/MS). Analytical methods demonstrated good precision (%RSD < 15%), acceptable accuracy (recoveries > 76% for most ARVDs), and strong linearity ($R^2 \geq 0.93$). While seasonal disparities were noted, site-based variations were the dominant influence on ARVD concentrations, potentially reflecting proximity to pollution sources and patterns of treatment in densely populated areas. The data revealed consistent contamination patterns, particularly for lopinavir (up to 64 µg/L) and highlight the need for improved monitoring of lopinavir, efavirenz, and ritonavir. Pseudo-persistent ARVD concentrations are likely driven by both environmental conditions and human activity. However, no clear seasonal or hydrological trends were observed. The findings suggest that localised and repeated but variable inputs rather than widespread environmental factors were the primary reasons of ARVD pollution in these specific freshwater systems. The priority rivers to be routinely sampled are the Hennops, Klip, and Jukskei rivers.

Keywords: seasonal variation; geospatial analysis; river pollution; antiretroviral drugs; South Africa.

3.1 Introduction

The widespread use of antiretroviral drugs (ARVDs) in South Africa's public health sector has raised growing concerns about their presence in aquatic environments (Chapter 2). ARVD contamination arises from various sources, including wastewater treatment plants (WWTPs), hospital, and household effluents, and improper disposal. Although WWTPs are intended to mitigate such pollution, of the 850 WWTPs in South Africa approximately 40% fail to meet regulatory standards, such as the South African Green Drop certification criteria, resulting in persistent pharmaceutical loads entering water bodies (Evans, 2023).

ARVDs enter aquatic environments through multiple pathways (Figure 3-1), including excretion after human consumption, improper disposal of unused medications, and discharges from hospitals and WWTPs. After ingestion, ARVDs are excreted via urine or faeces either unchanged or as active metabolites into wastewater systems (Ncube *et al.*, 2018) (Table 3-1). However, conventional WWTPs are not explicitly designed to effectively remove pharmaceuticals and primarily rely on basic physical-chemical processes (Akinawo *et al.*, 2023; Samer, 2015). As a result, many ARVD residues persist and are discharged into rivers via treated effluent, making WWTPs significant point sources of contamination (Talvitie *et al.*, 2015; Szymonik *et al.*, 2017).

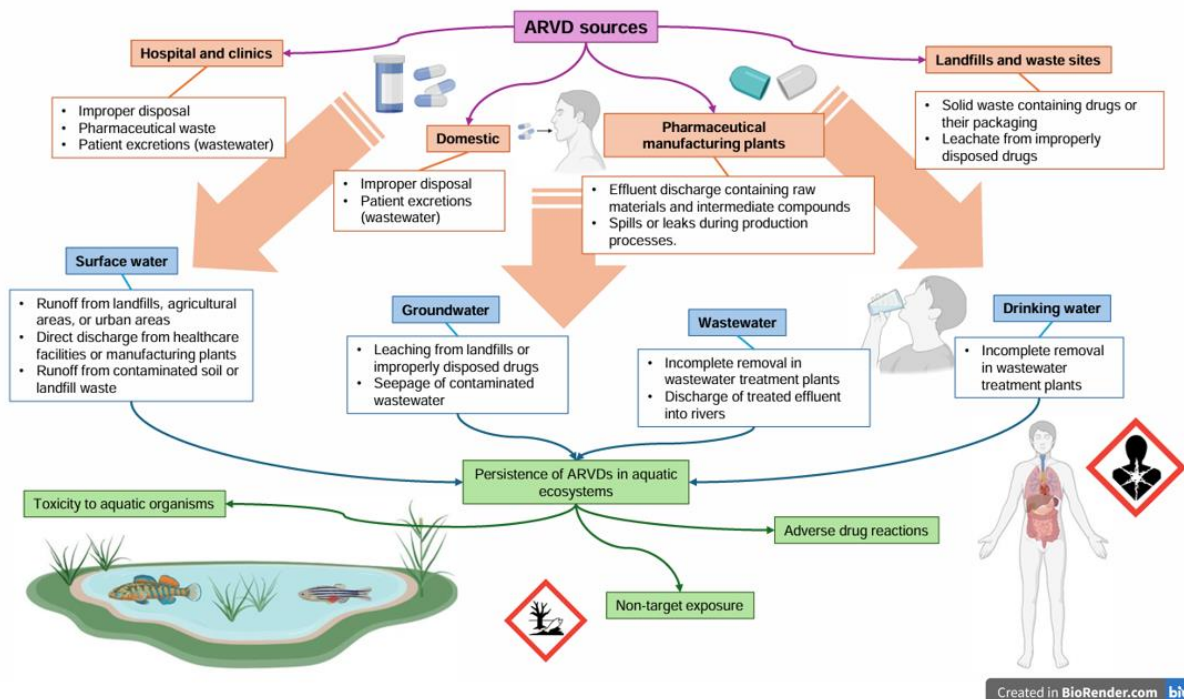


Figure 3-1: Potential sources and pathways of antiretroviral drugs entering the environment, particularly water systems, and their subsequent effects.

Table 3-1: Excreted amounts of unchanged and metabolites of targeted antiretrovirals (ARVDs) to be quantified in the current study (PubChem, 2025).

ARVD	Unchanged (metabolites)	
	Urine	Faeces
Efavirenz (EFV)	<1% (14–34%)	16–61%
Lopinavir (LPV)	2.2% (10.4±2.3%)	19.8% (82.6±2.5%)
Nevirapine (NVP)	<5%	-
Ritonavir (RTV)	3.5% (11.3±2.8%)	33.8% (86.4±2.9%)
Zidovudine (ZDV)	29% (45%)	-

Additional sources include leaking sewage infrastructure, industrial effluent, and runoff from informal settlements. Structural faults in sewage systems allow untreated wastewater to infiltrate surface and groundwater (Held *et al.*, 2006; Iloms *et al.*, 2020). In densely populated informal settlements with limited sanitation, illicit waste often reaches nearby rivers directly or by means of landfills and stormwater runoff (Abbott, 2002; Verster and Bouwman, 2020; Ziajahromi *et al.*, 2017), further exacerbating ARVD pollution in aquatic ecosystems.

The physicochemical properties of ARVDs (Table 3-3), such as water solubility and resistance to degradation, influence their environmental persistence and mobility (Mosekiemang *et al.* 2019; Wang *et al.*, 2023). Each class of ARVDs exhibits different chemical properties that have different behavioural effects in the environment (Table 2-3; Table 3-3). Environmental conditions such as temperature, pH, and microbial activity of water and soil systems can also affect the biodegradation and transformation of ARVDs (Abafe *et al.*, 2018; Adeola and Forbes, 2022; Bavumiragira and Yin, 2022; Chojnacka *et al.*, 2022).

Regional disparities in hydrology, flow regimes, and seasonal climatic conditions further complicate the environmental dynamics of ARVDs by influencing their transport, dilution, and accumulation in river systems. These climatic and weather-related factors play a pivotal role in determining the spatial and temporal distribution of ARVD contamination. In high-rainfall or flood-prone regions, for example, runoff and leaching from landfills and fields can introduce ARVDs into nearby surface and groundwater systems, potentially increasing their presence but could also cause the dilution of the concentration of ARVDs (Lam *et al.*, 2004).

Arid or semi-arid regions are often associated with lower water volumes and theoretically less contaminant mobility. Furthermore, high evaporation rates can lead to increased drug concentrations in surface waters due to less dilution and water loss especially in closed systems (Lam *et al.*, 2004; Osorio *et al.*, 2012). Subsequently, although climate and weather shapes transport mechanisms like runoff and leaching, the dilution and concentration effects can vary substantially depending on flow regimes, catchment hydrology, and the persistence of distinct compounds to name a few (Petrie *et al.*, 2015).

The distribution of ARVDs in the environment is also affected by population density and land-use practices. Regions with a high population density are expected to have a greater production and discharge of wastewater, resulting in higher concentrations of ARVDs in surface waters and sediments (Vogt *et al.*, 2020).

In accordance with Rogowska *et al.* (2022), the occurrence of pharmaceuticals in the environment is a result of both their usage and disposal. Establishing a direct linkage between environmental occurrence and consumption of a pharmaceutical substance is inherently multifarious due to a variety of factors (Aus der Beek *et al.*, 2016).

Examples of known temporal changes in pharmaceutical usage patterns and, subsequently environmental concentrations, range from seasonal shifts in human population profiles (Mandaric *et al.*, 2017; Vatovec *et al.*, 2016) to isolated popular events that result in noticeable spikes (Lindholm-Lehto *et al.*, 2016).

While it is arduous to directly determine the source pathways of ARVDs in aquatic environments, elevated concentrations may suggest certain patterns. These patterns, particularly when observed in proximity to urban wastewater outlets or healthcare facilities, may reflect broader trends in clinical prescriptions, patient adherence, and/or systemic inefficiencies in pharmaceutical waste management practices (Archer *et al.*, 2017). Environmental monitoring, combined with healthcare usage data, offers valuable indirect insight into these trends (Aus der Beek *et al.*, 2016; Kasprzyk-Hordern *et al.*, 2009).

In South Africa, however, national ARVD consumption data are not sufficiently detailed to enable accurate regional or seasonal analysis. Available reports (e.g., Schellack *et al.*, 2017) show increasing public sector use of fixed-dose combinations (FDCs) and protease inhibitors (PIs), while private sector use generally declined (Table 3-2). Emerging drugs such as dolutegravir may shift future patterns (Schellack *et al.*, 2017). Nonetheless, the lack of detailed consumption data limits predictive modelling and risk assessment, highlighting a need for more transparent and standardised pharmaceutical usage reporting.

Table 3-2: Quantitative ARVD consumption (dosage units) by drug class in South Africa (2013 vs. 2015) based on a situational analysis adapted from Schellack *et al.* (2017).

ATC code	Antiretroviral class	Total QTY ^a units 2013	Total QTY ^a units 2015	CAGR ^b (2013–2015)	Market share (2015)
<i>Private sector</i>					
J05AE	PI	102 749	59 806	–24%	1.69%
J05AF	NRTI	256 537	202 173	–11%	5.71%
J05AG	NNRTI	584 759	409 065	–16%	11.55%
J05AR	Combination ARVDs	2 415 447	2 855 217	9%	80.64%
J05AX	Other antivirals	8 347	14 217	31%	0.40%
	Total Units	3 367 839	3 540 478	3%	100%
<i>Public sector</i>					
J05AE	PI	1 528 000	3 395 000	49%	0.06%
J05AF	NRTI	2 672 240 000	554 208 000	–54%	10.21%
J05AG	NNRTI	976 696 000	681 832 000	–16%	12.56%
J05AR	Combination ARVDs	1 187 336 000	4 188 540 000	88%	77.15%
J05AX	Other antivirals	36 000	1 170 000	470%	0.02%
	Total Units	4 837 836 000	5 429 145 000	6%	100%

a = Total QTY units: total number of antiviral units (tablets, vials, capsules, etc.) was determined by considering the pack size (units per pack) and the quantity of packs awarded in the contract to arrive at quantity per unit; b = was used as it is a measure of market growth over multiple time periods for two sectors; ATC = anatomical therapeutic chemical; CAGR = compound annual growth rate; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; QTY = quantity.

To evaluate the risks and develop relief strategies for ARVD contamination, the intricate interactions between the environment and ARVDs with, the economic and social factors that propel drug use and disposal must be considered. This often leads to interdisciplinary research and stakeholder engagement to determine regulatory measures and advanced pharmaceutical waste management (Vogt *et al.*, 2020). Integrating these sciences can aid in providing a more holistic approach to addressing ARVD pollution.

The aim of this investigation is to assess seasonal variation and spatial disparities in ARVD concentrations in rivers, evaluate potential patterns, and identify areas or rivers at greatest potential risk. The study further explores how site-specific characteristics, environmental conditions, and human-related factors, such as the presence and performance of WWTPs, influence ARVD dynamics in South Africa. Comparable regional studies, such as that by Nantaba *et al.* (2023), which assessed the spatial distribution and ecotoxicological risks of pharmaceuticals in Lake Victoria sediments, emphasise the value of geographically resolved monitoring approaches to identify contamination hotspots and support evidence-based environmental management in freshwater ecosystems across African.

Findings from this chapter offer further insights into the environmental burden of ARVDs and provide an evidence base for future monitoring priorities, risk mitigation strategies, and policy development for pharmaceutical pollution control.

3.2 Materials and methods

The present study was approved by the NWU Ethics Committee (NWU-00002-23-A1). The seasonal variation in ARVD concentrations was investigated by statistical analyses of laboratory results obtained from multiple sampling events conducted over time. Samples were collected from the same sites over five different months (March, April, May, August, and October 2018). This multiple-sampling regime was adopted to capture fluxes in contaminant concentrations over time and identify potential seasonal effects. The selected months encompasses both wet and dry periods in the region, allowing for assessment of temporal variation.

Sample locations cover four key river systems in Gauteng province, South Africa (Crocodile, Hennops, Jukskei, and Klip) each of which plays an essential role in pollution dynamics and water quality within the region. A total of 31 sampling sites were selected based on the framework established by Petersen *et al.* (2017), which identified these areas as prospective sources of contaminants of concern, released due to their proximity to WWTPs, medical centres, high population density, and environmental vulnerability.

Site distribution included ten locations along the Jukskei River (Jukskei 1–10), nine along the Hennops River (Hennops 1–9), nine along the Klip River (Klip 1–9), and three along the Crocodile River (Crocodile 1–3) (Figure 3-2). The unequal number of sites across rivers reflects practical and scientific considerations. Site selection was guided by accessibility, safety, and representativeness of key pollution zones. For more detail see Annexures G (Table 7-11).

Sampling points along each river were strategically positioned to reflect a downstream gradient or key pollution discharge areas, including WWTP outlets and densely populated catchments, allowing for the assessment of site-based accumulation, dilution, and potential seasonal washout of ARVDs. Samples were collected from the same locations during five different months to capture temporal fluctuations and identify potential seasonal effects. The resulting laboratory data were subjected to statistical analysis to evaluate both spatial and seasonal trends in ARVD concentrations.

Site-based variation was further investigated by comparing ARVD concentrations relative to hydrological features such as confluences, upstream-downstream site placements, and proximity to known pollution sources. By overlaying sample site coordinates with land-use data and infrastructure locations (e.g., WWTPs, hospitals; Figure 3-2), the study aimed to better contextualize contamination patterns and trace the likely sources of observed pollutants.

Although parts of the dataset were generated in 2018, ARVDs are pseudo-persistent pollutants with ongoing use and limited degradation, implying their environmental levels remain comparatively stable over time. The data thus provides a valuable baseline for temporal comparison in future studies.

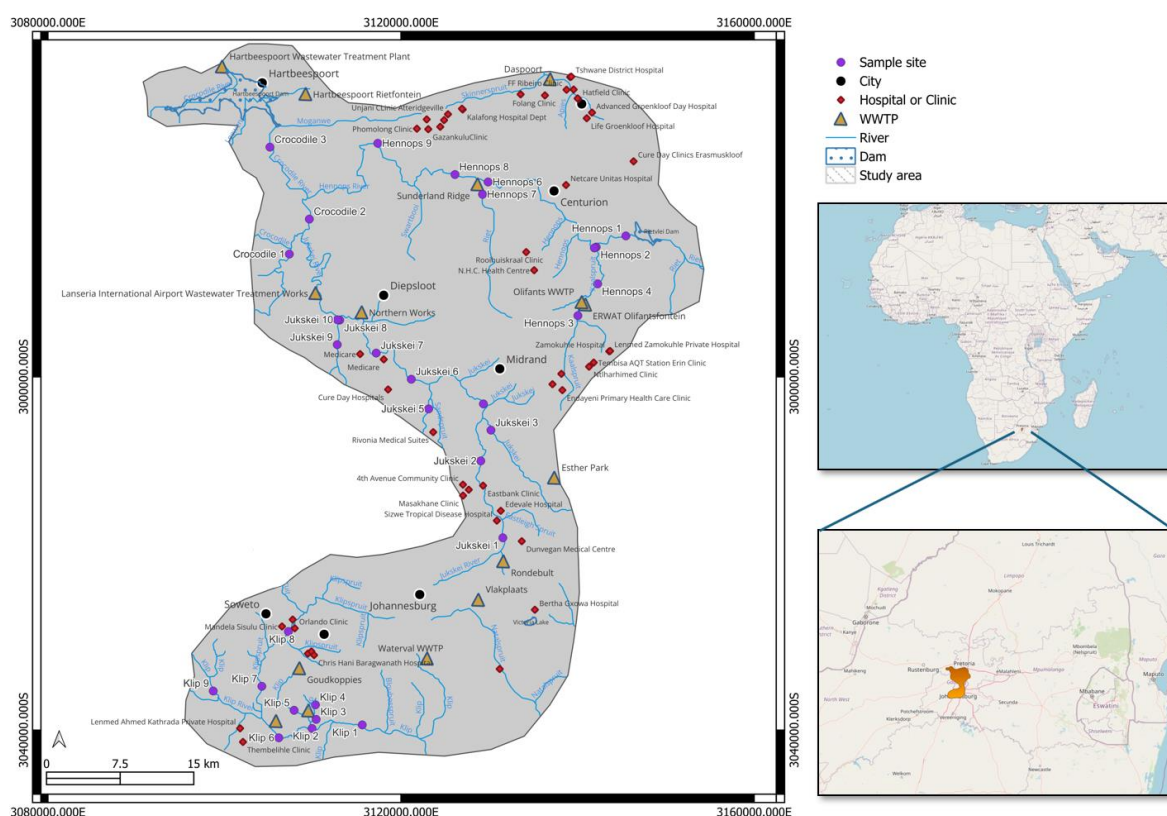


Figure 3-2: Study area and sample sites as located in Gauteng, South Africa.

3.2.1 Sample collection and handling

Samples were collected according to the USEPA standard sampling procedure for pharmaceuticals (USEPA, 2010) using pre-cleaned 1 L Schott bottles. The sampling method was intended to prevent contamination and ensure sample quality. Clean, non-powdered

nitrile gloves were used throughout the sampling process to prevent contamination (Wood *et al.*, 2015). None of the samplers was on any viral medications at the time of sampling (Horn *et al.*, 2022). Field blanks were not included in this study due to logistical constraints associated with multi-site fieldwork and the extensive number of sampling points. Human contact was kept to a minimum to minimise contamination. Sample bottles were lowered mid-depth in the river using a steel extension pipe, rinsed three times with river water, and then sealed. Aqueous grab samples were taken directly from water sources, ensuring a small space was left in the containers to allow for mixing and thermal expansion (e.g. expansion during freezing).

Additional physicochemical parameters (e.g., pH, temperature, turbidity, conductivity, and dissolved oxygen) were not measured in the field, as the study aimed primarily to characterise the spatial and temporal distribution of ARVDs, rather than general water quality dynamics. No additives were added to the samples, as lowering the pH could degrade the target compounds (Ferrer and Thurman, 2012). Moreover, at the time of sampling, turbidity was not recorded, as the target compounds were not known to adsorb to suspended solids (Horn *et al.*, 2022). To ensure reproducibility and accuracy, all samples were collected in triplicate.

During both collection and transport, all samples were immediately placed into a cooler box to protect samples from UV light and to minimise degradation (Peng *et al.*, 2014; Prasse *et al.*, 2010). Immediately after collection, samples were placed in iced cool boxes and kept at temperatures of approximately $\leq 4^{\circ}\text{C}$ during transport to the laboratory. Upon arrival, they were transferred to a refrigerator maintained at 4°C to ensure continued stability (Peng *et al.*, 2014). Extraction of ARVDs from the collected samples was done within 24 hours of collection to ensure sample integrity and reliability of results. The samples remained sealed until they were spiked with a surrogate standard prior to extraction (Horn *et al.*, 2022). From that point onward, laboratory blanks were incorporated into the process.

3.2.2 Chemicals

Samples were analysed to determine the presence of the following ARVDs: efavirenz (EFV), lopinavir (LPV), nevirapine (NVP), ritonavir (RTV), and zidovudine (ZDV) (Table 3-3). Analytical reference standards ($\geq 99\%$ purity) from the United States Pharmacopeial Convention, European Pharmacopoeia, British Pharmacopoeia, and the World Health Organisation were obtained from Stargate Scientific (Johannesburg, South Africa) in crystalline form. Deuterated fluconazole (FLZ-d4) (CAS#1124197-58-5) from Toronto Research Chemicals, Canada, was used as a surrogate standard.

This selection was made since it is a representative of the pharmaceutical class as no ARVD-specific deuterated or isotope-labelled standards were available at the time of analysis (Horn *et al.*, 2022).

Acetonitrile and methanol of spectrometry grade, provided by Honeywell Burdick and Jackson, were purchased from Anatech, South Africa. Acetic and formic acids (98% solutions for mass spectrometry) were acquired from Fluka (Sigma, Germany), as were ammonium formate and ammonium acetate. Deionised water was procured from an in-house ELGA water purification system. Hydrophilic-lipophilic balance (low) (HLB-L) SPE disks (47 mm) were obtained from Atlantic (Horizon Technology, Salem, NH, USA). Individual stock solutions for each ARVD compound were made gravimetrically by weighing 10 mg of each compound and dissolving it in 10 g (7.9 mL) of methanol, ensuring a concentration of 1 mg/mL. These stock solutions were prepared in amber glass vials with solid screw caps. These primary stock solutions were then used to produce standard mix solutions at concentrations of 1, 10, and 100 ng/mL. These were not used as calibration standards (see Table 3-6), but rather to assess compound stability under different conditions (−20°C, 4°C, and 19°C). During the preparation of the stock solutions, an Excel spreadsheet was utilised to calculate the required volume or mass of solvent for the desired concentration. The surrogate standard, FLZ-d4, was prepared to provide a concentration of 5 µg/mL.

3.2.3 Extraction of ARVDs

The techniques employed by Ferrer and Thurman (2012) for pharmaceuticals served as the foundation for the extraction of target ARVDs from water. Water samples were spiked with 500 ng/mL internal standard (FLZ-d4). Using the SPE-DEX system (Horizon Technology, Salem, NH, USA), automated SPE was used to concentrate the target compounds 2 000 times. Oasis HLB-L (47 mm, Horizon Technology) disks were used since they are effective at extracting analytes with a range of polarities and acid/base properties at varying pHs (Pedrouzo *et al.*, 2011). These discs are used in USEPA method 1694 for the examination of medicines and personal care products. Using 30-second soak durations and 15-second dry times, the HLB extraction discs were twice conditioned with methanol. This was followed by another 10-second methanol conditioning with a two-second dry time. Further water conditioning was performed twice, with a 10-second soak period, a 2-second dry time, and no dry time on the second cycle. After passing the water sample through the disc, it was allowed to air-dry for three minutes. Three methanol cycles were used to elute the desired compounds.

The third cycle eluted for one minute and then had a one-minute dry period, while the previous two cycles eluted for three minutes and then had a 20-second dry time. A mild stream of nitrogen gas was used to concentrate the eluent until near dryness. Methanol (500 μ L) was used to reconstitute the samples.

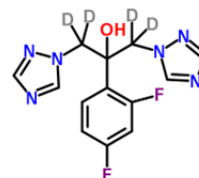
Table 3-3: Physiochemical properties of the target compounds to be analysed.

Compound	CAS#	Molecular formula	Mono-isotopic mass	Structure	Water solubility (mg/mL)	pKA (base; acid)	Log Kow	K _{oc} (mL/g)	Henry's Law constant (atm-cu m/mol at 25 °C)	
ARVD	EFV	155213-67-5	C ₁₄ H ₉ ClF ₃ NO ₂	315.03		0.093	-1.5; 12.52	4.69	17×10 ³	7×10 ⁻⁹
	LPV	192725-17-0	C ₃₇ H ₄₈ N ₄ O ₅	628.36		7.7x10 ⁻⁶	-1.5; 6.26	5.94	1.0×10 ⁶	4.3×10 ⁻²⁸
	NVP	129618-40-2	C ₁₅ H ₁₄ N ₄ O	266.12		0.705	-5.06; 10.37	2.5	-	3.3×10 ⁻¹⁷
	RTV	155213-67-5	C ₃₇ H ₄₈ N ₆ O ₅ S ₃	720.3		1.26x10 ⁻³	2.84; 13.68	6.27	-	2.7×10 ⁻³³
	ZDV	30516-87-1	C ₁₀ H ₁₃ N ₅ O ₄	267.1		7.7x10 ⁻⁶	-3; 9.96	0.05	-	2.0×10 ⁻²²

Internal standard

FLZ-d4

C₁₃H₈D₄F₂N₆O 310.13



- - - -

CAS = chemical abstracts service number; EFV = efavirenz; FLZ-d4 = deuterated fluconazole; Koc = soil organic carbon-water partition coefficient; LPV = lopinavir; Log Kow = logarithm of the octanol-water partition coefficient; pKa = acid dissociation constant. NVP = nevirapine; RTV = ritonavir; ZDV = zidovudine. Information obtained from <https://www.ncbi.nlm.nih.gov/pccompound/>, <https://www.drugbank.ca/drugs/>, and <https://comptox.epa.gov/dashboard/dsstoxdb/> accessed June 2024.

3.2.4 Instrumentation

An Agilent 6540 Accurate Mass Quadrupole-Time-Of-Flight/Mass Spectrometry (Q-TOF/MS) (G6540A) (Agilent Technologies, Santa Clara, CA, USA) was connected to a 1290 Infinity Binary pump (G4220A), 1290 Infinity Autosampler (G4226A), and 1290 Infinity Thermostatted Column Compartment (G1316C) as part of the ultra-high-performance liquid chromatography (UPLC) system.

Positive and negative electrospray ionisation (ESI) enhanced with Agilent Jet Stream (AJS) technology was used to desolvate and ionise the samples. The instrument was set to extended dynamic range (2 GHz), and the Q-TOF was configured to scan between 50 and 950 m/z. Quantitative Analysis for Q-TOF (version B.05.01), MassHunter Data Acquisition (version B.05.00), and MassHunter Qualitative Analysis (version B.05.00) were the software programs used. Daily mass axis calibration of the Q-TOF instrument was performed using tuning mixes (G1969-85000, Agilent) under both positive and negative ionisation modes. A reference solution containing compounds with known protonated masses of 121.050873 [M+H]⁺ and 922.009798 [M+H]⁺ was continuously infused to ensure accurate mass calibration throughout the analysis.

The polarity of the ARVDs investigated varies (Table 3-3) which affects the separation method applied. The Poroshell 120 Bonus-RP columns (Agilent, 2.1×100mm 2.7 µm) and an Agilent ZORBAX C18 (stationary phase) were initially examined in the present study. However, the Bonus-RP column kept at 25°C produced the best peak separation and was used for subsequent analyses. The injection volume was 1 µL.

Table 3-4: Optimised liquid chromatography and mass spectrometry parameters used for analysis.

Parameters	Positive ionisation		Negative ionisation	
Injection volume	1 µL			
Column	Poroshell 120 Bonus-RP column (Agilent, 2.1 x 100 mm, 2.7 µm)			
Column temperature	25°C			
Flow rate	0.6 mL/min		0.7 mL/min	
Mobile phase A	Water + 0.05% formic acid		Water + 0.1% acetic acid	
Mobile phase B	ACN + 0.05% formic acid		ACN + 0.1% acetic acid	
Gradient (min)	A (%)	B (%)	A (%)	B (%)
0/0	90	10	95	5
8.5/4.5	90	10	95	5
8.6/4.6	50	50	90	10
13/7	50	50	90	10
13.3/7.1	0	100	50	50
14.3/11	0	100	50	50
15/11.1	90	10	0	100
12.2	-	-	0	100
Post run-time	2 min		13.8 min	
Total run-time	17 min		15 min	
Drying gas temperature	275°C		275°C	
Drying gas flow	10 L/min		10 L/min	
Nebuliser pressure	45 psi		45 psi	
Sheath gas temperature	400°C		400°C	
Sheath gas flow	10 L/min		10 L/min	
VCap	3000 V		3000 V	
Nozzle voltage	0 V		300 V	
Fragmentor	130 V		130 V	
Skimmer	48 V		48 V	
OCT RF Vpp	750 V		750 V	

Table 3-5: Retention times and m/z values of ARVDs and internal standard (IS) analysed.

	Compounds	Retention time (min)	m/z
Negative ionisation	Efavirenz	10.0	314.027
	Zidovudine	3.6	266.092
	Fluconazole-d4 (IS)	6.3	309.124
Positive ionisation	Lopinavir	11.1	629.373
	Nevirapine	7.5	267.125
	Ritonavir	10.7	721.319
	Fluconazole-d4 (IS)	3.8	311.125

3.2.5 Quality control and quality assurance

3.2.5.1 Matrix-matched external calibration curve

To account for matrix effects during quantification, an external matrix-matched calibration curve (spiked post-extraction) was utilised to control for possible matrix-suppression and -enhancement effects. Deionised water (18.2 MΩ) was supplemented with 0.7 mM NaHCO₃, 2 mM CaCl₂·2H₂O, 0.5 mM MgSO₄·7H₂O, and 75 μM KCl to make artificial freshwater according to International Organization for Standardization (ISO) 6341 (ISO, 2012), to replicate natural occurring water as closely as possible for the matrix-matched calibrations. To mimic natural organic components, 25 mature freshwater snails (*Bulinus tropicus*) reared under clean laboratory environments were added to 1 L of the ISO-prepared water for approximately 24 h. Five percent of this snail-infused water was combined with ISO water to create the matrix-matched water. After extracting 1 L of this matrix, it was concentrated using nitrogen gas, reconstituted in methanol, and spiked with ARVDs, fluconazole, and d4-fluconazole to reach a final volume of 0.5 mL. This process produced matrix-matched calibrations.

The concentrations for the calibration curve were established based on the anticipated levels of ARVDs in the environment and the performance of the instrument. During extraction, samples were concentrated two thousand times. This should be taken into consideration regarding the calibration range. To create a range that would encompass all the ARVD concentrations in various samples, the calibration levels were initially varied for every sampling event. The calibration ranges are specified in the results section. Dilutions originated from different stocks, rather than being serially prepared. To determine the reportable range, these requirements were repeated across three separate validation attempts to ensure the robustness of the method (Westgaard, 2008). To avoid carry-over, they were administered in ascending concentrations, separated by blank injections.

3.2.6 Data analysis

$$X_{\text{ARVD}} = \left(\frac{(\text{Native/Stable isotope}) - c}{m} \right) \times \text{ISO}_{\text{conc}} \quad (3-1)$$

Where:

X_{ARVD}	= calculated analyte concentration
Native	= native abundance
Stable isotope	= stable isotope abundance
c	= calibration curve y-intercept

m = slope of calibration curve
 ISO_{conc} = internal standard concentration

It is important to emphasise the necessity of proper interpretation of the final ARVD results (LOD and LOQ) concerning the critical value (x_c) in the current study. If the measured concentration (x) value was as follows:

$x < x_c$ = analyte not detected; concentration is < LOD

$x_c < x < LOQ$ = analyte detected; concentration is < LOQ

$x > LOQ$ = analyte detected, concentration can be quantified with dependable accuracy and precision (Rajaković *et al.*, 2012).

For statistical analyses, values < LOQ were assigned as $\frac{1}{2}$ LOQ to avoid biases related to non-detects while maintaining a conservative estimation. This approach ensures data comparability and conserves statistical power without overestimating or underestimating concentrations (Rajaković *et al.*, 2012).

3.2.7 Precision

The repeatability of the method was assessed by analysing three QC samples spiked with low, medium, and high concentrations of analytes. Precision was estimated using the formula (Eq. (3-2):

$$\%RSD = \left(\frac{\text{mean of the standard deviations of QCs}}{\text{mean of QCs}} \right) \times 100 \quad (3-2)$$

For good repeatability, the %RSD should be below 15% (Schoeman *et al.*, 2015). Intra- and interlaboratory precision was evaluated by analysing the QC samples over three consecutive days in triplicate. Interlaboratory precision was satisfactory (%RSD < 15%) for most compounds.

3.2.8 Accuracy

Accuracy was evaluated by calculating the mean recovery of spiked compounds at three different concentrations in the analysed matrix. The formula used was Eq. (3-3):

$$\% \text{ accuracy} = \left(\frac{\text{mean value}}{\text{true value}} \right) \times 100 \quad (3-3)$$

Nevirapine, and ZDV demonstrated acceptable accuracy, with recoveries > 76%. However, RTV and LPV showed lower recoveries at 63% (Table 3-6).

3.2.9 Linearity

The linearity of the calibration curve was evaluated by calculating the R^2 value for each set of analyses (Table 3-6). Good linearity is denoted by an R^2 value as close to one as possible, with a minimum acceptable value of 0.9 (Miller and Miller, 2018). R^2 values close to one indicate a strong linear relationship between the measured values and the concentrations or amounts of the target compounds. All targeted compounds demonstrated excellent linearity, with R^2 values of 0.93 or higher across all analyses. Confirming the reliability of the calibration curve for quantifying the compounds in these analyses.

3.2.10 Limit of detection (LOD)/ Limit of quantification (LOQ)

Using linear regression analysis, the uncertainties of the calibration curve can be employed to determine the method's LOD and LOQ from an external matrix-matched calibration curve. Based on the equation $y = mx + c$, the LOD is determined as $3 \cdot S_a/b$, and the LOQ as $10 \cdot S_a/b$, where S_a is the standard deviation of the intercept (abundance) and b is the slope of the calibration curve (Schoeman *et al.*, 2015) (Table 3-6).

Table 3-6: Quality control and quality assessment (QC/QA) results for sampling of the Hennops, Jukskei, Klip, and Crocodile rivers.

	Linear range (µg/L)	Linearity	LOD (µg/L)	LOQ (µg/L)	Accuracy (%)	Interday precision %RSD	Intraday precision %RSD
EFV	0, 5, 10, 20, 40, 80, 160, 330, 660, 1000	0.972	386	1286	196	14.1	16.9
LPV	0, 5, 10, 20, 40, 80, 160, 330, 660, 1000	0.999	74	248	63	7.8	29.6
NVP	0, 5, 10, 20, 40, 80, 160, 330, 660, 1000	0.980	322	1074	76	4.1	5.5
RTV	0, 5, 10, 20, 40, 80, 160, 330, 660, 1000	0.938	578	1926	63	7.4	30.7
ZDV	0, 5, 10, 20, 40, 80, 160, 330, 660, 1000	0.979	333	1111	80	13.7	6.4

EFV = efavirenz; LOD = limit of detection; LOQ = limit of quantification; LPV = lopinavir; NVP = nevirapine; RSD = relative standard deviation; RTV = ritonavir; ZDV = zidovudine.

3.2.11 Statistical methods of investigation

Statistical analyses were conducted in multiple steps to account for the nature of the data. Normality was assessed to determine whether parametric or non-parametric tests were appropriate. The D'Agostino and Pearson test was used for large sample sets and Kolmogorov-Smirnov or Shapiro-Wilk test in smaller sample sets ($\alpha = 0.05$). For non-parametric data (that did not meet the assumptions of normality), Mann-Whitney statistical tests were used to compare independent groups of data. In contrast, for data that was normally distributed, parametric tests were applied.

These included one-way analysis of variance (ANOVA) for comparing multiple groups and repeated measures two-way ANOVA when comparing related groups or repeated observations. Where these tests indicated statistical significance of differences, a post-hoc test was conducted to identify which groups differed from each other. Spearman's rank correlation coefficient (Spearman's ρ) analyses were performed to identify monotopic relationships between variables. A significance level of $\alpha = 0.05$ was applied for all statistical tests.

Some graphs present a subset of processed data used for statistical analysis. Values $< \text{LOD}$ were substituted with zero, and values $< \text{LOQ}$ were replaced with $\frac{1}{2} \text{LOQ}$ (Helsel, 2011). Consequently, not all sample sites are displayed in the graph, as only sites with sufficient quantifiable data were included (e.g. Figure 3-3).

3.2.12 Spatial interpolation

The IDW interpolation technique was used to create a spatial map of ARVD distribution in water using the QGIS software version 3.40.3-Bratislava. This approach is regarded as one of the most popular deterministic interpolation techniques, aside from Splines and Kriging, due to its ease of use, adaptability and capacity to manage unevenly spaced data (Oyinkuro and Rowland, 2018).

This method of estimating an unknown value at a location using certain known values and related weighted values is known as deterministic spatial interpolation and uses a linear-weighted combination set of sample points to determine cell values (Arman *et al.*, 2025; Fatima *et al.*, 2022; Nistor *et al.*, 2020; Yang *et al.*, 2020). For more detail see Annexures G.

3.3 Results

3.3.1 Tracking antiretroviral trends in South African river systems

Quality control and assessment results demonstrated acceptable analytical performance across all five ARVDs (Table 3-6). All compounds exhibited strong linearity ($R^2 \geq 0.94$) over a wide calibration range (0–1000 $\mu\text{g/L}$), with LPV showing the highest linearity ($R^2 = 0.999$). LOD and LOQ varied among ARVDs, with LPV presenting the lowest LOD (74 $\mu\text{g/L}$), while RTV had the highest (578 $\mu\text{g/L}$). Accuracy ranged from 63% (RTV and LPV) to 96% (EFV). Interday and intraday precision (%RSD) were largely within acceptable limits ($< 15\%$), except for higher variability in EFV, RTV, and LPV's intraday measurements, which exceeded 15%, representing possible inconsistencies during extraction or instrument response.

The presence of ARVDs in water samples was examined by calculating the mean concentrations for each drug across the different river systems and sampling sites (see Annexures F to Table 7-10). Analysis of ARVD concentrations across several months revealed varying concentration ranges: March ($<\text{LOD}$ –63.7 $\mu\text{g/L}$), April ($<\text{LOD}$ –16.2 $\mu\text{g/L}$), May ($<\text{LOD}$ –6.61 $\mu\text{g/L}$), August ($<\text{LOD}$ –46.8 $\mu\text{g/L}$), and October ($<\text{LOD}$ –13 $\mu\text{g/L}$). Across all sampling months, LPV consistently exhibited the highest quantifiable concentrations in surface water. The highest overall ARVD concentration was recorded at Hennops sample site 6, where LPV reached a concentration of 63.7 $\mu\text{g/L}$.

Comparing ARVD concentrations between river systems, the highest concentrations per system were recorded at the following sites, all for LPV: Hennops sample site 6 (63.7 $\mu\text{g/L}$); Klip sample site 3 (46.8 $\mu\text{g/L}$); Jukskei sample site 10 (23.1 $\mu\text{g/L}$); and Crocodile sample site 2 (19.5 $\mu\text{g/L}$). Comparisons between river systems with the greatest average ARVD concentrations revealed that the Hennops River had the highest ARVD burden, especially in March (7.8 $\mu\text{g/L}$) and August (4.9 $\mu\text{g/L}$), followed closely by the Klip River in August (7.6 $\mu\text{g/L}$).

The relative abundance and composition of ARVDs across sites and rivers are displayed in (Figure 3-3). Drug composition revealed that certain ARVDs had higher concentrations at certain sites. From the composition analysis, it is clear that LPV (orange bars) appeared to be the most abundant drug across almost all sample sites and months, with notably higher concentrations than the other drugs.

EFV displayed higher concentrations in the months of August and October, whereas RTV displayed higher concentrations in March and August (Figure 3-3). In March and August, LPV concentrations were highest across numerous sample sites, representative of a possible increased ARVD input during these months. April and May demonstrated reduced LPV concentrations, but the presence of the other drugs remained fairly stable. October had lower ARVD concentrations overall. This variation suggests potential environmental or seasonal influences on ARVD distribution in surface water. Further statistical tests were performed to investigate the significance of seasonal influences.

Correlation analyses were performed to see if there were any time trends. The Spearman's correlation test illustrated the strength and direction of the relationship between variables over time (months) without assuming linearity or normality. A moderate to strong positive correlation was found for March, April, August, and October (> 0.61 ; Table 3-7). Trends in these months could be linked. A high correlation between March and August (0.68) was found which might suggest seasonal or cyclical patterns. The month of May appears to be an outlier, having weak or no correlations with the other months.

Table 3-7: Results of the Spearman correlation test. Data in bold present a strong positive correlation.

	Mar	Apr	May	Aug	Oct
Mar	1	0.608	-0.019	0.680	0.616
Apr	0.608	1	0.408	0.608	0.541
May	-0.019	0.408	1	0.183	0.006
Aug	0.680	0.608	0.183	1	0.398
Oct	0.616	0.541	0.006	0.398	1

The results of the repeated measures (RM) two-way ANOVA provided insights into the effects of two factors (sampling site and month) on the variability of ARVDs in water samples, as well as their interaction. There was no significant interaction between the sampling sites (e.g. Hennops 1 vs. Klip 9) and month (e.g. May vs. August) factors. The F-statistic for the interaction term was 1.02, with $p = 0.42$, suggesting no significant effect from the interaction between factors. The effects of the sampling sites and month factors are therefore independent of each other. Sampling sites do not significantly vary across different months. The p-value for sampling sites was 0.09 ($p > \alpha$; $F = 2.74$). This suggests that the sampling sites do not have a significant effect on the ARVDs quantified in surface water and that the variation in ARVDs cannot be attributed to sampling sites alone.

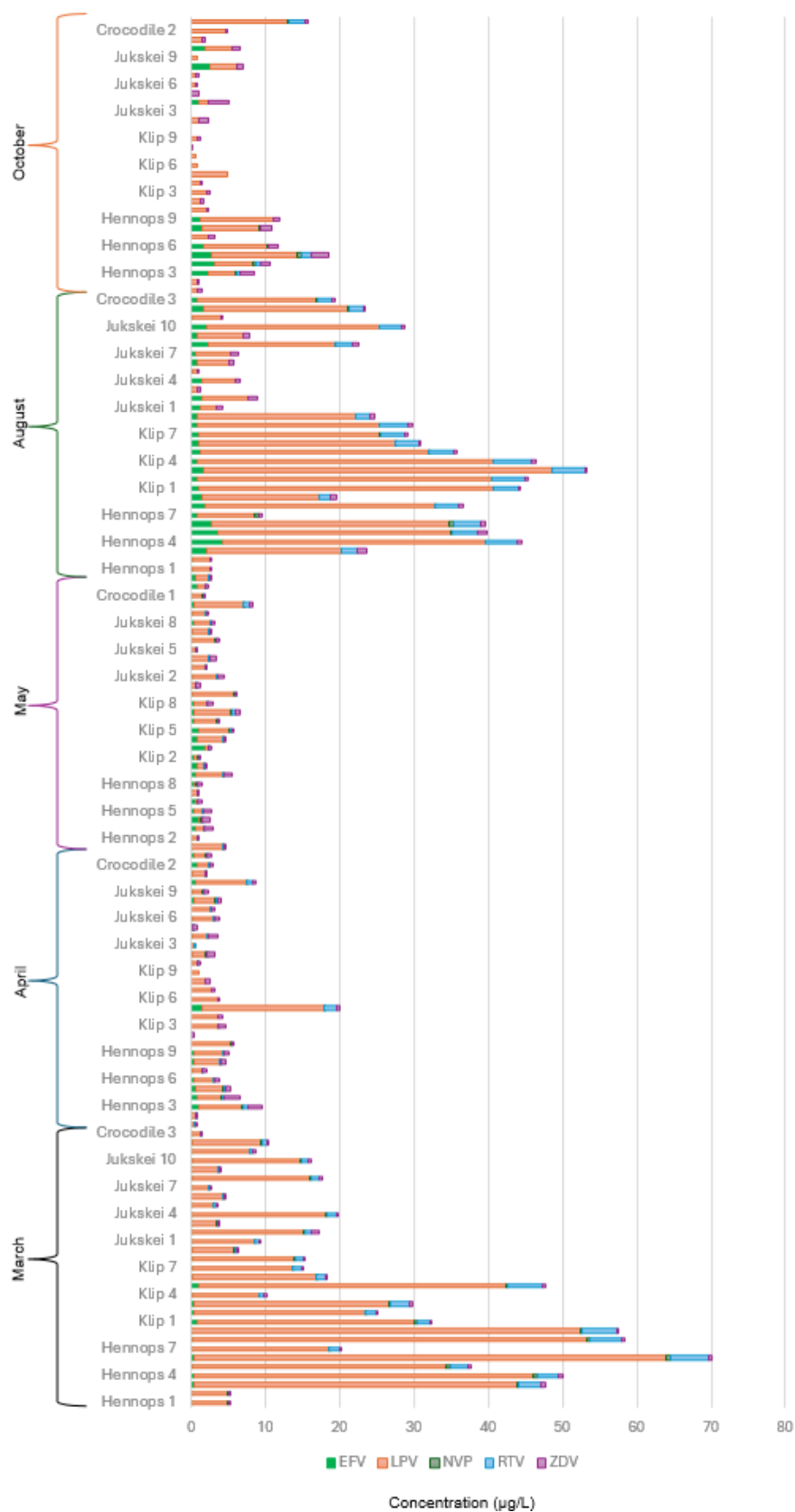


Figure 3-3: Antiretroviral drug concentration profiles across five months (March, April, May, August, and October) at different sampling sites. Data shown are based on

processed concentrations used for statistical analysis. Only sites with sufficient quantifiable data are displayed. EFV = efavirenz, LPV = lopinavir, NVP = nevirapine, RTV = ritonavir, and ZDV = zidovudine.

The results also indicated no significant effect of the time (month) factor on ARVD concentrations ($p = 0.6$; $F = 0.7$). The differences in ARVD concentrations across several months were therefore not meaningful. The data revealed substantial individual variability, which suggests that individual differences explain a large proportion (48%) of the total variation in the data. Given that sphericity was not assumed, the Geisser-Greenhouse correction was applied to the sample site effect, yet the adjusted significance level remained non-significant ($p = 0.09$).

Subsequently, there was no strong evidence to conclude that the sample site and time factors had significantly influenced the ARVD concentrations, either independently or interactively. Inter-individual differences (subject effect) accounted for nearly half of the total variance, implying that individual sample site factors had major contributing factors. This suggests that the variation in ARVD concentrations was greatly due to site-specific differences, rather than differences between months or the interaction between site and month. Additional investigations into these individual characteristics are warranted.

3.3.2 Temporal and spatial distribution of ARVD contamination in water

To obtain useful ARVD water contamination information, the spatial and temporal distribution trends were investigated.

3.3.3 IDW interpolation

The IDW approach makes the assumption that the concentrations of nearer unsampled sites are closer than sites further away (Haldar *et al.*, 2020). IDW was utilised in this investigation because ARVD concentrations in water are assumed to change continuously and are notably influenced by nearer monitoring stations. Additional layers were included in the spatial analysis (e.g. proximity to WWTPs) to identify any other environmental correlations (see Figure 3-4 to Figure 3-8).

3.3.3.1 Spatial patterns

There appears to be somewhat of a spatial pattern in the Hennops River system, with ARVD hotspots (concentrations > 1.6 µg/L), especially at Hennops sample Sites 3 and 4 as well as at Sites 8 and 9. The month of May, once again being somewhat of an outlier, only showed hotspots at Hennops sample Sites 1 and 9.

The Crocodile River system revealed to have no substantial predicted ARVD concentrations over several months, with the exception of October (based on the colder IDW colour scheme concentrations which were generally < 0.5 µg/L). Crocodile River sample Site 3 and the surrounding area of Hartbeespoort were predicted to have high ARVD concentrations in October. Crocodile River sample Site 2 also had moderate drug contamination predicted in August.

The downstream gradient of sample sites in the Jukskei River system allowed for the assessment of ARVD accumulation and potential seasonal washout effects. Notably, upstream samples in the Jukskei River remained relatively stable with no meaningful ARVD pollution. Yet, downstream at the Jukskei sample site 8–9, the data consistently depicted higher drug concentrations. March was predicted to have the least drug pollution concentrations compared with other months. However, Mann-Whitney tests revealed that there were no significant differences in drug concentrations between the upstream and downstream samples ($p > 0.5$).

The Klip River system also revealed consistent hotspots over several months, particularly at Klip River sample Site 5. Apart from a few exceptions, the upstream concentrations of drugs were greater than the downstream concentrations. For example, Klip River sample Sites 2 and 3 generally had lower concentrations than Klip River sample Site 5. However, non-parametric tests revealed no significant differences in drug concentrations between the upstream and downstream samples (Mann Whitney test; $P > \alpha$; $P = 0.09$ – 0.45).

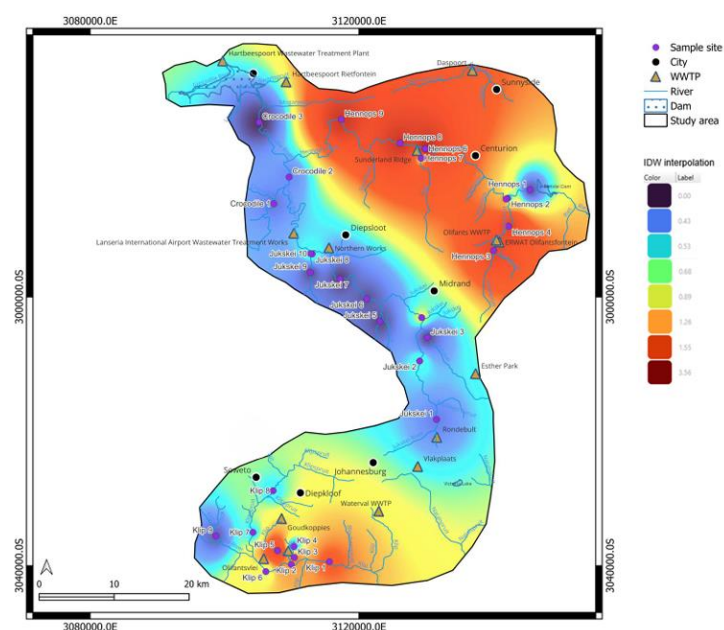


Figure 3-4: Inverse Distance Weighted (IDW) interpolation maps showing the spatial distribution of antiretroviral drugs sampled in March across the study area. The maps use a blue-to-red colour gradient, where blue represents lower values and red indicates higher values ($\mu\text{g/L}$).

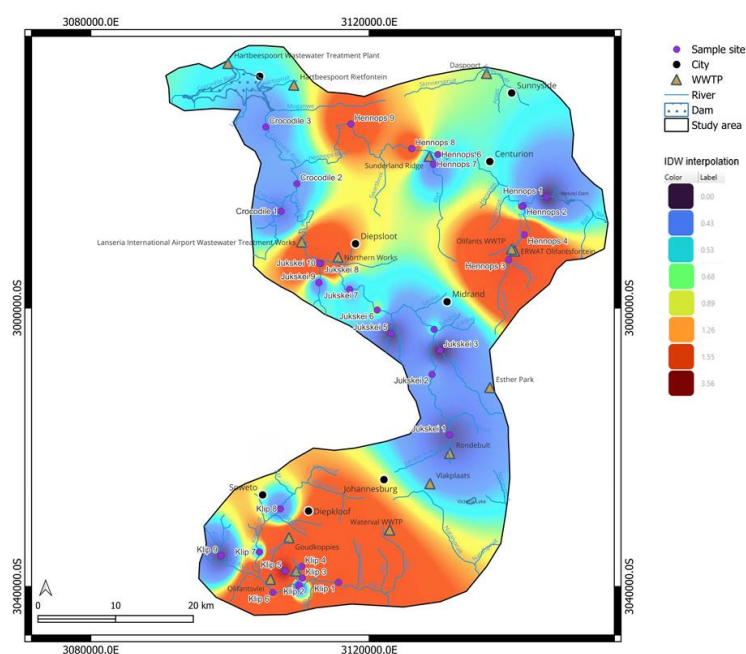


Figure 3-5: Inverse Distance Weighted (IDW) interpolation maps showing the spatial distribution of antiretroviral drugs sampled in April across the study area. The maps use a blue-to-red colour gradient, where blue represents lower values and red indicates higher values ($\mu\text{g/L}$).

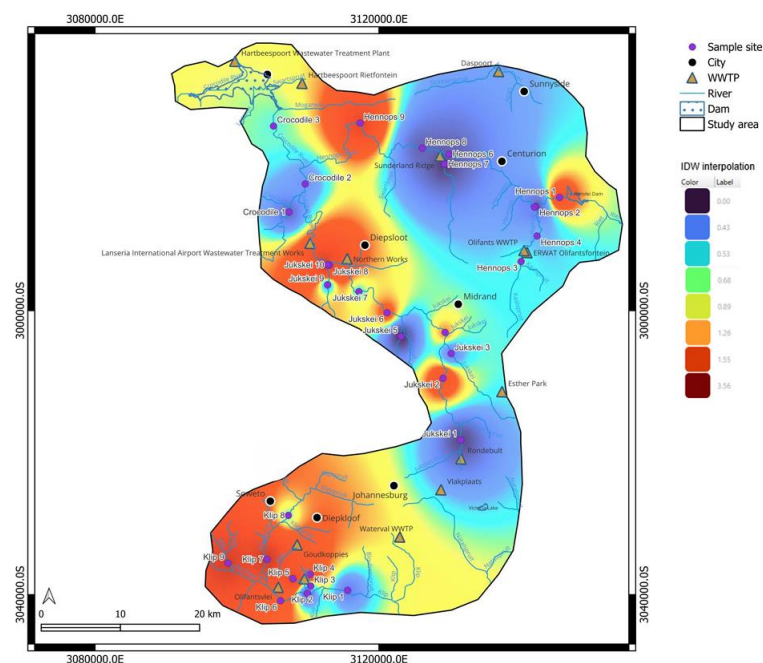


Figure 3-6: Inverse Distance Weighted (IDW) interpolation maps showing the spatial distribution of antiretroviral drugs sampled in May across the study area. The maps use a blue-to-red colour gradient, where blue represents lower values and red indicates higher values ($\mu\text{g/L}$).

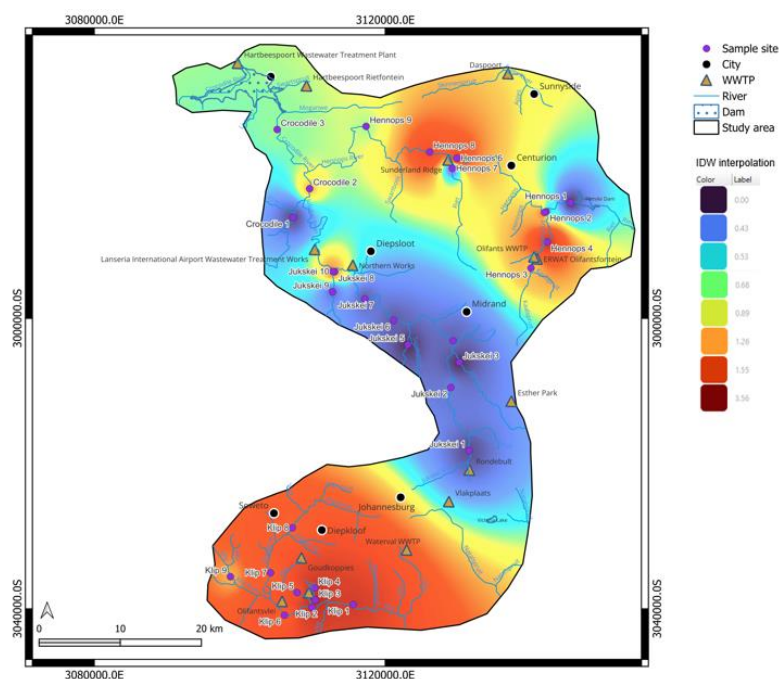


Figure 3-7: Inverse Distance Weighted (IDW) interpolation maps showing the spatial distribution of antiretroviral drugs sampled in August across the study area. The maps use a blue-to-red colour gradient, where blue represents lower values and red indicates higher values ($\mu\text{g/L}$).

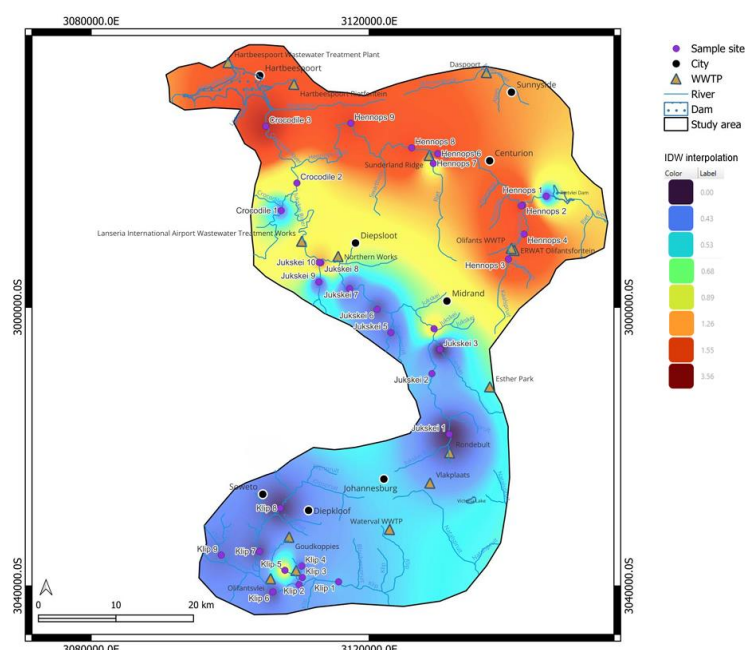


Figure 3-8: Inverse Distance Weighted (IDW) interpolation maps showing the spatial distribution of antiretroviral drugs sampled in October across the study area. The maps use a blue-to-red colour gradient, where blue represents lower values and red indicates higher values ($\mu\text{g/L}$).

3.4 Discussion

For the present study area, November to March is considered the wet season due to higher rainfall and more thunderstorms. April to May (Autumn) is a transition period where rainfall decreases, leading into the dry season of June to August (winter). September to October is the start of another transition period where rainfall increases. Elevated precipitation during the wet season may result in an increased dilution of ARVD in aquatic systems; however, it also contributes to increased runoff from informal urban settlements and wastewater treatment facilities (Mosekiemang *et al.*, 2019). In contrast, during the dry season, diminished river flow may yield higher concentrations of ARVD owing to the decreased dilution effect.

The concentrations of EFV and ZDV were higher during the dry season compared with the wet season. In contrast, NVP exhibited higher concentrations in the wet season than in the dry season (Figure 3-3). This concurs with studies reporting rainfall as an important factor influencing pharmaceutical concentrations in wastewater and receiving water (Kasprzyk-Hordern *et al.*, 2009). The concentrations of RTV and LPV varied inconsistently between the wet and dry seasons.

The fluctuating concentrations of RTV and LPV across seasons suggest influence of several variable factors, including wastewater input, degradation rates, or adsorption/desorption dynamics in sediments and organic matter.

Mosekiemang *et al.* (2019) documented increased 3TC, FTC and 12-hydroxy nevirapine concentrations in the dry compared with the wet season in the Western Cape of South Africa, which aligns with the current study. However, the authors reported an increase in EFV concentrations and its metabolites (8,14-dihydroxy efavirenz) in the wet compared with the dry season, which is in contradiction with the results for EFV in the current study. According to Mosekiemang *et al.* (2019) this resulted from low inflows in the dry season, promoting in-pipe settling of compound-bearing solids, as contrasted with the wet season where high flow rates facilitate suspension and transmission of compound-bearing solids to WWTPs (Peng *et al.*, 2014; Schoeman *et al.*, 2017).

The observed higher concentrations of NVP during the wet season could potentially be attributed to factors such as increased runoff, variation in wastewater discharge, and/or increased solubilisation during heavy rainfall. Heavy rainfall can increase the solubilisation of residual pharmaceutical compounds present in sediments or soils (Nantaba *et al.*, 2024), causing an increase in surface waters concentrations during the wet season. Wood *et al.* (2015) examined the chlorination behaviour and environmental fate of NVP in South African surface waters. The authors found that the concentration varied with the type of aquatic environment, alike Schoeman *et al.* (2017).

Lopinavir emerged as the most abundant drug across almost all sample sites and months, with notably higher concentrations than the other drugs (Figure 3-3). LPV is usually co-formulated with RTV to boost plasma levels (Wang *et al.*, 2023). In South Africa, lopinavir/ritonavir (LPV/r) has been used extensively for ART, historically in first-line and second-line HIV treatment regimens (Vogt *et al.*, 2020). Although LPV was found to be the most abundant ARVD, it does not necessarily concur with recent prescription and usage data. In public-sector treatment programmes, the use of LPV/r has declined sharply, and it is now considered a third-choice option. It is mainly used in exceptional cases rather than routine therapy. For example, the 2020 SAHCS guidelines outlined a clear hierarchy for selecting PIs, prioritising DRV first, followed by ATV, and placing LPV as a third option. However, the 2023 guidelines take a firmer stance, strongly advising against the use of LPV in nearly all situations due to concerns about its side effect profile and treatment burden (Nel *et al.*, 2023).

As a key component of these therapies, LPV is only partially metabolised in the human body. Metabolic data indicate that 2% of LPV is excreted unchanged in urine, with 10% excreted as metabolites, while 20% is excreted unchanged in faeces, alongside 83% as metabolites (Table 3-1). Due to its limited bioavailability, LPV is more prevalent in wastewater than ARVDs that undergo more efficient absorption and metabolism.

Lopinavir is not readily biodegradable, with a biodegradability probability of 0.91 (Drugbank, 2023). This limited degradability may account for its accumulation (Abafe *et al.*, 2018). LPV also has a low removal efficiency in WWTPs (Krasucka *et al.*, 2022). Its high logK_{ow} value (> 5.9; Table 3-1) indicates strong lipophilicity, leading to extensive adsorption onto suspended solid particles in wastewater (Wang *et al.*, 2023). This process facilitates its accumulation in sewage sludge rather than being effectively degraded or removed from the system.

Furthermore, LPV's hydrophobic nature, high molecular weight, and strong protein binding affinity contribute to its persistence in aquatic environments (Table 3-3). These properties make it less susceptible to conventional wastewater treatment processes. Therefore, high concentrations could be detected in effluent (Wang *et al.*, 2023). Its hydrophobic nature also suggests that it can bind to sediments, which might cause a gradual release and prolonged detection in river systems. Studies suggest that WWTPs have trouble in effectively eliminating LPV, resulting in its accumulation in effluent and subsequent discharge into rivers (Horn *et al.*, 2022). Lopinavir has been detected in several environmental matrices, especially in sub-Saharan Africa, where its concentrations are remarkably high (Ngwenya and Musee, 2023). The stability of this drug in the environment is alarming because it can persist in water ways and pose risks to aquatic organisms (Kudu *et al.*, 2022).

The high detection frequency of LPV across sampled rivers may partly reflect the enhanced sensitivity of the analytical method toward this compound. As indicated in Table 3-6, LPV exhibited the lowest LOD (74 µg/L) and LOQ (248 µg/L) among the ARVDs assessed. This methodological advantage enabled the identification of even trace concentrations of LPV, potentially inflating its representation in the dataset relative to other compounds with higher detection and quantification thresholds. Certain drugs with lower LOD/LOQs could have fallen below the threshold for detection. This analytical bias might have led to potential overrepresentation of LPV and should be considered when interpreting prevalence trends in the data.

3.4.1 Spatial and temporal trends of ARVD water analysis over five-months

3.4.1.1 Hennops River

The Hennops River originates in the southeast of Centurion, near Tembisa and Kempton Park. It flows through Centurion, transversing formal and informal residential, commercial, industrial, and recreational areas. The Hennops River ultimately feeds into the Crocodile River, which eventually empties into the Hartbeespoort Dam. The Crocodile River continues to flow northwest, eventually feeding into the Limpopo River, from there to the Indian Ocean through Mozambique. It lies near two major WWTPs, namely Olifantsfontein and Sunderland Ridge. The Olifantsfontein WWTP treats ca. 105 ML wastewater per day primarily from Tembisa and Ivory Park and discharges into the Kaal Spruit, later merging with the Olifants Spruit to form the Hennops River (Horn *et al.*, 2022). The Sunderland Ridge WWTP receives wastewater from Midrand and Centurion (65 ML/day) and discharges directly into the Hennops (Roux *et al.*, 2010).

High concentrations of ARVDs were consistently detected across multiple sites over several months (Figure 3-4 to Figure 3-8), particularly downstream of Sunderland Ridge WWTP. Site 8, located 2.5 km downstream of the WWTP, exhibited higher ARVD concentrations compared to upstream Site 7 (which is 1.1 km upstream of the Sunderland WWTP in the Rietspruit River), confirming the WWTP as a major contamination source. Similar to the findings of Horn *et al.* (2022). Nonetheless, no significant differences were observed between upstream and downstream concentrations (Mann-Whitney; $p = 0.08\text{--}0.54$). This suggests complex hydrological interactions, likely mediated by the Rietspruit River confluence and site-specific processes such as dilution, sedimentation, and photodegradation.

Site 6, located 3.5 km upstream from Site 8, recorded the highest ARVD concentrations, particularly for LPV (63.7 µg/L in March), suggesting direct or localised contamination sources such as malfunctioning sewer systems or runoff from informal settlements and urban infrastructure. Overloaded WWTPs, ineffective waste removal (Abafe *et al.*, 2018; Horn *et al.*, 2022), and limited infrastructure in high-density areas (e.g., Centurion, Tembisa, Ivory Park) (Masuku, 2015) further exacerbate contamination. The Sunderland Ridge WWTP's documented non-compliance with discharge standards and overcapacity operation, as reported in the 2022 Green Drop report, could justify the persistently high ARVD residues found in the Hennops River (DWS, 2022).

Horn *et al.* (2022) also noted a different ARVD compound signature upstream from the downstream sites in the same river. Greater concentrations in urbanised areas are in line with global findings showing higher antiviral detection frequencies in urban > suburban > rural river sections (Peng *et al.*, 2014).

Robson *et al.* (2017) reported a higher EFV concentration upstream of a WWTP at 0.01 µg/L than downstream (< LOQ) in the Luvuvhu River in the Limpopo province, South Africa. However, the present study consistently found higher EFV concentrations downstream of a WWTP at Hennops sample Site 8 compared with upstream (Site 7). This implies that, unlike in the Luvuvhu River study (Robson *et al.*, 2017), the WWTP is a major source of EFV pollution rather than a removal site. Other authors have also reported higher EFV concentrations in effluent-receiving water (Abafe *et al.*, 2018; Mosekiemang *et al.*, 2019; Xolo and Mahlambi, 2024; Wood *et al.*, 2015). The disparity between Robson *et al.* (2017) and the current study highlights site-specific differences in WWTP efficiency and hydrological conditions.

3.4.1.2 Crocodile River

The Crocodile River, along with its principal tributaries, the Hennops and Jukskei rivers, transverse through highly industrialised and densely populated regions between the metropolitan of Johannesburg and the Hartbeespoort Dam. The river system drains a region facing severe water stress due to demands from mining, agriculture, industry, and urban development (Taylor *et al.*, 2005). Several streams, including the Braamfontein Spruit, Modderfontein, and Sandspruit, feed into the Jukskei River before it empties into the Crocodile River. Of these, the Modderfontein stream experiences minimal anthropogenic impact due to its location within a nature reserve (Moropa, 2015).

The IDW investigations at Crocodile sample Site 3 and surrounding Hartbeespoort area suggests a localised increase, possibly linked to poor water quality and pollutant inputs from upstream sources, particularly WWTP effluent and urban runoff entering via the Hennops River (Dennis and Dennis, 2019). The moderate contamination at Crocodile sample Site 2 (Figure 3-7) might reflect pollution transported from the Jukskei River (e.g. Site 10), where elevated concentrations were detected.

River flow variations such as the reduction in flow during dry months, can alter the river's dilution capacity which can in turn affect any pollutant inputs and persistent drug concentrations. Precipitation-driven dilution can thus influence ARVD concentration dynamics, similar to patterns observed in other river systems (e.g., Pearl River, China; Peng *et al.* 2014).

Furthermore, sediment interactions play a role in the Crocodile River's contaminant profile (Figure 3-8). Resuspension events, likely triggered by changing water levels or biotic activity (Archer *et al.*, 2017), may remobilise previously deposited ARVD residues from sediments. This pseudo-persistence is supported by studies documenting ARVD retention in sediments with potential for re-release during hydrological disturbances (Kairigo *et al.* 2020), sustaining their pseudo-persistence in surface waters. The temporal and spatial variability of ARVDs in the Crocodile River is believed to be shaped by a confluence of variables, including urban runoff, WWTP outputs, seasonal hydrology, sediment remobilisation, and legacy contamination.

3.4.1.3 Jukskei River

The Jukskei River watershed supports over 1.25 million people, many of whom live in informal settlements such as Diepsloot and Alexandra Townships, with strained infrastructure beyond its intended capacity (Neswiswi, 2014; Fitchett, 2017). Originally built for 70,000 people, the Alexandra township now houses an estimated four times that number (CGJM, 2000), leading to frequent sewer blockages and overflows into the nearby river (Makuya, 2022). Many riverbank communities rely on inadequate sanitation facilities like portable toilets, resulting in direct sewage discharge, further exacerbated by stormwater runoff during heavy rainfall and flash floods (DWS, 2023).

A major potential pollution source is the Northern Johannesburg WWTP (Johannesburg's largest WWTP) which treats over 460 million litres of wastewater daily (Ally and Campbell, 2021; Kings, 2016). Reports indicate repeated discharges of untreated sewage from this plant into the Jukskei River, likely due to infrastructure failures or hydraulic overloading at the WWTP (Makuya, 2022; Webster and Iqani, 2024).

The downstream sample sites in the Jukskei River system revealed higher concentrations of ARVDs than upstream sample sites. The Northern Johannesburg WWTP is adjacent to the river. It is ca. 2.4 km from the Jukskei sample Site 8, and 2.6 km from sample Site 10. The Northern Johannesburg WWTP treats wastewater from a dense urban population, where ARVD use is high due to South Africa's extensive HIV/AIDS treatment programmes (Juta *et al.*, 2024; Nardell *et al.*, 2024). Overflow events during the rainy season might contribute to the increased pharmaceutical loading (Lee *et al.* 2022; Phillips *et al.*, 2012).

Gradual dilution and attenuation might explain why concentrations decrease further downstream of sample Sites 8 and 10. In contrast, seasonal dilution during high rainfall periods, such as March (Figure 3-4), may temporarily reduce observed ARVD levels, whereas dry months like August and October (Figure 3-7 to Figure 3-8) can lead to concentration build-up due to reduced river flow (DWS, 2023). Overall, the Jukskei River's pollutant profile is shaped by inadequate sanitation infrastructure, WWTP inefficiencies, high urban density, and seasonal hydrological variability.

3.4.1.4 Klip River

The Klip River is among the most vulnerable catchments to anthropogenic pollution in Gauteng Province, primarily due to high population density, proximity to numerous WWTPs, and the concentration of over one-third of the province's medical facilities within their vicinity (Peterson *et al.*, 2017). The Klip River starts in the southern region of Johannesburg and flows southward through Soweto, ultimately into the Vaal River a crucial drinking water source (Mothetha, 2016; Vermaak, 2009). Several informal settlements line the banks of the Klip River and its main tributaries, the Klipspruit and Harrington Spruit, potentially contributing to pollution. The river receives more than 500 ML/day of effluent daily from three WWTPs (Vermaak, 2009): Goudkoppies, Bushkoppies, and Olifantsvlei, all situated within a 5 km radius of sampling Site 5.

Despite the directional flow of effluent from Goudkoppies and Bushkoppies WWTPs into the Klip River, no statistically significant differences in ARVD concentrations were detected between upstream and downstream sites (Mann-Whitney; $p = 0.09\text{--}0.45$). However, higher concentrations upstream, particularly at Site 5, suggest localised discharge of poorly treated effluent, especially from Goudkoppies WWTP, which lies only 4.2 km upstream. The 2022 Green Drop report confirmed that Goudkoppies WWTP failed all effluent quality parameters, while Bushkoppies was hydraulically overloaded and failed one category (Evans, 2023; DWS, 2022). Inspection revealed widespread infrastructure failures, including non-functional degritters, aerators, and disinfection systems, that compromise treatment effectiveness. (DWS, 2022).

Land-use and hydrological processes affect seasonal variation in water quality (Pakoksung *et al.*, 2025). Urbanisation often exacerbates pollution levels due to augmented wastewater discharge and runoff (Vidon *et al.*, 2008). Additional factors such as tributary inflows, groundwater recharge, and natural attenuation mechanisms (e.g. sorption, microbial degradation, photolysis) may partially explain reductions in ARVD concentrations downstream

(Abafe *et al.*, 2018; Adeola and Forbes, 2022; Chojnacka *et al.*, 2022). Nonetheless, the cumulative impact of multiple failing WWTPs and nearby informal settlements with direct waste discharge remains an important contributor to ARVD contamination in the Klip River system.

3.4.2 Environmental factors

The lack of significant sample site vs. month interaction ($p = 0.42$) and month effects ($p = 0.6$) indicated that temporal variation is not the primary factor influencing ARVD concentrations patterns, indicating relatively stable drug concentrations across locations and time points (months). This could suggest either uniform distribution of ARVDs across sample sites or that other environmental variables (e.g., seasonal rainfall, point source discharge) might have a stronger influence. According to Adeola and Forbes (2022), nonpoint or diffuse source pollution of ARVDs are major contributing factors to the contamination of surface water in Africa.

Nearly half of the total variance was attributed to individual differences, implying significant variability introduced by local environmental conditions, ARVD-specific behaviour, differences in sample collection, or measurement irregularities. Episodic events stormwater runoff and sewer overflows during transitional hydrological periods (e.g., March and August) may explain observed peaks in ARVDs, particularly LPV. These months coincide with changes in rainfall intensity and river flow, which are known to affect pollutant transport and dilution (Pourfallah Koushali *et al.*, 2021; Prathumratana *et al.*, 2008).

Seasonally driven differences in ARVD degradation may also contribute to concentration patterns. Higher thermal and photo-degradation is expected for analytes in summer months (Holton *et al.*, 2022). Phototransformation was identified as the fundamental removal mechanism for ABC, ZDV, and FTC, with respective half-lives ($t_{1/2}$, photo = 1.6–25 h), while 3TC is predominantly driven by slower microbial degradation processes ($t_{1/2}$, bio = 120 h) (Prasse *et al.*, 2015). Consequently, environmental conditions such as sunlight, water depth, and turbidity shape compound persistence, particularly in winter, when reduced photolysis may lead to higher ARVD concentrations (as seen in August) (Holton *et al.*, 2022).

Phototransformation rates of ARVDs and their biotransformation products appear unaffected by prior microbial exposure, implying that partially treated effluent can still contain photodegradable residues (Prasse *et al.*, 2015).

This may account for persistent ARVD signatures in surface waters, even downstream of WWTPs. However, due to the limited number of studies, drawing conclusive links between temperature and ARVD persistence remains challenging.

3.4.2.1 Uncertainties

Uncertainty is an inherent aspect of water quality research due to varying methods and data quality under different environmental conditions (Umwali *et al.*, 2021). In the present study, uncertainties stem primarily from the selection and distribution of sampling sites, which can influence measured concentrations, particularly in dynamic river systems with high spatial and temporal variability.

The use of grab sampling introduces limitations, as it captures water at a single time and location, offering only a snapshot of conditions. This method may fail to detect transient contamination peaks, especially for antiretroviral drugs (ARVDs), which can fluctuate considerably due to effluent discharges or rainfall events (Cristóvão *et al.*, 2021). These limitations could result in the under- or overestimation of actual drug concentrations. While practical and commonly used, grab sampling may not fully represent pollutant dynamics, and composite sampling is recommended for more accurate and continuous monitoring.

However, the above-mentioned uncertainties related to site selection are not believed to have a substantial impact on this specific study. This is because the primary objective was not to provide an exact representation of the area's ARVD pollution levels but rather to offer insights that inform policymakers about potential future scenarios based on current knowledge. Therefore, future research should prioritise minimising uncertainties to enhance the understanding of water quality vulnerabilities in relation to different land-use types, such as intensive urbanisation. Differences in urban, peri-urban, and rural sites will likely reflect varying concentrations of ARVD pollution, influenced by population density, and wastewater management proficiency.

3.4.2.2 Risk specific trends

One of the first conundrums of researching variations in ARVDs is the concentrations reported. Reported concentrations of drugs such as ARVDs do not readily state if the detected concentrations or analysed doses are harmful. Inherently, the acute use of these drugs in water is anticipated to be harmless to human health, given that the reported concentrations are far lower than the dosages applied in medical treatments.

Nonetheless, Nugnes *et al.* (2024) reported that certain ARVDs are harmful to certain species. Some species may be more sensitive to ARVD toxicity than others. Even though environmental concentrations might not be toxic enough to cause harm to human health, it may cause irreversible damage to aquatic species, irrespective of a short ingestion time or low exposure concentrations. Subsequently, the development of ecological thresholds is vital to determine the scope of ARVD pollution and the associated risks.

The consequences of systematic human exposure to low doses of ARVDs have been a frequent topic of discussion (Horn *et al.*, 2020; Horn *et al.*, 2022), yet little is known about it, potentially due to the ethical problem raised by it. Is it ethical to disclose information to the general public about ARVD environmental toxicity when not much is known about its harmful effects? Disclosing inadequate information could cause undue panic. The ethical implications of the research question place great emphasis on a comprehensive and all-encompassing investigation into the historic, current, and future state of ARVD pollution in SA.

In the event that the current or future state of ARVD pollution is found to threaten environmental and human health, it will raise serious issues. It would mean a considerable increase in financial costs for state monitoring, health inspection stations, water safety laboratories, and analytical companies. In addition to increasing the efficiency of wastewater purification, the reconstruction of existing WWTPs, and the incorporation of alternative or new technologies to improve drinking water standards.

Given the excessive financial burden associated with addressing such a problem, prospective investors should focus on research that would set limitations on the most recurrent and toxic ARVDs and the aptitude of the biological and geological environments to remove these substances. This extends to the distribution of ARVDs as well as their spatial and temporal variability. As designing a monitoring network will rely on a comprehensive geochemical, geological, and hydrogeological review (Rozman *et al.*, 2015).

For example, certain ARVDs could be significantly attenuated in groundwater and degrade into various intermediates and metabolites. These intermediates can be biologically active as well but not identified by analytical methods set to analyse for the primary ARVD. Antiretroviral drug pollution subsequently requires interdisciplinary cooperation between specialists in organic chemistry and environmental scientists, as it necessitates new research and laboratory analysis.

Certain risk exposures, such as ARVD pollution, are highly correlated with economic and social development. As economies grow, access to healthcare (such as ARVDs) increases, causing more PLWH to consume ARVDs, leading to higher drug residues in wastewater. Nonetheless, the incremental effectiveness of specific policies (such as austere wastewater treatment regulations) has yet to be fully recognised in this and other studies. If government actions (e.g. awareness campaigns, WWTP upgrades, and programmes) have aided in reducing overall ARVD pollution, it is unclear to what extent.

Bending this developmental curve is feasible by the abrupt accelerations in the decline of certain risk factors, such as with improved WWTP technologies, ARVD removal rates, and stronger policies regulating drug disposal and water treatment standards. Analysis of exemplars, countries with lower ARVD risks, albeit due to lower exposure levels or technological advancements, could yield further insights into ARVD pollution and potentially lead to faster progress in reducing pollution consequences. Regulations can potentially drive behavioural changes that contribute to long-term environmental benefits. Herin lies the opportunity for South Africa to lead other African nations in the study of ARVD contamination and the development of effective policies and strategies to combat predictable risks.

3.5 Conclusions

Spatial analysis effectively mapped the distribution of ARVD contaminations, revealing both point and non-point source pollution patterns across the river systems. The IDW method provided valuable insight into the spatial dynamics of contamination, with findings shaped by a complex interplay of seasonal effects, pollution sources, and hydrological transport. High ARVD concentrations were consistently recorded at specific downstream sites, particularly for LPV, suggesting possible accumulation effects or repeated upstream inputs.

Site-specific patterns were dominant over seasonal variation and largely driven by proximity to pollution sources such as WWTPs, with the Crocodile River generally showing lower contamination levels. The frequent detection of LPV in highly contaminated rivers corresponds with increased public sector use of protease inhibitors, reflecting broader treatment trends (Schellack *et al.*, 2017). EFV and RTV displayed some seasonal shifts, potentially linked to variations in healthcare service load or patient adherence, as has been suggested by both local and international studies (Mandaric *et al.*, 2017).

Lopinavir emerged as the most critical ARVD, with consistently high concentrations across all sites and months. Urgent assessment of LPV is valid given its persistence, toxicity, and dominance across all sample sites and months (Czech *et al.*, 2022). EFV and RTV also require monitoring due to observed seasonal peaks and associated toxicological risks (Robson *et al.*, 2017), while ZDV and NVP presented more stable and lower levels of concern. The study therefore observed spatial-temporal patterns. ARVD hotspots were closely associated with WWTP discharges, and hydrological processes like low-flow conditions promoting accumulation (dry season) and high-flow events leading to resuspension and dilution (wet season). However, statistical analysis indicated that spatially fixed pollution sources were the predominant drivers of contamination patterns.

Urban areas with high ART coverage, particularly near major WWTPs like Northern Johannesburg, showed elevated ARVD concentrations in wastewater. The current study emphasises the value of integrating national ARVD consumption data with environmental monitoring to identify and categorise contamination hotspots. Still, the lack of spatially and temporally verified consumption data limits predictive modelling efforts and highlights the need for routine site-specific prescribing data. The promise of prevention through risk modification is not being realised. regulatory focus should shift toward proactive monitoring of LPV, EFV, and RTV in river ecosystems. Priority should be given to targeted sites (e.g., Hennops 6, Klip 5, Jukskei 8–9) and sampling during peak contamination months (March and August) to inform policy, management strategies, and future research.

3.6 Recommendations

- Limitations in sampling strategy (e.g., sampling frequency, detection limits) could have obscured some differences and should be revised in future studies. It is recommended that further data be collected over a longer period and for at least two wet and dry seasons.
- Future work should consider using time-flow-proportional composite samplers or passive samplers instead of grab sampling, which better integrate pollutant fluctuations over time and reduce temporal bias in water-quality assessments.
- The development of standard protocols for sampling wastewater, accounting for diurnal variations and residence time distribution is advocated.
- Different advanced wastewater treatment methods should be assessed to improve removal efficiencies.
- Future studies can also be correlated to specific hydrological data. Comprehending rainfall, river flow, and anthropogenic activities can strengthen the interpretation.

- Further studies could incorporate additional covariates to refine analysis. Further investigation into the sources of variation could improve data interpretation.
- Long-term ecological effects of LPV exposure should be investigated due to its dominance and persistence. The potential bioaccumulation of EFV and RTV should also receive further investigation, particularly for aquatic organisms.
- The IDW technique is dependent on the density of sampling points. Sparse data points may lead to less reliable interpolation in certain areas. It is recommended that further studies adequately spread sampling points over study areas. Interpolation methods can also be compared in future studies, for example Kriging or other geostatistical models could be used to compare and validate the IDW results.
- Future studies could focus on modelling the hydrodynamic transport of ARVDs to better understand and predict accumulation zones.
- A central limitation in this investigation is the absence of true patient consumption data. It is recommended that ARVD usage trends be standardised using defined daily doses (DDD) and expressed as DDDs per 1000 inhabitants per day (DID) for comparability across regions (Schellack *et al.*, 2017). Where this is not feasible, procurement or tender data may be used as a proxy, provided consistent metrics are applied.

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3.7 References

- Abafe, O.A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., Pietruschka, B. and Martincigh, B.S. 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, 200:660–670.
- Abbott, J. 2002. An analysis of informal settlement upgrading and critique of existing methodological approaches. *Habitat International*, 26(3):303–315.
- Adeola, A.O. and Forbes, P.B. 2022. Antiretroviral drugs in African surface waters: prevalence, analysis, and potential remediation. *Environmental Toxicology and Chemistry*, 41(2):247–262.
- Akinnawo, S.O., Ayadi, P.O. and Temitope, M. 2023. Chemical coagulation and biological techniques for wastewater treatment. *Ovidius University Annals of Chemistry*, 34(1):14–21. <https://doi.org/10.2478/auoc-2023-0003> Date of access: 6 Mei 2024.
- Ally, S.H. and Campbell, R.B. 2021. *The status of wastewater as an untapped resource in South Africa*. WRC (Water Research Commission) Report No. TT 860/21. ISBN 978-0-6392-0284-6. <https://wrcwebsite.azurewebsites.net/wpcontent/uploads/mdocs/TT%20860%20final%20web.pdf> Date of access: 15 Feb. 2025.
- Aminot, Y., Fuster, L., Pardon, P., Le Menach, K. and Budzinski, H. 2018. Suspended solids moderate the degradation and sorption of waste water-derived pharmaceuticals in estuarine waters. *Science of the Total Environment*, 612:39–48.
- Archer, E., Petrie, B., Kasprzyk-Hordern, B. and Wolfaardt, G.M. 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere*, 174:437–446.
- Arman, N.Z., Aris, A., Salmiati, S., Rosli, A.S., Foze, M.F. and Talib, J. 2025. Water quality assessment of Johor River Basin, Malaysia, using multivariate analysis and spatial interpolation method. *Environmental Science and Pollution Research*, 32(4):1766–1782.

Aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A. and Küster, A. 2016. Pharmaceuticals in the environment—Global occurrences and perspectives. *Environmental Toxicology and Chemistry*, 35(4):823–835.

Azuma, T., Ishida, M., Hisamatsu, K., Yunoki, A., Otomo, K., Kunitou, M., ... Mino, Y. 2017. A method for evaluating the pharmaceutical deconjugation potential in river water environments. *Chemosphere*, 180:476–482.

Azzouz, A. and Ballesteros, E. 2013. Influence of seasonal climate differences on the pharmaceutical, hormone and personal care product removal efficiency of a drinking water treatment plant. *Chemosphere*, 93(9):2046–2054.

Bakare, A.A., Akinseye, K.M., Aminu, B.A., Ofoegbu, F.C., Anifowose, S.O., Abruda, S., ... Adetona, O.M. 2020. Genetic and reproductive toxicity of lamivudine, tenofovir disoproxil fumarate, efavirenz and their combination in the bone marrow and testicular cells of male mice. *Annals of Science and Technology*, 5(1):1–10.

Bavumiragira, J.P. and Yin, H. 2022. Fate and transport of pharmaceuticals in water systems: A processes review. *Science of The Total Environment*, 823:153635.

Bezy, V., Chaimbault, P., Morin, P., Unger, S.E., Bernard, M.C. and Agrofoglio, L.A. 2006. Analysis and validation of the phosphorylated metabolites of two anti-human immunodeficiency virus nucleotides (stavudine and didanosine) by pressure-assisted CE-ESI-MS/MS in cell extracts: Sensitivity enhancement by the use of perfluorinated acids and alcohols as coaxial sheath-liquid make-up constituents. *Electrophoresis*, 27(12):2464–2476.

Boxall, A.B., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., ... Ankley, G.T. 2012. Pharmaceuticals and personal care products in the environment: what are the big questions?. *Environmental Health Perspectives*, 120(9):1221–1229.

Cavalcante, G.I.T., Chaves Filho, A.J.M., Linhares, M.I., de Carvalho Lima, C.N., Venâncio, E.T., Rios, E.R.V., ... de França Fonteles, M.M. 2017. HIV antiretroviral drug Efavirenz induces anxiety-like and depression-like behavior in rats: evaluation of neurotransmitter alterations in the striatum. *European Journal of Pharmacology*, 799:7–15.

CGJM (Council for Greater Johannesburg Metropolitan). 2000. Report on the interactive planning workshop for Johannesburg. *World Bank, Upgrading Urban Communities: A Resource Framework*. Washington, DC: World Bank.

<https://web.mit.edu/urbanupgrading/upgrading/case-examples/overview-africa/alexandra-township.html>

Charizopoulos, N., Zagana, E. and Psilovikos, A. 2018. Assessment of natural and anthropogenic impacts in groundwater, utilizing multivariate statistical analysis and inverse distance weighted interpolation modeling: the case of a Scopia basin (Central Greece). *Environmental Earth Sciences*, 77:1–18.

Chen, F.W. and Liu, C.W. 2012. Estimation of the spatial rainfall distribution using inverse distance weighting (IDW) in the middle of Taiwan. *Paddy and Water Environment*, 10:209–222.

Chojnacka, K., Skrzypczak, D., Izydorczyk, G., Mikula, K., Szopa, D., Moustakas, K. and Witek-Krowiak, A. 2022. Biodegradation of pharmaceuticals in photobioreactors—a systematic literature review. *Bioengineered*, 13(2):4537–4556.

Cristóvão, M.B., Bento-Silva, A., Bronze, M.R., Crespo, J.G. and Pereira, V.J. 2021. Detection of anticancer drugs in wastewater effluents: Grab versus passive sampling. *Science of The Total Environment*, 786:147477.

Czech, B., Krzyszczak, A., Boguszevska-Czubara, A., Opielak, G., Joško, I. and Hojamberdiev, M. 2022. Revealing the toxicity of lopinavir-and ritonavir-containing water and wastewater treated by photo-induced processes to *Danio rerio* and *Allivibrio fischeri*. *Science of the Total Environment*, 824:153967.

D'Avolio, A., Sciandra, M., Siccardi, M., Baietto, L., de Requena, D.G., Bonora, S. and Di Perri, G. 2008. A new assay based on solid-phase extraction procedure with LC-MS to measure plasmatic concentrations of tenofovir and emtricitabine in HIV infected patients. *Journal of Chromatographic Science*, 46(6):524–528.

Dennis, I.N.G.R.I.D. and Dennis, S.R. 2019. Social paradigm shift required to counter the eutrophication of the Hartbeespoort Dam in South Africa. *WIT Transactions on Ecology and the Environment*, 239:159–172.

Drugbank, 2023. Drugbank: Drugs. [online] <https://go.drugbank.com/> Date of access: Jan. 2023.

Evans, J. 2023. Inside Joburg's wastewater treatment works — the complex process of keeping drinking water safe. Daily Maverick. <https://www.dailymaverick.co.za/article/2023-06-26-inside-joburgs-wastewater-treatment-works-the-complex-process-of-keeping-drinking-water-safe/> Date of access: 18 Jan. 2025.

Fatima, S.U., Khan, M.A., Alamgir, A., Mahmood, N. and Sulman, N. 2022. Multivariate and spatial methods-based water quality assessment of Chu Tran Valley, Gilgit Baltistan. *Applied Water Science*, 12(6):129.

Ferrer, I. and Thurman, E.M. 2012. Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. *Journal of Chromatography A*, 1259:148–157.

Fitchett, A. 2017. SuDS for managing surface water in Diepsloot informal settlement, Johannesburg, South Africa. *Water SA*, 43(2):310–322.

Glaser, C., Schwientek, M. and Zarfl, C. 2019. Designing field-based investigations of organic micropollutant fate in rivers. *Environmental Science and Pollution Research*, 26:28633–28649.

Gomes, M.P., Kubis, G.C., Kitamura, R.S.A., Figueredo, C.C., da Silva Nogueira, K., Vieira, F., ... Juneau, P. 2022. Do anti-HIV drugs pose a threat to photosynthetic microorganisms? *Chemosphere*, 307:135796.

Gwenzi, W., Simbanegavi, T.T. and Rzymiski, P. 2023. Household disposal of pharmaceuticals in low-income settings: practices, health hazards, and research needs. *Water*, 15(3):476.

Haldar, K., Kujawa-Roeleveld, K., Dey, P., Bosu, S., Datta, D.K. and Rijnaarts, H.H. 2020. Spatio-temporal variations in chemical-physical water quality parameters influencing water reuse for irrigated agriculture in tropical urbanized deltas. *Science of the Total Environment*, 708:134559.

Held, I., Wolf, L., Eiswirth, M. and Hötzl, H. 2006. Impacts of sewer leakage on urban groundwater: review of a case study in Germany. In: Hötzl, H., Zehnsdorf, A. and Häfner, F. (eds). *Urban groundwater management and sustainability*. Dordrecht: Springer Netherlands:189–204.

Helsel, D.R. 2011. *Statistics for censored environmental data using Minitab and R*. Vol. 77. Hoboken, NJ: John Wiley and Sons.

Hodam, S., Sarkar, S., Marak, A.G., Bandyopadhyay, A. and Bhadra, A. 2017. Spatial interpolation of reference evapotranspiration in India: Comparison of IDW and Kriging methods. *Journal of the Institution of Engineers (india): Series A*, 98:511–524.

Holton, E., Archer, E., Fidal, J., Kjeldsen, T., Wolfaardt, G. and Kasprzyk-Hordern, B. 2022. Spatiotemporal urban water profiling for the assessment of environmental and public exposure to antimicrobials (antibiotics, antifungals, and antivirals) in the Eerste River Catchment, South Africa. *Environment International*, 164:107227.

Horn, S., Vogt, B., Pieters, R. and Bouwman, H. 2020. Impact of Potential COVID-19 Treatment on South African Water Sources Already Threatened by Pharmaceutical Pollution. *Environmental Toxicology and Chemistry*.

Horn, S., Vogt, T., Gerber, E., Vogt, B., Bouwman, H. and Pieters, R. 2022. HIV-antiretrovirals in river water from Gauteng, South Africa: Mixed messages of wastewater inflows as source. *Science of The Total Environment*, 806:150346.

Iloms, E., Ololade, O.O., Ogola, H.J. and Selvarajan, R. 2020. Investigating industrial effluent impact on municipal wastewater treatment plant in Vaal, South Africa. *International journal of environmental research and public health*, 17(3):1096.

ISO (International Organization for Standardization). 2012. *Water quality: Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test*. ISO 6341.

<https://cdn.standards.iteh.ai/samples/54614/bc25430113c94dcab3ccdb6e6a097e6c/ISO-6341-2012.pdf> Date of access: 27 Mar. 2023.

Juta, P.M., Jansen van Vuuren, J.M. and Mbaya, K.J. 2024. A multidisciplinary approach for people with HIV failing antiretroviral therapy in South Africa. *Southern African Journal of HIV Medicine*, 25(1):1–11.

Kairigo, P., Ngumba, E., Sundberg, L.R., Gachanja, A. and Tuhkanen, T. 2020. Contamination of surface water and river sediments by antibiotic and antiretroviral drug cocktails in low and middle-income countries: occurrence, risk and mitigation strategies. *Water*, 12(5):1376.

Kasprzyk-Hordern, B., Dinsdale, R.M. and Guwy, A.J. 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research*, 43(2):363–380.

Khouni, I., Louhichi, G. and Ghrabi, A. 2021. Use of GIS based Inverse Distance Weighted interpolation to assess surface water quality: Case of Wadi El Bey, Tunisia. *Environmental Technology and Innovation*, 24:101892.

Kings, S. 2016. Northern Works sewage nightmare turns Jukskei black. *Mail and Guardian*. <https://mg.co.za/article/2016-11-03-00-sewage-spill-turns-jukskei-black/> Date of access: 18 Jan. 2024.

Kowlaser, S., Barnhoorn, I. and Wagenaar, I. 2022. Developmental abnormalities and growth patterns in juvenile *Oreochromis mossambicus* chronically exposed to efavirenz. *Emerging Contaminants*, 8:83–89.

Krasucka, P., Rombel, A., Yang, X.J., Rakowska, M., Xing, B. and Oleszczuk, P. 2022. Adsorption and desorption of antiviral drugs (ritonavir and lopinavir) on sewage sludges as a potential environmental risk. *Journal of Hazardous Materials*, 425:127901.

Kudu, I., Pillay, V. and Moodley, B. 2022. Antiretrovirals (ARVs) in the environment. *Emerging Freshwater Pollutants*, 227–239.

Lam, M.W., Young, C.J., Brain, R.A., Johnson, D.J., Hanson, M.A., Wilson, C.J., ... Mabury, S.A. 2004. Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry*, 23(6):1431–1440.

Lee, J., Beck, K. and Bürgmann, H. 2022. Wastewater bypass is a major temporary point-source of antibiotic resistance genes and multi-resistance risk factors in a Swiss river. *Water Research*, 208:117827.

Letseka, T., Richards, H., Tutu, H., Simatele, M.D., James, L. and Chimuka, L. 2023. A combination of chemical analysis and stakeholder's participation in addressing the Hennops River pollution in Gauteng Province, South Africa. Water Research Commission. WRC Report No. 3109/1/23. ISBN 978-0-6392-0567-0.

Lindholm-Lehto, P.C., Ahkola, H.S., Knuutinen, J.S. and Herve, S.H. 2016. Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland. *Environmental Science and Pollution Research*, 23:7985–7997.

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

Makuya, T.S. 2022. *The long-term impact of Northern Wastewater Treatment Works effluent on macroinvertebrates and water quality in the Jukskei River*. Mahikeng: North-West University. (Dissertation – PhD).

Mandaric, L., Diamantini, E., Stella, E., Cano-Paoli, K., Valle-Sistac, J., Molins-Delgado, D., ... Sabater, S. 2017. Contamination sources and distribution patterns of pharmaceuticals and personal care products in Alpine rivers strongly affected by tourism. *Science of the Total Environment*, 590:484–494.

Mantzafleri, N., Psilovikos, A. and Blanta, A. 2009. Water quality monitoring and modeling in Lake Kastoria, using GIS. Assessment and management of pollution sources. *Water Resources Management*, 23:3221–3254.

Masuku, T. 2015. A case study of the ivory park community work programme. An evaluation of the Community Work Programme's impact on violence prevention. Johannesburg: CSV. <https://idl-bnc-idrc.dspacedirect.org/server/api/core/bitstreams/bf73116a-0363-40c8-9d2d-9841f09b70f6/content> Date of access: 18 Feb. 2024.

McGrane, S.J. 2016. Impacts of urbanisation on hydrological and water quality dynamics, and urban water management: a review. *Hydrological Sciences Journal*, 61(13):2295–2311.

Mehdi, H., Lau, S.C., Synyshyn, C., Salena, M.G., McCallum, E.S., Muzzatti, M.N., ... Kidd, K.A. 2021. Municipal wastewater as an ecological trap: Effects on fish communities across seasons. *Science of The Total Environment*, 759:143430.

Meintjes, G., Black, J., Conradie, F., Cox, V., Dlamini, S., Fabian, J., Maartens, G., Manzini, T., Mathe, M., Menezes, C. and Moorhouse, M. 2014. Adult antiretroviral therapy guidelines 2014: guideline. *Southern African Journal of HIV Medicine*, 15(4):121–143.

Miller, J. and Miller, J.C., 2018. *Statistics and chemometrics for analytical chemistry*. Harlow: Pearson Education.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H. and Halm-Lemeille, M.P. 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. *Environmental Science and Pollution Research*, 23:4992–5001.

Moropa, A. 2015. Environmental studies of the Klip-Bosmontspruit, Jukskei River Alexandra and upper Klip Soweto water management units. Unit C: Jukskei River Alexandra water management unit. <http://ntcenvironmental.co.za/assets/coj-jukskei-alexandra-water-management-unit-rehabilitation-plan---final.pdf> Date of access: 25 Jan. 2023.

Mosekiemang, T.T., Stander, M.A., and de Villiers, A. 2019. Simultaneous quantification of commonly prescribed antiretroviral drugs and their selected metabolites in aqueous environmental samples by direct injection and solid phase extraction liquid chromatography-tandem mass spectrometry. *Chemosphere*, 220:983–992.
<https://doi.org/10.1016/j.chemosphere.2018.12.205>

Motayo, B.O., Usen, U., Folarin, B.O., Okerentugba, P.O., Innocent-Adiele, H.C. and Okonko, I.O. 2013. Prevalence and seasonal variations of HIV 1 and 2 infection among children in Abeokuta, South West Nigeria. *Journal of Microbiology Research*, 3(3):107–110.

Mothetha, M.L. 2016. *Evaluation of the impacts of municipal wastewater treatment in the receiving environment: a case study of the Olifantsvlei wastewater treatment plant in the Gauteng province, South Africa*. Pretoria: University of South Africa. (Thesis – MSc.).

Nantaba, F., Wasswa, J., Kylin, H., Bouwman, H., Palm, W.U. and Kümmerer, K. 2024. Spatial trends and ecotoxic risk assessment of selected pharmaceuticals in sediments from Lake Victoria, Uganda, East Africa. *Science of The Total Environment*, 906:167348.

Nardell, M.F., Govathson, C., Mngadi-Ncube, S., Ngcobo, N., Letswalo, D., Lurie, M., Miot, J., Long, L., Katz, I.T. and Pascoe, S. 2024. Migrant men and HIV care engagement in Johannesburg, South Africa. *BMC Public Health*, 24(1):435.

Nawn, R. 2004. *The water quality and associated problems of the Hennops River and proposed rehabilitative measures*. Johannesburg: University of Johannesburg. (Thesis – MSc.).

Ncube, S., Madikizela, L.M., Chimuka, L. and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. *Water research*, 145:231–247.

Nel, J., Wattrus, C., Osih, R. and Meintjes, G. 2023. 2023 Southern African HIV clinicians society adult antiretroviral therapy guidelines: What's new?. *Southern African Journal of HIV Medicine*, 24(1).

Neswiswi, A.B. 2014. Development of water quality index (WQI) for the Jukskei river catchment, Johannesburg. Johannesburg: University of Witwatersrand. (Thesis – MSc.).

Ngwenya, P. and Musee, N. 2023. Modelling ecological risks of antiretroviral drugs in the environment. *Environmental Chemistry and Ecotoxicology*, 5:145–154.

Nistor, M.M., Rahardjo, H., Satyanaga, A., Hao, K.Z., Xiaosheng, Q. and Sham, A.W.L. 2020. Investigation of groundwater table distribution using borehole piezometer data interpolation: Case study of Singapore. *Engineering Geology*, 271:105590.

Nugnes, R., Orlo, E., Russo, C., Lavorgna, M. and Isidori, M. 2024. Comprehensive eco-geno-toxicity and environmental risk of common antiviral drugs in aquatic environments post-pandemic. *Journal of Hazardous Materials*, 480:135947.

Nurmi, T. and Kiljunen, T. 2024. Quantifying the Seasonal Variation of Environmental Pharmaceutical Residues. *ChemRxiv*. <https://chemrxiv.org/engage/api-gateway/chemrxiv/assets/orp/resource/item/6628d4af418a5379b093e12b/original/quantifying-the-seasonal-variation-of-environmental-pharmaceutical-residues.pdf> Date of access: 4 Mar. 2025.

Oseke, F.I., Anornu, G.K., Adjei, K.A. and Eduvie, M.O. 2021. Assessment of water quality using GIS techniques and water quality index in reservoirs affected by water diversion. *Water-Energy Nexus*, 4:25–34.

Osorio, V., Marcé, R., Pérez, S., Ginebreda, A., Cortina, J.L. and Barceló, D. 2012. Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. *Science of the Total Environment*, 440:3–13.

Oyinkuro, O. and Rowland, E. 2018. Spatial groundwater quality assessment by WQI and GIS in Ogbia LGA of Bayelsa State, Nigeria. *Asian Journal of Physical and Chemical Sciences*, 4(4):1–12.

Pakoksung, K., Inseeyong, N., Chawaloeshonsiya, N., Punyapalakul, P., Chaiwiwatworakul, P., Xu, M. and Chuenchum, P. 2025. Seasonal dynamics of water quality in response to land use changes in the Chi and Mun River Basins Thailand. *Scientific Reports*, 15(1):7101.

Pedrouzo, M., Borrull, F., Marcé, R.M. and Pocurull, E. 2011. Analytical methods for personal-care products in environmental waters. *TrAC Trends in Analytical Chemistry*, 30(5):749–760.

Peng, X., Wang, C., Zhang, K., Wang, Z., Huang, Q., Yu, Y. and Ou, W. 2014. Profile and behavior of antiviral drugs in aquatic environments of the Pearl River Delta, China. *Science of the Total Environment*, 466:755–761.

Petersen, F., Dabrowski, J.M. and Forbes, P.B. 2017. Identifying potential surface water sampling sites for emerging chemical pollutants in Gauteng Province, South Africa. *Water SA*, 43(1):153–165.

Petrie, B., Barden, R., and Kasprzyk-Hordern, B. 2015. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Research*, 72:3–27.

Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T. and Wall, G.R. 2012. Combined sewer overflows: an environmental source of hormones and wastewater micropollutants. *Environmental Science and Technology*, 46(10):5336–5343.

Pourfallah Koushali, H., Mastouri, R. and Khaledian, M.R. 2021. Impact of precipitation and flow rate changes on the water quality of a Coastal River. *Shock and Vibration*, 2021(1):6557689.

Prasse, C., Schlüsener, M.P., Schulz, R. and Ternes, T.A. 2010. Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance? *Environmental Science and Technology*, 44(5):1728–1735.

Prasse, C., Wenk, J., Jasper, J.T., Ternes, T.A. and Sedlak, D.L. 2015. Co-occurrence of photochemical and microbiological transformation processes in open-water unit process wetlands. *Environmental Science and Technology*, 49(24):14136–14145.

Prathumratana, L., Sthiannopkao, S. and Kim, K.W. 2008. The relationship of climatic and hydrological parameters to surface water quality in the lower Mekong River. *Environment International*, 34(6):860–866.

Pubchem. National Center for Biotechnology Information. <https://pubchem.ncbi.nlm.nih.gov>
Date of access: 7 Jan. 2025.

Radouane, E.M., Chahlaoui, A., Maliki, A. and Boudellah, A. 2023. Assessment and modeling of groundwater quality by using water quality index (WQI) and GIS technique in meknes aquifer (Morocco). *Geology, Ecology, and Landscapes*, 7(2):126–138.

- Rajaković, L.V., Marković, D.D., Rajaković-Ognjanović, V.N. and Antanasijević, D.Z. 2012. The approaches for estimation of limit of detection for ICP-MS trace analysis of arsenic. *Talanta*, 102:79–87.
- Robson, L., Barnhoorn, I.E.J., and Wagenaar, G.M. 2017. The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56: 225–232 <https://doi.org/10.1016/j.etap.2017.09.017>
- Rogowska, J. and Zimmermann, A. 2022. Household pharmaceutical waste disposal as a global problem—a review. *International Journal of Environmental Research and Public Health*, 19(23):15798.
- Roux, S., Oelofse, S.H. and De Lange, W.J. 2010. *Can SA afford to continue polluting its water resources?* With special reference to water pollution in two important catchment areas. Science real and relevant conference, Pretoria.
- Rozman, D., Hrkál, Z., Eckhardt, P., Novotná, E. and Boukalová, Z. 2015. Pharmaceuticals in groundwaters: a case study of the psychiatric hospital at Horní Beřkovice, Czech Republic. *Environmental Earth Sciences*, 73:3775–3784.
- Samer, M. 2015. Biological and Chemical Wastewater Treatment. *Wastewater Treatment Engineering*, 1–50.
- Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Wilson, C.J. and Solomon, K.R. 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. *Regulatory Toxicology and Pharmacology*, 39(2):158–183.
- Schellack, N., Benjamin, D., Brink, A., Duse, A., Faure, K., Goff, D., ... Pople, T. 2017. A situational analysis of current antimicrobial governance, regulation, and utilization in South Africa. *International Journal of Infectious Diseases*, 64:100–106.
- Schoeman, C., Mashiane, M., Dlamini, M. and Okonkwo, O.J. 2015. Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography and Separation Techniques*, 6(4):1–10.

Schoeman, C., Dlamini, M. and Okonkwo, O.J. 2017. The impact of a Wastewater Treatment Works in Southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. *Emerging Contaminants*, 3(2):95–106.

Schunck, F. and Liess, M. 2023. *Persistent disruption of interspecific competition after ultra-low esfenvalerate exposure*. [Preprint] arXiv. <https://arxiv.org/abs/2307.03605> Date of access: 19 Jun. 2025.

Shukla, K., Kumar, P., Mann, G.S. and Khare, M. 2020. Mapping spatial distribution of particulate matter using Kriging and Inverse Distance Weighting at supersites of megacity Delhi. *Sustainable Cities and Society*, 54:101997.

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

Talvitie, J., Heinonen, M., Pääkkönen, J.P., Vahtera, E., Mikola, A., Setälä, O. and Vahala, R. 2015. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Science and Technology*, 72(9):1495–1504.

Taylor, J.C., Harding, W.R., Archibald, C.G.M. and Van Rensburg, L. 2005. Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957: a re-analysis of diatom count data generated by B.J. Choinoky. *Water SA*, 31(2):237–246.

Tong, A.Y., Peake, B.M. and Braund, R. 2011. Disposal practices for unused medications around the world. *Environment International*, 37(1):292–298.

Umwali, E.D., Kurban, A., Isabwe, A., Mind'je, R., Azadi, H., Guo, Z., ... Gasirabo, A. 2021. Spatio-seasonal variation of water quality influenced by land use and land cover in Lake Muhazi. *Scientific Reports*, 11(1):17376.

USEPA (United States Environmental Protection Agency). 2007. Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. Washington, DC: Office of Water, United States Environmental Protection

Agency (EPA-821-R-08-002). [Online]. https://www.epa.gov/sites/default/files/2015-10/documents/method_1694_2007.pdf Date of access: 3 Apr. 2025.

USEPA (United States Environmental Protection Agency). 2010. *Stability of Pharmaceuticals, Personal Care Products, Steroids, and Hormones in Aqueous Samples, POTW Effluents, and Biosolids*. EPA-820-R-10-008. [Online]. https://www.epa.gov/sites/production/files/2015-09/documents/ppcp-holding-time-study_2010.pdf Date of access: 3 Apr. 2020.

USEPA (United States Environmental Protection Agency). 2019. *Field Equipment Cleaning and Decontamination at the FEC (ASBPROC-206-R4)*. <https://www.epa.gov> Date of access: 28 Jan. 2023.

Vatovec, C., Phillips, P., Van Wagoner, E., Scott, T.M. and Furlong, E. 2016. Investigating dynamic sources of pharmaceuticals: Demographic and seasonal use are more important than down-the-drain disposal in wastewater effluent in a University City setting. *Science of the Total Environment*, 572:906–914.

Vermaak, V. 2009. *A geomorphological investigation of the Klip river wetland, south of Johannesburg*. Johannesburg: University of Witwatersrand. (Thesis – MSc.).

Verster, C. and Bouwman, H. 2020. Land-based sources and pathways of marine plastics in a South African context. *South African Journal of Science*, 116(5-6):1–9.

Vidon, P., Tedesco, L.P., Pascual, D.L., Campbell, M.A., Casey, L.R., Wilson, J. and Gray, M. 2008. Seasonal changes in stream water quality along an agricultural/urban land-use gradient. In *Proceedings of the Indiana Academy of Science*, 117(2):107–123.

Vieno, N., Hallgren, P., Wallberg, P., Pyhälä, M., Zandaryaa, S. and Baltic Marine Environment Protection Commission. 2017. *Pharmaceuticals in the aquatic environment of the Baltic Sea region: a status report. Vol. 1*. Paris: UNESCO Publishing.

Vivanti, A., Soheili, T.S., Cucuini, W., Luce, S., Mandelbrot, L., Lechenadec, J., ... Blanche, S. 2015. Comparing genotoxic signatures in cord blood cells from neonates exposed in utero to zidovudine or tenofovir. *AIDS*, 29(11):1319–1324.

Vogt, B., Bouwman, H., Bezuidenhout, C., Horn, S., Vogt, T., Bothma, L., Gerber, E., van Aswegen, D., Blom, K., Fouché, D. and Potgieter, J. 2020. *Quantification, Fate and Hazard Assessment of HIV-ARVs in Water Resources*. Water Research Commission: Pretoria, South Africa. WRC Project K5/2594.

Wang, Y., Fan, L., Crosbie, N. and Roddick, F.A. 2020. Photodegradation of emerging contaminants in a sunlit wastewater lagoon, seasonal measurements, environmental impacts and modelling. *Environmental Science: Water Research and Technology*, 6(12):3380–3390.

Wang, R., Luo, J., Li, C., Chen, J. and Zhu, N. 2023. Antiviral drugs in wastewater are on the rise as emerging contaminants: A comprehensive review of spatiotemporal characteristics, removal technologies and environmental risks. *Journal of Hazardous Materials*, 457:131694.

Webster, J. and Iqani, M. 2024. Johannesburg's shitty little river: faecal discourse and discontent regarding the Jukskei. *Social Dynamics*, 50(1):109–127.

Westgaard, J.O., Barry, P.L., Quam, E.F. and Ehrmeyer, S.S. 2008. *Basic Method Validation: Training in Analytical Quality Management for Healthcare Laboratories*. Madison, WI: Westgard Quality Corporation.

Whitmire, M., Ammerman, J., De Lisio, P., Killmer, J., Kyle, D., Mainstone, E., ... Zhang, T. 2011. LC-MS/MS bioanalysis method development, validation, and sample analysis: points to consider when conducting nonclinical and clinical studies in accordance with current regulatory guidances. *Journal of Analytical and Bioanalytical Techniques* 4(2).

WHO (World Health Organization). Collaborating Centre for Drug Statistics Methodology. 2016. *ATC classification index with DDDs*. Oslo, Norway: World Health Organization. https://atcddd.fhi.no/atc_ddd_index_and_guidelines/atc_ddd_index/

Wood, T.P., Duvenage, C.S.J., and Rohwer, E. 2015. The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution*, 199:235–243. <https://doi.org/10.1016/j.envpol.2015.01.030>

Xiao, T., Muhammad, A.M., Pan, X., Wang, Y., Guangming, H. and Tang, Z. 2022. Spatial distribution and risk identification of arsenic contamination in water and soil through GIS-

based interpolation techniques in Jiangnan Plain, Central China. *Frontiers in Environmental Science*, 10:1001862.

Xolo, T. and Mahlambi, P. 2024. Molecularly imprinted polymers as solid-phase and dispersive solid-phase extraction sorbents in the extraction of antiretroviral drugs in water: adsorption, selectivity and reusability studies. *Journal of Analytical Science and Technology*, 15(1):11.

Yang, W., Zhao, Y., Wang, D., Wu, H., Lin, A. and He, L. 2020. Using principal components analysis and IDW interpolation to determine spatial and temporal changes of surface water quality of Xin'anjiang river in Huangshan, China. *International Journal of Environmental Research and Public Health*, 17(8):2942.

Ziajahromi, S., Neale, P.A., Rintoul, L. and Leusch, F.D. 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. *Water Research*, 112:93–99.

Zisou, C. and Psilovikos, A. 2012. Monitoring and Modeling of the Physicochemical Parameters Geographic Distribution in Lake Karla using GIS. In: *2nd Hellenic Conference of "EYE-EEDYP"*. 1250–1261.

CHAPTER 4 FROM CURE TO CONTAMINANT: MIXTURE AND ENVIRONMENTAL RISK ASSESSMENT OF ANTIRETROVIRAL DRUGS IN SOUTH AFRICAN WATERS

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Abstract

The extensive use of antiretroviral drugs (ARVDs) for therapeutic purposes in South Africa has generated concerns about their persistence and ecotoxicological effects on freshwater ecosystems. This study aimed to assess the ecological risks posed by ARVDs in surface water and waste water effluent. Maximum measured environmental concentrations (MECs) for 14 ARVDs that were previously found in South African water sources were evaluated using risk quotient (RQ) methods, a prioritisation index (P_{index}), and a RQ hybrid model that amalgamates concentration addition for ARVDs with shared mechanisms and response addition for those exhibiting independent modes of action. The ecological risk was assessed and compared between algae, crustaceans, and fish. The study identified effluent as a major source of ARVD contamination, with several compounds, particularly efavirenz, lopinavir, and zidovudine, surpassing ecological high-risk thresholds. Prioritisation analysis revealed LPV, ZDV, and EFV as the highest environmental concern ARVD based on frequency of exceedance and ecological risk. The hybrid-mixture model demonstrated that different classes of ARVDs contributed considerably to the mixture risk, with probabilities of adverse effects nearing 100% in some effluent samples, suggesting a high possibility of ecological harm. The results highlight the need to improve wastewater treatment efficiency and integrate mechanistic models into regulatory frameworks. Given current uncertainties and data gaps, an adaptive risk assessment approach is recommended to inform future pharmaceutical monitoring and environmental policy.

Keywords: HIV; AIDS; antiretroviral drugs; environmental risk assessment; mixture toxicity; water contamination.

4.1 Introduction

Antiretroviral drugs (ARVDs), central to HIV/AIDS (human immunodeficiency virus/ acquired immunodeficiency syndrome) treatment (Ramana *et al.*, 2014), function by targeting distinct stages of the HIV life cycle including viral entry, reverse transcription, integration, and maturation (Figure 4-1). Their widespread use in Southern Africa, while critical for public health, has resulted in their continuous release into aquatic environments through wastewater, eliciting concerns over their environmental occurrence and prospective ecotoxicological effects (Adeola and Forbes, 2022; Nqwenya and Musee, 2023; Reddy *et al.*, 2023).

The heterogeneous pharmacokinetic profiles of ARVDs (including elimination pathways, half-life, and protein-binding capacities) illustrate the intricacy of their pharmacodynamic properties and emphasises the necessity for tailored therapeutic strategies (Table 4-1). The pseudo-persistent nature (continuous release due to high usage) of ARVDs in surface and wastewater across South Africa raises concerns for both environmental and public health (Horn *et al.*, 2022). ARVDs are designed to inhibit viral replication and alter cellular processes in humans (Figure 4-1). These compounds can retain their bioactivity when discharged into the environment where they may unintentionally affect non-target organisms. Their persistence and potency can disrupt aquatic ecosystems with potential consequences for biodiversity, ecosystem functioning and the safety of water resources.

Comparable concerns have been raised elsewhere in Africa, for example pharmaceuticals have been detected in sediment from Lake Victoria (Uganda), with spatial distribution patterns signifying ecotoxicological risks to aquatic ecosystems (Nantaba *et al.*, 2024). Notably, several ARVDs, including EFV and NVP, along with their metabolites (such as 12-hydroxy-nevirapine and 8,14-dihydroxy efavirenz), exhibit non-biodegradability, contributing to environment persistence (Mosekiemang *et al.*, 2019; Madikizela *et al.*, 2020). This regional evidence underscores the widespread concern of pharmaceutical pollution across African freshwater ecosystems.

The ecological risks associated with ARVDs (Figure 4-2) are increasingly recognised as ecotoxicological threats, particularly given their continuous input into aquatic ecosystems (Horn *et al.*, 2022). ARVDs display diverse toxicity profiles across aquatic taxa (Minguez *et al.*, 2016; Omotola *et al.*, 2021; Sanderson *et al.*, 2004). Compounds such as LPV, RTV, and EFV have been identified as hazardous to aquatic species (Czech *et al.*, 2022; Ngwenya and Musee, 2023).

Fish species appear especially susceptible to the toxic effects of ARVDs, with multiple species exhibiting organ-level pathologies and developmental disturbances. For example, *Clarias gariepinus* and *Cyprinus carpio* exhibited extensive liver damage exposed to EFV and NVP (Rimayi *et al.*, 2018), with EFV inducing steatosis and necrosis at concentrations as low as 20.6 ng/L (Robson *et al.*, 2017). Similarly, NVP caused severe histopathological changes such as vacuolation, necrosis, fibrosis, and inflammation in Mozambique tilapia (*Oreochromis mossambicus*), alongside elevated hepatosomatic indices (Nibamureke *et al.*, 2019). Beyond hepatic effects, Gorgulho *et al.* (2018) reported that TDF exposure disrupted zebrafish (*Danio rerio*) kidney integrity, causing nuclear delocalization, tubular dilatation, and epithelial disarray, suggesting early-stage acute tubular injury linked to mitochondrial morphological disturbances.

In addition to organ-specific effects, ARVD exposure has been associated with a range of developmental and morphological abnormalities across fish species. Efavirenz treatment resulted in reprotoxic effects in *D. rerio* embryos including increased mortality, skeletal malformations, delayed hatching, and reduced locomotor activity. Oestradiol levels and bone density in Mozambique tilapia were altered when exposed to 10.3–20.6 µg/L (Zizioli *et al.*, 2023; Kowlaser *et al.*, 2022). Lopinavir (880 µg/L) also caused deformities in *D. rerio*, including scoliosis and pericardial oedema following light-induced dilution (Czech *et al.*, 2022). The same study found that RTV's predicted no effect concentration values were toxic to *D. rerio* by increasing the incidence of deformities.

Behavioural assays revealed anxiogenic and depressive-like effects of EFV in *D. rerio* and rats (*Rattus norvegicus*), correlated with apoptosis and neural impairment (Cavalcante *et al.*, 2017; Zizioli *et al.*, 2023). While many studies reported a typical sigmoidal concentration-toxicity relationship, others observed atypical patterns, suggesting potential complexities in toxicological responses in species such as *O. mossambicus* (Robson *et al.*, 2017; Nibamureke *et al.*, 2019; and Czech *et al.*, 2022). This could point to unique dynamics or complexities in those particular studies.

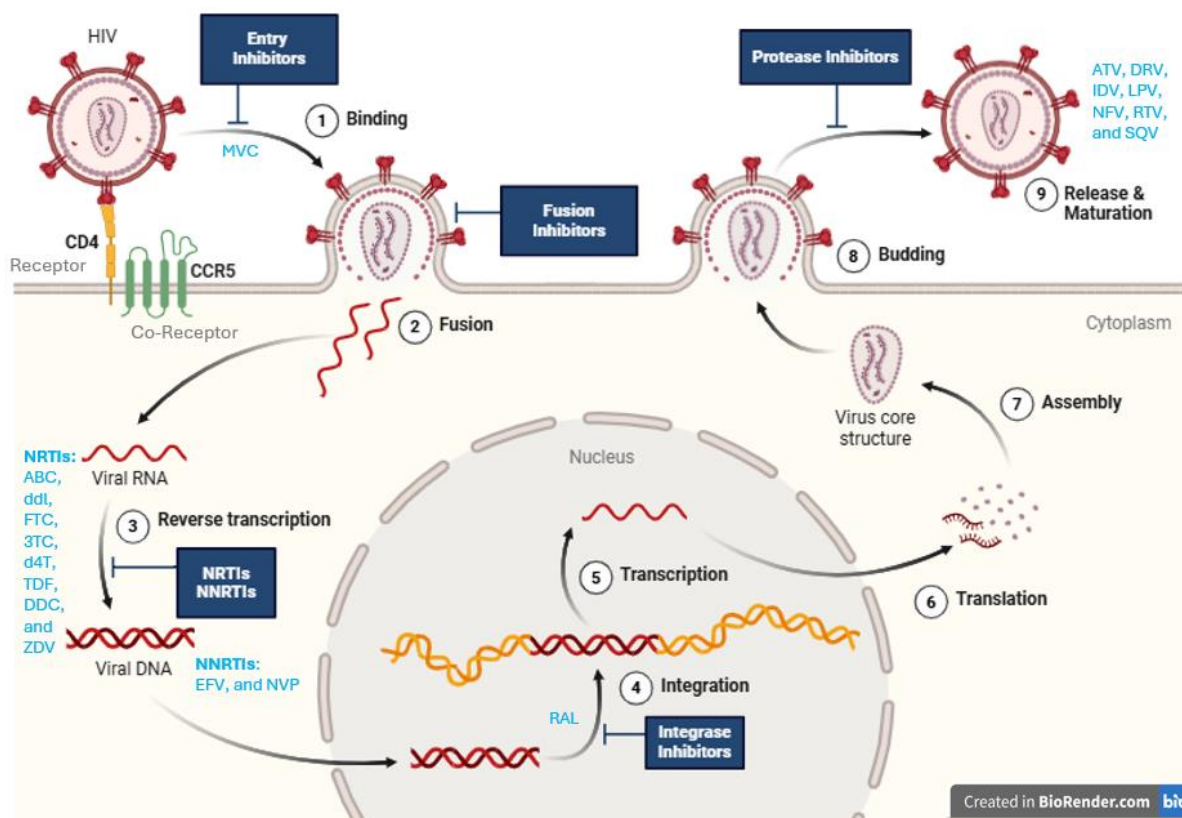


Figure 4-1: Sites of mechanisms of action for different antiretroviral drugs investigated in the current study (denoted in light blue colour) at different steps in the viral life cycle.

Lopinavir, TDF, EFV, and RTV have demonstrated considerable ecotoxicological effects across multiple aquatic species. LPV (880 µg/L) has been associated with cardiotoxicity in zebrafish embryos (Czech *et al.*, 2022), while TDF (3 mg/L) induces hemocytopenia in the freshwater mollusc *Biomphalaria glabrata* by suppressing amoebocyte production can be considered a toxic compound at concentrations between 1 mg/L and 10 mg/L (Souza-Silva *et al.*, 2023). EFV exposure leads to neurodevelopmental damage in zebrafish, including apoptosis and disrupted angiogenesis (Zizioli *et al.*, 2023).

Ritonavir was identified as highly toxic to planktonic rotifer species (*Brachionus calyciflorus*) and water fleas (*Ceriodaphnia dubia*), causing oxidative stress and DNA damage at 0.001 µg/L (Nugnes *et al.*, 2024). Biochemical disruptions caused by TDF in the cladoceran *Daphnia magna* include alterations in catalase and glutathione s-transferase activities (Mahaye and Musee, 2022). Genotoxicity studies suggest potential DNA fragmentation linked to nucleotide pool imbalances induced by nucleoside reverse transcriptase inhibitors (NRTIs) in cord blood cells and mice cells (Bakare *et al.*, 2020; Vivanti *et al.*, 2015).

Table 4-1: Pharmacokinetic properties and mechanisms of action of different antiretroviral drugs (ARVDs).

ARVD	Mechanism of action	Elimination (non-hepatic)	Physiological half-life (hours)	Protein binding capacity	Reference
ABC	Inhibits reverse transcriptase. Guanosine analogue.	1.2% urine, 16% faeces	1.54±0.63	50%	Liu and Chen, 2016
ATV	Protease inhibitor.	13% urine, 79% faeces	7	86%	Wood, 2008
ddl	Inhibits reverse transcriptase. Adenosine analogue.	18% urine	1.5	< 5%	De Clercq, 2005; Mason and Darryl, 2015
DRV	Inhibits viral protease.	13.9% urine, 79.5% faeces	15 (Ritonavir boosted)	95%	Liaño and Tenorio, 2008
EFV	Non-nucleoside reverse transcriptase inhibitor.	Mostly by urine	40–55	99.5–99.75%	Costa and Vale, 2022
FTC	Inhibits reverse transcriptase. Cytidine analogue.	86% urine, 14% faeces	10	< 4%	Desai, 2016
IDV	Inhibits viral protease.	< 20% urine	1.8±0.4	60%	Pollak and Parmar, 2024
3TC	Inhibits reverse transcriptase. Cytidine analogue.	5.2±1.4% urine	5–7	< 36%	Weston, 2007
LPV	Inhibits viral protease.	10.4±2.3% urine, 82.6±2.5% faeces	6.9±2.2	> 98%	Cvetkovic and Goa, 2003
MVC	Blocks viral entry.	-	14–18	76%	Paintsil and Cheng, 2019
NFV	Inhibits viral protease.	1–2% urine, 87% faeces	3.5–5	> 98%	Pai and Nahata, 1999
NVP	Non-nucleoside reverse transcriptase inhibitor.	< 3% urine	45	60%	Schauer <i>et al.</i> , 2014
RAL	Inhibits viral integrase enzyme.	urine and faeces	9	83%	Mouscadet, and Tchertanov, 2009
RTV	Inhibits viral protease.	11.3±2.8% urine, 86.4±2.9% faeces	3–5	98–99%	Kempf <i>et al.</i> , 1995
d4T	Inhibits reverse transcriptase. Thymidine analogue.	40% urine	0.8–1.5	Negligible	Piscitelli <i>et al.</i> , 1999; Scholar, 2007
SQV	Protease inhibitor.	1–3% urine, 81–88% faeces	-	98%	Pereira and Vale, 2022
TDF	Inhibits reverse transcriptase.	Mostly by urine	32	7.2%	Schauer <i>et al.</i> , 2014

DDC	Inhibits reverse transcriptase. Cytidine analogue.	60–80% urine	2	< 4%	Weston and Ile, 2007
ZDV	Inhibits reverse transcriptase. Thymidine analogue.	29% urine	1.1	30–38%	Edwards <i>et al.</i> , 2023

3TC = lamivudine; ABC = abacavir; ATV = atazanavir; DDC = zalcitabine; d4T = stavudine; ddl = didanosine; DRV = darunavir; EFV = efavirenz; FTC = emtricitabine; IDV = indinavir; LPV = lopinavir; MVC = maraviroc; NFV = nelfinavir; NVP = nevirapine; RAL = raltegravir; RTV = ritonavir; SQV = saquinavir; TDF = tenofovir; ZDV = zidovudine.

Comprehensive toxicological studies and environmental risk assessments remain scarce, particularly regarding their persistence, chronic exposure potential, and fate in water systems (Ncube *et al.*, 2018). These knowledge gaps are critical to address, especially in high-use regions such as South Africa, where chronic exposure may exert compounded ecotoxicological effects on freshwater species. The current study conducted a screening-level risk assessment of ARVDs using effluent water as a worst-case proxy for ecological hazard identification bodies (Al Aukidy, 2012; Ferrari *et al.*, 2004; Spilsbury *et al.*, 2024). The second aim was to prioritise ARVDs for mitigation strategies and inform eco-pharmacovigilance efforts.

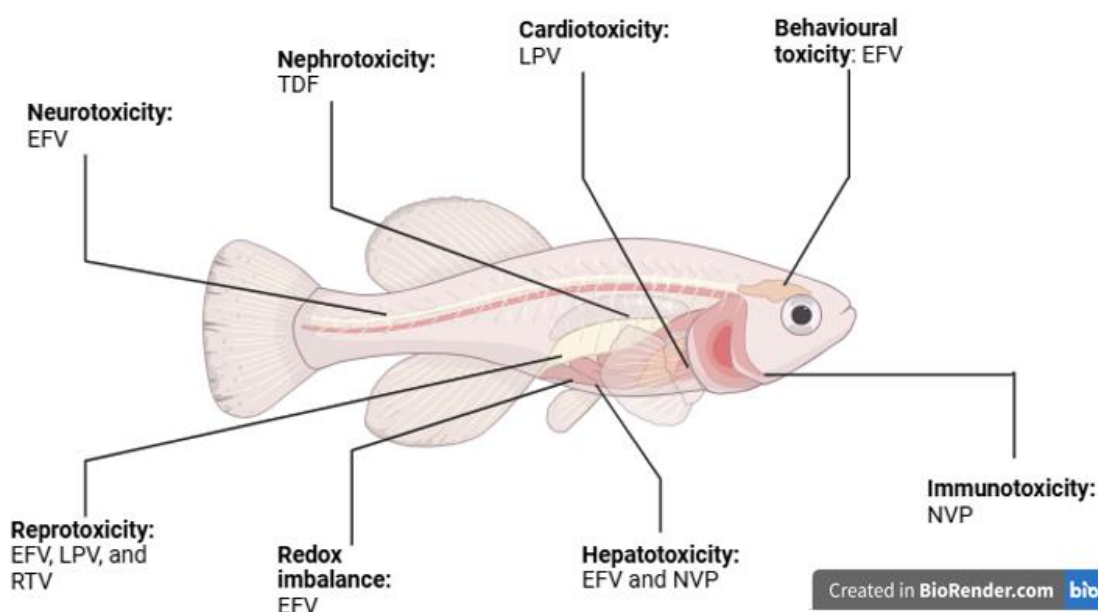


Figure 4-2: Schematic summary of the toxicological effects of some antiretroviral drugs on different physiological systems in fish. Created with Biorender (<https://BioRender.com>).

4.2 Methods

The present study was approved by the NWU Ethics Committee (NWU-00002-23-A1). A detailed literature review was conducted of peer-reviewed publications (2015–2024) reporting ARVD concentrations in South African surface waters and wastewater effluents (Annexure B–D). From 24 qualifying studies, data on maximum ARVD concentrations, sampling locations, and site-specific details from 386 sampling points were compiled. Statistical analysis included normality testing (D'Agostino, Pearson and Shapiro-Wilk) and appropriate non-parametric comparisons (Wilcoxon signed-rank, Mann-Whitney U, Kruskal-Wallis with Dunn's multiple comparisons post-tests). Statistical significance was set at $p < 0.05$, and all analyses were conducted using GraphPad Prism version 10.4.2.

4.2.1 Ecological risk assessment (ERA)

Quantified ARVD data were consolidated for ecological risk assessment (ERA) focusing on 14 out of the 19 ARVDs (Table 2-4; Chapter 2) due to limited ecotoxicity data for five ARVDs. The potential ecological risk was investigated via risk quotients (RQ) based on the measured environmental concentrations (MEC) and the predicted no effects concentration (PNEC) quality values. Toxic potencies of ARVDs to non-target organisms were primarily sourced from the FASS knowledgebase (<https://www.fass.se/>).

Due to absence of local ecotoxicological data, standard non-native organisms were used as proxies in this assessment, acknowledging potential limitations in sensitivity representation. However, our assessment of hazard is not significantly influenced by species' geographical distribution and habitat (Maltby *et al.*, 2005). The present ERA incorporated acute and chronic toxicity data across three different trophic levels; algae as primary producers, crustaceans as primary consumers; and fish as secondary consumers. The ARVDs of greatest concern were determined via a prioritisation index (P_{index}) (Section 4.2.2). The effects of the combined mixture (RQ) of ARVDs were also evaluated (Section 4.2.3).

4.2.1.1 Risk quotients (RQs)

Risk quotients, following European Union (EU) guidelines (Tran *et al.*, 2019), were calculated using maximum measured environmental concentrations (MEC) rather than predicted values, to offer a more realistic environmental assessment (Eq. (4-1)). Preliminary assessment ranks were developed by classifying ARVDs based on specific thresholds. A value of $RQ \geq 1$ designates a high risk, $1 > RQ \geq 0.1$ a medium risk, while $0.1 > RQ \geq 0.01$ designates a low or *di minimis* risk ($0.01 > RQ > 0$) (Grung *et al.* 2008).

4.2.1.2 PNEC estimation

The predicted no effect concentrations (PNECs) were estimated using available acute toxicity data (e.g., LC₅₀ or EC₅₀) divided by assessment factors (AFs) (Eq. 4-2) (Ngumba *et al.* 2016). Chronic toxicity data, expressed as no observed effect concentrations (NOECs), were used in the absence of acute data, following European Medicines Evaluation Agency (EMA) and the European Chemicals Agency (ECHA) guidelines (ECHA, 2008; EMA, 2006). See Annexure H for more detail. Preference was given to empirical ecotoxicity data over estimations such as Quantitative Structure-Activity Relationship (QSAR) values. For ARVDs or trophic levels lacking measured toxicity data, the Ecological Structure-Activity Relationships Program (ECOSAR) v1.11 model provided conservative acute toxicity estimates using the lowest value to reflect worst-case scenarios.

$$RQ_i = \frac{MEC_i}{PNEC_i} \quad (4-1)$$

$$PNEC_i = \frac{\min (EC_{50} \text{ or } LC_{50}, \text{NOEC fish, invertebrate, algae})_i}{AF} \quad (4-2)$$

Where:

PNEC_{*i*} = predicted no effect concentration;

EC₅₀ = median effective concentration;

LC₅₀ = median lethal concentration;

AF = assessment factor;

RQ_{*i*} = risk quotient;

MEC_{*i*} = maximum measured environmental concentration;

i = chemical (*i*) analysed in a water sample.

4.2.2 Prioritisation index (P_{index})

The prioritisation index (P_{index}) was used to identify ARVDs posing the greatest environmental concern by combining the frequency of PNEC exceedances across sites as well the maximum RQ (RQ_{max}) observed (Eq. (4-2)(4-4) (Desbiolles *et al.*, 2018; Liu *et al.*, 2020). The P_{index} results were interpreted as follows: P_{index} > 1 implies high risk due to widespread detection and high ecological risk; P_{index} < 1 implies a lower priority concern based on ecological risk and/or limited exceedance; P_{index} ≈ 1 suggests moderate concern.

$$F = \frac{n}{N} \times 100\% \quad (4-3)$$

$$P_{\text{index}} = RQ_{\text{max}} \times F \quad (4-4)$$

Where:

F = frequency of PNEC exceedance;

n = number of sites with concentrations above PNEC;

N = total number of sampling sites for a chemical;

P_{index} = prioritisation index; and

RQ_{max} = risk quotient calculated based on maximum concentration.

4.2.3 Combined risk quotients ($\sum RQ$) via CA-RA hybrid model

Antiretroviral drugs function collectively by targeting multiple stages of the HIV replication cycle, though they do not share identical mechanisms of action (Table 4-1; Figure 4-1). To assess their combined ecotoxicological impact, mathematical models are employed (De Zwart and Posthuma, 2005). Concentration addition (CA) concerns mixtures of compounds with the same mechanisms of action, such as reverse transcriptase inhibitors (RTIs), including NRTIs and NNRTIs (Figure 4-1). In contrast, response addition (RA) is correlated to independent joint action and is used to predict the combined effects of toxicants with different toxic mechanisms of action (De Zwart and Posthuma, 2005).

To most accurately predict the combined mixture effects of ARVDs, the current study adopted a hybrid model (Backhaus and Faust, 2012; Cedergreen *et al.*, 2008; De Zwart and Posthuma, 2005; Zilber and Messier, 2024). This model (referred to as the CA-RA hybrid model in the current study) distinguishes between ARVDs and applied the appropriate additive framework based on shared as well as distinct mechanisms of action. The combined RQ ($\sum RQ$) for RTIs was calculated by summing the individual RQs of each RTI (Eq. (4-5) and (4-6), as follows:

$$RQ_i = \sum_{i=1}^n \frac{MEC_i}{PNEC_i} \quad (4-5)$$

$$RQ_{\text{RTI}} = \sum_{i=1}^n RQ_i \quad (4-6)$$

Where:

MEC_i = measured environmental concentration of RTI_{*i*};

$PNEC_i$ = predicted no-effect concentration for RTI_{*i*};

RQ_{RTI} = risk quotient for chemical *i* for each reverse transcriptase inhibitor (RTI).

For ARVDs acting on different mechanisms (e.g., protease inhibitors, integrase inhibitors, and entry blockers), the RA model is more appropriate, since their actions are assumed to be independent (4-7) to (4-9). Under RA, combined ecological risk was calculated by converting each drug's RQ into a probability of effect (4-10), then aggregating these probabilities to estimate the overall risk (Eq. (4-10) (De Zwart and Posthuma, 2005; USEPA, 1986).

$$RQ_{Proteas} = \frac{MEC_{Proteas}}{PNEC_{Proteas}} \quad (4-7)$$

$$RQ_{Integrase} = \frac{MEC_{Integrase}}{PNEC_{Integrase}} \quad (4-8)$$

$$RQ_{Entry} = \frac{MEC_{Entry}}{PNEC_{Entry}} \quad (4-9)$$

$$P_i = \frac{RQ_i}{1 + RQ_i} \quad (4-10)$$

Where:

P_i = the individual probability of an adverse effect from compound (*i*) or drug group;

RQ_i = risk quotient for compound (*i*) or drug group.

The overall probability of an adverse effect from the mixture (P_{mix}) was then calculated by multiplying the probabilities of no effect for each drug class and subtracting the result from one (Eq.(4-11). Risk is assumed to increase with the RQ but is bounded by a maximum probability of one (100% effect). The combined risk is considered small if the P_{mix} is small (> 0.5). If the P_{mix} is close to one, there is a high likelihood of adverse effects from the combined mixture. The threshold value for the current study was set at $P_{mix} > 0.5$.

$$P_{mix} = 1 - \prod_{i=1}^n (1 - P_i) \quad (4-11)$$

In the case of ARVDs, this translates to:

$$P_{mix} = 1 - (1 - P_{RTI})(1 - P_{Proteas})(1 - P_{Integrase})(1 - P_{Entry}) \quad (4-12)$$

Where:

P_{mix} = the probability of an adverse effect due to the combined mixture;

P_i = the individual probability of an adverse effect from compound (*i*) or drug group approximated by its RQ.

4.3 Results

Figure 4-3 highlights the variability of MECs across different ARVDs quantified in surface water. Drugs such as ABC, LPV, and RTV exhibited higher MEC median and mean concentrations, suggesting a greater occurrence, while NFV, didanosine (ddl), and DDC had much lower concentrations with minimal variability. Wide concentration ranges in 3TC and ABC, along with outliers in EFV, LPV, NVP, RTV, and ZDV, point to potential instances of elevated contamination in some areas.

Figure 4-4 presents discrepancies in MECs in effluent water. ABC, 3TC, NVP, and EFV showing the highest median MECs, while IDV and MVC exhibited the lowest mean and median concentrations. 3TC displayed a particularly wide range spanning several orders of magnitude. NVP and EFV displayed desperate outliers, indicating infrequent raised concentrations in some effluent samples at certain sites. Wide interquartile ranges across several ARVDs reflect changing concentrations across samples.

Most ARVDs had MECs far greater than what were predicted for the compounds (Figure 4-5 and Table 4-3). PECs greatly underestimated environmental concentrations. ATV was the only drug with a lower median MEC in effluent than its PEC, while EFV showed slight overestimation of 1.5-fold (3.9 µg/L vs. 2.6 µg/L). MECs for ddl, 3TC, SQV, and TDF were an order greater; MVC and ZDV were two orders; ABC, DRV, NVP, and RTV were three; and LPV was ca. four orders of magnitude greater than the PEC. A Spearman rank-correlation test revealed moderate positive, yet no statistically significant correlation (Spearman's $\rho = 0.37$, $p = 0.25$) between PECs and MECs.

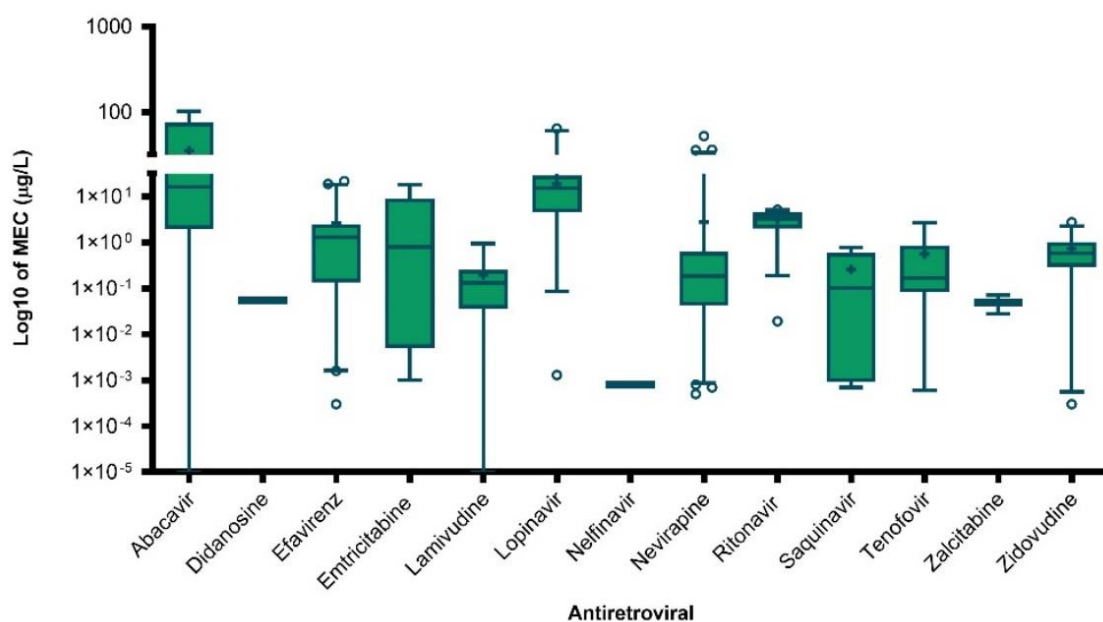


Figure 4-3: Measured environmental concentration (MEC) of selected antiretroviral drugs in surface water (whiskers: 5, 95 percentiles; + mean values; dots represent outliers).

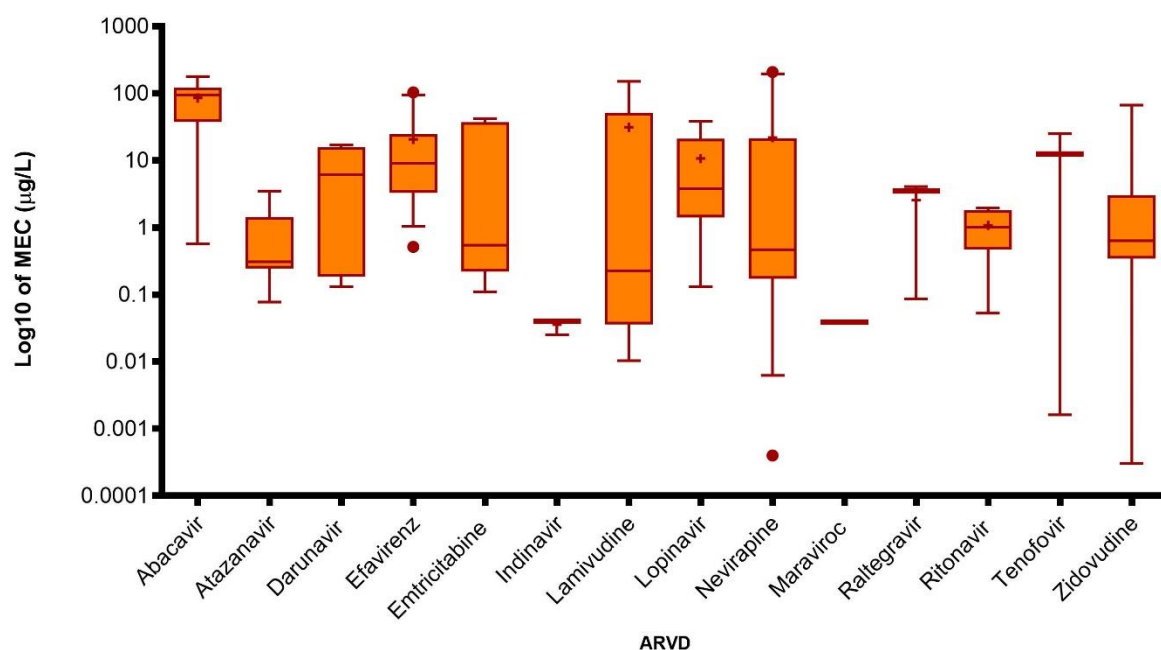


Figure 4-4: Measured environmental concentration (MEC) of selected antiretroviral drugs (ARVDs) in effluent (whiskers: 5, 95 percentiles; + mean values; dots represent outliers).

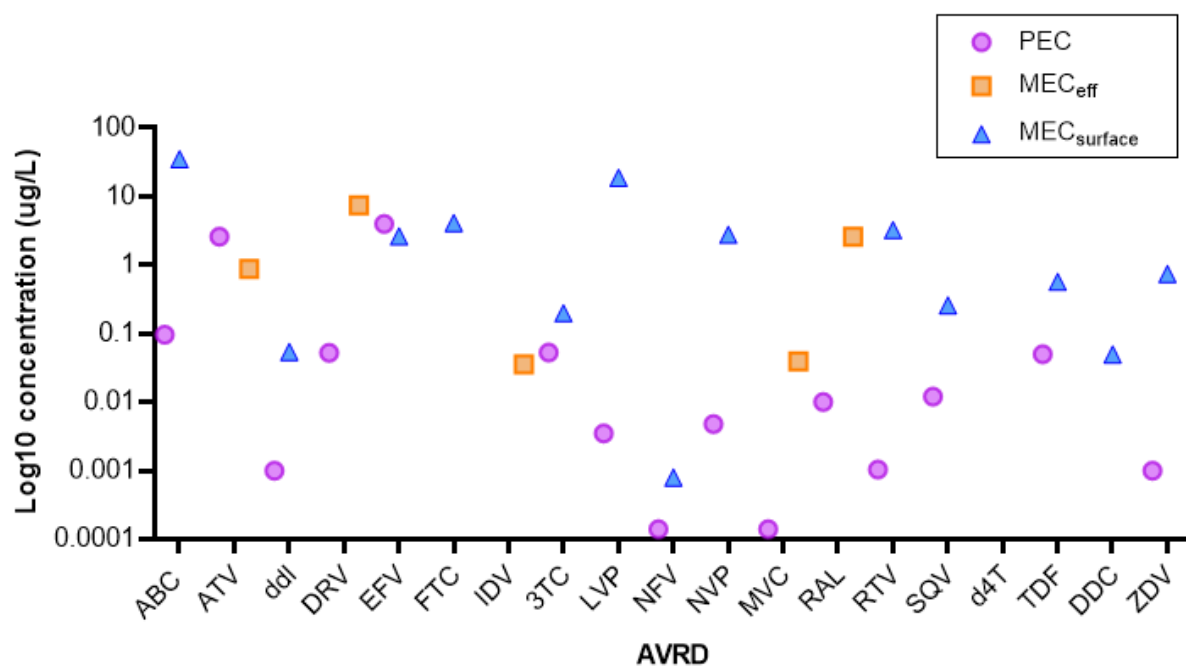


Figure 4-5: Predicted environmental concentrations (PECs; dots) and median measured environmental concentrations (MEC; yellow and blue triangles, respectively) of antiretroviral drugs (ARVDs) for surface (MEC_{surface}) and effluent water (MEC_{eff}).

Table 4-3 illustrates the diverse environmental risks posed by ARVDs, with some compounds (e.g., EFV, RTV, and LPV) expected to accumulate in the tissue of aquatic organisms based on their high bioaccumulation potential ($BCF \geq 500$ or LogD_{ow} (log octanol-water distribution) ≥ 4), presenting a prolonged risk if they are transferred further along the food web. ZDV, MVC, and DRV demonstrated lower bioaccumulation despite moderate to high persistence. While most ARVDs displayed low mobility and strong binding to organic matter, NFV stands out for its high predicted mobility, suggesting a potential for rapid transport through aquatic systems and into groundwater.

Table 4-2: Predicted effect concentration (PEC), predicted no effect concentration (PNEC) and mean measured environmental concentrations (MEC), maximum risk quotients (RQ_{max}), bioaccumulation, persistence, and mobility of ARVDs.

ARVD	Type	PEC ^a (µg/L)	MEC _{surface} (µg/L)	MEC _{eff} (µg/L)	PNEC _{surface} ^a (mg/L)	RQ _{max}	Bioaccumulation ^c	Persistence ^b	Mobility ^b
ABC	NRTI	0.096	35.01		0.56	0.18	Low	Low	
ATV	PI	2.6		0.87	0.41		Low	Low	Not mobile
ddl	NRTI	0.001	0.054		0.21	0.0003	Low		
DRV	PI	0.053		7.37	0.26		Low	Moderate	
EFV	NNRTI	3.93	2.62		0.003	8.35	High		Not mobile
FTC	NRTI		4.1		0.061	0.29			
IDV	PI			0.035	0.001				
3TC	NRTI	0.053	0.197		0.097	0.001	Low	Low	
LPV	PI	0.004	18.71		0.002	30.59	High		
MVC	Entry-inhibitor	0.000		0.039	0.001		Low	High	
NFV	PI		0.001		0.000	0.03			Highly mobile
NVP	NNRTI	0.005	2.78		4.3	0.012	Low	Low	
RAL	Integrase inhibitor	0.01		2.56	0.38		Low	Low	
RTV	PI	0.001	3.23		0.03	0.17	High	Low	
d4T	NRTI		0.26		0.112				
SQV	PI	0.012			0.112	0.007	Low	Low	Not mobile
TDF	NRTI	0.05	0.57		0.009	0.3	Low		
DDC	NRTI		0.05		4.7	1.5E-05			
ZDV	NRTI	0.001	0.74		0.001	5.01	Low	High	

a = Cid *et al.* (2021), FASS (2023; 2025), Roche (2023); b = ECHA (2023), PubChem (2023); c = Bioaccumulation hazard statements were interpreted from Organisation for Economic Cooperation and Development test 308 (OECD, 2002), tests 301, 302B (OECD, 1992a,b), 302C (OECD, 2009) and FASS (2023) guidelines, as follows: low bioaccumulation = bioconcentration factor (BCF) <500 or log octanol-water distribution ratio (DOW; at pH 7) <4; high bioaccumulation = BCF ≥500 or log DOW (at pH 7) ≥4; high persistence = 50% degradation time (DT50) >120 or no ready or inherent biodegradation; moderate persistence or inherent biodegradation = DT50 ≤120; low persistence or ready biodegradability = DT50 ≤32. Mobility is classified based on the log organic-carbon partition coefficient (log KOC) as either very mobile = logKoc <3, mobile = logKoc <4, and otherwise not mobile. Cells

colour coded by magnitude of parameter (i.e., orange = high risk, mobility, persistence, etc.; blue = medium risk or persistence, etc; light grey = low risk, mobility, persistence, etc.). White cells present data that was not available.
NRTI = nucleoside reverse transcriptase; NNRTI = non-nucleoside reverse transcriptase; PI = protease inhibitor.

4.3.1 PNEC estimation

To derive PNECs (Table 4-3) for LVP, NFV, d4T, and TDF, an AF of 1000 was used, as at least one short-term L(E)C₅₀ from each of the three trophic levels was available. For ABC, ATV, ddl, DRV, EFV, MVC, NVP, RAL, SQV, DDC, and ZDV, an AF of 10 was used, since long-term results from three trophic levels were available.

For IDV and RTV, an AF of 50 was used, based on long-term ecotoxicological data available for two trophic levels: a primary consumer (*Daphnia magna*, a freshwater cladoceran) and a secondary consumer (*Lepomis macrochirus*, a freshwater fish). Although data were also available for *Mysis diluviana*, its trophic position overlaps with *D. magna*, justifying the use of two trophic levels rather than three.

The AFs were applied to the lowest value for toxicity. For the ARVDs, 3TC and FTC, an AF of 100 was used because results for one long-term NOEC from only one trophic level were available, namely primary producer (*Selenastrum capricornutum*) and primary consumer (*Pimephales promelas*). Among the ARVDs, the highest PNEC was for DDC followed by NVP. The lowest PNEC was for NFV, IDV, and MVC.

Table 4-3: The predicted no effect concentration (PNEC) for selected antiretroviral drugs, listed according to trophic level of the most sensitive species. Ecotoxicity data were obtained from FASS^a and ECOSAR^b databases.

ARVD	Aquatic organism	Species	Period	End point	Protocol	Concentration (mg/L)	PNEC (mg/L)
ATV	Green algae	<i>Pseudokirchneriella subcapitata</i>		NOEC	OECD 201	4.1	0.41
EFV	Green algae	<i>Selenastrum capricornutum</i>	12 d	NOEC	FDA 4.01	0.026	0.003
3TC	Green algae	<i>Selenastrum capricornutum</i>	72 h	NOEC	OECD 201	96.9	0.097
NVP	Green algae	<i>Selenastrum capricornutum</i>	10 d	NOEC	FDA, TAD 4.01	43	4.3
RAL	Green algae	<i>Selenastrum capricornutum</i>	96 h	NOEC	OECD 201	3.8	0.38
SQV	Green algae	<i>Raphidocelis subcapitata</i>	72 h	NOEC	OECD 201	1.12	0.112
ZDV	Green algae	Unspecified species		ChV		0.01	0.001
ABC	Crustacean	<i>Ceriodaphnia dubia</i>	7 d	NOEC	EPA 1002	5.6	0.56
DRV	Crustacean	<i>Daphnia magna</i>		NOEC	OECD 202	2.6	0.26
IDV	Crustacean	<i>Daphnia magna</i>		ChV		0.04	0.001
MVC	Crustacean	Mysid		ChV		0.008	0.001
NFV	Crustacean	Mysid		ChV		0.027	0.000
DDC	Crustacean	<i>Daphnia magna</i>		ChV		47.2	4.7
RTV	Crustacean	<i>Daphnia magna</i>	48 h	EC ₅₀	FDA TAD 4.08	1.5	0.03
d4T	Crustacean	<i>Artemia salina</i>	24 h	IC ₅₀	ISO 6341	111.82	0.112
ddl	Fish	Unspecified species		ChV		2.1	0.21
FTC	Fish	<i>Pimephales promelas</i>	28 d	NOEC	OECD 210	6.1	0.061
LPV	Fish	<i>Lepomis macrochirus</i>	96 h	LC ₅₀		2.1	0.002
TDF	Fish	<i>Pimephales promelas</i>	32 d	NOEC	OECD 210	9	0.009

a = www.fass.se ; b = USEPA (2023); ChV = chronic value (the geometric mean of the NOEC and the LOEC); d = days; FDA = United States Food and Drug Administration; h = hours; IC₅₀ = inhibitory concentration 50%; ISO = International Organisation for Standardisation; LOEC = lowest observed effect concentration; NOEC = no observed effect concentration; OECD = Organization for Economic Co-operation and Development. Blank cells = data was not available.

4.3.2 Risk quotients (RQs)

Significant differences between the median RQ values for each trophic level in surface water were observed: algae ($p < 0.0001$; $H = 156.2$); crustaceans ($p < 0.0001$; $H = 154.7$); and fish ($p < 0.0001$; $H = 164.7$). Significant differences were also observed between the RQs of ARVDs in surface water samples for algae, crustaceans, and fish ($p < 0.05$). Overall, the ERA showed the following (Figure 4-6 to Figure 4-8 and compact letter display (CLD) analysis):

- For algae (Figure 4-6), LPV, EFV, and ZDV posed high risk ($RQ \geq 1$), with LPV standing out as the most toxic to algal communities. TDF and d4T showed inconsistent effects, suggesting uncertainty in their risk profiles.
- For crustaceans (Figure 4-7), LPV presented the greatest risk, while EFV and RTV exhibited moderate risk ($0.1 \leq RQ < 1$). Groupings showed variable risk profiles, with DDC, ZDV, and NVP among the least hazardous.
- For fish (Figure 4-8), LPV again ranked highest in median RQs, followed by EFV and RTV ($0.1 \leq RQ < 1$). ABC, TDF, NFV, and ddl showed fluctuating risk levels, while ZDV, FTC, and d4T had the lowest risk potential of ecotoxicological risk to fish compared to other ARVDs.

Most ARVDs had tightly clustered RQs, indicating consistent toxicity across different sampling points. However, TDF and d4T, showed greater variability in response among algae, crustaceans, and fish, reflecting uncertainty of the true ecological risks presented by these ARVDs.

Distinct ecological risks across trophic levels were found for effluent water (Figure 4-6 to Figure 4-8). For algae, EFV, LPV, and ZDV had mean RQ values depicting high risk ($RQ \geq 1$), with ZDV reaching the highest observed RQ (Figure 4-6). Tenofovir also exceeded the high-risk threshold. However, TDF showed a notable uncertainty of the true mean, while 3TC and ABC posed low risk ($0.1 > RQ \geq 0.01$). Despite a robust dataset for NVP ($n = 31$), results showed low to *di minimis* risk.

For crustaceans (Figure 4-7) and fish (Figure 4-8), the RQ results were fairly similar. Efavirenz ($RQ = 1.3, 3$) and LPV ($RQ = 4, 5.1$) had tightly clustered mean RQ values depicting high risk for crustaceans and fish, respectively. However, TDF and ZDV showed great irregularity. Abacavir and TDF exceeded medium-risk thresholds for crustaceans, while DRV, IDV, MVC, and RTV posed lower risks (Figure 4-7). In fish (Figure 4-8) FTC was the only drug calculated

at medium risk. All the other drugs posed low to *di minimis* risk. Statistical tests confirmed significant differences in median RQ values across trophic levels: algae ($p < 0.0001$; $H = 89.5$); crustacean ($p < 0.0001$; $H = 99.5$); and fish ($p < 0.0001$; $H = 101.8$), and between ARVDs in effluent within each trophic level ($p < 0.05$).

The CLD analyses further identified EFV, LVP, and TDF as consistently high-risk for algae, crustaceans, and fish, while ZDV and NVP showed variable but generally lower risks. Overlapping CLD groupings for several ARVDs, including DRV, RTV, and RAL, suggest moderate but statistically indistinct risk profiles across taxa.

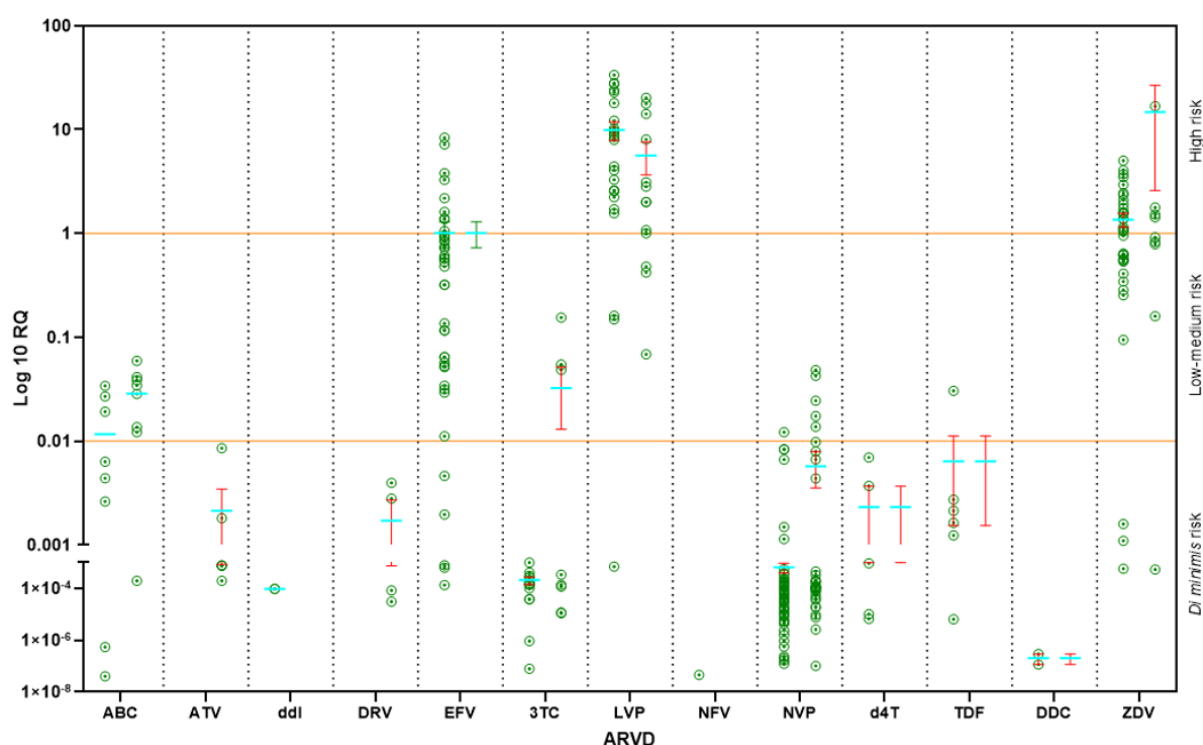


Figure 4-6: Log10 risk quotient (RQ) values of antiretroviral drugs (ARVDs) detected in surface water (first data set per compound) and effluent (second data set per compound) for algae. Individual data points are shown as circles, bars represent the standard error of the mean, and the orange lines indicate risk threshold values.

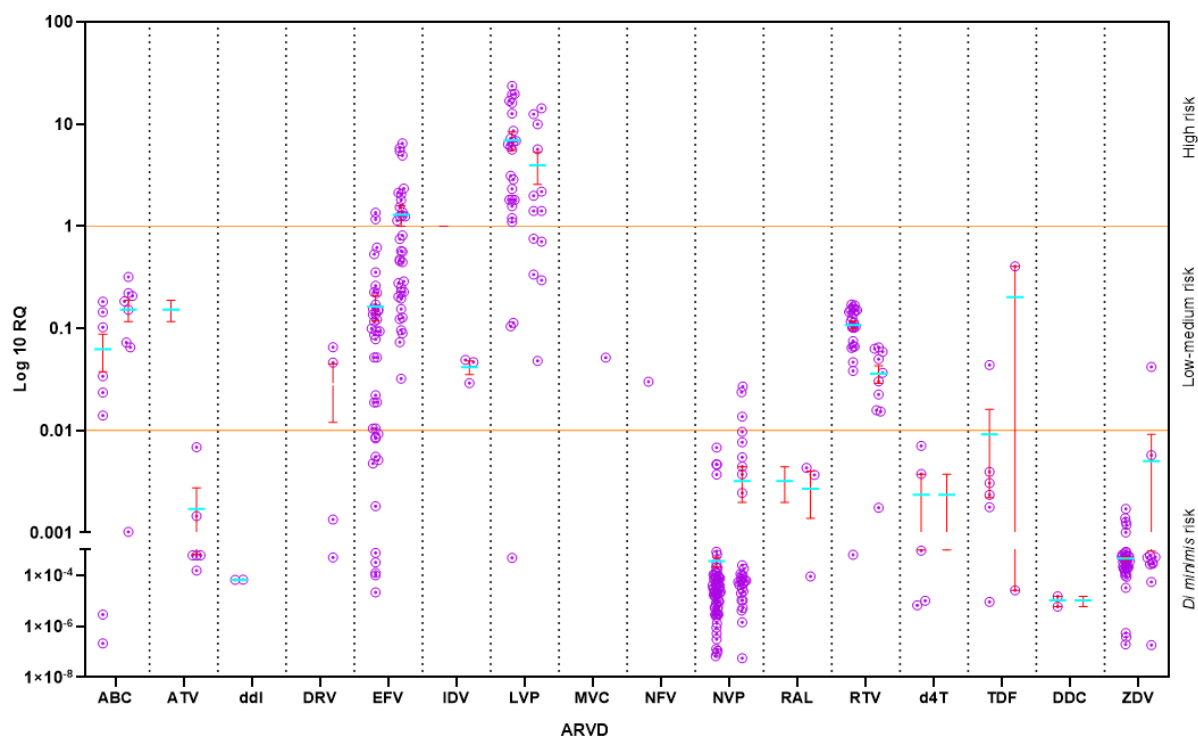


Figure 4-7: Log₁₀ risk quotient (RQ) values of antiretroviral drugs (ARVDs) detected in surface water (first data set per compound) and effluent (second data set per compound) for crustaceans. Individual data points are shown as circles, bars represent the standard error of the mean, and the orange lines indicate risk threshold values.

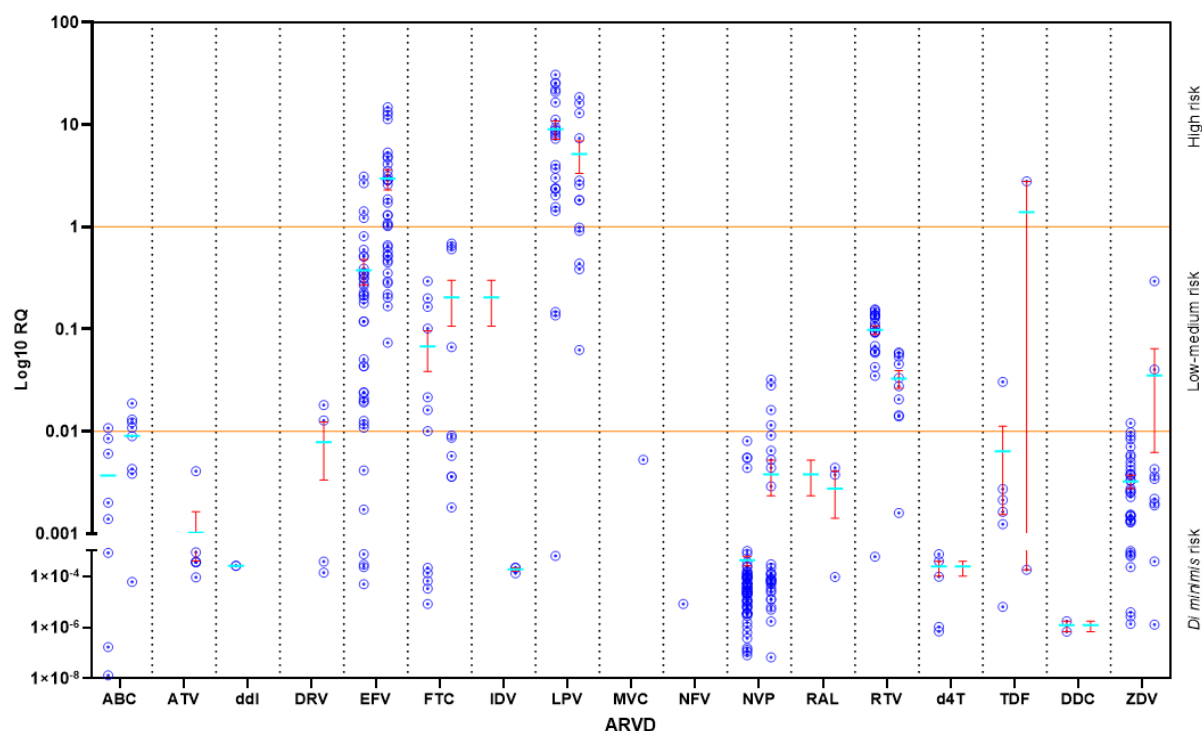


Figure 4-8: Log10 risk quotient (RQ) values of antiretroviral drugs (ARVDs) detected in surface water (first data set per compound) and effluent (second data set per compound) for fish. Individual data points are shown as circles, bars represent the standard error of the mean, and the orange lines indicate risk threshold values.

Figure 4-9 revealed that the majority of ARVDs exhibit RQ_{max} values below the threshold for medium-risk, suggesting a low environmental risk in surface water sources. Exceptions included EFV, ZDV, and LPV, which exceeded the medium-risk threshold and posed high risk for aquatic organisms in surface water.

In contrast, effluent water exhibited RQ_{max} values above the low-risk threshold, suggesting varying degrees of environmental risk. ABC, 3TC, and FTC posed medium risk, while ZDV, TDF, LPV, and EFV presented considerable risks towards aquatic organisms. Although a Wilcoxon signed-rank test showed no statistically significant difference in RQ values between surface water and effluent samples ($n = 10$ pairs; $p = 0.1$), a strong Spearman correlation ($r_s = 0.89$; $p = 0.0006$) suggests consistent contamination patterns across water types.

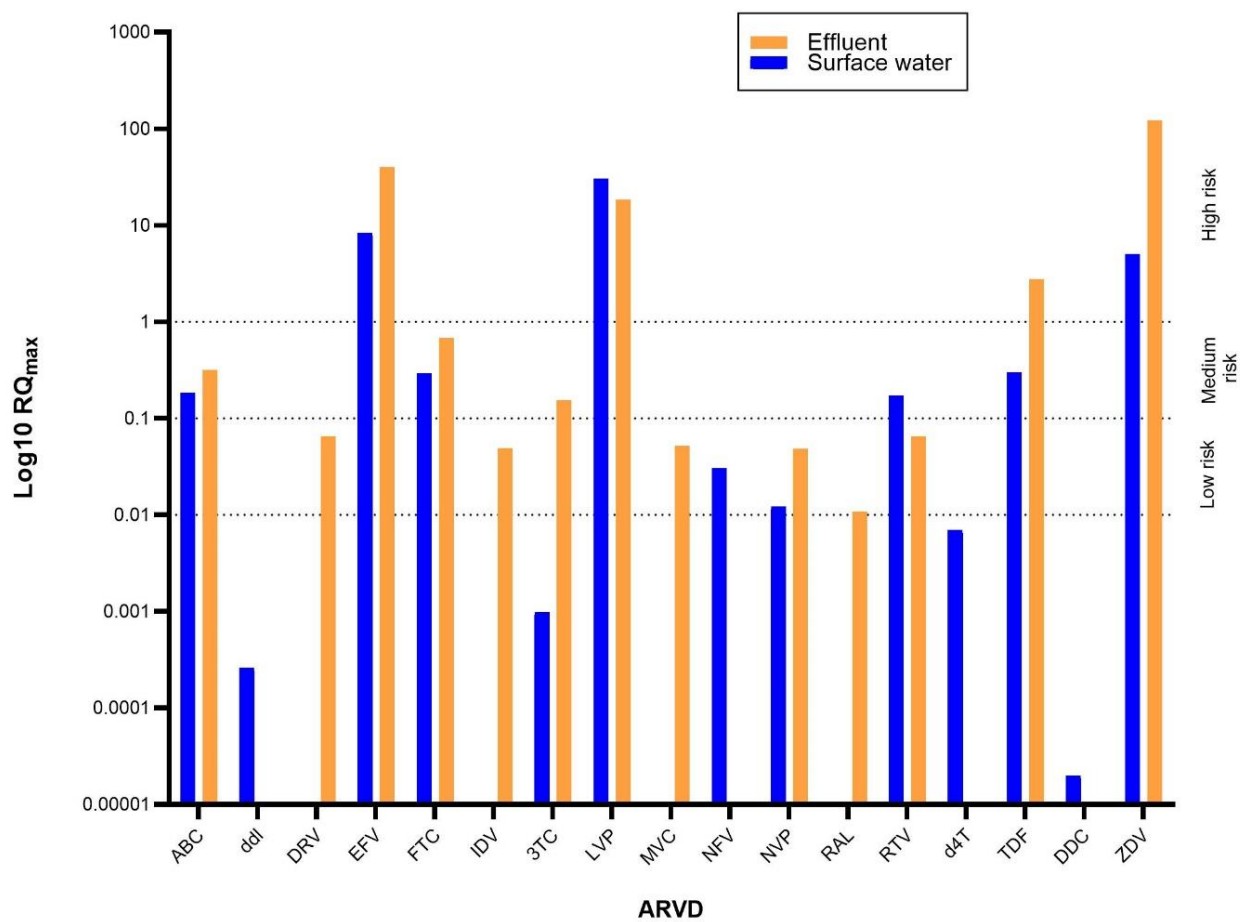


Figure 4-9: Maximum log₁₀ risk quotient (RQ_{max}) of antiretroviral drugs (ARVDs) in surface water and effluent based on the most sensitive trophic level, where an RQ ≥ 1 designates high risk, 1 > RQ ≥ 0.1 a medium risk, and 0.1 > RQ ≥ 0.01 designates a low or *di minimis* risk (0.01 > RQ > 0).

4.3.3 Combined risk quotients (ΣRQ)

The results from the CA-RA hybrid model (Table 4-4) demonstrated significant ecological risk from ARVD mixtures in both surface water and effluent. In surface waters, ΣRQ_{RTI} yielded a maximum value of 14.2 with a combined probability (P_{mix}) of 99.8% for adverse effects. The median ΣRQ_{RTI} for surface water was considerably lower at 1.6, indicating the presence of high values affecting the risk profile in surface water. However, the central risk remains much lower. Non-RTIs in surface water posed high risks compared to RTIs (Table 4-4), yielding a maximum $\Sigma RQ_{Non-RTI}$ of 30.8, alongside a P_{mix} of 0.998 (99.79%) but is also much higher than the median value ($\Sigma RQ_{Non-RTI} = 7.4$). This discrepancy between maximum and median values potentially suggests the presence of localised contamination hotspots, predominantly from non-RTI compounds, rather than uniform contamination.

Effluent waters exhibited even greater risks, with maximum $\sum RQ_{RTI} > 167$ and median $\sum RQ_{RTI}$ values of 6.2. The resulting P_{mix} was 99.97%, suggesting near-certain detrimental effects due to combined ARVD contamination in these waters. Notably, risk contributions in effluent were dominated by RTIs, whereas non-RTIs posed greater risks in surface water.

Effluent also had probabilities > 0 for all the ARVD classes (P_{RTI} , $P_{Integrase}$, P_{Entry} , and $P_{Protease}$) unlike surface water, where integrase and entry inhibitor drug classes showed no predicted toxicity. Although median values were comparatively lower than the maximum values, they still represented a substantial risk for combined mixtures ($P_{mix} > 95\%$).

Table 4-4: CA-RA hybrid model results for the mixture risk assessment of antiretroviral drugs in South Africa waters.

	Surface water		Effluent water	
	Max	Median	Max	Median
RTIs (CA)				
$\sum RQ_{RTI}$	14.16	1.62	166.24	6.20
P_{RTI}	0.93	0.62	0.99	0.86
Non-RTIs (RA)				
$\sum RQ_{Non-RTI}$	30.79	7.41	18.72	1.99
$P_{Integrase}$	0	0	0.01	0.01
P_{Entry}	0	0	0.05	0.05
$P_{Protease}$	0.97	0.88	0.95	0.66
P_{mix}	1.0	0.96	1.0	0.96
$P_{mix} (\%)$	99.79	95.46	99.97	95.54

$\sum RQ$ = combined risk quotients of mixture; CA = concentration addition; non-RTI = non reverse transcriptase inhibitor; P_{Entry} = probabilities for entry inhibitors; $P_{Integrase}$ = probabilities for integrase inhibitors; and $P_{Protease}$ = probabilities for protease inhibitors; P_{RTI} = probabilities for reverse transcriptase inhibitors; RA = response addition; RTI = reverse transcriptase inhibitor.

4.3.4 Prioritisation index (P_{index})

Ranked P_{index} values (Table 4-5) showed LPV, EFV, and ZDV as the top three concerning ARVDs in surface water (P_{index} : 501–2 937), followed by TDF, RTV, and FTC. Despite a lower RQ_{max} compared to ABC, d4T displayed a 60% PNEC exceedance frequency, ranking it above ABC and NVP. The remaining compounds had no recorded instances of exceedance ($F = 0$; $P_{index} = 0$).

Table 4-5: Ranked prioritisation index (P_{index}) for selected antiretroviral drugs (ARVDs) in surface water.

Surface water					
ARVD	n	N	F (%)	RQ_{max}	$P_{\text{index}} > 0$
LVP	24	25	96	30.6	2936.7
EFV	36	40	90	8.4	751.2
ZDV	36	36	100	5.01	501.2
TDF	5	6	83	0.3	25.1
RTV	21	22	95	0.17	16.4
FTC	4	12	33	0.29	9.8
d4T	3	5	60	0.01	0.4
ABC	0	8	0	0.18	
NVP	0	4	0	0.05	
3TC	0	14	0	0.001	
ddl	0	2	0	2.6×10^{-4}	
DDC	0	2	0	1.5×10^{-5}	
$RQ \geq 1$ = high risk					
$1 > RQ \geq 0.1$ a medium risk					
$0.1 > RQ \geq 0.01$ = a low risk					
$0.01 > RQ > 0$ = <i>di minimis</i> risk					

F = frequency of exceedance; n = number of sites with concentrations > PNEC; N = total number of sites

Effluent P_{index} rankings (Table 4-6) yielded TDF, ZDV, EFV, and LPV as top-priority. ZDV had the highest prioritisation ($P_{\text{index}} = 11\,026$; $F = 90\%$), followed by EFV ($P_{\text{index}} = 3\,973$; $F = 100\%$), which remained ecologically significant despite a lower RQ_{max} value compared with ZDV. LPV also presented a consistently high ecological risk and frequency of exceedance (100%). Medium-risk compounds such as ABC and 3TC ranked lower than low-risk drugs (MVC, IDV, and RTV), likely due to a lack of exceedance ($F = 0\%$). Emtricitabine ranked highest of the medium-risk ARVDs due to its combined exceedance ($F = 40\%$) and RQ_{max} values.

Table 4-6: Ranked prioritisation index (P_{index}) for selected antiretroviral drugs (ARVDs) in effluent.

Effluent					
ARVD	n	N	F (%)	RQ _{max}	$P_{\text{index}} > 0$
ZDV	9	10	90	122.51	11026.3
EFV	35	35	100	39.74	3973.8
LVP	13	13	100	18.5	1846.8
TDF	1	2	50	2.78	139.1
FTC	4	10	40	0.7	27.3
MVC	1	1	100	0.05	5.2
IDV	3	3	100	0.05	4.9
RTV	6	10	60	0.07	3.9
ABC	0	8	0	0.32	
ATV	0	4	0	0.01	
DRV	0	4	0	0.07	
3TC	0	8	0	0.16	
NVP	0	31	0	0.05	
RAL	0	3	0	0.01	
RQ ≥ 1 = high risk					
1 > RQ ≥ 0.1 a medium risk					
0.1 > RQ ≥ 0.01 = a low risk					
0.01 > RQ > 0 = <i>di minimis</i> risk					

F = frequency of exceedance; n = number of sites with concentrations > PNEC; N = total number of sites.

4.4 Discussion

There is a significant research gap in the availability of chronic studies regarding ARVDs. Granted, the use of chronic toxicity tests with numerous species would be ideal for conducting ERAs for every ARVD, but it is not pragmatic to expect such an experimental endeavour due to time and financial constraints. Therefore, the assessment of intrinsic ecotoxicological properties of ARVDs should be accompanied by the usage of standardised acute tests in conjunction with protective AF. In the absence of chronic data, it is advantageous to compare the PNECs calculated for compounds with both acute and chronic data to reveal potential compounds of concern.

This approach could aid in prioritising established compounds for chronic testing when only acute data are readily available as well as in ascertaining those types of ARVDs for which acute toxicity data are suitable substitutes for chronic toxicity data. In addition to limiting the number of tested organisms, this is an important factor in the ethical framework.

The variability in the environmental presence of ARVDs in surface water is illustrated in Figure 4-3. This variability could be due to differences in ARVD persistence, local variations in usage, dilution, instrumentation sensitivity, methods of detection, and geographical factors. As previously shown by Ding *et al.* (2017), it is probable that the presence of ARVDs at lower trophic levels can be transferred to higher-level organisms, similar to other pharmaceutical drugs (such as propranolol or roxithromycin).

Aquatic invertebrates, such as daphnids, are at ecological risks from continuous exposure to ARVDs as environmental contaminants, particularly at sub-lethal doses (Mahaye and Musee, 2022). In turn, the disturbance in wellbeing of aquatic invertebrates may result in adverse consequences such as ecological function imbalances because daphnids are primary consumers, acting as a bridge between primary producers (algae) and tertiary consumers (fish). Predicting the potential hazards of ARVDs to human health will be greatly improved by first understanding their effects at lower trophic levels.

4.4.1 MEC and PEC

Measured environmental concentrations of ARVDs frequently exceeded PECs and regulatory thresholds underscoring the need for region-specific risk assessments (Cid *et al.*, 2021; Coetsier *et al.*, 2009; Ngwenya and Musee, 2023). Figure 4-5 and Table 4-3 showed that most MECs were far greater than their corresponding PECs, with eight ARVDs exceeding the EMEA limits ($PEC > 0.01 \mu\text{g/L}$). However, these inconsistencies between MEC and PEC were drug dependent and not uniform (e.g. ATV; Figure 4-5) due to limited data availability and potentially variable pharmacokinetics or environmental degradation rates that require deeper fate analysis.

This discrepancy, particularly pronounced for LPV and RTV, which were underestimated by up to four orders of magnitude, highlights the limitations of using foreign PEC models such as those from Sweden (FASS database) in the South African context. Due to the excessive ARVD treatment programme and consumption in South Africa, localised PEC calculation results are expected to be more comparable with far less underestimation than true measurements. The current findings align with prior studies indicating that certain second-line ARVDs, despite low PECs, pose considerable ecological risks due to high toxicity and low PNECs ($< 1 \mu\text{g/L}$) (Cid *et al.*, 2021; Ngwenya and Musee, 2023).

Persistent detection of compounds such as EFV, NVP, and ABC further supports concerns about their pseudo-persistent nature and environmental stability. EFV, RTV, and LPV also exhibit high bioaccumulation potential ($BCF \geq 500$ or $\text{LogD}_{\text{OW}} \geq 4$), while others such as ZDV and MVC are environmentally persistent despite lower bioaccumulation risks. Although most ARVDs appear immobile in aquatic systems, the overall lack of data on bioaccumulation, persistence, and mobility highlights a critical gap in current risk frameworks.

4.4.2 Risk quotients (RQs)

The ERA revealed that several ARVDs, especially EFV, LPV, RTV, and ZDV, posed significant risks to aquatic organisms across trophic levels, largely due to high MECs and low PNECs. The results of the current study depicted MECs being higher than PECs, with some compounds (e.g., LPV and NVP) showing high-risk even when PNECs were not remarkably low (Table 4-3). This denotes that actual exposure, not inherent toxicity, is the prevailing risk driver in high-use regions such as South Africa, a crucial point for future risk mitigation strategies. A low PNEC does not necessarily equate to a high risk, as risk is also a function of exposure parameters (Vestel *et al.*, 2016). The variability in PNECs (Table 4-3) also reflects differences in the AFs applied during acute and chronic toxicity calculations (Kumari and Kumar, 2022).

Comparable studies, such as that of Kuroda *et al.* (2021), predicted high ecological risks ($\text{RQ} > 1$) for LPV and RTV in river waters, primarily based on modelled concentrations (PEC) following increased antiviral usage during the COVID-19 pandemic. In contrast, the present study reports RQs derived from MECs, with several ARVDs (including LPV and EFV) exceeding thresholds for chronic risk in surface waters. These empirical findings therefore substantiate and, in some cases, exceed modelled risk estimates (section 4.4.1), highlighting that actual exposure in regions with sustained ARVD usage (e.g. for HIV treatment) may surpass predicted scenarios.

While effluent water was assumed to be more contaminated than surface water and generally had higher RQ values than surface water, the difference was not statistically significant ($p = 0.1$). This may be due to pseudo-persistence, environmental accumulation, and local variability in dilution, degradation, and bioavailability, which can elevate surface water concentrations.

For some ARVDs (e.g. LPV, ATV, RAL, and RTV) surface water exceeded effluent RQs, potential due to an overlooked environmental fate mechanism such as build-up to persistence (Mosekiemang *et al.* 2019; Wang *et al.*, 2023) (Figure 4-6 to Figure 4-8). Heterogeneity across species sensitivities and environmental conditions complicates definitive conclusions.

Statistically significant variability among trophic levels in the present study likely reflects species-specific sensitivity and mode-of-action differences between ARVDs (Vestel *et al.*, 2016). Algae and daphnids are typically more sensitive to oxidative stress and membrane-disrupting compounds, which explains their higher RQs for EFV and LPV (Almeida *et al.*, 2021).

Algae generally emerged as the most sensitive trophic level. The most critical ARVDs identified were EFV and LPV based on their high RQ values across multiple species. EFV presented the most alarming ecological risk, corroborated by its profound toxicity towards *R. subcapitata* and *C. dubia*, with an EC₅₀ value of 26 µg/L for *C. dubia* and an IC₅₀ value of 34 µg/L for *R. subcapitata*, both within environmentally relevant ranges (Almeida *et al.*, 2021). The findings of the current study for algae corroborated the results by Ngwenya *et al.* (2025), who also reported EFV (Kenya) and ZDV (Kenya, Zambia, and Germany) as exceeding the high-risk threshold. This convergence highlights the global persistence and ecotoxicological relevance of these ARVDs in surface waters.

LPV exhibited considerable concern, particularly affecting fish and daphnids, though its impact was comparatively less severe than EFV. Comparable RQ values for EFV and LPV were reported by Almeida *et al.* (2021). In contrast, drugs such as 3TC, MVC, and TDF exhibited lower RQ values and were deemed less concerning. These findings align with prior research (Almeida *et al.*, 2021; Ngwenya and Musee, 2023).

For fish, LPV and EFV showed the highest median RQs ($0.1 \leq \text{RQ} < 1$) in the present study, comparable to the EFV-driven high risks observed in Kenyan waters and the RTV-related high risk reported in a French study (Ngwenya *et al.*, 2025). These similarities suggest that although absolute concentrations vary by region, EFV, LPV, and RTV consistently emerge as key ecological risk drivers across climatic and geographical contexts.

According to EU Directive 93/67/EEC (EC, 2003), EFV is categorised as a very toxic substance, while 3TC and ZDV are classified as toxic. Ojemaye and Petrik (2019) reported the RQ values measured in selected commercially exploited, wild-caught small and medium-

sized pelagic fish species and their organs attained from Kalk Bay harbour, Cape Town, South Africa. However, 3TC was not detected in any of the studied fish species; therefore, the RQ value reported was zero, which is similar to that reported by Ngumba *et al.* (2016). ZDV displayed pronounced toxicity to daphnids but limited impact on fish, validating findings by Ngwenya and Musee (2023), who ranked ZDV's risk as highest for algae, followed by crustaceans and then fish.

Nevirapine, previously reported by Ngumba *et al.* (2016) as hazardous across three aquatic taxa in the Nairobi River Basin, exhibited negligible risk in the current study. RTV and LPV frequently demonstrated high RQs across trophic levels in studies by Kumari and Kumar (2022) and Escher *et al.* (2011) in hospital effluents, with RTV emerging as the major contributor, due to low EC_{50}/LC_{50} values (< 0.1 mg/L). As per the EU directive 93/67/EEC, 1996 (EC, 2003), drugs such as RTV with $EC_{50} < 0.1$ mg/L are considered very toxic to aquatic organisms (Kumari and Kumar, 2022).

Generally, RQ_{max} values remained below the medium-risk threshold, implying that the overall ecological risk of ARVDs in surface water is relatively low (Figure 4-9). However, continued monitoring is suggested, specifically for ARVDs with high RQ_{max} values exceeding the high-risk thresholds, to prevent concentrations from reaching ecologically harmful levels. Surface water regularly showed the highest RQ_{max} values across the majority of ARVDs, with numerous drugs, such as EFV, NFV, and NVP, surpassing the medium-risk threshold. This suggests that surface water ecosystems are more susceptible to ARVD contamination, perhaps due to higher drug concentrations or longer drug persistence in these environments.

Ngwenya and Musee (2023) reported higher RQ results for surface water in Eswatini (Swaziland) regions with limited WWTP access than in regions with access to WWTPs. EFV, LPV, and RTV were identified to present medium to high risk, similar to the findings in the current study. According to a European study by Aminot *et al.* (2018), the aerobic physicochemical characteristics of water affect the persistence of certain ARVDs. Aerobic physicochemical conditions have been found to influence ARVD degradation rates (Aminot *et al.*, 2018), with ABC, RTV, 3TC, and SQV displaying short half-lives (< 5 days), while ZDV and NVP displayed stability even under WWTP processes.

Similar to the findings in coastal Brazilian waters (Ramos *et al.*, 2025), EFV, LPV, and RTV emerged as the dominant risk drivers in the current study. However, the higher RQ values observed in South African surface waters likely reflect greater ARVD loading and lower dilution compared to marine systems, suggesting that exposure intensity, rather than intrinsic toxicity, is the prevailing determinant of ecological risk.

The higher RQ_{max} values LPV, RTV and ddl in surface water draw attention to the need for targeted or specialised monitoring and possible regulatory measures to mitigate the ecological impacts of ARVDs on algae, crustaceans, and fish (Figure 4-9). The variation in RQ_{max} values among the different sources further indicates the importance of considering the environmental context when assessing the risks and hazards posed by these drugs.

The chemical, physical, and biological factors of each localised ecosystem inherently vary; therefore, it is expected that the different water sources will exert different risks. LPV, EFV, TDF, and ZDV, displayed substantial risks to aquatic organisms. These ARVDs could bioaccumulate and cause chronic toxicity in aquatic settings as evidenced by their high RQ_{max} values in effluent samples. The results highlight the need for improved ARVD removal efficiencies and treatment technologies in WWTPs, particularly for these drugs.

There is a noticeable lack of ecotoxicity data at various trophic levels for all ARVDs. Species sensitivity distributions could refine RQ estimation, particularly for less studied ARVDs like DDC or ddl. Local ecotoxicity data is also limited. This raises the possibility that complete or true risk may be considerably underestimated due to their omission. However, making definite assessments would be premature in the absence of information regarding the extent to which these ARVDs may impact overall risk.

When compared internationally, variation in RQ magnitudes across studies (e.g., Ngwenya *et al.*, 2025; Kuroda *et al.*, 2021; Ramos *et al.*, 2025) may stem from regional pharmaceutical consumption patterns, treatment efficiencies, sampling periods, and analytical detection limits. The variability across studies underscores the necessity of region-specific ERA frameworks that incorporate local usage patterns, hydrological conditions, and treatment technologies to improve global comparability and contextual relevance of ARVD risk assessments.

4.4.3 Combined risk quotients (ΣRQ)

Numerous studies have assessed the ecotoxicological effects of ARVDs on aquatic organisms (Ojemaye and Petrik, 2019; Kumari and Kumar, 2022; Ngwenya and Musee, 2023). Nonetheless, a critical gap remains in understanding the synergistic effects of drug mixtures, as many compounds exhibit enhanced toxicity when combined (Li *et al.*, 2019). The ongoing debate over the scientific validity and pragmatic worth of predicting cumulative risks or the effects of mixtures emphasise the importance of this investigation.

We chose to investigate this issue because the combined effects of ARVDs with similar modes of action are expected to remain a subject of scientific and regulatory interest warranting further exploration. Conventional ERAs often fail to consider the combined effects of pharmaceuticals (Backhaus, 2016). However, various approaches exist to account for the heightened risk, such as the summation of RQs (Backhaus and Faust, 2012; Liu *et al.*, 2020; Rorije *et al.*, 2022).

Given the prevalence of ARVDs with overlapping mechanisms of action, the current study opted to employ a hybrid model offering a more nuanced and biologically pertinent evaluation of mixture toxicity. This hybrid model provided a more advanced evaluation of ARVD mixture risks by recognising that drugs targeting the same enzyme (e.g., RTIs) demonstrate additive effects, whereas drugs with different mechanisms operate independently (e.g., entry inhibitors). This differentiation permits more precise risk predictions, especially for intricate pharmaceutical mixtures. Furthermore, by integrating RA for ARVDs with varying (yet comparable) mechanisms of action, the hybrid model addresses potential non-additive interactions that a mere summation of RQs might neglect.

The results demonstrate considerable variability in the risks of combined ARVD contamination among aquatic environments, with surface water and effluent waters exhibiting the highest contributions to risk from both RTIs and non-RTIs based on the worst-case scenarios. The CA-RA model revealed that ARVDs mixtures pose probable ecological risks that often surpassed standard probability thresholds (Table 4-4).

Both the maximum and median P_{mix} values for effluent and surface water surpassed the threshold value, indicating a high probability of adverse ecological effects stemming from the combined mixture of ARVDs. Since $P_{mix} > 0.5$ was used in the current study to signify ecological risk, these results suggest that the mixture effects in both matrices are expected to

be of environmental concern, particularly at ARVD concentrations are highest. These high ΣRQ and P_{mix} values observed in effluent and surface waters support growing consensus that localised pharmaceutical hotspots, rather than median concentrations, are more ecologically consequential (Ramos *et al.*, 2025).

While CA models are effective for mixtures with similar modes of action, they may underestimate risks when synergistic interactions or complex dynamics are involved (Jang *et al.*, 2022). Conversely, RA models may overestimate the predicted effects for partial agonists because of the way they aggregate individual responses (Medlock Kakaley *et al.*, 2019). Despite these limitations, CA models are generally preferred for predicting combination toxicity, though they too may underestimate the potency of mixtures by an order of magnitude or more (Jang *et al.*, 2022).

The findings of the present study align with recent literature that also uses mechanism-based mixture risk assessment approaches (Ramos *et al.*, 2025). Similar to the CA-RA hybrid model used here, Ramos *et al.* (2025) have demonstrated that categorising ARVDs by their mode of action enables more accurate ecological risk prediction in Brazilian seawater. These parallels support the robustness of the hybrid model in describing both additive and independent effects of ARVD mixtures, strengthening its relevance for environmental monitoring.

Nonetheless, the combined effects of these chemicals may be greatly influenced by factors that are not generally taken into account in these models, such as transport, metabolism, excretion, secondary modes of action, compartmentalisation, and absorption kinetics (Cedergreen *et al.*, 2008). Subsequently, to properly reflect the complexities of drug interactions within combinations, existing risk assessment models need to be refined to enhance model accuracy and ecological relevance.

4.4.4 Prioritisation index

The P_{index} considers the PNEC, MEC, and FD as useful tools for ranking ARVDs according to their environmental impact. Unlike conventional RQ metrics, the P_{index} allows the identification of high-risk compounds that are both frequently detected and ecologically hazardous, while deprioritising those that are rare or present minimal risk. Several compounds had P_{index} scores above the threshold ($P_{index} > 1$), warranting further investigation (Liu *et al.*, 2020). In surface water, ARVDs should be prioritised in accordance with the following order of concern: LPV > EFV > ZDV > TDF > RTV > FTC > d4T. Whereas in effluent, the prioritisation differs slightly, with ARVDs ranked as follows: ZDV > EFV > LPV > TDF > FTC > MVC > IDV > RTV.

The ranked P_{index} results show clear prioritisation patterns, with LPV, EFV, and ZDV emerging as the highest ecological risks across both water sources. These drugs represent concentrations potentially harmful to non-target organisms, with RQ_{max} values > 1 and P_{index} values $>> 0$. Several ARVDs showed minimal or no detectable environmental risk as observed for P_{index} of zero. Risk mitigation and environmental monitoring should therefore primarily focus on ZDV, EFV, and LPV due to their anticipated higher environmental impact.

Studies corroborate the significant ecological threats posed by LPV, EFV, and ZDV. There is growing apprehension over psychoactive drugs since they are designed to alter human behaviour and have adverse consequences that could also impact fitness-related traits in free-living animals (Arnold *et al.*, 2014). Given the psychoactive traits of EFV, this finding is particularly concerning for aquatic organisms.

Comprehending pharmacokinetic-pharmacodynamic relationships and resistance profiles are critical for optimising ARVD therapy (Hoetelmans, 2001). Approaches to improve treatment outcomes include "protease inhibitor boosting". The boosting of protease inhibitors like LPV with low-dose RTV to enhance pharmacokinetic profiles may also contribute to environmental risks since intricate drug interactions in multi-drug regimens can raise toxicity risks (Hoetelmans, 2001; Back *et al.*, 2000).

The high ecological risk posed by LPV and RTV is consistent with previous studies (Escher *et al.*, 2011; Kuroda *et al.*, 2021). High RQ values in effluent-receiving water have also been reported for the metabolites of LPV, as well as a medium risk for the parent compound and metabolites of RTV in surface water (Kuroda *et al.*, 2021). It is noteworthy that ZDV was ranked first in wastewater, yet only third in surface water.

This discrepancy may be attributed to the different behaviours and degradation rates of ZDV in various water bodies (Table 4-3). Natural attenuation mechanisms such as dilution, biodegradation, and adsorption onto sediments are likely to reduce ZDV concentrations in surface water more effectively than do other ARVDs like EFV and LPV (Wang *et al.*, 2023). Therefore, ZDV poses a high risk in wastewater, but its impact diminishes in more natural water systems.

The dilution of surface water contributes to reducing the concentrations of ARVDs, especially in areas where the influx of water lessens the input from contaminated sources (see section 3.4.2). In contrast, wastewater effluent exhibits higher ARVD concentrations and subsequent

environmental risk because the dilution is minimal, and the presence of organic matter may increase the persistence of certain drugs. The variability in ARVD behaviour across different aquatic environments emphasise the significance of region-specific environmental assessments and emphasises the complex dynamics that influence ARVD risk levels in different contexts. Regional differences in WWTP practices and the prevalence of rural and urban untreated sewage discharges in the different study areas could also explain the discrepancies observed. Collectively, these results reinforce the value of using composite indices (P_{index}) for more accurate prioritisation of ARVD contaminants.

4.4.5 Limitations and assumptions

The current study presents certain limitations and assumptions that should be considered when interpreting the results.

- The scarcity of measured chronic toxicity data for ARVDs in PNEC calculations led to reliance on ECOSAR predictions in some cases, which introduced model-based uncertainties. These QSAR-derived estimates may not always accurately reflect real-world conditions or species-specific responses.
- The hybrid model has limitations: it assumes non-RTI drugs act independently, which may not be true due to biological systems where indirect interactions can occur. It also depends on precise PNECs and MECs, often unavailable for all ARVDs or environments. Converting RQs to effect probabilities has the concern of oversimplifying complex dose-response relationships in mixtures and should be reflected.
- The P_{index} calculation relies on the maximum concentration observed at any sampling site. This approach can overestimate risk if the maximum concentration is an outlier or not representative of typical environmental levels. It may bias the prioritisation towards chemicals with sporadic high values rather than those with consistent risks.
- Although the application P_{index} is broadly acknowledged, it fails to consider the temporal length or frequency of sampling. Consequently, transient observations of contamination levels may fail to accurately represent prolonged trends or seasonal variations in ARVD concentrations, potentially resulting in misguided risk prioritisation.
- This study relied on experimental ecotoxicological data from the literature on most ARVDs. However, when multiple data points existed for the same species and compound, the lowest toxicity value was used to ensure a worst-case scenario approach. Although this is a conservative approach, it might not always represent average or likely real-world conditions, which could affect the approximated risk generated.

4.5 Conclusions

In conclusion, the study stresses the serious environmental risks presented by ARVDs in aquatic ecosystems, with emphasis on the high bioaccumulation and toxicity potential of drugs such as EFV, LPV, and ZDV. Given the elevated risk of MECs of ARVDs in surface water and effluent, WWTPs show inefficiencies in removing ARVDs, suggesting a need for improved ecological risk assessments and monitoring, particularly for drugs that persist or bioaccumulate. The hybrid model used in the current study, which integrates CA for drugs sharing a common target and RA for those acting independently, provides an enhanced and mechanistically clarified approach to predicting the combined mixture effects of ARVDs. The ranked P_{index} results in the present study clearly indicated that LPV, ZDV, and EFV pose the greatest possibility of ecological risk amongst the selected ARVDs for surface and effluent water sources in South Africa.

At this stage, the full scientific comprehension of ecosystem ramifications due to single compounds at low concentrations still appears to be constrained. Ultimately, the environmental regulation of ARVDs (and other pharmaceuticals) should be guided by a transparent, inclusive decision-making process where the benefits and risks are cautiously balanced. Moreover, regulations should not only reflect the pertinent scientific evidence but also acknowledge the inherent uncertainties and knowledge gaps associated with ARVD research. An adaptive regulatory framework is required to account for new information such as prioritisation results or changing circumstances over time. This safeguards that decisions are made sensibly while also being open to revision as our knowledge progresses. In essence, this approach should promote informed, balanced decision-making that evolves with societal input and scientific progress.

4.6 Recommendations

By addressing these recommendations, future studies can better reflect real-world conditions and provide more actionable insights for environmental risk management of ARVDs.

- Chronic toxicity tests for ARVDs using representative local aquatic species should be conducted to obtain site-specific ecotoxicological data.
- It is recommended that future studies apply probabilistic models, such as Bayesian inference, to estimate concentrations below the LOD more realistically.
- Dynamic mixture toxicity studies are required. Experimental studies should be conducted to validate additive and independent action assumptions for both RTIs and non-RTIs.
- It is recommended that the hybrid model be refined by incorporating guidance from real-world data and controlled mixture experiments to capture non-linear dose-response relationships.

Future studies should include temporal monitoring programs. Long-term monitoring at regular intervals should be performed to provide a better understanding of average exposure levels and to accurately reflect maximum and median concentrations. A weighting factor that reflects the frequency and duration of contamination events can be used to refine the P_{index} .

4.7 References

- Adeola, A.O. and Forbes, P.B. 2022. Antiretroviral drugs in African surface waters: prevalence, analysis, and potential remediation. *Environmental Toxicology and Chemistry*, 41(2):247–262.
- Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M. and Barcelò, D. 2012. Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. *Science of the Total Environment*, 438:15–25.
- Almeida, L.C., Mattos, A.C., Dinamarco, C.P.G., Figueiredo, N.G. and Bila, D.M. 2021. Chronic toxicity and environmental risk assessment of antivirals in *Ceriodaphnia dubia* and *Raphidocelis subcapitata*. *Water Science and Technology*, 84(7):1623–1634.
- Arnold, K.E., Brown, A.R., Ankley, G.T. and Sumpter, J.P. 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1656):20130569.
- Back, D.J., Khoo, S.H., Maher, B. and Gibbons, S.E. 2000. Current uses and future hopes for clinical pharmacology in the management of HIV infection. *HIV Medicine*, 1(1):3–15.
- Backhaus, T. 2016. Environmental risk assessment of pharmaceutical mixtures: demands, gaps, and possible bridges. *The AAPS Journal*, 18(4):804–813.
- Backhaus, T. and Faust, M. 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environmental Science and Technology*, 46(5):2564–2573.
- Bakare, A.A., Akinseye, K.M., Aminu, B.A., Ofoegbu, F.C., Anifowose, S.O., Abruda, S., ... Adetona, O.M. 2020. Genetic and reproductive toxicity of lamivudine, tenofovir disoproxil fumarate, efavirenz and their combination in the bone marrow and testicular cells of male mice. *Annals of Science and Technology*, 5(1):1–10.
- Cavalcante, G.I.T., Chaves Filho, A.J.M., Linhares, M.I., de Carvalho Lima, C.N., Venâncio, E.T., Rios, E.R.V., ... de França Fonteles, M.M. 2017. HIV antiretroviral drug Efavirenz

induces anxiety-like and depression-like behavior in rats: evaluation of neurotransmitter alterations in the striatum. *European Journal of Pharmacology*, 799:7–15.

Cedergreen, N., Christensen, A.M., Kamper, A., Kudsk, P., Mathiassen, S.K., Streibig, J.C. and Sørensen, H. 2008. A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. *Environmental Toxicology and Chemistry*, 27(7):1621–1632.

Cid, R.S., Roveri, V., Vidal, D.G., Dinis, M.A.P., Cortez, F.S., Salgueiro, F.R., Toma, W., Cesar, A. and Guimarães, L.L. 2021. Toxicity of antiretrovirals on the Sea Urchin *Echinometra lucunter* and its predicted environmental concentration in seawater from Santos Bay (Brazilian Coastal Zone). *Resources*, 10(11):114.

Coetsier, C.M., Spinelli, S., Lin, L., Roig, B. and Touraud, E. 2009. Discharge of pharmaceutical products (PPs) through a conventional biological sewage treatment plant: MECs vs PECs? *Environment International*, 35(5):787–792.

Costa, B. and Vale, N. 2022. Efavirenz: history, development and future. *Biomolecules*, 13(1):88.

Cvetkovic, R.S. and Goa, K.L. 2003. Lopinavir/Ritonavir. *Drugs*, 63(8):769–802.
<https://doi.org/10.2165/00003495-200363080-00004>

Czech, B., Krzyszczak, A., Boguszevska-Czubara, A., Opielak, G., Joško, I. and Hojamberdiev, M. 2022. Revealing the toxicity of lopinavir-and ritonavir-containing water and wastewater treated by photo-induced processes to *Danio rerio* and *Allivibrio fischeri*. *Science of the Total Environment*, 824:153967.

De Clercq, E. 2005. Emerging anti-HIV drugs. *Expert Opinion on Emerging Drugs*, 10(2):241–274.

De Zwart, D. and Posthuma, L. 2005. Complex mixture toxicity for single and multiple species: proposed methodologies. *Environmental Toxicology and Chemistry*, 24(10), 2665–2676.

Desai, C. 2016. *Meyler's Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions*. 16th ed. Elsevier: Amsterdam.

Desbiolles, F., Malleret, L., Tiliacos, C., Wong-Wah-Chung, P. and Laffont-Schwob, I. 2018. Occurrence and ecotoxicological assessment of pharmaceuticals: is there a risk for the Mediterranean aquatic environment? *Science of the Total Environment*, 639:1334–1348.

Díaz-Cruz, M.S. and Barceló, D. 2008. Trace organic chemicals contamination in ground water recharge. *Chemosphere*, 72(3):333–342.

Ding, H., Wu, Y., Zhang, W., Zhong, J., Lou, Q., Yang, P. and Fang, Y. 2017. Occurrence, distribution, and risk assessment of antibiotics in the surface water of Poyang Lake, the largest freshwater lake in China. *Chemosphere*, 184:137–147.

EC (European Commission). 2003. Council Directive 67/548/EEC on the approximation of laws, regulations, and administrative provisions relating to the classification, packaging, and labelling of dangerous substances. *Official Journal of the European Communities*, L196, 16.8.1967:1–98

ECHA (European Chemicals Agency). 2008. *Guidance on information requirements and chemical safety assessment. Chapter R8: Characterisation of dose (concentration)–response for human health*. Guidance for the implementation of REACH. [Online] https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf Date of access: 7 Apr. 2023.

ECHA (European Chemicals Agency). 2023. *Information on chemicals*. [Online] Available at: <https://echa.europa.eu/information-on-chemicals> Date of access: 17 Apr. 2023.

Edwards, Z., Ingold, C.J. and Azmat, C.E. 2023. Zidovudine. StatPearls Publishing, Treasure Island (FL). <http://europepmc.org/books/NBK554419> Date of access: 6 Jun 2023.

Elias, A., Nelson, B., Oputiri, D. and Geoffrey, O.B.P. 2013. Antiretroviral toxicity and oxidative stress. *American Journal of Pharmacology and Toxicology*, 8(4):187.

EMA (European Medicines Agency). 2006. *Guideline on the Limits of Genotoxic Impurities*. EMA/CHMP/QWP/251344/2006. London: Committee for Medicinal Products for Human

Use (CPMP). [Online]. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-limits-genotoxic-impurities_en.pdf Date of access: 14 May 2024.

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

FASS. 2023. Environmental classification of pharmaceuticals. *Guidance for pharmaceutical companies*. 2012 V 2.0. [Online]. www.fass.se

FASS. 2025. FASS: The Swedish Medicines Information Portal. [online] Stockholm: LIF – The Research-Based Pharmaceutical Industry. [Online]. www.fass.se Date of access: 3 Jul. 2025.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxéaus, N., Giudice, R.L., Pollio, A. and Garric, J., 2004. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental toxicology and chemistry*, 23(5):1344–1354.

Gorgulho, R., Jacinto, R., Lopes, S.S., Pereira, S.A., Tranfield, E.M., Martins, G.G., ... Pintado, P. 2018. Usefulness of zebrafish larvae to evaluate drug-induced functional and morphological renal tubular alterations. *Archives of toxicology*, 92(1):411–423.

Grung, M., Källqvist, T., Sakshaug, S., Skurtveit, S. and Thomas, K.V. 2008. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. *Ecotoxicology and Environmental Safety*, 71(2):328–340.

Hoetelmans, R. 2001. Pharmacological exposure and the development of drug resistance in HIV. *Antiviral Therapy*, 6(2_suppl):37–47.

Horn, S., Vogt, T., Gerber, E., Vogt, B., Bouwman, H. and Pieters, R. 2022. HIV-antiretrovirals in river water from Gauteng, South Africa: Mixed messages of wastewater inflows as source. *Science of The Total Environment*, 806:150346.

Jang, S., Ford, L.C., Rusyn, I. and Chiu, W.A. 2022. Cumulative risk meets inter-individual variability: probabilistic concentration addition of complex mixture exposures in a population-based human in vitro model. *Toxics*, 10(10):549.

Kempf, D.J., Marsh, K.C., Denissen, J.F., McDonald, E., Vasavanonda, S., Flentge, C.A., ... Kong, X.P. 1995. ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans. *Proceedings of the National Academy of Sciences*, 92(7):2484–2488.

K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H. and Demeestere, K. 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere*, 149:238–244.

Kowlaser, S., Barnhoorn, I. and Wagenaar, I. 2022. Developmental abnormalities and growth patterns in juvenile *Oreochromis mossambicus* chronically exposed to efavirenz. *Emerging Contaminants*, 8:83–89.

Kumari, M. and Kumar, A. 2022. Environmental and human health risk assessment of mixture of Covid-19 treating pharmaceutical drugs in environmental waters. *Science of the Total Environment*, 812:152485.

Kuroda, K., Li, C., Dhangar, K. and Kumar, M. 2021. Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. *Science of the Total Environment*, 776:145740.

Lapworth, D.J., Baran, N., Stuart, M.E. and Ward, R.S. 2012. Emerging organic contaminants in groundwater: a review of sources, fate and occurrence. *Environmental Pollution*, 163:287–303.

Li, Y., Niu, X., Yao, C., Yang, W. and Lu, G. 2019. Distribution, removal, and risk assessment of pharmaceuticals and their metabolites in five sewage plants. *International Journal of Environmental Research and Public Health*, 16(23):4729.

Liaño, J.P. and Tenorio, C.H. 2008. Chemical characteristics, mechanism of action and antiviral activity of darunavir. *Infectious Diseases and Clinical Microbiology*, 26:3–9.

Liu, N., Jin, X., Yan, Z., Luo, Y., Feng, C., Fu, Z., ... Giesy, J.P. 2020. Occurrence and multiple-level ecological risk assessment of pharmaceuticals and personal care products (PPCPs) in two shallow lakes of China. *Environmental Sciences Europe*, 32:1–10.

Liu, Z. and Chen, X. 2016. Simple bioconjugate chemistry serves great clinical advances: albumin as a versatile platform for diagnosis and precision therapy. *Chemical Society Reviews*, 45(5):1432–1456.

Madikizela, L.M., Ncube, S. and Chimuka, L. 2020. Analysis, occurrence and removal of pharmaceuticals in African water resources: A current status. *Journal of Environmental Management*, 253:109741.

Mahaye, N. and Musee, N. 2022. Effects of two antiretroviral drugs on the crustacean *Daphnia magna* in river water. *Toxics*, 10(8):423.

Maltby, L., Blake, N., Brock, T.C. and Van den Brink, P.J. 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. *Environmental Toxicology and Chemistry*, 24(2):379–388.

Mason, T.B. and Darryl, C. 2015. Myopathies of systemic disease. In: *Neuromuscular Disorders of Infancy, Childhood, and Adolescence*. Academic Press.
<https://doi.org/10.1016/C2013-0-00077-1>

Medlock Kakaley, E., Cardon, M.C., Gray, L.E., Hartig, P.C. and Wilson, V.S. 2019. Generalized concentration addition model predicts glucocorticoid activity bioassay responses to environmentally detected receptor-ligand mixtures. *Toxicological Sciences*, 168(1):252–263.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H. and Halm-Lemeille, M.P. 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. *Environmental Science and Pollution Research*, 23:4992–5001.

Mosekiemang, T.T., Stander, M.A., and de Villiers, A. 2019. Simultaneous quantification of commonly prescribed antiretroviral drugs and their selected metabolites in aqueous

environmental samples by direct injection and solid phase extraction liquid chromatography - tandem mass spectrometry. *Chemosphere*, 220:983–992.

<https://doi.org/10.1016/j.chemosphere.2018.12.205>

Mouscadet, J.F. and Tchertanov, L. 2009. Raltegravir: molecular basis of its mechanism of action. *European Journal of Medical Research*, 14:1–12.

Nantaba, F., Wasswa, J., Kylin, H., Bouwman, H., Palm, W.U. and Kümmerer, K. 2024. Spatial trends and ecotoxic risk assessment of selected pharmaceuticals in sediments from Lake Victoria, Uganda, East Africa. *Science of The Total Environment*, 906:167348.

Ncube, S., Madikizela, L.M., Chimuka, L. and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. *Water Research*, 145:231–247.

Ngqwala, N.P. and Muchesa, P. 2020. Occurrence of pharmaceuticals in aquatic environments: A review and potential impacts in South Africa. *South African Journal of Science*, 116(7-8):1–7.

Ngumba, E., Gachanja, A. and Tuhkanen, T. 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of the Total Environment*, 539:206–213.

Ngwenya, P. 2022. *Ecological risk modelling of antiretroviral drugs in the aquatic environment*. Pretoria: University of Pretoria. (Dissertation – M.Sc.).

Ngwenya, P. and Musee, N. 2023. Modelling ecological risks of antiretroviral drugs in the environment. *Environmental Chemistry and Ecotoxicology*, 5:145–154.

Ngwenya, P., Tabana, L.S., Tichapondwa, S.M. and Chirwa, E.M. 2025. Occurrence, Ecotoxicity, and Photocatalytic Remediation of Antiretroviral Drugs in Global Surface Water Matrices. *Catalysts*, 15(4):381.

Nibamureke, U.M.C., Barnhoorn, I.E.J. and Wagenaar, G.M. 2019. Nevirapine in African surface waters induces liver histopathology in *Oreochromis mossambicus*: A laboratory exposure study. *African Journal of Aquatic Science*, 44(1):77–88.

Nugnes, R., Orlo, E., Russo, C., Lavorgna, M. and Isidori, M. 2024. Comprehensive eco-genotoxicity and environmental risk of common antiviral drugs in aquatic environments post-pandemic. *Journal of Hazardous Materials*, 480:135947.

OECD. Organisation for Economic Co-operation and Development. 1992a. *Test no. 301: Ready biodegradability*. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing. <https://doi.org/10.1787/9789264070349-en>

OECD. Organisation for Economic Co-operation and Development. 1992b. *Test no. 302B: Inherent Biodegradability: Zahn-Wellens/EVPA Test*. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing. <https://doi.org/10.1787/9789264070387-en>

OECD. Organisation for Economic Co-operation and Development. 2002. *Test no. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing. <https://doi.org/10.1787/9789264070523-en>

OECD. Organisation for Economic Co-operation and Development. 2009. *Test no. 302C: Inherent Biodegradability: Modified MITI Test (II)*. OECD Guidelines for the Testing of Chemicals, Section 3. Paris: OECD Publishing. <https://doi.org/10.1787/9789264070400-en>

Ojemaye, C.Y. and Petrik, L. 2019. Occurrences, levels and risk assessment studies of emerging pollutants (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish samples from Kalk Bay harbour, South Africa. *Environmental Pollution*, 252:562–572.

Omotola, E.O., Genthe, B., Ndlela, L. and Olatunji, O.S. 2021. Environmental risk characterization of an antiretroviral (ARV) lamivudine in ecosystems. *International Journal of Environmental Research and Public Health*, 18(16):8358.

Pai, V.B. and Nahata, M.C. 1999. Nelfinavir mesylate: a protease inhibitor. *Annals of Pharmacotherapy*, 33(3):325–339.

Paintsil, E. and Cheng, Y.C. 2019. Antiviral agents. In: Schmid *Encyclopedia of Microbiology*: 4th ed. Oxford: Academic Press, 176–187.

Pereira, M. and Vale, N. 2022. Saquinavir: From HIV to COVID-19 and cancer treatment. *Biomolecules*, 12(7):944.

Piscitelli, S.C., Kelly, G., Walker, R.E., Kovacs, J., Falloon, J., Davey Jr, R.T., ... Polis, M.A. 1999. A multiple drug interaction study of stavudine with agents for opportunistic infections in human immunodeficiency virus-infected patients. *Antimicrobial Agents and Chemotherapy*, 43(3):647–650.

Pollak, E.B., and Parmar, M. 2024. Indinavir. In StatPearls. StatPearls Publishing, LLC. Treasure Island, FL: StatPearls Publishing. [Online]
<https://www.ncbi.nlm.nih.gov/books/NBK535416/> Date of access: 14 Aug. 2023.

PubChem. 2023. National Centre for Biotechnology Information. PubChem Annotation Record for Maraviroc, Source: Hazardous Substances Data Bank (HSDB).
<https://pubchem.ncbi.nlm.nih.gov> Date of access: 3 Jan. 2023.

Ramana, L.N., Anand, A.R., Sethuraman, S. and Krishnan, U.M. 2014. Targeting strategies for delivery of anti-HIV drugs. *Journal of Controlled Release*, 192:271–283.

Ramos, L.T., Roveri, V., Toma, W., Kiyotani, R.B., de Assis Junior, W.R.A., Metropolo, A.P., Dos Santos, G.A., ... Guimarães, L.L. 2025. Predicted environmental concentration (PEC), environmental risk assessment (ERA) and prioritization of antiretroviral drugs (ARVs) in seawater from Guarujá (Brazilian coastal zone). *Marine Environmental Research*, 204:106964.

Reddy, K., Renuka, N., Kumari, S., Ratha, S.K., Moodley, B., Pillay, K. and Bux, F. 2023. Assessing the potential for nevirapine removal and its ecotoxicological effects on *Coelastrella tenuithea* and *Tetrademus obliquus* in aqueous environment. *Environmental Pollution*, 317:120736.

Rimayi, C., Odusanya, D., Weiss, J.M., de Boer, J., and Chimuka, L. 2018. Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa. *Science of the Total Environment*, 627:1008–1017.
<https://doi.org/10.1016/j.scitotenv.2018.01.263>

Robson, L., Barnhoorn, I.E.J. and Wagenaar, G.M. 2017. The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56:225–232.

Roche. 2023. Roche official website: Research and product information. Basel: F. Hoffmann-La Roche Ltd. A. [Online] <https://www.roche.com> Date of access: 13 Jun. 2023.

Rorije, E., Wassenaar, P.N., Slootweg, J., van Leeuwen, L., van Broekhuizen, F.A. and Posthuma, L. 2022. Characterization of ecotoxicological risks from unintentional mixture exposures calculated from European freshwater monitoring data: Forwarding prospective chemical risk management. *Science of the Total Environment*, 822:153385.

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Wilson, C.J. and Solomon, K.R. 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. *Regulatory Toxicology and Pharmacology*, 39(2):158–183.

Schauer, G.D., Huber, K.D., Leuba, S.H. and Sluis-Cremer, N. 2014. Mechanism of allosteric inhibition of HIV-1 reverse transcriptase revealed by single-molecule and ensemble fluorescence. *Nucleic Acids Research*, 42(18):11687–11696.

Scholar, E. 2007. HIV Non-Nucleoside Reverse Transcriptase Inhibitors. In: Enna, S.J., Bylund, D.B. (Eds.), *x Pharm: The Comprehensive Pharmacology Reference*. Amsterdam: Elsevier, 1–3.

Souza-Silva, G., Souza, C.R., de Jesus Pereira, C.A., dos Santos Lima, W., Mol, M.P.G. and Silveira, M.R. 2023. Toxicological evaluation of antiretroviral Tenofovir Disoproxil Fumarate on the mollusk *Biomphalaria glabrata* and its hemocytes. *Science of The Total Environment*, 891:164484.

Spilsbury, F., Kisieliu, V., Bester, K. and Backhaus, T. 2024. Ecotoxicological mixture risk assessment of 35 pharmaceuticals in wastewater effluents following post-treatment with ozone and/or granulated activated carbon. *Science of the Total Environment*, 906:167440.

Tran, N.H., Hoang, L., Nghiem, L.D., Nguyen, N.M.H., Ngo, H.H., Guo, W., ...Ta, T.T. 2019. Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Hanoi, Vietnam. *Science of the Total Environment*, 692:157–174.

USEPA (United States Environmental Protection Agency). 1986. *Guidelines for the health risk assessment of chemical mixtures*. Washington, DC: U.S. Environmental Protection Agency. [Online]. https://www.epa.gov/sites/default/files/2014-11/documents/chem_mix_1986.pdf Date of access: 16 May 2023.

USEPA (United States Environmental Protection Agency). 2023. ECOSAR (Ecological Structure–Activity Relationships) v2.2. Washington, DC: Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency. [Online]. <https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model> Date of access 15 May 2023.

Vivanti, A., Soheili, T.S., Cuccuini, W., Luce, S., Mandelbrot, L., Lechenadec, J., ... Blanche, S. 2015. Comparing genotoxic signatures in cord blood cells from neonates exposed in utero to zidovudine or tenofovir. *AIDS*, 29(11):1319–1324.

Vestel, J., Caldwell, D.J., Constantine, L., D'Aco, V.J., Davidson, T., Dolan, ... Straub, J.O. 2016. Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. *Environmental Toxicology and Chemistry*, 35(5):1201–1212.

Wang, R., Luo, J., Li, C., Chen, J. and Zhu, N. 2023. Antiviral drugs in wastewater are on the rise as emerging contaminants: A comprehensive review of spatiotemporal characteristics, removal technologies and environmental risks. *Journal of Hazardous Materials*, 457:131694.

Weston, M.D. 2007. Lamivudine. In: Enna, S.J., Bylund, D.B. (eds.), *xPharm: The Comprehensive Pharmacology Reference*. Amsterdam: Elsevier, 1–4.
<https://doi.org/10.1016/B978-008055232-3.62010-0>

Weston, M.D. and Ile, K. 2007. Zalcitabine. In: Enna, S.J., Bylund, D.B. (eds.), *xPharm: The Comprehensive Pharmacology Reference*. Amsterdam: Elsevier, 1–7.
<https://doi.org/10.1016/B978-008055232-3.62874-0>

Wood, R. 2008. Atazanavir: its role in HIV treatment. *Expert Rev Anti-Infect Ther* 6 (6):785–796. <https://doi.org/10.1586/14787210.6.6.785>

Zilber, D. and Messier, K. 2024. Reflected generalized concentration addition and Bayesian hierarchical models to improve chemical mixture prediction. *PLOS ONE*, 19(3):e0298687.

Zizioli, D., Ferretti, S., Tiecco, G., Mignani, L., Monti, E., Castelli, F., ... Zanella, I. 2023. Comparison of Efavirenz and Doravirine Developmental Toxicity in an Embryo Animal Model. *International Journal of Molecular Sciences*, 24(14):11664.

CHAPTER 5 HUMAN HEALTH HAZARD ASSESSMENT OF ANTIRETROVIRAL DRUGS IN SOUTH AFRICAN WATERS

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Abstract

The increasing detection of antiretroviral drugs (ARVDs) in South African water sources raises pressing concerns for human health, particularly among vulnerable populations. The current study presents a comprehensive human health hazard assessment (HHHA) of 20 ARVDs quantified in surface water, wastewater, and treated drinking water, focusing on ingestion as the primary exposure route. Wastewater was included to reflect realistic and worst-case exposure scenarios in South Africa, given frequent discharges of untreated or partially treated sewage into rivers used by informal settlements. Two hazard assessment (HA) approaches were employed: HA1, based on acceptable daily dose and reference dose (ADD/RfD), to capture worst-case scenarios; and HA2, using chronic daily intake and acceptable daily intake (CDI/ADI), to reveal broader regulatory thresholds. HA1 identified abacavir (ABC), efavirenz (EFV), lopinavir (LPV), and zidovudine (ZDV) as exceeding the hazard quotient (HQ) safety threshold ($HQ > 1$), with adults and pregnant women most affected, specifically in effluent and surface water. Statistically significant differences (Kruskal-Wallis, $p < 0.0001$) were observed among ARVDs and sub-populations in surface and wastewater, with high variability and uncertainty in wastewater. Despite advanced treatment, EFV, LPV, ritonavir, and zalcitabine had median $HQ > 1$ for adults in drinking water. EFV and LPV also presented hazards to pregnant women and children (99th percentile $HQ > 5$). Abacavir exhibited the highest toxic potential, while EFV and LPV revealed widespread concern across sources. Hazard index analysis showed cumulative hazard potentials well above thresholds, suggesting mixture effects. The reliability function analysis showed frequent exceedance ($> 70\%$) of non-carcinogenic thresholds, particularly for EFV, LPV, and ZDV, even in treated drinking water. Conversely, HA2 reported $HQ < 1$ across all scenarios, suggesting negligible hazard under standard exposure scenarios. This divergence highlights the importance of reference value selection and the value of dual-assessment frameworks: HA1 captured episodic, high-end

exposure concerns; while HA2 supports chronic, population-level protection under standard environmental conditions.

Keywords: antiretroviral drugs; hazard quotient; human health; hazard assessment; water sources.

5.1 Introduction

Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) continues to be a substantial public health challenge in South Africa, with one of the highest prevalence rates (13.9%) and highest total number of people living with HIV/AIDS (8.45 million) worldwide (South Africa Government, 2024). There are several routes through which pharmaceuticals such as antiretroviral drugs (ARVDs) are released into the environment, but the fundamental one is human or animal excretion following therapeutic use (Daughton, 2003; Sorell, 2016). Considerable amounts are excreted in primary—unmetabolised—form into raw sewage and wastewater treatment systems (Adeola and Forbes, 2022). Unfortunately, the conventional wastewater treatment processes only partially remove these drugs and some ARVDs even accumulate in wastewater treatment sludge (Sorell, 2016).

Both the ecotoxicological risk of ARVDs to aquatic organisms and their spatio-temporal distribution in surface, effluent, and influent waters across several South African catchments have been established (Ngwenya and Musee, 2023; Ripanda *et al.*, 2022). These findings raised concern for potential risks to human health, especially given that contaminated water bodies are used by vulnerable communities for drinking, recreation, and agriculture (Kunene and Mahlambi, 2023). Then again, recent findings by Bub *et al.* (2025) showed that, even on a continental scale, lack of systematic chemical monitoring leads to substantial underestimation of environmental risks. Less than 1% of potentially hazardous chemicals are actually monitored. Conventional and monotonous methods often cannot detect compounds at concentrations relevant to ecological and toxicological concern (Bub *et al.*, 2025).

In many areas of South Africa, including parts of Gauteng and the Hartbeespoort catchment for example, millions of litres of raw or partially treated sewage enter river systems daily due to ageing infrastructure, sewer overflows, and unregulated discharges (Adeola and Forbes, 2022; Webster and Iqani, 2024). Wastewaters, leachates from non-engineered landfills, on-site sanitation systems, shallow groundwater systems, and receiving surface waters are therefore suspected to be potential hotspot reservoirs of ARVDs (Adeola and Forbes, 2022).

In the particular context of South Africa, informal settlements and rural populations often rely directly on these contaminated water sources making a human health hazard assessment (HHHA) both timely and urgent (Mangesi and Mngeni, 2024; Ncube *et al.*, 2018).

Exposure pathways for ARVDs are primarily related to water activities, including drinking, food preparation, bathing, and recreational activities. Other exposure pathways may be through food if the water used for watering crops is contaminated with ARVDs, and the chemicals persist on the crops (Kunene and Mahlambi, 2023). ARVDs, while therapeutically beneficial, can pose unsolicited health risks when unintentionally ingested through environmental exposure. Continued use of these drugs may produce unexpected interactions with other drugs, disturb endocrine systems, cause adverse effects on the central and peripheral nervous systems, hepatotoxicity, nephrotoxicity, or lead to the emergence of drug resistance, to name a few. Vulnerable groups may also be more susceptible, including pregnant women, children, and people with underlying health conditions (Abers *et al.*, 2014; Ncube *et al.*, 2018; Verhelst *et al.*, 2002).

Historically, ARVD research has been focused on the safety and efficacy in treating HIV/AIDS. However, there is a general lack of extensive studies on the environmental fate, persistence, acute and chronic effects of these drugs at trace levels in water sources (Adeola and Forbes, 2022). Research into the presence of ARVDs is limited but increasing, and research gaps such as the cumulative effects of chronic low-dose exposure and interaction with other environmental contaminants, which could lead to additive or synergistic health effects (Ukić *et al.*, 2019) to name a few, have not yet been addressed. This lack of information reinforces the urgency of our investigation.

The current study builds on the previous findings by conducting a comprehensive HHHA of ARVDs detected in South African waters (Chapters 4 and 5), aimed at evaluating the potential non-carcinogenic hazards to different population groups. The assessment utilises two distinct approaches. The first approach is based on acceptable daily dose (ADD) and reference dose (RfD) estimation using therapeutic data. The second is the CDI/ADI approach which is based on chronic daily intake (CDI) and acceptable daily intake (ADI) thresholds. By integrating literature-based toxicological thresholds, exposure modelling, and mixture analysis, this study aims to: quantify the non-carcinogenic hazard potential of ARVDs in different water sources, evaluate the robustness and variability of hazard assessment (HA) tools, and provide evidence for more context-specific and precautionary approaches to water quality monitoring specifically for South Africa.

5.2 Materials and methods

The present study was approved by the NWU Ethics Committee (NWU-00002-23-A1). Health risk assessment (Table 5-1) can be considered either qualitatively or quantitatively to evaluate risks to human health (Zhang *et al.*, 2023). In the context of this chapter, the term HHHA is used deliberately, regardless of the application of mathematical approaches in risk assessment, such as hazard quotients (HQs). HQs were rather used as part of a semi-quantitative HA framework, allowing for screening-level evaluation of potential health effects. This conservative terminology aligns with established guidance in the literature, which advises that when data limitations, uncertainty, or resource constraints prevent a full probabilistic or dose–response-based analysis, the term hazard is more appropriate than risk to guide priority-setting and inform policy decisions (Coleman and Marks, 1999; Popov *et al.*, 2016; Zhang *et al.*, 2023).

Hazard classification and identification refer to the process of determining whether exposure to a particular stressor is expected to lead to an increased incidence of certain adverse health outcomes (USEPA, 2022). It involves recognising environmental contaminants (such as ARVDs) that may pose a health risk, quantifying their environmental concentrations, and evaluating their potential threat to human health. It also includes characterising the types of toxicity associated with the contaminants of concern and assessing the conditions under which these toxic effects may occur in exposed individuals (NRC, 1994).

The current study adopts this approach by identifying ARVDs of concern, evaluating the intrinsic potential of ARVDs to cause adverse human health effects under certain exposure scenarios. While HQs provide a numerical comparison between projected exposure and reference thresholds, they do not account for full probabilistic distributions or detailed exposure variability across populations (Zhang *et al.*, 2023).

The present study also focuses on single-point estimates for input parameters (deterministic modelling) rather than distributions, such as in probabilistic risk modelling due to data limitations. Therefore, in line with best practices, this assessment is positioned as a hazard assessment, rather than a full health risk assessment to accurately reflect the degree of analysis and associated uncertainty.

Table 5-1: Review of human health risk and hazard assessment definitions.

Definitions	Reference
Human health risk assessment (HHRA) is the process of evaluating the nature and potential severity of adverse health effects in people who may be exposed to chemical substances present in contaminated environmental sources, now or at some point in the future.	USEPA, 2025
HHRA is a structured, multi-phase process that involves four key steps. • Hazard identification: determining whether a substance has the potential to cause harmful health effects in any circumstance; • Hazard characterization: evaluating the probability and severity of adverse effects at different exposure levels; • Exposure assessment: assessing the extent of the substance individuals or populations are exposed to; • Risk characterisation: integrating the hazard characterisation and exposure information to assess the overall risk of adverse health outcomes, particularly for the most vulnerable populations.	Chartres <i>et al.</i> , 2019
HHRA involves a scientific assessment of the hazardous characteristics of environmental agents and the degree to which humans are exposed to those agents.	NRC, 1994
HHRA is a process designed to quantify or estimate the risk to a specific organism, system, or (sub)population by evaluating exposure to a particular agent, taking into account the agent's inherent properties, the characteristics of the target system, and any associated uncertainties.	WHO, 2008
Risk is an estimate of the effect of an adverse health outcome when exposed to a hazard.	Singh, 2015
A hazard is any natural or man-made substance, chemical, physical, or biological agent, that is capable of causing an adverse health outcome in certain circumstances.	Chartres <i>et al.</i> , 2019
Hazard analysis is the process that commences with the identification of a hazard or hazards and proceeds into an estimate of the severity of harm or damage that could result if the potential of an incident or exposure occurs.	ANSI/ASSP Z590.3-2011(R2016)
Hazard assessment is a process that commences with the identification of a hazard or hazards and proceeds into an approximation of the severity of harm or impairment that could result if the potential of an incident or exposure occurs. Hazard assessment identifies the potential for harm under specific conditions.	Popov <i>et al.</i> 2016

ANSI/ASSP = American National Standards Institute/American Society of Safety Professionals; USEPA = United States Environmental Protection Agency

Hazard identification is the first step in the assessment, which is then followed by exposure assessment, dose-response assessment, and risk characterisation (Gerba 2019; USEPA 2004). For the HA, the present study includes both acute and chronic exposure scenarios, ensuring that short-term and immediate risks are not disregarded. The process of HHRA for ARVDs was therefore systematically conducted following a structured framework that integrates multiple steps to quantify potential hazards (Figure 5-1). Table 5-2 contains a comparative summary of key parameters used between approaches.

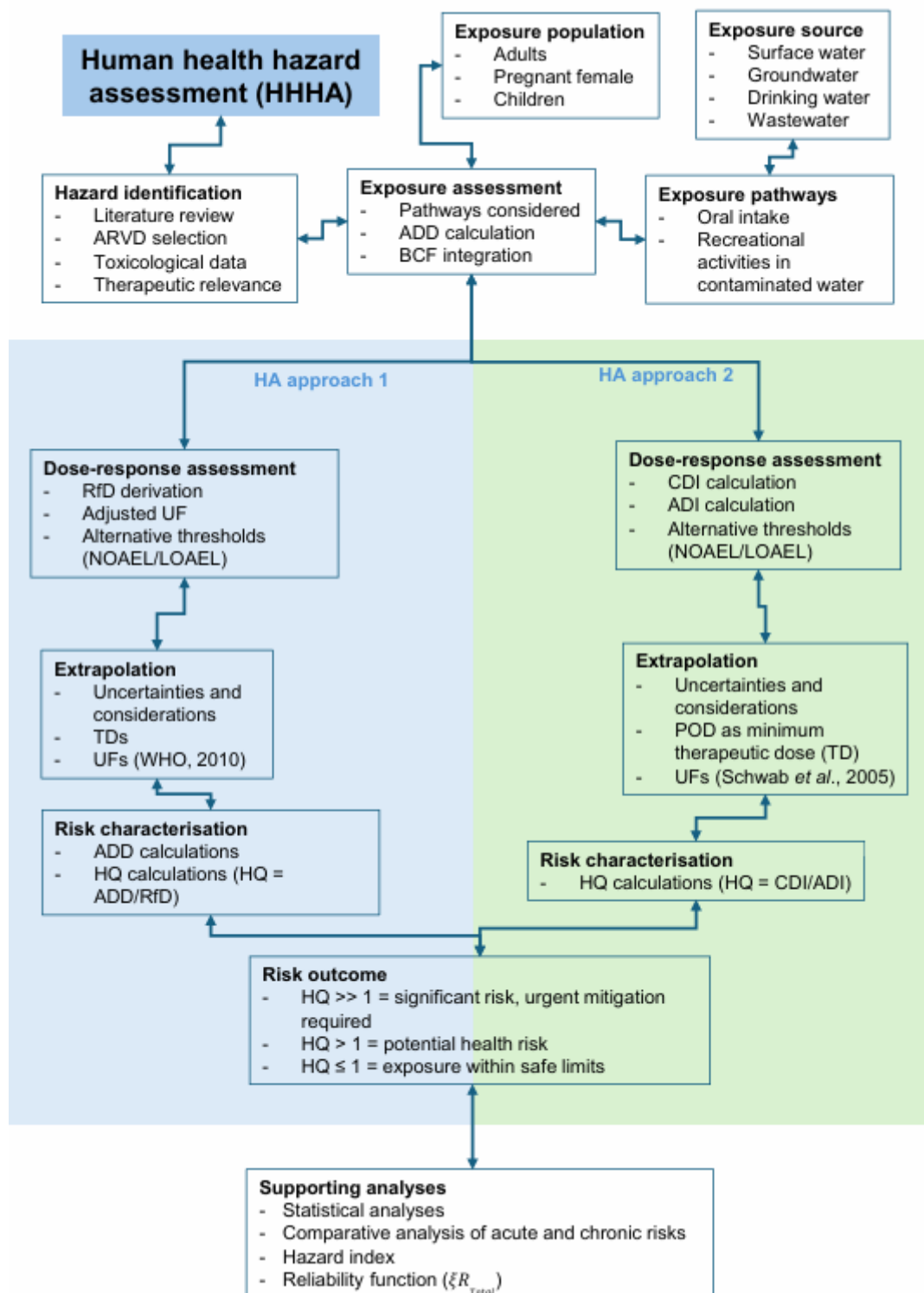


Figure 5-1: Process followed for the human health hazard assessment (HHHA) of antiretroviral drugs.

Table 5-2: Comparison of key parameters, assumptions, and applications in hazard assessment approaches used in the current study.

Component	Hazard assessment 1 (HA1)	Hazard assessment 2 (HA2)	Reference
Hazard identification	Comprehensive literature review; toxicological information compiled from peer-reviewed literature, databases and regulatory agencies (e.g., TOXNET ^b , PubChem ^b , USEPA, FDA).	Same as HA1	Annexures A – D
Sub-populations assessed	(i) children (< 18 years), (ii) adults (≥ 18 years) and (iii) pregnant adults (≥ 18 years)	Same as HA1	Table 5-4; USEPA, 2011
Water sources assessed	Surface-, effluent, influent, drinking water	Same as HA1	
Exposure type	Episodic, worst-case (acute)	Chronic, cumulative (long-term)	Sharma <i>et al.</i> , 2019
Bioconcentration	Not considered	Bioconcentration factors (BFC) applied to fish tissue	Table 5-5;
Dose metric	Average Daily Dose (ADD)	Chronic Daily Intake (CDI)	Table 5-4; Table 5-13; Kumar and Xagorarakis, 2010
Reference value	Reference Dose (RfD)	Acceptable Daily Intake (ADI)	Table 5-3; Table 5-7; Table 5-6; Cai <i>et al.</i> , 2019; Schwab <i>et al.</i> , 2005
Source of thresholds	Derived from therapeutic dose divided by uncertainty factors.	Derived from NOAEL/LOAEL or minimum therapeutic dose divided by composite uncertainty factors.	Table 5-7; Table 5-3; Chiu <i>et al.</i> , 2018; USEPA, 2005
Hazard quotient (HQ) interpretation^a			
HQ < 1	Low hazard potential.		
HQ = 1	Threshold of concern; no additional margin (safety buffer) for increased exposure.		
HQ > 1	Hazard potential; harm or adverse effects are possible. Need for mitigation strategies to reduce exposure and ensure safety.		
HQ >> 1	Significant hazard concern; substantial potential for adverse effects. Urgent mitigation strategies needed to reduce exposure and ensure safety.		

a = Kumar and Xagorarakis, 2010; b = <https://pubchem.ncbi.nlm.nih.gov/>; FDA = Food and Drug Administration; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level; UF = uncertainty factor; USEPA = United States Environmental Protection Agency

5.2.1 Justification for dual-method approach

The decision to use two distinct approaches was justified by the need to address scientific uncertainties and enhance the robustness of the HHHA for ARVDs in South African water systems. Monitoring gaps also mean that deterministic screening tools like HQs are even more essential as first-line hazard screening, given probabilistic modelling cannot proceed without reliable occurrence data, reflecting the concerns raised by Bub *et al.* (2025) for more comprehensive detection frameworks to support robust aquatic risk assessments.

This dual HA approach enables cross-validation of the results, emphasising consistencies or discrepancies in risk characterisation. This comparative perspective strengthens confidence in the results and identifies any limitations inherent to a single method. Environmental exposure to ARVDs involves many uncertainties related to their distribution, degradation, and bioaccumulation, to name a few. Using two approaches eases the limitations of each method by providing complementary insights and reducing the overall uncertainty in the assessment.

5.2.1.1 Absence of toxicological data for emerging contaminants

A cardinal challenge in HA of pharmaceutical pollutants, particularly in low- and middle-income countries, is the limited availability of chronic toxicity data and official health-based guideline values. For many ARVDs, no regulatory RfDs or ADIs exist, and toxicological data are often fragmented or derived from therapeutic contexts (Schwab *et al.*, 2005; Bull, 2011). This creates uncertainty in hazard characterisation and assessment (Kumar *et al.*, 2010). Using two methods allows for hazard estimation under different data constraints, leveraging both regulatory and pharmacological data sources where needed.

5.2.1.2 Cross-validation and sensitivity checking

Employing both methods allows for internal cross-validation, where consistency or divergence in outcomes can reveal how sensitive HQs are to underlying assumptions. If both methods yield similar hazard outcomes, it strengthens the reliability of the assessment. However, where results diverge, it highlights the importance of critical evaluation of method-specific assumptions, such as intake rates, uncertainty factors, and default safety thresholds (Dorne and Renwick, 2005).

5.2.1.3 Differences in point of departure selection

Each method used a different point of departure (POD). The RfD is often derived from no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) in toxicological studies, applying uncertainty factors to establish a daily exposure threshold unlikely to cause harm over a lifetime (Zhang *et al.*, 2023). The CDI/ADI method uses therapeutic dose (TD) fractions or alternative extrapolations. Uncertainties exist with the choice of endpoint and thus with the estimation of ADI values (Kumar *et al.*, 2010), as seen in studies by Schwab *et al.* (2005) and Webb *et al.* (2003).

These differences in POD source affect HQ outcomes. The RfD method may overestimate safety due to reliance on limited animal NOAELs and default uncertainty factors (Kumar *et al.*, 2010), which only sets thresholds rather than quantify risk probabilities and possibly omit human susceptibilities to variation (Chiu *et al.*, 2018). Contrarywise, the CDI/ADI method may yield more representative estimates in pharmaceutical-exposure contexts, yet results vary significantly depending on whether they were derived from therapeutic, ecological, or microbiome endpoints (Kumar *et al.*, 2010; Webb *et al.*, 2003). This emphasises their dependence on endpoint selection as well as assumptions about therapeutic relevance and human sensitivity (Kumar *et al.*, 2010; Kumar and Xagorarakis, 2010).

5.2.2 Justification for exposure route

Exposure assessment is based on exposure scenarios, which describe pathways, the origins of contaminants, and exposure routes during the process (WHO, 2004). In the South African context, it is particularly important to assess a broad range of exposure scenarios, including those involving wastewater, to ensure the full spectrum of conceivable human health hazards is captured. Media and institutional reports indicate that over five billion litres of raw sewage are generated daily, with approximately 4.2 billion litres discharged directly into rivers without treatment (Turton, 2022; Webster and Iqani, 2024). A formal inquiry conducted by the South African Human Rights Commission confirmed extensive raw sewage contamination in the Vaal River, a vital drinking water source, constituting a *prima facie* human rights violation affecting an estimated 19 million people (Sibanyoni, 2021).

Effluent from WWTPs represents a critical intermediate between raw sewage and receiving surface waters. In well-functioning systems, effluent is expected to pose minimal risk. However, in many South African municipalities, effluent is only partially treated or discharged untreated due to overloading, electricity outages, or mechanical failures (Webster and Iqani, 2024). Including effluent in the HA acknowledges the realistic exposure scenarios experienced

by downstream users of receiving water bodies. While influent water (raw, untreated sewage) is not typically a direct human exposure route under regulated conditions, it serves two fundamental roles in the current HHHA. Firstly, it represents a worst-case concentration scenario, offering insight into maximum environmental loads of ARVDs entering aquatic systems. Secondly, in unregulated settings, such as informal settlements or during system overflow incidents or failures from dysfunctional WWTPs, influent can enter natural water bodies directly, becoming a plausible exposure route for nearby rural communities.

Regarding exposures routes inhalation and dermal absorption of ARVDs are considered negligible due to their physicochemical properties. Antiretroviral drugs are characterised by low vapour pressure and limited volatility (Silva *et al.* 2017; Xie *et al.*, 2003), as well as large molecular mass and high hydrophilicity, resulting in minimal dermal permeability (Edagwa *et al.*, 2017; Palanisamy *et al.*, 2021; Pedreiro *et al.*, 2016).

These properties are consistent across all sub-populations, ensuring that the assumptions for excluding these pathways are valid regardless of age, health status, or activity level. Consequently, the current study appropriately focuses on ingestion pathways, which pose the most direct and potential measurable hazard for ARVD exposure. The ingestion of contaminated water during recreational activities as pathway is well-supported by peer-reviewed literature as a significant and realistic route of unintentional pharmaceutical exposure in aquatic environments (Duarte *et al.*, 2019; Ngunbane *et al.*, 2023).

5.2.3 Hazard assessment approach 1 (HA1)

5.2.3.1 Dose-response assessment

Reference doses represent estimations of daily exposure levels that are not likely to cause adverse health effects over a lifetime. Alternative thresholds from toxicological studies were considered when RfDs were not available (e.g. NOAEL or LOAEL). However, the majority of RfDs in the present study were determined based on TDs (Eq. (5-1) divided by uncertainty factors (UFs). While toxicity data are generally central to RfD estimations, in the absence of data, TDs are used (Bull, 2011). The provided standard TD (mg/day) was converted to a dose per unit body mass (mg/kg/day) by dividing it by the average body mass (Table 5-3).

$$\text{TD (mg/kg/day)} = \frac{\text{therapeutic dose (mg/day)}}{\text{average body mass (kg)}} \quad (5-1)$$

Since no published RfDs were available for ARVDs, the RfD values in the current study were calculated by dividing the TD (mg/kg/day) by total UF. Composite UFs are 1 000 and used by default to reduce the likelihood of real harm being underestimated (Chiu *et al.*, 2018; USEPA, 2005) (Table 5-3).

Table 5-3: Reference doses calculated for antiretrovirals (ARVDs).

ARVD		Therapeutic dose (mg/day)		Reference dose for non-carcinogenic effects mg/kg/d (safety factors included)	
		Adults	Children	Adults	Children
Abacavir	ABC	600	240–460*	0.009	0.024
Atazanavir	ATV	400	200–300*	0.006	0.013
Didanosine	ddl	400	200–240*	0.006	-
Dolutegravir	DTG	50	50	0.0008	0.003
Darunavir	DRV	800	350–800*	0.012	-
Efavirenz	EFV	600	200–600*	0.009	0.02
Emtricitabine	FTC	200	200*	0.003	-
Indinavir	IDV	1600	-	0.024	-
Lamivudine	3TC	300	300*	0.004	0.008
Lopinavir	LPV	800	300	0.012	0.03
Nelfinavir	NFV	2 250	500–750*		-
Nevirapine	NVP	400	300	0.006	-
Maraviroc	MVC	1200	100–600*	0.018	-
Raltegravir	RAL	800	800–1200*	0.012	-
Ritonavir	RTV	700	700–800	0.011	-
Saquinavir	SQV	1600	-	0.024	-
Stavudine	d4T	60	60*	0.001	-
Tenofovir	TDF	300	300*	0.005	0.008
Zalcitabine	DDC	2.25	-		-
Zidovudine	ZDV	300	240	0.005	0.024

* dose dependent on body mass of children. - not prescribed to children; no reference dose determined

5.2.3.2 Risk characterisation

Dose-response and exposure data were integrated to characterise the potential human health hazard posed by ARVDs in water. Hazard quotients were calculated by dividing ADD values by RfD values (to calculate the ratio of estimated exposure to the toxicity reference values). The ADD was calculated for adults and children via the following formula (Eq. (5-2). See Table 5-4.

$$ADD = \frac{C \times IR \times ED \times EF}{BM \times AT} \quad (5-2)$$

Where:

C = concentration of each ARVD detected in surface water (µg/L);

IR = ingestion rate;

ED = exposure duration;

EF = exposure frequency;

BM = average body mass of a South African; and

AT = average lifetime.

A HQ was determined (Eq. (5-3), and the exposed individuals were deemed safe if the HQ < 1 (Cai *et al.*, 2019).

$$HQ = ADD/RfD \quad (5-3)$$

Table 5-4: Parameters used to determine the average daily dose (ADD) according to population variables^{a,h}.

Abbreviation	Parameter	Unit	Adults	Pregnant adults	Children ^a
BM	Body mass ^b	Kg	66.5	80	31.8
LE	Life expectancy ^d	Years	59.3 (M) 64.6 (F)	64.6 (F)	62.5
AT	Average lifetime	LE x 365 d	22 922	23 689	22 813
EF	Exposure frequency	Days per year	365	365	365
ED	Exposure duration	Years	30	30	6
IR	Ingestion rate of water ^e	L/d	1.41	1.41	0.48
DWI	Drinking water intake ^e	mL/d	7.17	7.17	6.43
AWI	Accidental water ingestion ^{f, g}	mL/d	100	100	100
FC	Fish consumption ^e	g/d	20.1	20.1	20.1

a Health Canada (2004); b Puoane *et al.* 2002; c Rasmussen *et al.* 2009; d Stats SA, 2022; e USEPA, 2011; f Donovan *et al.*, 2008; g Wong *et al.*, 2009; h = USEPA (2004); F = female; M = male.

5.2.4 Hazard assessment approach 2 (HA2)

5.2.4.1 Bioconcentration factors (BFC)

Bioconcentration of ARVDs in fish tissue was estimated using bioconcentration factors (BFC). The BCF for each ARVD was estimated using the approach developed by Meylan *et al.* (1999), an approach used in other pharmaceutical studies (Cunningham *et al.*, 2009; Kumar and Xagorarakis, 2010; Schwab *et al.*, 2005). Bioconcentration factor values were calculated using Eq. (5-4 to (5-6, presented in Table 5-5.

For ionic compounds:

$$\text{Log } K_{ow} < 5; \log \text{ BCF} = 0.50 \quad (5-4)$$

For non-ionic compounds:

$$\text{Log } K_{ow} < 1; \log \text{ BCF} = 0.50 \quad (5-5)$$

$$\text{Log } K_{ow} 1-7; \log \text{ BCF} = 0.77 \log K_{ow} - 0.70 \quad (5-6)$$

For ionic ARVDs, a fixed BCF within a specific K_{ow} range was used to estimate the bioaccumulation potential. The $\log K_{ow}$ values, which specify the lipophilicity of the compounds, were observed to vary across the ARVDs, indicating different tendencies to partition into organic phases versus remaining in aqueous environments. High $\log K_{ow}$ values correspond to increased potential for bioaccumulation in organisms, which is further supported by the calculated $\log \text{ BCF}$ and BCF values (Table 5-5).

Table 5-5: Ranked bioconcentration factors (BCF) for selected antiretroviral drugs (ARVDs).

ARVD	Chemical structure	$\log K_{ow}$	$\log \text{ BCF (L/kg)}$	BCF(L/kg)
NFV	Non-ionic (Neutral)	5.46	3.5	3193.01
LPV	Non-ionic (Neutral)	5.42	3.47	2974.4
RTV	Non-ionic (Neutral)	5.03	3.17	1489.7
MVC	Non-ionic (Neutral)	4.42	2.7	505.13
EFV	Non-ionic (Neutral)	4.15	2.5	312.97
NVP	Non-ionic (Neutral)	3.89	2.3	197.38
IDV	Non-ionic (Neutral)	3.49	1.99	97.12
ATV	Non-ionic (Neutral)	2.9	1.53	34.12
DRV	Non-ionic (Neutral)	2.9	1.53	34.12
ABC	Non-ionic (Neutral)	1.62	0.55	3.53
SQV	Ionic	4.7	0.5	3.16
RAL	Non-ionic (Neutral)	-0.01	0.5	3.16
FTC	Non-ionic (Neutral)	-0.43	0.5	3.16
d4t	Non-ionic (Neutral)	-0.72	0.5	3.16
3TC	Non-ionic (Neutral)	-0.93	0.5	3.16
ddl	Non-ionic (Neutral)	-1.24	0.5	3.16
DDC	Ionic	-1.3	0.5	3.16
TDF	Non-ionic (Neutral)	-1.57	0.5	3.16
ZDV	Ionic	-7.05	0.5	3.16

BCF = bioconcentration factor; K_{ow} = octanol–water partition coefficient. The K_{ow} values were collected from PubChem, CompTox Chemical Dashboard and EpiSuite (USEPA, 2024a; 2024b).

High BCF values (> 1000 L/kg) imply a greater potential for bioaccumulation due to a compound's high lipophilicity, whereas low BCF values (< 10 L/kg) imply limited accumulation. NFV (3 193 L/kg), LPV (2 974 L/kg), and RTV (1 489 L/kg) exhibited the highest BCFs, indicating a greater potential for bioaccumulation and apprehension. In contrast, most other ARVDs, particularly those with low logK_{ow} values (< 0), such as ZDV, TDF, DDC, and ddl, showed very low BCFs (~3.16 L/kg), suggesting minimal risk of accumulation in fish tissue.

5.2.5 Dose response

5.2.5.1 Chronic daily intake (CDI)

Chronic daily intake of an ARVD during unintentional recreational activity and direct ingestion of water was assessed using Eq. (5-7) (Kumar and Xagorarakis, 2010). In this equation, IR denotes daily drinking water intake for direct ingestion and to the daily accidental ingestion of surface water. The BCF represents the bioconcentration factor of an ARVD in fish, and BW represents the body mass of the designated sub-population.

$$CDI = \frac{(IR + BCF \times FR) \times CW}{BW} \quad (5-7)$$

Where:

CDI = chronic daily intake (µg/kg/day);

BCF = bioconcentration factor (L/kg);

FR = daily consumption of fish (kg/day);

CW = ARVD concentration (µg/L);

BW = body weight (kg);

IR = daily water intake (L/day).

5.2.5.2 Acceptable daily intake (ADI)

The ADI is the identified amount of a chemical to which an individual can be exposed to daily over a lifetime without suffering any adverse health effects (Sorell, 2016). ADI calculations assumes that biological responses decrease to a threshold no-effect level below which no effects occur as the dose is reduced. While pharmaceuticals undergo risk–benefit analyses during development where some therapeutic risk is acceptable (FDA, 1999), yet accidental environmental exposure (such as via contaminated water) offers no therapeutic benefit. The ADI of an ARVD is the value of the daily intake determined not to cause any deleterious health effects from direct exposure in a population, including all sensitive sub-populations (Schwab *et al.*, 2005).

The ADIs were estimated using minimum TDs as surrogate POD, often considered equivalent to a LOAEL (Bercu *et al.*, 2008). To approximate a NOAEL from a LOAEL, a relevant UF was used as part of the ADI calculation (Table 5-6). Uncertainty factors are applied, as detailed in Eq. (5-8 to (5-10, to reduce the POD to a dosage where there is tenable certainty that no effect will occur (ATSDR, 1996; Dourson *et al.*, 1996; WHO, 1994). The ADI values were calculated using Eq. (5-8 to (5-10 (Cunningham *et al.*, 2009; Schwab *et al.*, 2005).

Where:

ADI = acceptable daily intake of ARVD (µg/kg/day);

POD = point-of-departure.

UF_{composite} = composite uncertainty factor which consists of UF₁, UF₂, UF₃, UF₄, and UF₅, and is calculated using Eq. (5-9).

$$ADI = \frac{1000 \times POD}{UF_{composite}} \quad (5-8)$$

$$UF_{composite} = \prod_{i=1}^5 UF_i \quad (5-9)$$

$$ADI = \frac{1000 \times POD}{UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5} \quad (5-10)$$

Values of individual UFs and their considerations are detailed in Table 5-7. These factors account for variations in individual susceptibility, extrapolation from clinical to general population, and potential long-term effects (Schwab *et al.*, 2005). Their use often results in ADIs several orders of magnitude lower than the thresholds associated with observable adverse effects (Sorell, 2016).

Risk assessment studies have employed different POD values based on either animal or human toxicity studies (USEPA, 2005). While conventionally, the NOAEL or LOAEL from toxicity studies are used as POD for ADI calculations, employing the minimum TD of an ARVD presents an alternative under specific conditions. Employing the TD provides a pragmatic approach to ensure that the calculated ADI aligns with established therapeutic margins while still allowing for therapeutic efficacy in human populations. Adeola and Forbes (2022), argued that data regarding relevant ecotoxicological endpoints are scarce with respect to biota and

human lethal dose or exposure. The authors further recommended that both the environmental concentrations and human TD should be considered to assess environmental exposure, biological effects, total bioavailable fraction, and dose-related response.

Accordingly, the present study uses the TD as a conservative, worst-case reference point while using measured environmental concentrations to reflect more realistic, population-level exposure scenarios. This twofold approach enables a contextualised assessment of both the upper-bound and actual hazard associated with ARVD exposure, acknowledging the limited availability of traditional toxicological endpoints and the capriciousness in environmental persistence and bioavailability of ARVDs.

Table 5-6: Considerations for estimation of acceptable daily intakes for different sub-populations.

ARVD	Therapeutic adults (mg/d)	Therapeutic child (mg/d)	POD _{adult} (mg/kg/day)	POD _{pregnant} (mg/kg/day)	POD _{child} (mg/kg/day)	UF ₁	UF ₂	UF ₃	UF ₄	UF ₅	ADI _{adult} (µg/kg/day)	ADI _{pregnant} (µg/kg/day)	ADI _{child} (µg/kg/day)
ABC	600	240–460*	9.02	7.5	7.55	3	3	1	3	3	111.4	92.6	93.2
ATV	300–400	200–300*	6.02	3.75	6.29	3	3	1	3	3	74.3	46.3	77.7
ddl	250–400	200–240*	3.76	3.13	6.29	3	1	1	3	3	139.2	115.7	232.9
DOL	50	50	0.75	0.63	1.57	1	3	1	3	3	27.85	23.15	58.2
DRV	800	350–800*	12.03	10	11.01	1	3	1	3	3	445.6	370.4	407.6
EFV	400–600	200–600*	6.02	5	6.29	1	3	1	1	3	668.3	555.6	698.8
FTC	200	200*	3.01	2.5	6.29	1	1	1	3	3	334.2	277.8	698.8
IDV	1600	-	24.06	20		3	3	3	3	3	99	82.3	0.0
3TC	300	300*	4.5	3.75	9.43	3	3	1	3	3	55.7	46.3	116.5
LPV	800	300	12.03	10	9.43	3	3	1	1	3	445.6	370.4	349.4
NFV	2250	500–750*	33.84	28.13	15.72	3	3	1	3	3	417.7	347.2	194.1
NVP	400	300	6.02	5	9.43	3	3	1	3	3	74.3	61.73	116.5
MVC	1200	100–600*	18.05	15	3.15	10	3	1	1	3	200.5	166.7	34.94
RAL	800	800–1200*	12.03	10	25.16	3	1	1	1	3	1336.7	1111	2795.3
RTV	700	700–800	10.53	8.75	22.01	3	1	1	3	3	389.9	324.1	815.3
SQV	1600	-	24.06	20		3	3	1	3	3	297	246.9	0
d4T	60	60*	0.9	0.75	1.89	3	3	1	3	10	3.34	2.8	6.99
TDF	300	300*	4.5	3.75	9.43	3	1	1	3	3	167.1	138.9	349.4
DDC	2.25	-	0.03	0.03		3	3	1	3	3	0.4	0.35	0.0
ZDV	600	240	9.02	7.5	7.55	3	3	1	3	3	111.4	92.6	93.2

ADI = acceptable daily intake; mcg = micrograms; POD = point of departure; UF = uncertainty factor; * = based on body mass.

Table 5-7: Extrapolation uncertainties and considerations for selection of uncertainty factors as adapted from Schwab *et al.* (2005).

Extrapolation uncertainties		Considerations for uncertainty factor selection	
LOAEL to NOAEL	UF ₁	10	Recommended when a NOAEL is not available.
		3	Recommended when the LOAEL is a therapeutic response, operative only in a disease state.
		1	Recommended when LOEL is associated with homeostatic response or an equivocal effect (LOEL = NOAEL).
Duration of exposure	UF ₂	10	Recommended when no relevant chronic data are available.
		3	Recommended when no chronic data are available, but PK or PD analyses suggest little persistence of compound or effect.
		1	Recommended when no chronic data are available, but PK and PD analyses suggest little persistence of compound and effect.
		1	Recommended when adequate chronic data are available.
Interspecies	UF ₃	10	Recommended when no human data are available unless considerations below apply.
		3	Recommended when ADME data are similar for multiple species, including humans or non-human primates.
		1	Used when derivation is based on human data.
Intra individual susceptibility	UF ₄	10	Recommended if NOAEL is from general adult population and/or animal study, with no multigenerational study of toxicity
		3	Recommended when effect is therapeutic and little difference between median and minimally effective dose.
		3	Recommend using adjusted LOEL, NOEL or therapeutic dose of a sensitive sub-population.
		1	Recommended when sufficient post-marketing data indicate absence of specific and particularly sensitive individuals or when using LOEL or NOEL for identified sensitive human population based on a large post-marketing study.
Data Quality	UF ₅	10, 3, or 1, or < than 1, recommended for professional judgement on quality of data available on a compound:	Critical studies used a small number of animals or groups (UF > 1).
			Results are poorly described or analysed (UF > 1).
			Data require route-to-route extrapolation to be relevant to the exposure condition (UF < 1 or > 1) depending on the relevance and relative sensitivity to the effect by alternate dosing routes).
			Important specialised studies not conducted (e.g., reproductive, teratogenicity, carcinogenicity) when positive genetic toxicity data is available (UF > 1).
			The absence of data is mitigated (UF < 1) by results on a compound of similar structure and responses.
			Non-standard study designs (UF > 1 or < 1 depending on the nature of the study).
			Esoteric or extreme effects (UF > 1 or < 1 depending on the nature of the study).
			NOEL is the highest dose tested (possibly a UF)

ADME = absorption, distribution, metabolism, and excretion; LOAEL = lowest observed adverse effect level; LOEL = lowest observed effect level; NOAEL = no observed adverse effect level; NOEL = no observed effect level;

pk = pharmacokinetic; PD = pharmacodynamic; UF = uncertainty factor.

5.2.6 Risk characterisation

An HQ was determined (Eq. (5-11)), and the exposed individuals were deemed safe if the $HQ < 1$ (Kumar and Xagorarakis, 2010).

$$HQ = CDI/ADI \quad (5-11)$$

Where:

HQ = Hazard quotient

ADI = acceptable daily intake ($\mu\text{g/kg/day}$)

CDI = Chronic daily intake ($\mu\text{g/kg/day}$)

5.2.7 Mixtures

In several studies, total non-carcinogenic metrics were calculated considering the risk associated with a chemical mixture (Guleria and Chakma, 2021; Henri *et al.*, 2015; Henri *et al.*, 2016). In a similar manner, the hazard index (HI) was calculated in the present study (Eq. (5-12)), representing the cumulative non-carcinogenic hazard posed by the chemical mixture of ARVDs (Dixit *et al.*, 2024).

$$HI_g(x) = \sum_{i=1}^n HQ_{i,g}(x) \quad (5-12)$$

Where:

$HI_g(x)$ = hazard index for population group (g) ($HI_{adults}(x)$, $HI_{pregnant}(x)$, or $HI_{children}(x)$) associated with a particular exposure scenario (x);

$HQ_{i,g}(x)$ = hazard quotient for compound (i) for group (g) in a specific exposure scenario (x).

n = total number of detected ARVDs in the mixture.

If $HI_g(x) > 1$, the cumulative exposure to the ARVD mixture exceeds the safe threshold for group (g), indicating a potential for non-carcinogenic adverse effects (Dixit *et al.*, 2024; Guleria and Chakma, 2021; Kaur *et al.*, 2020). If $HI_g(x) \leq 1$, the cumulative exposure is within acceptable limits for group (g), and no significant non-carcinogenic hazard is expected from the ARVD mixture in water source (x). The HI was calculated from the HA approach 1 HQs.

5.2.8 Reliability function

To gain insight into the reliability and safety of the ARVD concentrations in water samples for non-carcinogenic effects, the reliability function was calculated (5-13). The reliability function (ξR_{Total}) of the cumulative hazard due to chemical mixtures represents probability of exceedance and the percentage of times the exposure scenarios exceeded the safe limit. It

reflects how often exposure concentrations surpass the regulatory safety threshold ($HQ > 1$) as in well-documented within mixture toxicity and risk science literature (Guleria and Chakma, 2021; Henri *et al.*, 2015; Kaur *et al.*, 2020; Li *et al.*, 2024).

$$\xi R_{Total} = \text{Prob}(HQ > 1) \quad (5-13)$$

$$\xi R_{Total} = \frac{\text{Number of scenarios where } HQ > 1}{\text{Total number of scenarios}} \quad (5-14)$$

While the HQ is a deterministic metric that indicates hazard under specific input conditions, the reliability functions provide a probabilistic interpretation of hazard exceedance. It accounts for uncertainty and variability in parameters such as exposure concentrations and RfDs. This approach results in a probabilistic hazard exceedance profile or frequency-based exceedance probability that complements deterministic assessments. The reliability function in the present study was calculated with the HQs determined by HA1.

5.2.9 Statistical analysis

Normality of the data was assessed using D'Agostino's K-squared test ($P = 0.05$). Since several p-values were below the significance threshold, the data were considered to significantly deviate from a normal distribution. As such, the data were treated as non-parametric, and homogeneity of variance was not assumed. The Kruskal–Wallis test was applied to investigate differences between data groups ($p < 0.05$). Specific statistically significant differences among groups were further investigated by a post-hoc Dunn's multiple comparison test ($p < 0.05$).

5.3 Results

5.3.1 Hazard assessment (HA1)

5.3.1.1 Risk characterisation

General trends

The results of the HQ analysis for the various ARVDs across different sub-populations for surface-, drinking-, and wastewater are summarised in Table 5-8 to Table 5-11. The current study showed inter-drug and inter-group variability and some trends. Adults and pregnant females predominantly experienced greater hazard profiles, while children exhibited lower median HQs across ARVDs and water sources. Nonetheless, several drugs (e.g., EFV, LPV, ABC) still exceeded median $HQ > 1$ for children, denoting vulnerability under certain exposure

scenarios. ABC consistently exhibited the highest HQ values across surface and wastewater, especially among adults (e.g., HQ_{sw} median: 19.5; 99th percentile: 121), representing considerable non-carcinogenic hazard. Pregnant females followed closely in ABC exposure, with children exhibiting lower, but still concerning HQ values, mostly at the 99th percentile (e.g., HQ 99th percentiles 14–108). Efavirenz, FTC, TDF, and LPV, repeatedly demonstrated high HQs above the threshold in various sources, with notable toxicity concerns for adults and pregnant females. The results also presented a trend for certain ARVDs (e.g., NFV, MRV, IDV, RAL, and RTV) which generally had low HQ values across sub-populations and sources.

Wide 95% CI values for certain drugs (e.g., TDF, and DDC) showed high variability and uncertainty in the data. Significant inter-drug HQ differences were observed in surface water ($p < 0.0001$), effluent ($H = 42.57$, $p < 0.0001$), and influent ($H = 44.2$, $p < 0.0001$). Post-hoc analyses revealed significant pairwise divergences (e.g., ABC vs. NVP; EFV vs. ZDV).

Drugs were further grouped based on median HQ disparities using letter-based classifications (CLD), illustrating heterogeneity in hazard potential. These results suggest variability in potential health hazard levels among ARVDs in water sources, with some drugs posing relatively higher concern for certain sub-populations. In contrast, HQ_{dw} analysis yielded no statistically significant inter-group differences ($p = 0.15$), suggesting consistent drinking water exposure profiles across sub-populations.

Surface water

Nevirapine, FTC, and LPV also had high HQ_{sw} values representative of toxic concern. 3TC, RTV, ZDV, and d4T were revealed to be hazardous for adult and pregnant females but not to children. Conversely, NFV, ddI, and RTV had low HQ_{sw} values across all sub-populations, indicating less concern regarding hazard potential compared with ABC, for example, which indicated harm (Table 5-8). Lopinavir and EFV were notably elevated among pregnant females and adults, indicating heightened susceptibility within these groups. Dunn's post-hoc test revealed significant HQ disparities between select ARVD pairs (e.g., LPV vs. EFV, 3TC, NVP, and ZDV; as well as NVP vs. RTV).

Drinking water

Table 5-9 presents HQ_{dw} data that revealed variable hazard potential across compounds and groups. EFV exhibited the highest hazard profile in adults (median: 7.4; range: 2.8–32; 99th percentile: 32), indicating a substantial potential hazard for adults, particularly at higher

exposure levels. Lopinavir also showed notable concern (median: 4.05; max: 8.1), while ZDV, DDC, RTV, and NVP had much lower HQ_{dw} values, indicating lower hazard potential. In pregnant females, EFV maintained the highest median HQ_{dw} (median: 5.7; 95% CI: 40.5), implying increased vulnerability relative to other sub-populations. The HQ_{dw} for children was lower throughout all ARVDs, with EFV having the highest median HQ_{dw} of 0.7, but the 99th percentile still pointed to potential toxic concern. LPV and ddl had moderate hazard in both pregnant females and children, albeit lower than EFV.

Abacavir, NFV, and d4T had HQ_{dw} of 0 across all sub-populations, indicating no apparent hazard from these drugs in drinking water. Wide 95% CI values for ddl, EFV, LPV, and NVP denoting considerable variability in the data. Tests revealed no significant differences between mean HQ_{dw} among sub-population ($p = 0.15$).

Wastewater

In the HQ_{eff} analysis across various sub-populations (Table 5-10), 3TC had the greatest HQ_{eff} for adults. However, 3TC had a median $HQ < 1$, suggesting no immediate concern (median: 0.5; max: 357). Nevirapine and TDF demonstrated considerable irregularity (NVP: up to 370; TDF: up to 408), despite low medians (0.6–30) and restrained CIs (95% CI < 80). Post-hoc analysis revealed several divergences between ARVDs, as denoted by differing group letters (compact letter display). For example, ABC, EFV, and ZDV differed significantly. Indinavir, showed significantly lower HQ_{eff} values compared with e.g., ABC and EFV.

In the HQ_{inf} analysis across various sub-populations (Table 5-11), drugs of concern based on the HQ_{inf} results included EFV, DRV, and ZDV. Nevirapine and TDF had high variability similar to effluent water, indicating potential hazard at peak exposure levels. Post-hoc analysis revealed several statistically significant differences in HQ_{inf} values among the ARVDs. Abacavir had significantly higher HQ_{inf} values compared with NVP, RAL, and MRV. The remaining ARVDs did not significantly differ, suggesting relatively uniform hazard potential across compounds.

Table 5-8: Hazard quotients (HQs) calculated for various sub-populations in surface water (HQ_{sw}).

<i>ARVD</i>	<u><i>Adult</i></u>				<u><i>Pregnant</i></u>				<u><i>Child</i></u>			
	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P
<i>ABC</i>	0.0–121	19.5	2.3–81	121	0.0–93	14.5	1.6–61.9	93	0.0–14.0	2.1	0.2–9.3	14
<i>ddl</i>	0.1–0.1	0.1	0.1–0.1	0.1	0.1–0.1	0.1	0.1–0.1	0.1				
<i>EFV</i>	0.0–25.8	1.55	1.4–4.9	25.8	0.0–19.7	1.2	1–3.7	19.7	0.0–2.5	0.15	0.1–0.5	2.5
<i>FTC</i>	0.0–63.9	2.85	0.8–28.5	63.9	0.0–48.8	2.2	0.6–21.7	48.8	0.0–4.2	0.15	0.0–31.8	4.2
<i>3TC</i>	0.0–2.2	0.3	0.1–0.8	2.2	0.0–1.7	0.2	0.1–0.6	1.7	0.0–0.1	0	-0.01–0.04	0.1
<i>LPV</i>	0.0–56.8	13.5	9.7–23.7	56.8	0.0–43.3	10.3	7.4–18.1	43.3	0.0–9.9	2.3	1.7–4.1	9.9
<i>NFV</i>	0	0			0	0			0	0		
<i>NVP</i>	0.0–93.3	0.3	0.8–9.0	93.3	0.0–71.2	0.25	0.6–6.9	71.2	0.0–8.1	0	0.07–0.8	8.1
<i>RTV</i>	0.0–5.2	3.1	2–3.4	5.2	0.0–4.0	2.4	1.5–2.6	4	0.0–0.3	0.19	0.1–0.2	0.3
<i>d4t</i>	0.0–9.2	1.2	-1.9–8.0	9.2	0.0–7.1	0.9	-1.5–6.1	7.1	0.0–0.6	0.1	-0.1–0.5	0.6
<i>TDF</i>	0.0–6.4	0.35	-1.3–3.9	6.4	0.0–4.9	0.3	-1–3	4.9	0.0–2.1	0.1	-0.4–1.3	2.1
<i>DCC</i>	8.9–22.5	15.7	-71–102	22.5	6.8–17.2	12	-54–78.1	17.2				
<i>ZDV</i>	0.0–3.3	0.7	0.6–1.1	3.3	0.0–2.5	0.5	0.4–0.9	2.5	0.0–0.5	0.1	0.1–0.2	0.5

CI = confidence interval; sw = surface water; p = percentile; blank cells = no data.

Table 5-9: Hazard quotients (HQs) calculated for various sub-populations in drinking water (HQ_{dw}).

<i>ARVD</i>	<u><i>Adult</i></u>				<u><i>Pregnant</i></u>				<u><i>Child</i></u>			
	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P
<i>ABC</i>	0	0		0	0	0			0	0		
<i>ddl</i>	0.0–43.9	0	-23–46	43.9	0.0–33.5	0	-18–35	33.5	0.0–5.2	0	-2.8–5.4	5.2
<i>EFV</i>	2.8–31.9	7.4	-24.8–53	31.9	2.1–24.4	5.7	-19–40.5	24.4	0.3–3.1	0.7	-2.4–5.1	3.1
<i>LPV</i>	0.0–8.1	4.05	-47–55.5	8.1	0.0–6.2	3.1	-36–42.5	6.2	0.0–1.4	0.7	-8.2–9.6	1.4
<i>NFV</i>	0	0		0	0	0			0	0		
<i>NVP</i>	0.0–0.8	0.05	-0.06–0.4	0.8	0.0–0.6	0.05	-0.04–0.3	0.6	0.0–0.1	0	-0.02–0.04	0.1
<i>RTV</i>	2	2		2	0.7	0.7		0.7				
<i>d4T</i>	0	0			0	0						
<i>DDC</i>	2.7	2.7		2.7								
<i>ZDV</i>	0.0–1.4	0.5	0.06–0.9	1.4	0.0–1.1	0.4	0.05–0.7	1.1	0.0–0.2	0.1	0.02–0.15	0.2

CI = confidence interval; dw = drinking water; p = percentile; blank cells = no data.

Table 5-10: Hazard quotients (HQs) calculated for various sub-populations in effluent water (HQ_{eff}).

<i>ARVD</i>	<u><i>Adult</i></u>				<u><i>Pregnant</i></u>				<u><i>Child</i></u>			
	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P
<i>ABC</i>	0.68–211.6	111.9	44–158.5	211.6	0.52–161.5	85.4	34–121	161.5	0.09–28.8	15.3	6.1–21.6	28.8
<i>ATV</i>	0.14–6.2	0.55	-0.9–4	6.2	0.11–4.8	0.4	-0.6–3.1	4.8	0.01–0.7	0.06	-0.1–0.4	0.7
<i>DRV</i>	0.12–15.16	5.51	-5–18.6	15.2	0.09–11.6	4.2	-4.2–14.2	11.6	0.01–1.4	0.5	-0.4–1.7	1.4
<i>EFV</i>	0.61–122.8	10.7	13–35.9	122.8	0.47–93.7	8.17	10–27.36	93.7	0.06–12	1.05	1.3–3.5	12
<i>FTC</i>	0.39–148.7	1.9	-3.3–91.7	148.7	0.3–113.5	1.5	-2.5–70	113.5	0.03–9.7	0.1	-0.2–6	9.7
<i>IDV</i>	0.01–0.02	0.02	0.0–0.03	0.02	0.01–0.01	0.01	0.01–0.01	0.01				
<i>3TC</i>	0.02–356.7	0.54	-30–179.8	357	0.02–272.2	0.4	-23.6–137.2	272.2	0.0–23.24	0.04	-2–11.7	23.2
<i>LPV</i>	0.12–34.28	3.4	2.3–16.8	34.3	0.09–26.2	2.6	1.7–12.8	262	0.02–6	0.6	0.4–2.9	6
<i>NVP</i>	0.0–370.4	0.84	9.6–78	370	0.0–282.6	0.6	7.3–59.5	282.6	0.0–32.2	0.07	0.8–6.8	32.2
<i>MRV</i>	0.02	0.02		0.02	0.02	0.02		0.02	0.01	0.01		0.01
<i>RAL</i>	0.08–3.66	3.1	-2.5–7.01	3.7	0.06–2.8	2.4	-2–5.4	2.8	0.0–0.2	0.16	-0.1–0.4	0.19
<i>RTV</i>	0.05–1.99	1	0.6–1.6	2	0.04–1.5	0.8	0.5–1.2	1.5	0.0–0.1	0.07	0.03–0.1	0.1
<i>TDF</i>	0.0–59.5	29.76	-348–407.8	59.5	0.0–45.4	22.7	-265–311.2	45.4	0.0–3.9	1.9	-22.7–26.6	3.9
<i>ZDV</i>	0.0–79.6	0.76	-8.2–27.3	79.6	0.0–60.8	0.6	-6.3–20.8	60.8	0.0–13	0.1	-1.3–4.5	13

CI = confidence interval; eff = effluent; p = percentile; blank cells = no data.

Table 5-11: Hazard quotients (HQs) calculated for various sub-populations in influent water (HQ_{inf}).

ARVD	<i>Adult</i>				<i>Pregnant</i>				<i>Child</i>			
	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P	Range	Median	95% CI	99 th P
ABC	0.34–967.7	330.5	154–648	967.7	0.26–738.4	252.2	118–495	738.4	0.04–108.1	36.9	17.3–72.5	108.1
ATV	0.11–6.2	1.44	-2.2–6.8	6.24	0.09–4.8	1.1	-1.7–5.2	4.8	0.01–0.65	0.15	-0.2–0.7	0.65
DRV	0.06–38.3	7.99	-14.9–42.1	38.3	0.05–29.3	6.1	-11–32.1	29.3	0.01–3.5	0.7	-1.3–3.8	3.5
EFV	0.01–166.9	11.4	12.7–39.7	166.9	0.01–127.4	8.71	9.7–30.3	127.4	0.0–16.32	1.1	1.2–3.9	16.3
FTC	1.07–613.5	82.2	-17–346.2	613.5	0.82–468.1	62.71	-13–264.2	468.1	0.07–39.98	5.36	-1.1–22.6	39.98
IDV	0.12–0.26	0.13	-0.2–0.36	0.26	0.09–0.2	0.1	-0.02–0.28	0.2				
3TC	0.02–204.5	4.5	-20.4–82.4	204.5	0.02–156	3.45	-15.6–62.9	156	0.0–13.3	0.3	-1.3–5.4	13.3
LPV	1.07–21.7	4.7	2–12.4	21.7	0.82–16.6	3.61	1.5–9.44	16.6	0.19–3.8	0.8	0.3–2.15	3.77
NVP	0.01–465.4	1.2	4–106.7	465.4	0.01–355.2	0.91	3–81.4	355.2	0.0–40.45	0.1	0.35–9.8	40.5
MRV	0.05–0.19	0.05	-0.1–0.3	0.2	0.04–0.15	0.04	-0.08–0.23	0.15	0.01–0.04	0.01	-0.02–0.06	0.04
RAL	0.05–15.2	3.17	-5.7–16.5	15.16	0.04–11.6	2.42	-4.4–12.6	11.57	0.0–0.79	0.16	-0.3–0.9	0.8
RTV	0.06–3.3	1.5	0.5–2.2	3.26	0.05–2.5	1.15	0.4–1.66	2.49	0.0–0.2	0.1	0.03–0.1	0.2
SQV	0.08	0.08		0.08	0.06	0.06		0.06				
TDF	0.24–115.1	0.6	-28–105.7	115.1	0.18–87.8	0.45	-21.7–80.6	87.8	0.02–7.5	0.04	-1.86–6.9	7.5
ZDV	1.46–63	8.2	-13.6–49.7	63	1.125–48.1	6.26	-10.4–37.9	48.1	0.24–10.27	1.3	-2.2–8.1	10.3

CI = confidence interval; inf = effluent; p = percentile; blank cells = no data.

5.3.2 Hazard assessment (HA2)

5.3.2.1 POD and ADI

The estimation of ADIs and PODs for different sub-populations are presented in Table 5-12. Various uncertainty factors (UF1–UF5) were applied to account for interspecies differences, variability among individuals, and data limitations. The POD range from 0.03 (for DDC) to 33.8 mg/kg/day (for NFV) (Table 5-12). The calculated ADIs, varied for the different ARVDs and sub-populations (0.0–2 795 µg/kg/day), highlighting the need for a tailored HA approach to safeguard vulnerable groups. Indinavir, d4T, ATV, and DDC had very low ADI values, whereas drugs such as RAL, EFV, RTV, and LVP had high ADI values.

5.3.2.2 CDI

The highest CDI values in drinking water were observed for LPV and EFV, especially in children, who had CDIs of 50 µg/kg/day and 4.9 µg/kg/day, respectively (Table 5-13). RTV also had a high CDI for children, though much lower than LPV and EFV, implying a consistent inherent toxic potential to a specific vulnerable population. Other ARVDs like ABC, ddl, and ZDV had relatively low CDI values, presenting none to limited potential hazard.

Similar findings were observed in surface water, with LPV, EFV and RTV again showing the highest CDI values, particularly in children (120 µg/kg/day for LPV, 4.3 ng/kg/day for EFV, and 4.8 ng/kg/day for RTV). Nevirapine presented high CDI values in surface water as well, especially in children (6.5 µg/kg/day), highlighting toxicity concerns from this source (Table 5-13).

The effluent water samples showed the highest CDI values overall, particularly for LPV, EFV, and NVP. For instance, children's CDI for LPV had 72 µg/kg/day, 20 µg/kg/day for EFV, while NVP showed 26 µg/kg/day (Table 5-13). Ritonavir also showed high CDI values in effluent, but only for children. The influent water samples showed similar results with high CDI values for LPV, EFV, and NVP. Moderately high results ($CDI_{inf} > 1$) for RTV were seen across all sub-populations, and suspected hazard was documented for ABC, but only for children.

Table 5-12: Considerations for estimation of acceptable daily intakes for different sub-populations.

ARVD	Therapeutic adults (mg/d)	Therapeutic child (mg/d)	POD _{adult} (mg/kg/day)	POD _{pregnant} (mg/kg/day)	POD _{child} (mg/kg/day)	UF ₁	UF ₂	UF ₃	UF ₄	UF ₅	ADI _{adult} (µg/kg/day)	ADI _{pregnant} (µg/kg/day)	ADI _{child} (µg/kg/day)
ABC	600	240–460*	9.02	7.5	7.55	3	3	1	3	3	111.4	92.6	93.2
ATV	300–400	200–300*	6.02	3.75	6.29	3	3	1	3	3	74.3	46.3	77.7
ddl	250–400	200–240*	3.76	3.13	6.29	3	1	1	3	3	139.2	115.7	232.9
DOL	50	50	0.75	0.63	1.57	1	3	1	3	3	27.85	23.15	58.2
DRV	800	350–800*	12.03	10	11.01	1	3	1	3	3	445.6	370.4	407.6
EFV	400–600	200–600*	6.02	5	6.29	1	3	1	1	3	668.3	555.6	698.8
FTC	200	200*	3.01	2.5	6.29	1	1	1	3	3	334.2	277.8	698.8
IDV	1600	-	24.06	20		3	3	3	3	3	99	82.3	0.0
3TC	300	300*	4.5	3.75	9.43	3	3	1	3	3	55.7	46.3	116.5
LPV	800	300	12.03	10	9.43	3	3	1	1	3	445.6	370.4	349.4
NFV	2250	500–750*	33.84	28.13	15.72	3	3	1	3	3	417.7	347.2	194.1
NVP	400	300	6.02	5	9.43	3	3	1	3	3	74.3	61.73	116.5
MVC	1200	100–600*	18.05	15	3.15	10	3	1	1	3	200.5	166.7	34.94
RAL	800	800–1200*	12.03	10	25.16	3	1	1	1	3	1336.7	1111	2795.3
RTV	700	700–800	10.53	8.75	22.01	3	1	1	3	3	389.9	324.1	815.3
SQV	1600	-	24.06	20		3	3	1	3	3	297	246.9	0
d4T	60	60*	0.9	0.75	1.89	3	3	1	3	10	3.34	2.8	6.99
TDF	300	300*	4.5	3.75	9.43	3	1	1	3	3	167.1	138.9	349.4
DDC	2.25	-	0.03	0.03		3	3	1	3	3	0.4	0.35	0.0
ZDV	600	240	9.02	7.5	7.55	3	3	1	3	3	111.4	92.6	93.2

ADI = acceptable daily intake; mcg = micrograms; POD = point of departure; UF = uncertainty factor; * = based on body mass; empty cells = no data available

Table 5-13: Chronic daily intake (CDI) of antiretroviral drugs for various sub-populations as sampled from drinking water (_{dw}), surface water (_{sw}), influent (_{inf}) and effluent (_{eff}).

ARVD	CDI _{dw}			CDI _{sw}			CDI _{eff}			CDI _{inf}		
	Child	Adults	Pregnant	Child	Adults	Pregnant	Child	Adults	Pregnant	Child	Adults	Pregnant
ABC	0	0	0	0	0.11	0.09	0.4	0.19	0.16	1.82	0.87	0.72
ATV				0	0	0	0.08	0.04	0.03	0.08	0.04	0.03
ddl	0	0	0	0	0	0						
DRV							0.37	0.18	0.15	0.93	0.45	0.37
EFV	4.87	2.33	1.94	4.3	2.05	1.71	20.44	9.78	8.13	27.78	13.28	11.04
FTC				0.04	0.02	0.02	0.09	0.04	0.03	0.35	0.17	0.14
IDV							0.01	0	0	0.04	0.02	0.02
3TC				0.01	0.00	0.05	0.3	0.14	0.12	0.3	0.08	0.07
LPV	50.48	24.14	20.07	119.74	57.26	47.6	72.29	34.57	28.74	72.29	21.89	18.2
MVC						0	0.02	0.01	0.01	0.11	0.05	0.04
NFV	0.01	0	0	0.01	0	0						
NVP	0.06	0.03	0.02	6.53	3.12	2.59	25.92	12.39	10.3	32.57	15.57	12.94
RAL										0.04	0.02	0.01
RTV	1.84	0.88	0.73	4.84	2.32	1.93	1.84	0.88	0.73	3.02	1.44	1.2
d4T	0	0	0	0	0	0						
SQV										0	0	0
TDF				0.01	0	0	0.05	0.03	0.02	0.10	0.05	0.04
DDC	0	0	0	0	0	0						
ZDV	0.01	0	0	0.01	0	0	0.14	0.07	0.05	0.11	0.05	0.04

Values in bold = hazard potential (HQ > 1); empty cells = no data available.

5.3.2.3 Risk characterisation

None of the ARVDs had HQ values > 1 (Table 5-14), according to the assessment of drinking water, surface water, effluent, and influent for all three sub-populations. This suggests that for all sub-populations across the various water sources, the possible non-carcinogenic hazards linked to these ARVDs are below the threshold of concern. The highest observed HQ values were documented for LPV in surface water towards children (0.34), and NVP in effluent water also towards children (0.22).

5.3.3 Mixtures

The $HI(x)$ values for ARVD mixtures varied widely across water sources and sub-populations (Figure 5-2). Under HA1, all sources exceeded $HI(x) > 1$, signifying cumulative exposures far surpassed safety thresholds and pose non-carcinogenic concerns for exposed populations. The highest HI values were observed in influent ($HI_{adults} = 8707$, $HI_{pregnant} = 6644$, and $HI_{children} = 850$) and effluent samples ($HI_{adults} = 4401$, $HI_{pregnant} = 3359$, and $HI_{children} = 426$, respectively), indicating significant hazard exceedance well above the threshold.

Surface water had more moderate HI values compared with wastewater, particularly for children ($HI_{children} = 176$), while drinking water presented the lowest but still concerning HI values ($HI_{adults} = 104$). Children consistently displayed lower absolute HI values (HA1) when compared with adults, yet remain highly vulnerable due to their lower body weight and higher relative water intake. Under HA2, all $HI(x) < 1$ (Figure 5-2). No significant differences in $HI(x)$ values across water sources were found ($H = 3.04$, $p = 0.23$).

Table 5-14: Hazard quotients of drinking water (_{dw}), surface water (_{sw}), influent (_{inf}) and effluent (_{eff}) for different sub-populations.

ARVD	HQ _{dw}			HQ _{sw}			HQ _{eff}			HQ _{inf}		
	Child	Adults	Pregnant	Child	Adults	Pregnant	Child	Adults	Pregnant	Child	Adults	Pregnant
ABC	0	0	0	0	0.001	0.001	0	0	0	0.02	0.008	0.008
ATV				0	0	0	0	0	0	0.001	0.001	0.001
ddl	0	0	0	0	0	0						
DRV							0	0	0	0.002	0.001	0.001
EFV	0	0	0	0.01	0	0	0.03	0.01	0.01	0.04	0.02	0.02
FTC				0	0	0	0	0	0	0	0	0
IDV							0	0			0	0
3TC				0	0	0	0	0	0	0.003	0.002	0
LPV	0.1	0.1	0.1	0.34	0.13	0.13	0.21	0.08	0.08	0.21	0.05	0.05
MVC				0	0	0	0	0	0	0.003	0	0
NFV	0	0	0	0	0	0	0	0	0			
NVP	0	0	0	0.06	0.04	0.04	0.22	0.17	0.17	0	0	0
RAL										0.3	0.04	0.2
RTV	0	0	0	0.01	0.01	0.01	0	0	0	0.004	0.004	0.004
d4T	0	0	0	0	0	0					0	0
SQV										0	0	0
TDF				0	0	0	0	0	0	0.001	0	0
DDC		0	0.0	0.01	0	0						
ZDV	0	0	0	0	0	0	0	0	0	0.001	0.001	0.001

Values in bold = hazard concern (HQ > 1) ; empty cells = no data available.

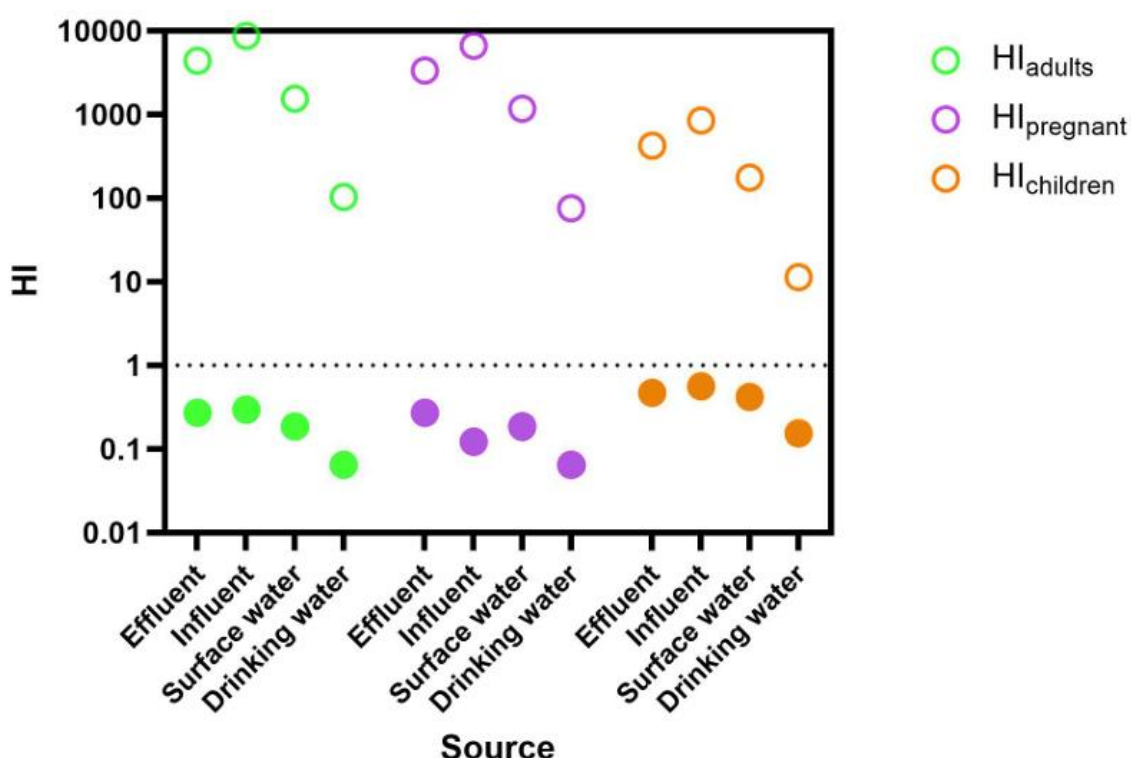


Figure 5-2: Hazard index (HI) values of antiretroviral drug mixtures for separate population groups in various water sources. Filled circles represent HI values calculated from hazard assessment 2; empty circles represent values from hazard assessment 1.

5.3.4 Reliability function

The reliability function (ξR_{Total}) indicated variable probabilities of non-carcinogenic hazard exceedance ($HQ > 1$) across water sources (Table 5-15). In surface water, ARVDs such as LPV (88%), RTV (81%), EFV, FTC, ABC, NVP, and ZDV exhibited high exceedance rates (41–75%). In drinking water, the only noteworthy exceedances were for EFV ($n = 3$; 100% reliability), DDC ($n = 1$; 100% reliability) and ZDV ($n = 3$; 43% reliability), suggesting potential hazard through even after presumed treatment.

Wastewater revealed the highest probability of exceedance for several ARVDs ($\xi R_{\text{Total}} > 70\%$) in water sources. Effluent samples showed great exceedance for EFV (35 occurrences, 100%), LVP (12 occurrences, 92%), and NVP (22 occurrences, 71%). Similarly, ABC and FTC demonstrated high reliability percentages (100% and 90%, respectively), despite fewer occurrences ($n \leq 9$). In influent, EFV (92%) and LPV (100%) again dominated, while FTC, ZDV, 3TC, and ABC also showed high reliability ($\geq 89\%$) despite fewer exceedances.

Table 5-15: Reliability function of the non-carcinogenic hazard potential (ξR_{Total}) for ARVDs in various water sources.

ARVD	Surface water		Drinking water		Effluent		Influent	
	HQ>1	ξR_{Total} (%)	HQ>1	ξR_{Total} (%)	HQ>1	ξR_{Total} (%)	HQ>1	ξR_{Total} (%)
ABC	6	75	0	0	8	100	10	91
ATV					4	67	2	50
ddl	0	0	1	25				
DRV					2	50	3	75
EFV	23	58	3	100	35	100	34	92
FTC	7	58			9	90	8	100
IDV					0		0	0
3TC	4	29			4	50	8	89
LVP	22	88	1	50	12	92	10	100
MRV					0	0	0	0
NFV	0	0	0	0				
NVP	27	41	1	13	22	71	14	61
RAL					2	67	3	75
RTV	22	81			7	70	6	75
SQV							0	0
d4T	3	60						
TDF	2	33			1	10	3	60
DCC	0	0	1	100				
ZDV	20	56	3	43	7	70	5	100

Bold values = 100% reliability; Empty cells = no data available.

5.4 Discussion

5.4.1 Hazard assessment (HA1)

The HQ results provided insights into the worst-case scenarios where the possibility of harm is greatest. The 99th percentile HQ values can aid regulators to protect not only the average individual but also those who are exposed to higher-than-average levels of concern. Those ARVDs with HQ values far greater than acceptable thresholds ($HQ \gg 1$), should be targeted for mitigation strategies and additional risk assessments to reduce exposure despite the majority of the population being within safer limits ($HQ < 1$).

The hierarchy of exposure severity followed the expected pattern: $HQ_{inf} > HQ_{eff} > HQ_{sw} > HQ_{dw}$, reflecting decreasing pharmaceutical load with increased water treatment. It is, however, disquieting that EFV, LPV, RTV, and DDC had median $HQ_{dw} > 1$ for adults in such meticulously treated water.

Efavirenz and LPV were identified to be hazardous in drinking water for pregnant females (median HQ_{dw}) and hazardous in extreme cases for children (99th percentile HQ_{dw} up to 5; Table 5-9).

The HQ analysis revealed considerable potential hazards, particularly for adults and pregnant women, with some concern for children. Abacavir consistently emerged as the ARVD with the highest toxicity potential, showing elevated HQ values across all sub-populations, specifically in adults and pregnant females. Efavirenz and LPV also demonstrated significant hazard potential, in drinking water and wastewater, suggesting concern at higher exposure concentrations.

Compounds such as TDF, NVP, and FTC showed high variability within some sub-populations, particularly adults and pregnant women, facing apparent hazard, especially at the upper exposure concentrations. In contrast, MRV, IDV, and certain other ARVDs, including ZDV and d4T, posed lower hazards across all groups, with children consistently showing the lowest HQs. Nevertheless, the analysis highlights the need for observation, as even lower HQs in children do not entirely disregard potential adverse effects, specifically in cases of overexposure.

Similar to this assessment, Ramírez-Morales *et al.* (2020) evaluated different pharmaceuticals' hazard potential across different water matrices in Costa Rica, documenting elevated HQs in both influent and treated effluent. The authors identified the five most hazardous compounds with median HQs ranging from 86 for fluoxetine to 600 for risperidone (psychiatric drugs). Such high HQ values in wastewater were also documented in the present study. The results emphasise the concern that raw sewage spills into surface water can introduce ARVD concentrations high enough to pose significant non-carcinogenic hazards, more than ever when untreated influent carries compounds with known high HQs such as ABC, FTC, and EFV.

Threshold values often indicated inflexion points or tipping points where risk levels transitioned from safe ($HQ \leq 1$) to unsafe ($HQ > 1$). In some cases, HQ values approached the threshold value. These inflexion points suggest that even small increases in ARVD concentrations in certain water sources could disproportionately increase hazard. For example, a slight increase in d4T (median $HQ_{sw} = 0.9$) (Table 5-8), could rapidly escalate the hazard potential from negligible to critical for pregnant women, illustrating the non-linear nature of threshold exceedance.

This analysis supports the idea that certain ARVDs, such as LPV and NVP, are more hazardous than others (such as RTV or ZDV) in surface water and that the toxic potential is not uniform across all ARVDs or population groups. This justifies prioritising specific compounds for environmental risk management and further research. Overall, ARVDs such as ABC, EFV, and TDF demand close attention due to their higher HQs, which could pose measurable health hazard concern to adults and pregnant women, with variable extents of toxic threat to children.

5.4.2 Hazard assessment (HA2)

5.4.3 Bioconcentration factor

The log BCF values suggest varying degrees of bioaccumulation potential, with some ARVDs showing significant propensity to accumulate in aquatic organisms. Although logK_{ow}-derived BCFs were used, they are less accurate than measured bioaccumulation values but are used given the lack of experimental data (Salvito *et al.*, 2020). The BCF further quantifies the potential of ARVDs to accumulate in tissue, particularly in fish, from the surrounding water. The high BCF values of LPV, NVP, and RTV reflect how much of these drugs are potentially absorbed and retained in the fish exposed relative to its concentration in water.

A study by Robson *et al.* (2017) on *Oreochromis mossambicus* exposed to EFV in SA focuses on the toxic effects of this ARVD on fish health, with significant liver damage and general health decline observed at environmentally relevant concentrations. The BCF for EFV determined in the current study (313 L/kg) supports the observed adverse health effects documented by Robson *et al.* (2017), as it indicates the potential for EFV to accumulate in fish tissue. Given that fish such as *O. mossambicus* (Mozambique tilapia) are part of local diets (Mashaphu *et al.*, 2025), the presence of bioaccumulated ARVDs in edible tilapia tissues raises concerns for human health, especially regarding chronic exposure to pharmacologically active substances through fish consumption.

5.4.4 Acceptable daily intake

Drugs assessed to have a high ADI value, such as RAL, EFV, RTV, and LVP (Table 5-12), suggest that they have a wide safety margin. Whereas drugs such as IDV, SQV, and DDC with very low ADI values suggest that they have a much narrower safety margin and potential for harm at low exposure concentrations (Ellis *et al.*, 2013).

Sub-populations are therefore suspected to have higher toxicity or sensitivity towards these drugs. Indinavir and SQV require a high TD to achieve their specific therapeutic effect. Higher therapeutic dosing may theoretically lead to greater environmental release and human exposure through water bodies.

In comparison, d4T and DDC have a much lower TD yet low ADI values, implying either greater potency or a narrower margin between their therapeutic and toxic doses (Tamargo *et al.*, 2015). The consumption of IDV, DDC, and SQV by children is concerning due to a lack of TD information and potential health hazards that could not be determined in the current study. Zalcitabine in adults and pregnant women require more cautious consumption given the low ADI values. Regarding pregnant women, the daily consumption of low amounts of d4T is also concerning, even though d4T is not currently prescribed in present ARVD treatment regimes.

Using the minimum TD integrates both toxicological and therapeutic perspectives. Since traditional POD values such as NOAEL or LOAEL derive from human or animal toxicity studies, they might not always accurately reflect the dose levels relevant to clinical use. The minimum TD, by contrast, is grounded in human clinical knowledge and provides a direct linkage between safety and therapeutic efficacy (Schwab *et al.*, 2005; Suh *et al.*, 2016).

This approach can be particularly useful for ARVDs, where the therapeutic window is narrow and the balance between efficacy and safety is critical (EMA, 2024; FDA, 2005; Soldin *et al.*, 2003). In general, the most sensitive TD was usually identified as the POD for the ADI calculations, similar to that by Webb *et al.* (2003). The comprehensive set of toxicology data from human clinical trials developed to support marketing as a human medicine allows substantial certainty in determining the POD for the ADI calculations. Furthermore, by incorporating uncertainty factors, the minimum TD can be adjusted to derive an ADI value that provides a conservative estimate of safe daily exposure.

This approach maintains a safety margin while leveraging clinically relevant data. Scientific literature supports the use of TDs in risk assessments under specific circumstances (Cunningham *et al.*, 2009; EPHC/NRMMC/AHMC, 2008; Schwab *et al.*, 2005; Webb *et al.*, 2003). Some studies have used the minimum TD as POD to calculate ADI value. For example, Schwab *et al.* (2005) used a minimum TD of acetaminophen (650 mg/d) as POD to calculate the ADI value for children and adults' sub-populations. This is particularly relevant for ARVDs, where TDs are well-established, and their safety profiles are extensively documented. This approach aligns with established practices in risk assessment and is supported by regulatory and scientific precedents (Bull *et al.*, 2011; Schwab *et al.*, 2005).

There exists a distinct positive correlation between the ADI and the total amount of active pharmaceutical ingredient (API) that ultimately enters the environment (Cunningham *et al.*, 2009). When the ADI is derived from the TD, a more potent or efficacious drug, which necessitates a smaller production volume, will have a lower environmental outcome.

Conversely, drugs that require higher TDs yield greater production volumes, thereby increasing the amount of API ultimately released into the natural environment (Daughton and Runoy, 2009). Therefore, the total environmental release of an ARVD API is genuinely inversely related to its potency.

However, certain characteristics of APIs might necessitate a more comprehensive risk assessment for potential human health impacts. For example, APIs in ARVDs that exhibit long-term persistence in the environment such as d4T, those that may impact non-target cardiovascular systems such as LPV/RTV, those with potential endocrine-disrupting effects such as EFV, ATV or RAL, or those that demonstrate a significant propensity for causing drug-drug interactions in vulnerable populations such as LPV/RTV, EFV and ATV, should be subjected to more thorough evaluation.

5.4.5 Chronic daily intake

The CDI analysis revealed continuous oral exposure to ARVDs across various sub-populations and water sources under defined long-term exposure scenarios, with children facing the highest intake, mainly for LPV, EFV, and RTV in drinking water. Surface water was suggested to be a profound source of LPV, NVP, RTV, and EFV, especially among children. Wastewater, representing a worst-case scenario, showed that LPV, NVP, RTV, and EFV had substantial exposure concerns similar to surface water. Abacavir exposure was notable only for children in influent water.

As a whole, surface water represents the most concerning source, followed by wastewater with drinking water still posing a significant intrinsic toxicity due to certain ARVDs. This assessment employed maximum concentrations to reflect the upper-bound scenarios; while conservative, these values might not reflect typical exposure levels.

5.4.6 Risk characterisation (HA2)

While there are variations in HQ values across ARVDs and water sources, none exceeded one, suggesting that the threat of non-carcinogenic effects is minimal under the current exposure scenarios. This aligns with the observed low environmental concentrations for most ARVDs (Abafe *et al.*, 2018). Their pharmacological specificity might not have the same

profound effects on the broader physiological systems that other classes of pharmaceuticals do (Schoen *et al.*, 2013). This selectivity, especially at low environmental concentrations, may reduce the toxic potential of unintentional effects during exposure.

For the most part, children displayed higher HQs than adults and pregnant women, attributable to greater water intake relative to body weight and physiological vulnerability (Duarte *et al.*, 2022; Kumar and Xagorarakis, 2010). The current study, as well as that of Duarte *et al.* (2022) affirm that age-based differences in the intake rates and body mass are vital in driving exposure assessments. This underlines the importance of including sensitive sub-populations in hazard assessments and stresses age-related vulnerability in hazard modelling.

The TD of an ARVD is closely related to safe exposure levels. Moreover, ARVDs with higher TDs, such as LPV, EFV, and NVP, were associated with slightly higher HQs, strengthening the relationship between drug potency, production volume, and environmental presence (Daughton and Runoy, 2009). This supports the concept that ARVDs requiring larger doses are inclined to result in greater environmental release, increasing the potential for chronic human exposure.

The bioconcentration in fish tissue is often not a significant route of exposure for human consumption (Cunningham *et al.*, 2009). Non-ionic ARVDs, tend to be less reactive and more stable in environmental systems compared with ionic compounds. Non-ionic ARVDs are potentially less likely to be absorbed by humans via passive diffusion across membranes, which could result in lower toxic potentials estimated in such HQ models. Similar findings were observed by Duarte *et al.* (2022) in the Vecht River, Germany, for pharmaceuticals. The authors reported that under current exposure scenarios, chronic health concerns to humans are low under average environmental conditions.

Duarte *et al.* (2022) found HQs ranging from 10^{-9} to 2.5. These HQs match the observed HQs of ARVDs, such as EFV, LPV, and NVP ($HQ < 0.3$). The absence of data for several ARVDs (RAL, DRV, DDC) in specific water sources or sub-populations exposes key gaps in current environmental monitoring and toxicological datasets. Addressing these data gaps will be vital for improving hazard assessments and developing a more comprehensive understanding of long-term toxic potential associated with ARVD exposure through water sources in South Africa.

5.4.7 Comparison between approaches

This comparative HAs enhanced the scientific depth of the current study by capturing both worst-case (acute or high-end) exposure scenarios and more realistic, chronic exposure trends. The different methods and assumptions of these approaches shaped contrasting hazard conclusions (see Annexures I, Table 7-13).

While the CDI/ADI approach (HA2) is generally regarded as more conservative for chronic, population-level protection in regulatory contexts (FDA, 1999), the ADD/RfD approach (HA1) in the present study yielded higher HQs due to its reliance on 99th percentile exposure estimates and potentially more rigorous RfDs. This exemplifies how practical conservatism can depend on input assumptions, emphasising the importance of contextualising hazard and risk outcomes based on both method design (theory-based) and practical exposure data (real-world results).

The HA1 was found to be more conservative in the current study design because it used safety thresholds derived from NOAEL or LOAEL values, which are based on detailed toxicological studies. Standardised UFs were applied to account for variability between individuals, adding an extra layer of precaution. The outcome is a very cautious hazard estimate designed to avoid any adverse effects, even in vulnerable populations. This approach identified ARVDs such as ABC, EFV, LPV, and TDF with HQ values well above one, particularly for adults and pregnant women, indicating health concerns. Median HQs were rather substantial, but extreme values (e.g., ABC's 99th percentile HQ of 968) were stressed for regulatory purposes and urgent mitigation strategies.

The HA2 was found to be less conservative (in practice) because it incorporated clinically relevant thresholds like the ADI. When comparing the ADI (Table 5-12) and the RfD (Table 5-3) values used in the present study, it was found that RfDs were consistently lower than ADIs for the majority of ARVDs. Consequently, the RfD-based HQ approach (HA1) produced higher HQ values, making it more protective in identifying potential hazard exceedance. Although ADIs are largely derived with protective safety margins, the RfDs used in this specific assessment were numerically 'stricter'. Highlighting once again the importance of evaluating reference value selection based on empirical data rather than theoretical assumptions alone, yet it is important to note that this finding was data-specific to the present study.

The HA2 approach concluded no significant health hazard across sub-populations and water sources under standard exposure conditions. This is based on the observation that even the highest HQ values remained below the threshold of concern. The assessment focused on overall trends and broader toxicological considerations, including the selective action of ARVDs on viral enzymes and their environmental persistence, which could limit both bioavailability and inherent toxicity.

This approach focused on chronic hazard evaluation by interpreting HQ values as indicative of long-term safety under 'typical' exposure conditions (Bull *et al.*, 2011; Schwab *et al.*, 2005). Furthermore, HA2 mitigated concerns over short-term exposure spikes by demonstrating that HQ values across all ARVDs and sub-populations remained well below one.

Cross-validation of the HAs revealed unexpected differences. It was expected that both methods would agree (negligible HQs for most ARVDs) which would have added confidence in safety conclusions. Yet, they diverged ($HQ \gg 1$ in HA1 vs. $HQ < 1$ in HA2). This divergence denoted that critical locations or sub-populations require additional assessment. Together, these approaches did offer some complementary insights. The HA1 captured the potential for episodic hazard exceedance and vulnerability between different populations in extreme cases that should not be overlooked, while HA2 supported broader regulatory assurance where hazard is minimal based on average exposure under most conditions.

Nevertheless, the dual approach would benefit from greater integration. For example, a tiered decision-making framework could guide when suspected hazard warrants the escalation from HA2 to HA1. The absence of full uncertainty analysis (probabilistic modelling) and data gaps for several ARVDs point to areas for methodological improvement. Despite these limitations, the dual HA assessment supports the practice of balancing conservativeness with contextual realism. It also supports aligning human health protection objectives with the intricacies of environmental pharmaceutical contamination in a changing South African context.

5.4.8 Hazard index (ARVD mixtures)

The HI results gave a rudimentary insight into whether exposure groups are safe from the cumulative effects of being exposed to all the ARVDs simultaneously in a specific water source, even if each ARVD alone (HQs) did not indicate hazardous consequences. The HI values for ARVD mixtures in wastewater were extraordinarily high and consistent with some international reports. Ramírez-Morales *et al.* (2020) reported high $\sum$ HQ values in Costa Rican urban wastewater for pharmaceutical mixtures ($\sum$ HQ = 1.0×10^4), demonstrating a similar magnitude of cumulative hazard. Compared with the Chinese meta-analysis reporting total target HQ (~ 0.002) for antibiotics in river water, the ARVD HI results were 5–6 orders of magnitude higher, highlighting that ARVD mixtures in South African surface water pose a serious non-carcinogenic hazard compared to the study by Li *et al.* (2024).

According to a WRC report in South Africa, cumulative HIs for ARVDs generally ranged in the same of order of magnitude as the current study with HI values up to 230 in the Hennops River (Vogt *et al.*, 2020). While some magnitudes differ, both studies support concern for non-carcinogenic hazards, especially in surface water. The results are consistent with other global assessments where $\sum$ HQ exceeded 1 in untreated effluents (Kaur *et al.*, 2020; Ramírez-Morales *et al.*, 2020).

These discrepancies underscore that, while low-level pharmaceutical contamination in rivers may present minimal risk, the concentrated ARVD mixtures in wastewater constitute a far more pressing local concern. This high HI values in the current study may stem from higher ARVD concentrations in South Africa compared to other countries and/or additive effects of multiple compounds. The observed threshold exceedance reinforces concerns about inefficient ARVD removal during wastewater treatment, as well as the contribution of raw sewage discharges and infrastructure failure, which remains a prominent challenge in many parts of the country.

Worldwide, regulatory frameworks for pharmaceuticals in water are still evolving. This regulatory gap is not unique as most countries, including South Africa, do not have enforceable limits for individual pharmaceuticals such as ARVDs nor their mixtures in water (Adeola and Forbes, 2022). The results of the current study are therefore essential in highlighting the risks of cumulative environmental exposure associated with ARVD contamination, especially given the high HIV/AIDS prevalence and considerable ARVD usage in ART, which will continue to lead to their eventual release (in assortments) into the environment through excretion and improper disposal.

As an interim measure, a HA may be performed for those drugs in the mixture for which sufficient exposure and health effects data are accessible. The data of the current study emphasise the necessity for local environmental management schemes to alleviate ARVD contamination, particularly in susceptible regions with poor sanitation infrastructure or high dependency on potentially contaminated surface water.

An important limitation is that the HI assumes additive effects but does not account for synergistic or antagonistic interactions between ARVDs with different mechanisms of action. This simplification limits interpretation for long-term human health. Real-world mixtures may display pairwise interaction effects, and additive HI may miscalculate combined toxicity unless interaction-modified HI methods are applied, such as in the study by Duarte *et al.* (2022).

The current study also contributes to the growing body of evidence about the collective toxicological effects of ARVDs in the environment and provides some insight into the cumulative non-carcinogenic hazards of mixtures. Yet, further monitoring data are required to adequately characterise the mixture's exposure and potential hazards (Adeola and Forbes, 2022; Vogt *et al.*, 2020). The results have implications for public health policy and environmental risk assessment frameworks, by suggesting a requirement for updated guidelines that account for cumulative mixture effects of ARVDs and a general need to address water safety in South Africa, mainly for sub-populations such as children and pregnant women who face greater vulnerabilities.

5.4.9 Reliability function

Analysing the reliability function for non-carcinogenic risks linked to ARVDs across various water sources reveals important insights into the effects of ARVD contamination on the environmental and public health. While no Monte Carlo simulation was performed, the reliability function in the current study echoes a frequency-based exceedance probability, estimated from observed HQ values across exposure scenarios. This provides an informative summary of the proportion of scenarios where the ARVDs present potential non-carcinogenic concern, even though it does not account for parameter uncertainty in a probabilistic sense.

The consistency of exceedance for EFV, DDC, LPV, and ZDV even in drinking water suggests inadequate treatment, or infrastructure failures, highlighting a critical gap in water safety for human consumption. The detection of ARVDs with high reliability percentages in water sources further supports the assertion that preferential flow pathways and untreated contaminant channels can transport hazardous compound mixtures beyond expected exposure points (Henri *et al.*, 2015).

As the drugs may bypass proper treatment or filtration through these preferential or untreated pathways, reaching populations in unexpected concentrations. Consequently, analysing wastewater probabilities provides a worst-case scenario, offering insight into the maximum environmental loads of ARVDs and their potential to infiltrate vulnerable water systems.

The consistency of exceedance (up to 88%) of these compounds (e.g. EFV, FTC, RTV, LVP, ABC, NVP, and ZDV) in surface water, which is often used for informal domestic use, further stress the need for monitoring frameworks that assess ARVD chronic toxicity and source-control measures. The long-term exposure of aquatic ecosystems to these ARVDs, as well as the possibility of human exposure through indirect ingestion pathways from contaminated water supplies, are concerns raised by these exceedances.

The study focuses on deterministic rather than a full probabilistic investigation approach. Although the reliability function added value to the research, it does not account for uncertainty in input parameters (e.g., exposure variability or toxicity thresholds), which would be better addressed by Bayesian models or Monte Carlo simulations (Henri *et al.*, 2025; Kaur *et al.*, 2020; Liu *et al.*, 2020).

5.5 Conclusions

The study conducted a comprehensive HHHA of selected ARVDs quantified in South African water sources, encompassing surface water, wastewater, and drinking water. The primary objectives were to evaluate potential health hazards by assessing both acute and chronic exposure pathways, with ingestion as the primary route of exposure. It provided a population-based HA which allowed for nuanced insights into vulnerable sub-populations, an aspect often neglected in South African studies. Interestingly, the two HA approaches yielded substantially different HQs, prompting further investigation into how methodological differences can significantly affect risk characterisation outcomes of ARVDs. This comparative analysis highlighted the importance of using multiple HA tools, particularly when dealing with emerging contaminants for which regulatory toxicological data may be limited or lacking (Bull, 2011; Schwab *et al.*, 2005).

Continuous environmental exposure to ARVDs, particularly ABC, EFV, and LPV, poses significant health hazards (HQs > 1), especially among vulnerable population such as adults and pregnant women, who showed great susceptibility to these compounds in surface and effluent water. The prolonged exposure could be more hazardous to these sensitive sub-populations. The comparative HA demonstrated how differing methodologies can produce

contrasting outcomes based on exposure assumptions and reference values. The first HA was more responsive to acute, worst-case scenarios, identifying potential toxicity for particular ARVDs and sub-populations, while the second HA exhibited chronic exposure safety associated with regulatory thresholds. The findings underscore the value of a dual-assessment strategy and the need to select reference values based on both empirical data and contextual exposure patterns.

The study acknowledged uncertainties, particularly due to data limitations, which could affect the accuracy of predictions. The HI results demonstrated hazards associated with cumulative ARVD exposure across all water sources, with HI values in wastewater samples well above safe thresholds. Contributing factors may include raw sewage discharge, and infrastructure failure challenges faced by South Africa. While children revealed lower HI values than adults and pregnant females, their greater physiological susceptibility remains alarming. Surface and drinking water also exceeded $HI > 1$, suggesting health hazards even post-treatment. The reliability analysis confirmed a high probability of harm ($> 70\%$ for EFV, LVP, ABC, and ZDV). Repeated exceedances in treated drinking water also suggest a plausible exposure route that requires further risk-based evaluations.

The study exposed critical data gaps such as insufficient toxicological profiles, incomplete environmental monitoring, and limited understanding of ARVD mixture effects. These gaps can be addressed through targeted research, robust surveillance systems, and improved coordination between scientists and regulators. Ultimately, this research contributes to the field of environmental health by demonstrating the practical and scientific value of comparative hazard approaches. It calls for a proactive, evidence-based strategy to manage ARVD contamination through eco-pharmacovigilance, improved treatment technologies, systematic monitoring programs, updated water quality guidelines, and inclusive public health interventions. In doing so, South Africa can better protect human and ecological health without compromising its commitment to life-saving HIV/AIDS treatment programs.

5.6 Recommendations

The study opted to assess health hazards from single ARVD exposure in various water sources. Currently, ARVDs are not explicitly included in the regulatory framework of South Africa's National Water Act (NWA) or Water Services Act (WSA). Yet, the findings of the current study support that ARVDs are emerging contaminants of concern. Given the current and foreseen significant ARVD treatment program, the consecutive presence of these drugs

in the water, context-specific exposure scenarios, and the inefficiency of traditional wastewater treatment plants to remove these drugs, regulatory action is required.

- It is recommended that regulatory systems specific to ARVDs and other pharmaceutical pollutants are established within South Africa's water quality management strategies.
- The regulatory framework should include plans for actions towards strategic intent of removing or addressing ARVD pollution in South African waters, especially towards vulnerable sub-populations such as children and pregnant women.
- Follow-up studies on acute exposure risks and scenarios (e.g., from accidental ingestion) are recommended (risk assessments). South Africa could benefit from preparation strategies to combat acute risks of pharmaceutical spills.
- Future studies should use empirical or model-based BCF data where available, as variability could alter risk estimations considerably. Dietary exposure, e.g., via fish consumption, also warrants investigation.
- Frequently detected and high probability ARVDs such as EFV require targeted risk management strategies.
- Advanced treatment technologies, such as reverse osmosis, advanced oxidation processes (AOPs), or granular activated carbon (GAC) should be considered as wastewater facilities to improve ARVD removal (Kang *et al.*, 2024).
- It is recommended that a tiered decision-support tool be developed. For example, use the CDI/ADI-based approach (HA2) for screening, when thresholds near or exceed 1 escalate to ADD/RfD-based approach (HA1). Such support tools can be used when dealing with vulnerable populations or hotspot contamination sites.
- High-risk areas dependent on untreated surface water should be prioritised for pilot interventions using site-specific interventions such as constructed wetlands, biofiltrations systems, ion exchange, or biochar sorption technologies for example (Fakioglu *et al.*, 2024), to mitigate these drugs.
- South African treatment facilities could adopt similar approaches tailored to local conditions.
- National water quality guidelines should be updated to incorporate cumulative risk assessments in the future. The outdated South African water framework guidance on mixture risk should also be replaced with a more comprehensive approach.
- Future cumulative risk assessments may help to identify ARVDs that greatly contribute to mixture risks as candidates for pollutant prioritisation, even if they do not exceed acceptable levels.

- South Africa could consider adapting elements of the European Water Framework Directive (Directive 2000/60/EC and 2008/105/EC for example) to support chemical mixture provisions, by prioritising high-risk zones identified in the current study.
- New pollutant prioritisation schemes should be applied to target drugs and sites that make substantial contributions to cumulative hazard.
- Improved and stronger feedback between monitoring data for pharmaceuticals and regulatory bodies is needed to close data gaps.

5.7 References

- Abafe, O.A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., Pietruschka, B. and Martincigh, B.S. 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, 200:660–670.
- Abers, M.S., Shandera, W.X. and Kass, J.S. 2014. Neurological and psychiatric adverse effects of antiretroviral drugs. *CNS Drugs*, 28:131–145.
- Adeola, A.O. and Forbes, P.B. 2022. Antiretroviral drugs in African surface waters: prevalence, analysis, and potential remediation. *Environmental Toxicology and Chemistry*, 41(2):247–262.
- Agency for Toxic Substances and Disease Registry (ATSDR). 1996. Minimal risk levels for priority substances and guidance for derivation. *Federal Register*, 61(101):25–775.
- ANSI/ASSP (American National Standards Institute/American Society of Safety Professionals). 2016. *Prevention through design guidelines for addressing occupational hazards and risks in design and redesign processes (ANSI/ASSP Z590.3-2011(R2016))*. Park Ridge, IL: American Society of Safety Professionals.
- Bercu, J.P., Parke, N.J., Fiori, J.M. and Meyerhoff, R.D. 2008. Human health risk assessments for three neuropharmaceutical compounds in surface waters. *Regulatory Toxicology and Pharmacology*, 50(3):420–427.
- Bub, S., Petschick, L.L., Stehle, S., Wolfram, J. and Schulz, R. 2025. Limitations of chemical monitoring hinder aquatic risk evaluations on the macroscale. *Science*, 388(6753):1301–1305.
- Bull, R.J., Crook, J., Whittaker, M. and Cotruvo, J.A. 2011. Therapeutic dose as the point of departure in assessing potential health hazards from drugs in drinking water and recycled municipal wastewater. *Regulatory Toxicology and Pharmacology*, 60(1):1–19.
<https://doi.org/10.1016/j.yrtph.2009.12.010>

Cai, K., Li, C., Song, Z., Gao, X. and Wu, M. 2019. Pollution and health risk assessment of carcinogenic elements As, Cd, and Cr in multiple media—A case of a sustainable farming area in China. *Sustainability*, 11(19):5208.

Chartres, N., Bero, L.A. and Norris, S.L. 2019. A review of methods used for hazard identification and risk assessment of environmental hazards. *Environment International*, 123:231–239.

Chiu, W.A., Axelrad, D.A., Dalaijamts, C., Dockins, C., Shao, K., Shapiro, A.J. and Paoli, G. 2018. Beyond the RfD: broad application of a probabilistic approach to improve chemical dose–response assessments for noncancer effects. *Environmental Health Perspectives*, 126(6):067009.

Coleman, M.E. and Marks, H.M. 1999. Qualitative and quantitative risk assessment. *Food Control*, 10(4-5):289–297.

Cunningham, V.L., Binks, S.P. and Olson, M.J. 2009. Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment. *Regulatory Toxicology and Pharmacology*, 53(1):39–45.

Daughton, C.G. 2003. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy. *Environmental health perspectives*, 111(5):757–774.

Daughton, C.G. and Ruhoy, I.S. 2009. Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers. *Environmental Toxicology and Chemistry*, 28(12):2495–2521.

Dixit, A., Pandey, H., Rana, R., Kumar, A., Herojeet, R., Lata, R., ... Sarkar, B. 2024. Ecological and human health risk assessment of pharmaceutical compounds in the Sirsa River of Indian Himalayas. *Environmental Pollution*, 347:123668.

Donovan, E., Unice, K., Roberts, J.D., Harris, M. and Finley, B. 2008. Risk of gastrointestinal disease associated with exposure to pathogens in the water of the Lower Passaic River. *Applied and Environmental Microbiology*, 74(4):994–1003.

Dorne, J.L.C.M. and Renwick, A.G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicological Sciences*, 86(1):20–26.

Dourson, M.L., Felter, S.P. and Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regulatory Toxicology and Pharmacology*, 24(2):108–120.

Duarte, D.J., Oldenkamp, R. and Ragas, A.M. 2022. Human health risk assessment of pharmaceuticals in the European Vecht River. *Integrated Environmental Assessment and Management*, 18(6):1639–1654.

Dufour, A.P., Evans, O., Behymer, T.D., and Cantú, R. 2006. Water ingestion during swimming activities in a pool: A pilot study. *Journal of Water and Health*, 4(4), 425–430.
<https://doi.org/10.2166/wh.2006.0026>

Edagwa, B., McMillan, J., Sillman, B. and Gendelman, H.E. 2017. Long-acting slow effective release antiretroviral therapy. *Expert opinion on drug delivery*, 14(11):1281–1291.

Ellis, P., Kenyon, M. and Dobo, K. 2013. Determination of compound-specific acceptable daily intakes for 11 mutagenic carcinogens used in pharmaceutical synthesis. *Regulatory Toxicology and Pharmacology*, 65(2):201–213.

EMA (European Medicines Agency). 2024. Medicines. [Online].
<https://www.ema.europa.eu/en/medicines> Date of access: 9 Sep. 2023.

EPHC/NRMMC/AHMC (Environmental Protection and Heritage Council, Natural Resource Management Ministerial Council and Australian Health Ministers' Conference). 2008. *Australian Guidelines for Water Recycling (Phase 2)*. Augmentation of Drinking Water Supplies. Canberra: National Water Quality Management Strategy.

Fakioglu, M., Ahrens, L. and Ozturk, I. 2024. A Review on Pharmaceuticals Removal from Wastewater Effluents by Single and Combined Ozone-Sorption Systems and Fate of Transformation Products. *Ozone: Science and Engineering*, 1–21.

FDA (United States Food and Drug Administration). 1999. *Benefit vs. Risk: how CDER approves new drugs. From test tube to patient: improving health through human drugs*. Center for Drug Evaluation and Research Special Report, 33–40.

FDA (United States Food and Drug Administration). 2005. *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*. [Online]. <https://www.fda.gov> Date of access: 9 Mar. 2024.

Gerba, C.P. 2019. Risk assessment. In: M. L. Brusseau, I.L. Pepper and C.P. Gerba, eds. *Environmental and pollution science*. 3rd ed. Amsterdam: Elsevier, 541–56.

Guleria, A. and Chakma, S. 2021. Fate and contaminant transport model-driven probabilistic human health risk assessment of DNAPL-contaminated site. *Environmental Science and Pollution Research*, 28(12):14358–14371.

Health Canada. 2004. *Canadian Exposure Factors Used in Human Health Risk Assessments*. Ottawa, ON: Health Canada. [Online]. <https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/canadian-exposure-factors-human-health-risk-assessments.html> Date of access: 3 Jul. 2024.

Henri, C.V., Fernàndez-Garcia, D. and de Barros, F.P. 2015. Probabilistic human health risk assessment of degradation-related chemical mixtures in heterogeneous aquifers: Risk statistics, hot spots, and preferential channels. *Water Resources Research*, 51(6):4086–4108.

Henri, C.V., Fernàndez-Garcia, D. and De Barros, F.P. 2016. Assessing the joint impact of DNAPL source-zone behaviour and degradation products on the probabilistic characterization of human health risk. *Advances in Water Resources*, 88:124–138.

Kang, Z., Duan, L. and Zahmatkesh, S. 2024. Optimizing removal of antiretroviral drugs from tertiary wastewater using chlorination and AI-based prediction with response surface methodology. *Science of The Total Environment*, 934:172931.

Kaur, L., Rishi, M.S. and Siddiqui, A.U. 2020. Deterministic and probabilistic health risk assessment techniques to evaluate non-carcinogenic human health risk (NHHR) due to

fluoride and nitrate in groundwater of Panipat, Haryana, India. *Environmental Pollution*, 259:113711.

Kumar, A. and Xagorarakis, I. 2010. Pharmaceuticals, personal care products and endocrine-disrupting chemicals in US surface and finished drinking waters: a proposed ranking system. *Science of the Total Environment*, 408(23):5972–5989.

Kumar, A., Chang, B. and Xagorarakis, I. 2010. Human health risk assessment of pharmaceuticals in water: issues and challenges ahead. *International Journal of Environmental Research and Public Health*, 7(11):3929–3953.

Kunene, P.N. and Mahlambi, P.N. 2023. Case study on antiretroviral drugs uptake from soil irrigated with contaminated water: Bio-accumulation and bio-translocation to roots, stem, leaves, and fruits. *Environmental Pollution*, 319:121004.

Li, C., Li, A., Hui, X., Wang, A., Wang, L. and Chang, S. 2024. Concentrations, probabilistic human and ecological risks assessment attribute to antibiotics residues in river water in China: Systematic review and meta-analysis. *Ecotoxicology and Environmental Safety*, 285:117022.

Mangesi, T. and Mngeni, A. 2024. Vulnerability of Rural Communities to Contaminated Water and Its Implications: A Case Study from KSD Local Municipality. *Environmental and Earth Sciences Research Journal*, 11(4).

Mashaphu, M.F., O'Brien, G.C. and Willows-Munro, S. 2025. Genetic assessment of farmed *Oreochromis mossambicus* populations in South Africa. *Peer J*, 13:18877.

Meylan, W.M., Howard, P.H., Boethling, R.S., Aronson, D., Printup, H. and Gouchie, S. 1999. Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. *Environmental Toxicology and Chemistry*, 18(4):664–672.

Ncube, S., Madikizela, L.M., Chimuka, L. and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. *Water Research*, 145:231–247.

Ngubane, Z., Dzwauro, B., Moodley, B., Stenström, T.A. and Sokolova, E. 2023. Quantitative assessment of human health risks from chemical pollution in the uMsunduzi River, South Africa. *Environmental Science and Pollution Research*, 30(55):118013–118024.

Ngwenya, P. and Musee, N. 2023. Modelling ecological risks of antiretroviral drugs in the environment. *Environmental Chemistry and Ecotoxicology*, 5:145–154.

NRC (National Research Council). 1994. Division on Earth, Life Studies, Board on Environmental Studies, Commission on Life Sciences and Committee on Risk Assessment of Hazardous Air Pollutants. *Science and Judgment in Risk Assessment*. Washington, DC: National Academies Press.

Palanisamy, V., Sanphui, P., Jyothi, V.G.S., Shastri, N.R., Bolla, G., Palanisamy, K., ... Vangala, V.R. 2021. Tuning diffusion permeability of an anti-retroviral drug, emtricitabine, via multicomponent crystallizations. *Crystal Growth and Design*, 21(3):1548–1561.

Pedreiro, L.N., Cury, B.S.F., Chaud, M.V. and Gremião, M.P.D. 2016. A novel approach in mucoadhesive drug delivery system to improve zidovudine intestinal permeability. *Brazilian Journal of Pharmaceutical Sciences*, 52:715–725.

Popov, G., Lyon, B.K. and Hollcroft, B.D. 2016. *Risk assessment: A practical guide to assessing operational risks*. Hoboken, NJ: John Wiley and Sons.

Puoane, T., Steyn, K., Bradshaw, D., Laubscher, R., Fourie, J., Lambert, V. and Mbananga, N. 2002. Obesity in South Africa: the South African demographic and health survey. *Obesity Research*, 10(10):1038–1048.

Ramírez-Morales, D., Masís-Mora, M., Montiel-Mora, J.R., Cambroner-Heinrichs, J.C., Briceño-Guevara, S., Rojas-Sánchez, C.E., ... Rodríguez-Rodríguez, C.E. 2020. Occurrence of pharmaceuticals, hazard assessment and ecotoxicological evaluation of wastewater treatment plants in Costa Rica. *Science of the Total Environment*, 746:141200.

Rasmussen, H.N., Scheier, M.F. and Greenhouse, J.B. 2009. Optimism and physical health: A meta-analytic review. *Annals of Behavioral Medicine*, 37(3):239–256.

Ripanda, A.S., Rwiza, M.J., Nyanza, E.C., Njau, K.N., Vuai, S.A. and Machunda, R.L. 2021. A review on contaminants of emerging concern in the environment: a focus on active chemicals in Sub-Saharan Africa. *Applied Sciences*, 12(1):56.

Robson, L., Barnhoorn, I.E.J. and Wagenaar, G.M. 2017. The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56:225–232.

Salvito, D., Fernandez, M., Jenner, K., Lyon, D.Y., de Knecht, J., Mayer, P., ... León-Paumen, M. 2020. Improving the environmental risk assessment of substances of unknown or variable composition, complex reaction products, or biological materials. *Environmental Toxicology and Chemistry*, 39(11):2097–2108.

Schoen, J.C., Erlandson, K.M. and Anderson, P.L. 2013. Clinical pharmacokinetics of antiretroviral drugs in older persons. *Expert Opinion on Drug Metabolism and Toxicology*, 9(5):573–588.

Schwab, B.W., Hayes, E.P., Fiori, J.M., Mastrocco, F.J., Roden, N.M., Cragin, D., Meyerhoff, R.D., Vincent, J.D. and Anderson, P.D. 2005. Human pharmaceuticals in US surface waters: a human health risk assessment. *Regulatory Toxicology and Pharmacology*, 42(3):296–312.

Sharma, B.M., Bečanová, J., Scheringer, M., Sharma, A., Bharat, G.K., Whitehead, P.G., Klánová, J. and Nizzetto, L. 2019. Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. *Science of the Total Environment*, 646:1459–1467.

Sibanyoni, J.B. 2021. *Final report of the Gauteng provincial inquiry into the sewage problem of the Vaal River*. Johannesburg: South African Human Rights Commission.

Silva, J.P.A., Figueirêdo, C.B.M., de Medeiros Vieira, A.C.Q., de Lyra, M.A.M., Rolim, L.A., Rolim-Neto, ... Soares-Sobrinho, J.L. 2017. Thermal characterization and kinetic study of the antiretroviral tenofovir disoproxil fumarate. *Journal of Thermal Analysis and Calorimetry*, 130:1643–1651.

Singh, A.K., 2015. *Engineered nanoparticles: structure, properties and mechanisms of toxicity*. Cambridge, MA: Academic Press.

Soldin, O.P., Elin, R.J. and Soldin, S.J. 2003. Therapeutic drug monitoring in human immunodeficiency virus/acquired immunodeficiency syndrome: *Quo vadis?* *Archives of pathology and laboratory medicine*, 127(1):102–105.

Sorell, T.L. 2016. Approaches to the development of human health toxicity values for active pharmaceutical ingredients in the environment. *The AAPS journal*, 18(1):92–101.

South African Government. 2024. *People of South Africa*. [Online].
<https://www.gov.za/about-sa/people-south-africa-0> Date of access: 9 Mar. 2024.

Stats SA, Statistics South Africa. 2022. Mid–year population estimates. Statistical release P0318. <https://www.statssa.gov.za/publications/P0318/P03182022.pdf> Date of access: 16 Sep. 2023.

Suh, H.Y., Peck, C.C., Yu, K.S. and Lee, H. 2016. Determination of the starting dose in the first-in-human clinical trials with monoclonal antibodies: a systematic review of papers published between 1990 and 2013. *Drug design, development and therapy*, 4005–4016.

Tamargo, J., Le Heuzey, J.Y. and Mabo, P. 2015. Narrow therapeutic index drugs: a clinical pharmacological consideration to flecainide. *European Journal of Clinical Pharmacology*, 71:549–567.

Turton, A. 2022. A tsunami of human waste inundates our rivers and dams, and it's a security issue. *News24*, 3 March. [Online]
<https://www.news24.com/news24/opinions/analysis/anthony-turton-a-tsunami-of-human-waste-inundates-our-rivers-and-dams-and-its-a-security-issue-20220303> Date of access: 7 Jul. 2025.

Ukić, Š., Sigurnjak, M., Cvetnić, M., Markić, M., Stankov, M.N., Rogošić, M., ... Bolanča, T. 2019. Toxicity of pharmaceuticals in binary mixtures: Assessment by additive and non-additive toxicity models. *Ecotoxicology and Environmental Safety*, 185, 109696.

USEPA. United States Environmental Protection Agency. 2002. *A review of the reference dose and reference concentration processes*. EPA/630/P-02/002F. Washington, DC: Risk Assessment Forum. [Online]. <https://www.epa.gov/sites/default/files/2014-12/documents/rfd-final.pdf> Date of access: 2 Jan. 2023.

USEPA. United States Environmental Protection Agency. 2004. *Risk assessment guidance for Superfund Volume I: Human health evaluation manual (Part E, supplemental guidance for dermal risk assessment)*. Washington, DC: United States Environmental Protection Agency.

USEPA. United States Environmental Protection Agency. 2005. Guidelines for carcinogen risk assessment. Risk Assessment Forum Series, Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf Date of access: 16 Sep. 2023.

USEPA. United States Environmental Protection Agency. 2011. Exposure factors handbook: 2011 ed. (final report). EPA/600/R-09/052F. Washington, DC: US Environmental Protection Agency. [Online]. <https://www.epa.gov/sites/default/files/2015-09/documents/efh-frontmatter.pdf> Date of access 2 May 2023.

USEPA. United States Environmental Protection Agency. 2022. Conducting human health risk assessments. [Online]. <https://www.epa.gov/risk/human-health-risk-assessment> Date of access 2 May 2024.

USEPA. United States Environmental Protection Agency. 2024a. *Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11*. Washington, DC: United States Environmental Protection Agency.

USEPA. United States Environmental Protection Agency. 2024b. CompTox Chemicals Dashboard. United States Environmental Protection Agency. [Online]. <https://comptox.epa.gov/dashboard> Date of access: 16 Jul. 2023.

USEPA. United States Environmental Protection Agency. 2025. Human Health Risk Assessment. [Online]. <https://www.epa.gov/risk/human-health-risk-assessment> Date of access: 6 Jul. 2025.

Verhelst, D., Monge, M., Meynard, J.L., Fouqueray, B., Mougenot, B., Girard, P.M., Ronco, P. and Rossert, J. 2002. Fanconi syndrome and renal failure induced by tenofovir: a first case report. *American Journal of Kidney Diseases*, 40(6):1331–1333.

Vogt, B., Bouwman, H., Bezuidenhout, C., Horn, S., Vogt, T., Bothma, L., Gerber, E., van Aswegen, D., Blom, K., Fouché, D. and Potgieter, J. 2020. *Quantification, Fate and Hazard Assessment of HIV-ARVs in Water Resources*. Pretoria: Water Research Commission. (WRC Report No. TT 813/20).

Webb, S., Ternes, T., Gibert, M. and Olejniczak, K. 2003. Indirect human exposure to pharmaceuticals via drinking water. *Toxicology letters*, 142(3):157–167.

Webster, J. and Iqani, M. 2024. Johannesburg's shitty little river: Faecal discourse and discontent regarding the Jukskei. *Social Dynamics*, 50(1):109–127.

WHO. World Health Organization. 1994. *Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits*. World Health Organization.

WHO. World Health Organization. 2004. World Health Organization and Inter-Organization Programme for the Sound Management of Chemicals, *IPCS risk assessment terminology*, (1).

WHO. World Health Organization. 2008. Human Health Risk Assessment Toolkit: Chemical Hazards. [Online].
https://apps.who.int/iris/bitstream/handle/10665/44458/9789241548076_eng.pdf Date of access: 8 Jul. 2025.

Wong, M., Kumar, L., Jenkins, T.M., Xagorarakis, I., Phanikumar, M.S. and Rose, J.B. 2009. Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. *Water Research*, 43(4):1137–1149.

Xie, M., Ziemba, T.M. and Maurin, M.B. 2003. Sublimation characterization and vapor pressure estimation of an HIV nonnucleoside reverse transcriptase inhibitor using thermogravimetric analysis. *American Association of Pharmaceutical Scientists PharmSciTech*, 4:99–108.

Zhang, S., Han, Y., Peng, J., Chen, Y., Zhan, L. and Li, J. 2023. Human health risk assessment for contaminated sites: A retrospective review. *Environment International*, 171:107700.

CHAPTER 6 A MULTI-CRITERIA MODEL FOR THE INTEGRATED PRIORITISATION OF ANTIRETROVIRAL DRUGS IN AQUATIC ENVIRONMENTS

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Abstract

Antiretroviral drugs (ARVDs), widely used in the treatment of HIV/AIDS (human immunodeficiency virus/ acquired immunodeficiency syndrome), are increasingly detected in aquatic environments, raising concerns about their potential human and ecological health risks. The current study developed and applied a multi-criteria decision analysis (MCDA) model to prioritise 19 clinically relevant ARVDs commonly used in South Africa, based on environmental occurrence, toxicity, and exposure data. Six weighted and normalised indicators were integrated to compute a final priority score (FPS) for each compound: hazard quotient score, hazard index, drinking water contribution, ecological risk score, priority index, and reliability function. Among the evaluated ARVDs, abacavir (ABC), lopinavir (LPV), emtricitabine (FTC), efavirenz (EFV), and nevirapine (NVP) emerged as highest-priority, with ABC demonstrating the greatest overall hazard potential across both human health and ecological risk metrics. The results emphasise the urgent need for targeted environmental monitoring and regulatory attention to specific ARVDs, especially in regions with high pharmaceutical usage and limited water quality management. This integrative model supports evidence-based prioritisation in water quality management, advancing both ecopharmacovigilance efforts and public health protection strategies in regions burdened by high pharmaceutical use, not just South Africa, but elsewhere as well. The model is adaptable, can include many more compounds and may serve as a decision-support tool for policymakers and environmental authorities globally.

Keywords: antiretroviral drugs; water contamination; human health hazard; ecotoxicological risk.

6.1 Introduction

Despite their critical role in managing HIV/AIDS (human immunodeficiency virus/ acquired immunodeficiency syndrome), antiretroviral drugs (ARVDs) are increasingly recognised as emerging contaminants in water systems. Designed to be pharmacologically potent and persistent (Ramana *et al.*, 2014), many ARVDs evade complete removal during conventional wastewater treatment (Adeola and Forbes, 2022), resulting in their accumulation in surface and drinking water systems. However, despite this growing environmental presence, ARVDs remain largely unregulated and understudied in terms of risk prioritisation and are not currently classified as priority substances under most international water quality frameworks (Bub *et al.*, 2025; Viana *et al.*, 2021).

This regulatory gap is specifically concerning in high-burden regions such as South Africa, where large-scale antiretroviral therapy (ART) programmes have contributed to the widespread environmental dissemination of ARVDs. Studies have confirmed the presence of these compounds in South African surface and drinking water (Abafe *et al.*, 2018; Horn *et al.*, 2022; Vogt *et al.*, 2020), raising concerns about their potential ecotoxicological effects and chronic exposure risks to vulnerable human populations (Abers *et al.*, 2014; Mangesi and Mngeni, 2024; Ncube *et al.*, 2018; Verhelst *et al.*, 2002).

Existing environmental risk assessments for pharmaceuticals often focus either on ecological or human health effects in isolation (Al Aukidy *et al.*, 2012; Guleria, and Chakma, 2021), limiting their capacity to holistically identify substances of highest concern. To address this limitation, the present study proposes an integrative multi-criteria decision analysis (MCDA) framework that combines multiple exposure and hazard endpoints into a unified prioritisation model. This approach enables the systematic ranking of ARVDs based on environmental occurrence, ecotoxicity, human health hazard, and the recurrence of exposure across water sources.

Integrated approaches are rarely published (Olson *et al.*, 2017; Spiesman and Speight, 2014) because of differences in risk evaluations (WHO, 2022) and data scarcity (Prüss-Ustün *et al.*, 2017). Accordingly, integrated frameworks for the assessment of the drinking water risk posed by emerging contaminants are needed (Hartman *et al.*, 2021). The primary objective of the present study was to apply the adapted MCDA model to prioritise 19 commonly used ARVDs in South Africa, identifying those posing the greatest combined risk to human and environmental health.

The model was informed by and expanded upon existing methodologies (Hartmann *et al.*, 2021; Kumar and Xagorarakis, 2010; Sang *et al.*, 2022; Woods *et al.*, 2016), with specific modifications to address the South African context and data availability. The resulting prioritisation serves to guide future environmental monitoring, regulatory efforts, and eco-pharmacovigilance strategies in water-stressed and pharmaceutically impacted regions.

6.1.1 Methods

The present study was approved by the NWU Ethics Committee (NWU-00002-23-A1) and employed a MCDA model to prioritise ARVDs according to a priority control list based on their combined ecological and hazard potential. The approach integrates multiple hazard and exposure metrics into a final prioritisation score (FPS) to support evidence-based decision-making (Clahsen *et al.*, 2020) in water quality management.

6.1.2 Data collection

Environmental occurrence data for ARVDs were obtained from published literature and validated analytical studies conducted between 2015 and 2023 in South Africa (Annexures A–Annexures D). Toxicological endpoints and health-based reference doses were compiled from peer-reviewed databases and regulatory documents. All relevant risk and index metrics used in this MCDA were derived from data and calculations presented in previous chapters (see Chapters 4–5). These values were integrated into the MCDA model as pre-calculated inputs representing compound-specific human hazard and ecological risk indicators.

6.1.3 Ecological risk scoring (ECS) and prioritisation index (P_{index})

Consistent with previous ecological risk assessments (Roveri *et al.*, 2022), the current study calculated the risk quotient (RQ) for algae, daphnids, and fish. For balanced ecological representation across trophic levels, average RQs were used. This treats all trophic levels equally and reflects overall risk without biasing the score toward one trophic level. Higher ECS values suggest greater potential toxicological risk to multiple aquatic species (algae, crustaceans, and fish). High ECS can also reflect continuously elevated RQ values across trophic levels, rather than a single outlier based on average values.

$$ECS(i) = \frac{RQ_{algae} + RQ_{crust} + RQ_{fish}}{3} \quad 6-1$$

Where:

$ECS(i)$ = final ecological risk score for compound (i), representing its average impact on aquatic organisms;

RQ_{algae} = risk quotient calculated for algae;

RQ_{crust} = risk quotient calculated for crustaceans;

RQ_{fish} = risk quotient calculated for fish.

To strengthen the model, the priority index (P_{index}) based on the frequency of RQ exceedance was included (Desbiolles *et al.*, 2018; Liu *et al.*, 2020). This index captures how often an ARVD exceeds the ecological risk threshold ($RQ > 1$) (Grung *et al.*, 2008) across multiple sites and sampling events.

6.1.4 Reliability scoring and inversion for priority ranking

The reliability function (ξR) was incorporated into the overall scoring model to reflect the probability and persistence of risk across water sources (Guleria and Chakma, 2021). Higher ξR values indicate greater potential for concern due to frequent exceedance of safety thresholds ($HQ > 1$) (Table 6-1), as supported in mixture toxicity literature (Henri *et al.*, 2015; Li *et al.*, 2024). A compound-specific average exceedance probability ($\xi R_{\text{total}(i)}$) was calculated across the four exposure pathways according to Eq. 6-2:

$$\xi R_{\text{total}(i)} = \frac{\xi R_{\text{sw}} + \xi R_{\text{dw}} + \xi R_{\text{eff}} + \xi R_{\text{inf}}}{4} \quad 6-2$$

Where:

$\xi R_{\text{total}(i)}$ = total reliability function for compound (i);

ξR_{sw} = reliability of surface water;

ξR_{dw} = reliability of drinking water;

ξR_{eff} = reliability of effluent;

ξR_{inf} = reliability of influent.

Table 6-1: The interpretation of reliability values and their implications for prioritisation.

$\xi R_{\text{total}(i)}$	Interpretation	Priority implication
≥ 0.8	Frequent exceedance	High priority
$0.5 \leq \xi R_{\text{total}(i)} < 0.8$	Occasional exceedance	Moderate priority
< 0.5	Rare/no exceedance	Low priority

$\xi R_{\text{total}(i)}$ = total reliability function for compound (i).

6.1.5 Hazard index score

According to USEPA (2005), cumulative non-cancer hazards should be represented as the sum of hazard index (HI) across exposure pathways. In the current study, this principle was

adapted by calculating the arithmetic mean of maximum HI values across different population and source combinations for each compound. This yielded a simplified composite hazard index score (HI_{score}). Allowing integration of population-level exposure scenarios into a prioritisation model. The HI_{score} for each compound (i) was calculated using E.q.6-3, based on the HI approach of Dixit *et al.* (2024).

$$HI_{score(i)} = \frac{1}{|X|} \sum_{x \in X}^n \max(HI_{i,x}) \quad 6-3$$

Where:

$HI_{score(i)}$ = mean hazard index for compound (i) across all sources (s);

$\max(HI_{i,x})$ = maximum hazard index for compound (i) via water source (x);

n = is the total number of sources (x) included in the calculation;

X = is the set of water sources (surface, drinking, effluent, influent);

$|X|$ = is the number of water sources in the set (here, $|X| = 4$).

This method provides a composite indicator of chronic non-carcinogenic hazard potential, taking into account differences in exposure and vulnerability among various population groups and water sources. By averaging the maximum HI values across all relevant combinations, this approach ensures that each pathway and sub-population is weighted equally, allowing for clear comparison and prioritisation across compounds.

6.1.6 Drinking water contribution

To assess the relative importance of drinking water as an exposure route in the overall human health hazard posed by each ARVD, a drinking water contribution (DWC) score was calculated. This score quantifies the proportion of total hazard attributable specifically to drinking water exposure (adapted from Sang *et al.*, 2020) and was computed for each compound (i) using Eq. 6-4. This formula assumes equal weighting of population groups.

$$DWC(i) = \frac{\sum_{g \in G} HQ_{g, drinking}^{(i)}}{\sum_{x \in X} \left(\sum_{x \in X} HQ_{g, x}^{(i)} \right)} \quad 6-4$$

Eq. 6-4 can be broken down into the following parts (Eq. 6-5 to 6-7):

$$HQ_{g, drinking}^{(i)} = \sum_{g \in G} HQ_{g, drinking}^{(i)} \quad 6-5$$

$$HQ_{total}^{(i)} = \sum_{g \in G} \left(\sum_{x \in X} HQ_{g,x}^{(i)} \right) \quad 6-6$$

$$DWC(i) = \frac{HQ_{g,drinking}^{(i)}}{HQ_{total}^{(i)}} \quad 6-7$$

Where:

$DWC_{(i)}$ = drinking water contribution for compound (i);

$HQ_{g,x}^{(i)}$ = hazard quotient value for group (g) and water source (x) for compound (i);

G = is the set of population groups (adults, children, pregnant women);

X = is the set of water sources (surface, drinking, effluent, influent);

HQ_{dw} = is the average hazard quotient for drinking water;

HQ_{total} = is the total hazard quotient for compound (i) across all water sources and population groups.

6.1.7 Hazard quotient (HQ) scoring

To capture the maximum potential health hazard posed by each ARVD under any exposure scenario, the HQ_{score} was defined as the maximum HQ observed across all population groups (g) and water sources (x). This approach reflects a worst-case or acute exposure scenario, consistent with the precautionary principle (Carolan, 2007) and methods outlined by Sang *et al.* (2022). The HQ_{score} was calculated as follows:

$$HQ_{score(i)} = \max \{ HQ_{g,x}^{(i)} \} \quad 6-8$$

Where:

$HQ_{score(i)}$ = hazard quotient score for compound (i);

Max = maximum hazard quotient value from the set;

$HQ_{g,x}^{(i)}$ = is the hazard quotient for compound (i), in population group (g), and exposure routes (x).

By selecting the maximum HQ value among all population-exposure combinations, this metric emphasises compounds with the highest observed hazard, regardless of how limited or localised the scenario may be. This ensures that ARVDs posing acute or disproportionate toxic potentials to specific vulnerable groups are not masked by averaged values. This worst-case-driven approach is aligned with public health prioritisation frameworks (Sharma *et al.*, 2019), where substances may warrant further control or monitoring if even a single exposure scenario presents a significant risk.

As shown in Sang *et al.* (2022), incorporating maximum estimates into prioritisation models strengthens their ability to identify compounds that might otherwise be underestimated under mean-based assessments.

6.1.8 Data normalisation and weighting

To ensure comparability across variables with different units and scales, all quantitative components contributing to the overall prioritisation score were normalised (Hartmann *et al.*, 2021) using min–max normalisation (Jahan and Edwards, 2015; Vafaei *et al.*, 2016). This standardisation technique scales values to a common range of 0 to 1, allowing for unbiased integration of metrics such as hazard indices, reliability scores, and drinking water contributions. The normalised value for each data point X was calculated using the Eq. 6-9:

$$\text{Normalised value} = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad 6-9$$

Where X is the original, unscaled value of the variable; $X_{\min}$ and $X_{\max}$ are the minimum and maximum observed values for that variable across all compounds. This transformation rescales all variables to a uniform range.

To integrate multiple variables of health and environmental hazard into a final priority score, each component was also assigned a weight factor (w_n) reflecting its relative importance in the context of the study's emphasis on human health relevance (Eq. 6-10). The weights were determined based on judgment, literature precedent, and the conceptual significance of each component in the prioritisation model (Hartmann *et al.*, 2021; Kumar and Xagorarakis, 2010; LeBreton and Tonidandel, 2008). The weights assigned to the six normalised components are shown in Table 6-2. The component weights w_1 – w_6 were set to reflect the study's emphasis on both human health and environmental relevance.

$$w_1 + w_2 + w_3 + w_4 + w_5 + w_6 = 1 \quad 6-10$$

Table 6-2: Weight factors assigned to each variable for a human health emphasis.

Component	Weight
HQ _{score} (<i>norm</i>)	0.3
HI(<i>norm</i>)	0.2
DWC(<i>norm</i>)	0.2
ECS(<i>norm</i>)	0.15
PII(<i>norm</i>)	0.1
ξR(<i>norm</i>)	0.05

The FPS for each ARVD was calculated as a weighted sum of six normalised components (Eq. 6-11 to 6-12):

$$FPS_{(i)} = w_1 \cdot ECS(norm) + w_2 \cdot PId(norm) + w_3 \cdot \xi R + w_4 \cdot HI(norm) + w_5 \cdot HQscore(norm) + w_6 \cdot DWC(norm) \quad 6-11$$

$$FPS_{(i)} = 0.3 \cdot ECS(norm) + 0.1 \cdot PId(norm) + 0.05 \cdot \xi R + 0.2 \cdot HI(norm) + 0.3 \cdot HQscore(norm) + 0.2 \cdot DWC(norm) \quad 6-12$$

Where:

$FPS_{(i)}$ = final priority score for compound (i);

(norm) = each component is normalised between 0 and 1 for comparability;

w_n = weight assigned to each variable.

6.2 Results

The FPS for each ARVD is illustrated in Table 6-3. This weighted aggregation enables the ranking of compounds based on combined health and environmental relevance, with higher scores indicating greater concern and higher priority for monitoring or control. Table 6-3 presents the ranked ARVD priority control list. The top five most hazardous ARVDs according to the priority control list were as follows: ABC > LPV > FTC > EFV > NVP.

Table 6-3: Ranked antiretroviral drug (ARVD) priority control list based on final priority scores (FPS) weighted and normalised data.

ARVD	ECS	P _{index}	ξR _{total}	HI	HQ _{score}	DWC	FPS	Ranking
ABC	0.003		0.76	1	1	1.1x10 ⁻⁶	0.539	1
LVP	1	0.04	0.94	0.096	0.06	0.108	0.409	2
FTC	0.003	2.6x10 ⁻⁴	0.71	0.618	0.634		0.35	3
EFV	0.146	1	1	0.265	0.17	0.207	0.34	4
NVP	3x10 ⁻⁴		0.53	0.7	0.48	0.002	0.312	5
ddl	4.7x10 ⁻⁶		0.07	0.034	0.045	0.991	0.222	6
DCC	1.9x10 ⁻⁷		0.29		0.024	1	0.22	7
3TC	1.1x10 ⁻⁵		0.48	0.42	0.37		0.219	8
NFV	3.4x10 ⁻⁴			1.1x10 ⁻⁶	0	0.81	0.162	9
ZDV	0.057	0.03	0.61	0.116	0.08	0.017	0.102	10
RTV	0.004	0.003	0.93	0.01	0.005	0.22	0.095	11
TDF	0.001		0.29	0.136	0.12		0.078	12
DRV	0	0.001	0.36	0.03	0.04		0.036	13
RAL			0.41	0.014	0.016		0.028	14
ATV		0.001	0.33	0.01	0.006		0.02	15
d4T	1.6x10 ⁻⁴		0.17	0.007	0.009	0.003	0.013	16
IDV			0	2x10 ⁻⁴	2.7x10 ⁻⁴		1.2x10 ⁻⁴	17
MRV			0	1.7x10 ⁻⁴	1.9x10 ⁻⁴		9.3x10 ⁻⁵	18
SQV	0		0	5.7x10 ⁻⁵	8.2x10 ⁻⁵		3.6x10 ⁻⁴	19

3TC = lamivudine; ABC = abacavir; ATV = atazanavir; bold values = highest score; d4T = stavudine; DCC = zalcitabine; ddl = didanosine; DRV = darunavir; DWC = drinking water contribution; ECS = ecological risk score; EFV = efavirenz; empty cells = no data available; FTC = emtricitabine; HI = hazard index; HQscore = hazard quotient score; IDV = indinavir; LPV = lopinavir; MRV = maraviroc; NFV = nelfinavir; NVP = nevirapine; P_{index} = prioritisation index; RAL = raltegravir; RTV = ritonavir; SQV = saquinavir; TDF = tenofovir; ZDV = zidovudine; ξR_{total} = total reliability function.

6.3 Discussion

This prioritisation approach ensured that both human health and environmental endpoints were considered in a specific South African context. By quantifying the recurrence of risk in everyday environmental monitoring data, the P_{index} complements indicators such as the HI and HQ. While HQ and HI reflect the potential severity of human health impacts, the P_{index} identifies compounds that constantly exceeded safety parameters, representative of persistent or widespread ecological risk.

This metric is particularly valuable for identifying compounds that pose lower-level but frequent risks, which might be overlooked if only single exposure assessments are considered. Together with the ξR and DWC, this model provided a comprehensive, precautionary framework that accounted for both acute and chronic risks, as well as exposure recurrence.

The ARVDs with the highest FPS (ABC > LPV > FTC > EFV > NVP) are those that simultaneously demonstrate foremost concern across multiple hazard dimensions. These compounds are the most hazardous to human health under standard and extreme exposure scenarios, those that contribute meaningfully through drinking water exposure pathways, and those that exhibit notable ecotoxicological potential in aquatic environments as well as observed environmental recurrence of risk.

Abacavir ranked 1st, with a high score across all human health-related indicators and FPS (Table 6-3). This compound has concerning maximum (HQ) and mean cumulative (HI) exposure potentials. Despite its low DWC, its toxicological profile and frequency of safety threshold exceedance (ξR_{total}) made it a top-priority. ABC's dominance supports its urgent consideration for regulatory focus and targeted environmental monitoring. ABC is one of the most potent nucleoside reverse transcriptase inhibitors (NRTIs) used in ART (Nannou *et al.*, 2020).

Notably, Swanepoel *et al.* (2015) were the only authors to document ABC, alongside ddl, NFV, and d4T, in drinking water samples. Despite its widespread and continuous clinical use, data on ABCs' presence in South African aquatic environments remain limited, with only a few studies documenting its occurrence in wastewater (Abafe *et al.*, 2018; Ngwenya and Mahlambi, 2023; Späth *et al.*, 2021; Xolo and Mahlambi, 2024).

Viana *et al.* (2022) support prioritising ABC due to its exclusive human use and frequent detection in Portuguese waters, advocating regulatory action and eco-pharmacovigilance. Their stance aligns with the current study's monitoring goals. The findings by Selwe *et al.* (2023) in wastewater in Botswana also provide strong regional support for the prioritisation of ABC within Southern African environmental monitoring frameworks.

FTC was also identified as a high-priority compound due to its hazard potential for human health and high probability of hazard exceedance across water sources, though it scored low in terms of environmental priority concerns. The lack of DWC data might have caused an underestimation of the actual exposure potential. To support precautionary action, drinking water pathways warrant further investigation. The ECS for each compound was calculated as a mean, thereby treating each trophic level equally and reflecting collective ecological impact without overemphasising a single species.

LPV, with an ECS of one, emerged as the most ecotoxic ARVD, suggesting considerable disruption potential in aquatic ecosystems. LPV and FTC should be a priority for environmental regulation, even if it is not the most hazardous ARVDs towards humans. EFV had a moderate ECS (0.15), yet it had the highest P_{index} , representative of a great environmental presence and frequent risk exceedance, highlighting its ecological stress potential. Similarly, EFV had a total reliability function of one, representing a high probability of hazard exceedance in all water sources.

Some ARVDs with known clinical importance (DRV, MRV, and RAL) had low prioritisation in this integrated model, likely due to low HQ values, limited environmental detection and toxic concerns. Nevertheless, data gaps or underreporting could have masked hazard potential. In contrast, several older generation ARVDs (ddl and DDC) warrant renewed scrutiny owing to high DWC scores and persistent legacy effects. Sang *et al.* (2022) highlighted the significance of drinking water pathways in disproportionate risk estimation, underlining the need to integrate DWC into prioritisation frameworks for regulatory relevance. Incorporating DWC into this prioritisation model ensured that ARVDs relevant to public health protection are appropriately emphasised, particularly in contexts where drinking water standards are a central focus of regulation.

Didanosine and DDC, introduced in South Africa in the early 2000s and 1990s respectively (FDA approval records), were widely used in second-line HIV treatment (Nassar *et al.*, 1993; Steegen *et al.*, 2014) before being phased out due to severe adverse effects including significant peripheral neuropathy (RxList, 2021). Despite their discontinued clinical use (DDC (Hivid®) was globally withdrawn by 2006) the elevated ranking in the top half of priority ARVDs reflects concerns regarding environmentally persistent ARVDs, the legacy effects arising from historical use, and the toxic potency of these substances. This emphasises the necessity of incorporating both bequest and currently utilised pharmaceuticals in monitoring frameworks.

6.4 Conclusions

This research introduces a comprehensive, integrated prioritisation model designed to identify ARVDs that pose the greatest concern for both human and ecological health within South African aquatic environments. By integrating multiple exposure and hazard indicators into a weighted multi-criteria model, the approach enabled evidence-based ranking of selected pharmaceutical contaminants. Abacavir emerged as the top-priority compound, warranting immediate consideration for inclusion in targeted environmental monitoring and regulatory assessments.

This approach aligns with international initiatives, such as the World Health Organization's guidance on pharmaceuticals in drinking water, the European Union's Water Framework Directive and Watch List mechanism, and the Organisation for Economic Co-operation and Development (OECD) recommendations on pharmaceutical pollution mitigation. It also supports Sustainable Development Goal 6, which advocates for improved water quality through pollution reduction and safe reuse.

Given the high ARVD burden in South Africa, it is recommended that the Department of Water and Sanitation incorporate such prioritisation tools into national water quality monitoring strategies. Doing so would bolster eco-pharmacovigilance efforts and facilitate the development of health-protective guidelines wherever used. Moreover, this model has the potential to inform regulatory decision-making on both legacy and emerging contaminants, thereby ensuring effective safeguarding of public health and ecosystem integrity.

6.5 References

- Abafe, O.A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., ... Martincigh, B.S. 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, 200:660–670.
- Abers, M.S., Shandera, W.X. and Kass, J.S. 2014. Neurological and psychiatric adverse effects of antiretroviral drugs. *CNS Drugs*, 28:131–145.
- Adeola, A.O. and Forbes, P.B. 2022. Antiretroviral drugs in African surface waters: prevalence, analysis, and potential remediation. *Environmental Toxicology and Chemistry*, 41(2):247–262.
- Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M. and Barcelò, D. 2012. Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. *Science of the Total Environment*, 438:15–25.
- Bub, S., Petschick, L.L., Stehle, S., Wolfram, J. and Schulz, R. 2025. Limitations of chemical monitoring hinder aquatic risk evaluations on the macroscale. *Science*, 388(6753):1301–1305.
- Clahsen, S.C., van Klaveren, H.S., Vermeire, T.G., Van Kamp, I., Garssen, B., Piersma, A.H. and Lebret, E. 2020. Understanding conflicting views of endocrine disruptor experts: a pilot study using argumentation analysis. *Journal of Risk Research*, 23(1):62–80.
- Carolan, M.S. 2007. The precautionary principle and traditional risk assessment: Rethinking how we assess and mitigate environmental threats. *Organization and Environment*, 20(1):5–24.
- Desbiolles, F., Malleret, L., Tiliacos, C., Wong-Wah-Chung, P. and Laffont-Schwob, I. 2018. Occurrence and ecotoxicological assessment of pharmaceuticals: is there a risk for the Mediterranean aquatic environment? *Science of the Total Environment*, 639:1334–1348.
- Dixit, A., Pandey, H., Rana, R., Kumar, A., Herojeet, R., Lata, R., ... Sarkar, B. 2024. Ecological and human health risk assessment of pharmaceutical compounds in the Sirsa River of Indian Himalayas. *Environmental Pollution*, 347:123668.

- Grung, M., Källqvist, T., Sakshaug, S., Skurtveit, S. and Thomas, K.V. 2008. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. *Ecotoxicology and Environmental Safety*, 71(2):328–340.
- Guleria, A. and Chakma, S. 2021. Fate and contaminant transport model-driven probabilistic human health risk assessment of DNAPL-contaminated site. *Environmental Science and Pollution Research*, 28(12):14358–14371.
- Hartmann, J., Chacon-Hurtado, J.C., Verbruggen, E., Schijven, J., Rorije, E., Wuijts, S., de Roda Husman, A.M., van der Hoek, J.P. and Scholten, L. 2021. Model development for evidence-based prioritisation of policy action on emerging chemical and microbial drinking water risks. *Journal of Environmental Management*, 295:112902.
- Henri, C.V., Fernández-Garcia, D. and de Barros, F.P. 2015. Probabilistic human health risk assessment of degradation-related chemical mixtures in heterogeneous aquifers: Risk statistics, hot spots, and preferential channels. *Water Resources Research*, 51(6):4086–4108.
- Horn, S., Vogt, T., Gerber, E., Vogt, B., Bouwman, H. and Pieters, R. 2022. HIV-antiretrovirals in river water from Gauteng, South Africa: Mixed messages of wastewater inflows as source. *Science of The Total Environment*, 806:150346.
- Jahan, A. and Edwards, K.L. 2015. A state-of-the-art survey on the influence of normalization techniques in ranking: Improving the materials selection process in engineering design. *Materials and Design (1980-2015)*, 65:335–342.
- Kumar, A. and Xagorarakis, I. 2010. Pharmaceuticals, personal care products and endocrine-disrupting chemicals in US surface and finished drinking waters: a proposed ranking system. *Science of the Total Environment*, 408(23):5972–5989.
- LeBreton, J.M. and Tonidandel, S. 2008. Multivariate relative importance: extending relative weight analysis to multivariate criterion spaces. *Journal of Applied Psychology*, 93(2):329.
- Li, C., Li, A., Hui, X., Wang, A., Wang, L. and Chang, S. 2024. Concentrations, probabilistic human and ecological risks assessment attribute to antibiotics residues in river water in China: Systematic review and meta-analysis. *Ecotoxicology and Environmental Safety*, 285:117022.

Liu, N., Jin, X., Yan, Z., Luo, Y., Feng, C., Fu, Z., ... Giesy, J.P. 2020. Occurrence and multiple-level ecological risk assessment of pharmaceuticals and personal care products (PPCPs) in two shallow lakes of China. *Environmental Sciences Europe*, 32:1–10.

Mangesi, T. and Mngeni, A. 2024. Vulnerability of Rural Communities to Contaminated Water and Its Implications: A Case Study from KSD Local Municipality. *Environmental and Earth Sciences Research Journal*, 11(4).

Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E. and Lambropoulou, D. 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. *Science of The Total Environment*, 699:134322.

Nassar, M.N., Chen, T., Reff, M.J. and Agharkar, S.N. 1993. Didanosine. In: H.G. Brittain, ed. *Analytical profiles of drug substances and excipients*. Vol. 22. San Diego: Academic Press.

Ncube, S., Madikizela, L.M., Chimuka, L. and Nindi, M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. *Water Research*, 145:231–247.

Ngwenya, N. and Mahlambi, P. 2023. Methods optimization and application: Solid phase extraction, ultrasonic extraction and Soxhlet extraction for the determination of antiretroviral drugs in river water, wastewater, sludge, soil and sediment. *Journal of Pharmaceutical and Biomedical Analysis*, 230:115358.

Olson, G., Wilczak, A., Boozarpour, M., Degraça, A. and Weintraub, J.M. 2017. Evaluating and prioritizing contaminants of emerging concern in drinking water. *Journal (American Water Works Association)*, 109(12):54–63.

Prüss-Ustün, A., Wolf, J., Corvalán, C., Neville, T., Bos, R. and Neira, M. 2017. Diseases due to unhealthy environments: an updated estimate of the global burden of disease attributable to environmental determinants of health. *Journal of Public Health*, 39(3):464–475.

Ramana, L.N., Anand, A.R., Sethuraman, S. and Krishnan, U.M. 2014. Targeting strategies for delivery of anti-HIV drugs. *Journal of Controlled Release*, 192:271–283.

Roveri, V., Guimarães, L.L., Toma, W. and Correia, A.T. 2022. Occurrence, ecological risk assessment and prioritization of pharmaceuticals and abuse drugs in estuarine waters along the São Paulo coast, Brazil. *Environmental Science and Pollution Research*, 29(59):89712–89726.

RxList. 2021. *Hivid (zalcitabine): Side Effects, Uses, Dosage, Interactions, Warnings*. Available at: <https://www.rxlist.com/hivid-drug.htm> Date of access: 16 Jul. 2025.

Sang, C., Yu, Z., An, W., Sørensen, P.B., Jin, F. and Yang, M. 2022. Development of a data driven model to screen the priority control pesticides in drinking water based on health risk ranking and contribution rates. *Environment International*, 158:106901.

Selwe, K.P., Sallach, J.B. and Dessent, C.E. 2024. Nontargeted screening of contaminants of emerging concern in the glen valley wastewater treatment plant, Botswana. *Environmental Toxicology and Chemistry*, 43(1):52–61.

Sharma, B.M., Bečanová, J., Scheringer, M., Sharma, A., Bharat, G.K., Whitehead, P.G., Klánová, J. and Nizzetto, L. 2019. Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. *Science of the Total Environment*, 646:1459–1467.

Späth, J., Arumugam, P., Lindberg, R.H., Abafe, O.A., Jansson, S., Fick, J. and Buckley, C.A. 2021. Biochar for the removal of detected micropollutants in South African domestic wastewater: a case study from a demonstration-scale decentralised wastewater treatment system in eThekweni. *Water SA*, 47(4):396–416.

Spiesman, A.L. and Speight, V.L. 2014. A risk-based methodology for contaminant prioritization. *Journal-American Water Works Association*, 106(3):E150–E159.

Steegen, K., Levin, L., Ketseoglou, I., Bronze, M., Papathanasopoulos, M.A., Carmona, S. and Stevens, W. 2014. High-level cross-resistance to didanosine observed in South African children failing an abacavir-or stavudine-based 1st-line regimen. *PLoS One*, 9(5):e97067.

Swanepoel, C., Bouwman, H., Pieters, R. and Bezuidenhout, C. 2015. *Presence, concentrations and potential implications of HIV-anti-retrovirals in selected water resources in South Africa*. Water Research Commission. WRC Report, 2144(1):14.

USEPA (United States Environmental Protection Agency). 2005. *Human Health Risk Assessment Protocol (HHRAP) for Hazardous Waste Combustion Facilities: Volume I – Human Health – Chapter 7: Characterizing Risk and Hazard*. Washington, DC: U.S. Environmental Protection Agency. Region 6 and Office of Solid Waste and Emergency Response.

Vafaei, N., Ribeiro, R.A. and Camarinha-Matos, L.M. 2016. Normalization techniques for multi-criteria decision making: analytical hierarchy process case study. In: *Doctoral conference on computing, electrical and industrial systems*. Cham: Springer International Publishing.

Verhelst, D., Monge, M., Meynard, J.L., Fouqueray, B., Mougenot, B., Girard, P.M., Ronco, P. and Rossert, J. 2002. Fanconi syndrome and renal failure induced by tenofovir: a first case report. *American Journal of Kidney Diseases*, 40(6):1331–1333.

Viana, P., Meisel, L., Lopes, A., De Jesus, R., Sarmento, G., Duarte, S., ... and Oliveira, M.C. 2021. Identification of antibiotics in surface-groundwater. A tool towards the ecopharmacovigilance approach: A portuguese case-study. *Antibiotics*, 10(8):888.

Vogt, B., Bouwman, H., Bezuidenhout, C., Horn, S., Vogt, T., Bothma, L., Gerber, E., van Aswegen, D., Blom, K., Fouché, D. and Potgieter, J. 2020. *Quantification, Fate and Hazard Assessment of HIV-ARVs in Water Resources*. Pretoria: Water Research Commission. (WRC Report No. TT 813/20).

WHO (World Health Organization). 2022. *Guidelines for drinking-water quality: incorporating the first and second addenda*. 4th ed. Geneva: World Health Organization.

Woods, M., Crabbe, H., Close, R., Studden, M., Milojevic, A., Leonardi, G., Fletcher, T. and Chalabi, Z. 2016. Decision support for risk prioritisation of environmental health hazards in a UK city. *Environmental Health*, 15(Suppl 1):S29.

Xolo, T. and Mahlambi, P. 2024. Molecularly imprinted polymers as solid-phase and dispersive solid-phase extraction sorbents in the extraction of antiretroviral drugs in water: adsorption, selectivity and reusability studies. *Journal of Analytical Science and Technology*, 15(1):11.

CHAPTER 7 CONCLUSIONS

7.1 Link to overall thesis objectives Introduction

This chapter provides a comprehensive summary of the thesis, synthesising the research conducted across all chapters and addressing the overarching hypothesis, sub-hypotheses, objectives, and aims.

Main hypothesis: “In South Africa, ARVDs in freshwaters pose an increasing ecological and public health hazard, especially in regions with intensive antiretroviral treatment rollout and inadequate environmental monitoring.”

Together, the sub-hypotheses in Table 7-1 substantiate the main hypothesis by providing a multi-layered view of how ARVD pollution arises, persists, and threatens ecological and public health, particularly in a resource-constrained and heavily HIV/AIDS-burdened regions of South Africa. Table 7-1 provides strong, layered support for the main hypothesis by establishing the presence and persistence of ARVDs; tracing patterns to regulatory gaps; and highlighting consequences on ecosystems and public health.

Subsequently, the research provides enough empirical and observational evidence to accept the main hypothesis.

Table 7-1: Summary of sub-hypotheses, outcomes, and rationales.

Hypothesis type	Statement	Accepted	Rationale
Proposition/observational (Chapters 2 and 4)	Current wastewater treatment technologies in South Africa are insufficient for removing ARVDs to environmentally safe levels.	Yes	Descriptive: Current WWTP technologies are ineffective at removing ARVDs to safe environmental concentrations. Data show 15 ARVDs in wastewater (0.3 ng/L–172 µg/L), some with negative removal efficiencies at WWTPs, exceeding safety thresholds. Most ARVDs had RQs > low-risk threshold, posing environmental risks in both effluent and influent. Existing waste water treatment technologies are not designed for pharmaceutical removal, and toxicity data confirms ecologically significant concentrations of ARVDs in surface water.
Proposition/observational (Chapter 2)	Absence of national monitoring guidelines results in inconsistent detection and reporting of ARVDs across provinces.	Yes	Descriptive: Literature review and interprovincial comparisons showed inconsistencies in data and regulation, with data gaps in nearly half of South Africa's regions and large variability in analyte concentrations. The review revealed a stark imbalance in the spatial distribution of ARVD monitoring efforts, with data available for only some of the provinces and disproportionately concentrated in a few high-density urban areas. The lack of a centralised regulatory framework appears to have resulted in ad hoc and inconsistent sampling efforts, which hinder comprehensive national risk assessments.
Testable H ₁ (Chapter 3)	ARVD concentration in river water will be higher at effluent-receiving and downstream sites compared to upstream sites.	No, H ₁ is rejected. H ₀ is accepted.	Empirical: Non-parametric tests (Mann-Whitney) revealed no significant differences between up- and downstream sites. Empirical data did not show consistent upstream-downstream gradients, with elevated concentrations only at certain discharge points. There is not enough evidence to support H ₁ . More data is required to prove the statement.
Testable H ₁ (Chapter 3)	Environmental factors such as temporal variation affect ARVD concentrations in South African waters.	No, H ₁ is rejected. H ₀ is accepted.	Empirical: Statistical analysis of temporal effects indicated that monthly variations observed were not statistically meaningful. The lack of significant sample site vs. month interaction (p = 0.42) and month-effects (p = 0.6) indicated that temporal variation is not the primary factor influencing ARVD concentrations and suggested other driving factors. There is not enough evidence to support H ₁ .

Testable H ₁ (Chapter 3)	ARVD concentrations vary significantly across different sites and river systems, showing spatial heterogeneity.	No, H ₁ is rejected. H ₀ is accepted.	Empirical: ARVD concentrations showed spatial trends and site-level variability across river systems, with certain hotspots (e.g., Klip River 5) consistently displaying high concentrations. Although, the observed concentration patterns and spatial interpolations support the presence of spatial heterogeneity in ARVD distributions, these differences were not statistically significant. As such, the hypothesis cannot be conclusively supported without more comprehensive data.
Testable H ₁ (Chapter 3)	ARVD concentrations in aquatic environments are affected by seasonal variations, reflecting hydrological factors (such as rainfall, river flow, and dilution capacity).	No, H ₁ is rejected. H ₀ is accepted.	Empirical: Although high ARVD concentrations were observed in March and August (2018), no significant month-effect was found. The limited temporal scope of the study and the presence of multiple overlapping pollution sources make it difficult to confidently attribute these patterns to hydrological processes due to seasonal variation alone. As such, the hypothesis cannot be conclusively supported without more comprehensive, long-term data across multiple wet and dry seasons.
Proposition/ observational (Chapter 4)	ARVDs in South African freshwaters pose environmental risks to aquatic ecosystems.	Yes	Descriptive: Supported by toxicity data and risk assessment results. MECs of several ARVDs exceed PNEC thresholds: RQ values for EFV, LPV, RTV, and ZDV indicate moderate to high ecological risk, especially in surface water. Ecological imbalances may occur due to the impact on primary consumers (daphnids) and primary producers (algae), with cascading effects on the entire aquatic food web.
Proposition / observational (Chapter 4)	ARVD mixtures exhibit combined ecotoxicological effects as predicted through a hybrid CA-RA model.	Yes	Descriptive: Supported by model predictions. The hybrid CA-RA model demonstrated additive effects of drugs with similar modes of action and the independent effects of compounds with different mechanisms, providing a more biologically relevant risk prediction. The model showed significant risk in surface water, effluent, and influent, particularly from RTIs and non-RTIs, with high probabilities (> 95%) of adverse effects, supporting the effectiveness of the hybrid model in predicting combined ecotoxicological effects of ARVD mixtures.
Proposition/ observational (Chapter 5)	ARVDs in South African freshwaters pose a measurable non-carcinogenic health hazard to different sub-populations.	Yes	Descriptive: Supported by toxicity data and hazard assessment results of chronic exposure in multiple scenarios. The HQ analysis in the HA1 revealed that several ARVDs (such as ABC, EFV, and LPV) posed considerable potential toxic concerns, particularly for adults and pregnant females with some concern for children. The reliability function analysis

			confirmed that several ARVDs pose frequent hazard exceedance across several water sources. EFV, LVP, ABC, FTC, NVP, and ZDV exhibited very high reliability values ($\geq 70\%$, often up to 100%) across surface- and wastewater. In a large proportion of sampled scenarios, these compounds therefore consistently exceeded safety thresholds ($HQ > 1$) suggesting a widespread and recurrent hazard.
Testable H_1 (Chapter 5)	Certain sub-populations are at greater concern from exposure to hazardous ARVDs in water sources.	Yes	Empirical: The HQ analysis highlighted significant (Kruskal-Wallis; $p < 0.0001$) variability in hazard concern across different sub-populations (adults, pregnant women, and children) in surface water. Pregnant women and children had notable high HQs from ABC, EFV, and LPV, suggesting these groups are more vulnerable to non-carcinogenic effects from ARVDs in contaminated water sources, especially in scenarios of high exposure levels.
Proposition/ observational (Chapter 5)	Mixtures of ARVDs may pose cumulative, unregulated non-carcinogenic hazards.	Yes	Descriptive: The consistently raised hazard index values across all water sources and sub-population confirmed that ARVD mixtures present cumulative, unregulated non-carcinogenic hazard concerns. All investigated water sources far surpassed safety thresholds and posed non-carcinogenic concerns for exposed populations. This suggests that these mixtures present substantial health hazards to exposed populations, including those living near or consuming contaminated water.
Proposition/ Observational (Chapter 6)	ARVDs of greatest concern can be prioritised using a weighted multi-criteria model incorporating both human health hazard and ecological risk indicators.	Yes	Descriptive: Specific ARVDs, notably ABC, LPV, and FTC, ranked high across various indicators of human health and environmental risk. These indicators encompassed HQ scores, drinking water contribution, hazard and prioritisation indices, ecological risk scores, and reliability of hazard exceedance. Their high final priority scores confirmed that the integrated scoring model effectively identified compounds of greatest concern (priority control list), supporting their prioritisation for monitoring, regulatory action, and interventions.

3TC = lamivudine; ABC = abacavir; ARVD = antiretroviral drug; CA-RA = concentration addition-response addition; DRV = darunavir; EFV = efavirenz; FTC = emtricitabine; H_0 = null hypothesis (represents the assumption that there is no effect or no difference); H_1 = alternative hypothesis (represents the assumption that there is an effect or a difference); HA1 = hazard assessment approach 1; HQ = hazard quotient; LPV = lopinavir; MEC = measured environmental concentration; NVP = nevirapine; P = probability; PNEC = predicted no effect concentration; RQ = risk quotient; RTI = reverse transcriptase inhibitor; RTV = ritonavir; WWTP = wastewater treatment plant; ZDV = zidovudine.

The findings of my study provide a scientifically robust foundation for shaping future regulatory policies on ARVD monitoring and management. This research reinforces the theoretical implications discussed earlier by demonstrating the pseudo-persistent presence and ecotoxicological relevance of ARVDs in treated freshwaters, thereby advocating for the implementation of improved and advanced treatment technologies. This may advance policy innovations that incentivise the integration of advanced technologies and strategic interventions aimed at safeguarding water quality and protecting public health.

The identified gaps in monitoring and reporting across South African provinces, as highlighted, further illustrates the complexity of implementing an equitable and effective pharmaceutical regulatory framework. Conditions and concentrations may have changed since sample collection. This relationship suggests underlying assumptions about institutional capacity and coordination, supporting the need for a standardised and centralised system for ARVD monitoring. Such a framework could ensure consistent sampling, data collection, and reporting, thus enabling more comprehensive and comparable ecotoxicological risk assessments across provinces and informing more nuanced management decisions.

Furthermore, the demonstrated risks posed by ARVDs, particularly the synergistic effects of drug mixtures, encourage a shift toward more holistic ecotoxicological assessments. This may echo prior arguments made by researchers who advocate for cumulative risk considerations in environmental policy. Current regulatory paradigms often focus on individual contaminants. However, this perspective complicates the initial interpretations by highlighting the underestimated risk of compound mixtures in surface water and effluent. Consequently, policy frameworks may need to be expanded. This could drive the development of new environmental risk assessment and water quality frameworks that integrate the complexities of ARVD mixtures, thereby aligning more closely with the realities of pharmaceutical pollution.

This shift toward cumulative risk assessment reveals a deeper layer of environmental justice, as the identification of elevated toxic concerns to specific sub-populations (e.g., pregnant women and children) due to ARVD exposure in contaminated water points to the urgent need for tailored regulation. Regulatory policies could require monitoring at critical exposure points, particularly in densely populated areas and locations proximal to WWTPs, reflecting a policy shift towards protecting vulnerable communities and preventing disproportionate risks to at-risk populations while also considering broader socio-economic factors.

My research introduced a comprehensive, integrated prioritisation model for identifying ARVDs of greatest concern to both human and ecological health within South African aquatic environments. By synthesising multiple exposure and hazard indicators into a weighted multi-criteria framework, the model enabled evidence-based ranking of selected compounds and found ABC as the top-priority contaminant. Its frequent exceedance of safety thresholds, alongside EFV and LPV, supports the case for revised environmental quality standards of pharmaceutical pollutants. More stringent, evidence-based water quality standards could be implemented to address risks proactively. This will ensure that ARVDs are monitored and managed at concentrations that minimise both ecological and human health risks.

Finally, the call for a more uniform and integrated approach to ARVD monitoring suggests the potential for this research to contribute to regional or international policy dialogues. This example further illustrates the complexity of transboundary pharmaceutical pollution and may catalyse data-sharing initiatives and collaborative governance frameworks aimed at harmonising efforts to manage ARVDs in the environment.

In summary, my findings provide compelling evidence for the need to update and refine existing policies related to wastewater treatment, ARVD monitoring, and public health protection. By advocating for better treatment technologies, consistent monitoring, comprehensive hazard and risk assessments, and stronger regulatory thresholds, my research can meaningfully influence the development of future regulatory policies aimed at safeguarding both the environment and public health from the risks posed by ARVD contamination.

7.2 Recommendations

Based on the results of my study, several risk management strategies and urgent eco-pharmacovigilance measures are recommended to mitigate the potential health hazards associated with ARVDs in water. These include the establishment of permissible concentration limits for ARVDs in aquatic environments, active engagement with relevant stakeholders, and the application of diverse risk assessment tools to pinpoint and prioritise high-risk areas. Moreover, the implementation of systematic monitoring programmes is key to track the presence and trends of ARVDs in water sources.

Strengthening public health interventions to raise awareness about the potential risks of contaminated water, particularly among vulnerable populations dependent on untreated or inadequately treated water, is also critical. Ongoing assessment of ARVD contamination can

assist the opportune refinement of treatment protocols to ensure they remain effective in addressing the challenges posed by these pseudo-persistent pollutants. Future studies should address: temporal and spatial monitoring suitable for the entirety of the country; ecotoxicological data to assess the chronic and mixture effects of ARVDs and their metabolites on aquatic organisms, especially under environmentally realistic exposure scenarios; degradation pathways and transformation products of ARVDs; socio-economic factors influencing pharmaceutical usage, disposal behaviour, and risk perception; the efficacy, cost-effectiveness, and scalability of advanced treatment methods to guide technology adoption in resource-limited settings.

Specific recommendations for a framework for studying ARVDs in resource-limited settings such as South Africa.

Prioritisation and defining the scope:

- Identify priority ARVDs using prescription trends, water monitoring data, persistence profiles, and prioritisation tools.
- Conduct a scoping review to identify knowledge gaps, assess existing methods or current approaches, and locate regional data deficiencies.
- Identify and engage key stakeholders in water, health, and environmental sectors (e.g., government, academia, and non-governmental organisations).

Sampling and analytical strategy:

- Create a risk-based or tiered sampling strategy (e.g., downstream waters, WWTP influent/effluent).
- Record both spatial (urban vs. rural, upstream vs. downstream) and seasonal (wet vs. dry) variability.
- When liquid chromatography–tandem mass spectrometry (LC-MS/MS) is not affordable, use less expensive screening techniques (such as immunoassays).
- If lab capacity is limited, concentrate on pollution hotspots or collect pool samples.
- Collaborate with institutions or regional labs in nations with greater resources.

Wastewater treatment efficacy:

- Conduct audits of WWTPs to determine operational constraints and treatment types.
- Determine removal efficiencies by comparing influent and effluent ARVD concentrations.
- Identify specific infrastructure and process limitations linked to poor ARVD removal.

Environmental and public health integration:

- Select ecotoxicological endpoints pertinent to local ecosystems (e.g., algae, invertebrates).
- Use existing toxicity thresholds or predictive models to estimate ecological risks.

- Link contamination data to human activities like drinking, bathing, or fishing.
- Identify vulnerable populations (e.g., rural communities, immunocompromised individuals).
- When evaluating health risks, take local socioeconomic factors into account.

Data evaluation and quality assurance:

- Use modified quality or risk-of-bias frameworks to assess previous research.
- Assess internal study validity (sample size, replication, controls, quality assurance/quality control).
- Harmonise data by standardising concentration units, reporting formats, and metadata (e.g., global positioning system location, sampling date).

Interdisciplinary synthesis and risk prioritisation:

- Create a comprehensive dataset by combining usage, toxicity, treatment, and environmental data.
- Develop spatial risk maps using geographic information system (GIS) tools.
- Prioritise ARVDs with high persistence, exposure potential, and ecological concern.

Communication, policy, and capacity building:

- Propose interim policy actions, such as routine monitoring at high-risk WWTPs or basic infrastructure upgrades.
- Train local scientists and technicians in sampling, analysis, and data interpretation.
- Disseminate findings in accessible formats (e.g., infographics, community reports in local languages).
- Collaborate with community health and environmental organisations to raise awareness.
- Public awareness on proper ARVD disposal should be increased. Educational campaigns should encourage people living with HIV to return unused medications to pharmacies, clinics, or take-back programs.

END

ANNEXURES A:

Table 7-2: Quantifiable concentrations of antiretrovirals in groundwater from South Africa.

ARVD	Max Con (µg/L)	Matrix	Location	Reference
EFV	0.005	Ground water	Hartbeespoort Dam catchment, North West	Rimayi <i>et al.</i> , 2018
NFV	0.0009	Ground water	Potchefstroom – Vyfhoek 831, North West	Swanepoel <i>et al.</i> , 2015
NVP	0.0005	Gound water	Potchefstroom – Wilgeboom, North West	Swanepoel <i>et al.</i> , 2015
	0.0025	Gound water	Wildfontein 1, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.0003	Gound water	Potchefstroom – Bult, North West	Swanepoel <i>et al.</i> , 2015
	0.0006	Gound water	Potchefstroom – Haaskraal, North West	Swanepoel <i>et al.</i> , 2015
	0.0053	Gound water	Potchefstroom – Vyfhoek 831 GW, North West	Swanepoel <i>et al.</i> , 2015
	0.0006	Gound water	Potchefstroom – Miederpark, North West	Swanepoel <i>et al.</i> , 2015
	0.0049	Gound water	Boskop Garage, North- West	Swanepoel <i>et al.</i> , 2015
	0.013	Ground water	Hartbeespoort Dam catchment, North West	Rimayi <i>et al.</i> , 2018
SQV	0.0013	Ground water	Potchefstroom – Wilgeboom GW, North West	Swanepoel <i>et al.</i> , 2015
d4T	0.0009	Ground water	Potchefstroom – Bult, North- West	Swanepoel <i>et al.</i> , 2015
	0.0003	Ground water	Potchefstroom – Vyfhoek 831, North-West	Swanepoel <i>et al.</i> , 2015
TDF	0.0024	Ground water	Boskop Garage, North West	Swanepoel <i>et al.</i> , 2015

Table 7-3: Quantifiable concentrations of antiretrovirals in drinking water from South Africa.

ARVD	Max Con (µg/L)	Matrix	Location	Reference
ABC	0.0005	Drinking water	Wolverdiend, Gauteng	Swanepoel <i>et al.</i> , 2015
ddl	0.0033	Drinking water	Orkney, North West	Swanepoel <i>et al.</i> , 2015
	0.0004	Drinking water	Potchefstroom, North West	Swanepoel <i>et al.</i> , 2015
	0.00061	Drinking water	Garsfontein Rd, Gauteng	Swanepoel <i>et al.</i> , 2015
EFV	24.6	Drinking water	Olifantsfontein DS1, Gauteng	Vogt <i>et al.</i> , 2020
	6.24	Drinking water	Sunderland US DW, Gauteng	Vogt <i>et al.</i> , 2020
	2.33	Drinking water	Zeekoegat DS 1 DW, Gauteng	Vogt <i>et al.</i> , 2020

LPV	26.85	Drinking water	Olifantsfontein DS1, Gauteng	Vogt <i>et al.</i> , 2020
	9.04	Drinking water	Zeekoegat DS 1 DW, Gauteng	Vogt <i>et al.</i> , 2020
NFV	0.0011	Drinking water	Krugersdorp, Gauteng	Swanepoel <i>et al.</i> , 2015
NVP	0.0035	Drinking water	Kutsong, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.0005	Drinking water	Krugersdorp, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.0007	Drinking water	Sunninghill, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.0001	Drinking water	Muiskraal, North West	Swanepoel <i>et al.</i> , 2015
	0.0003	Drinking water	Klerksdorp, North West	Swanepoel <i>et al.</i> , 2015
	0.46	Drinking water	Olifantsfontein DS1, Gauteng,	Vogt <i>et al.</i> , 2020
	0.038	Drinking water	Waterval DS1 DW, Gauteng,	Vogt <i>et al.</i> , 2020
	0.21	Drinking water	Zeekoegat DS 1 DW, Gauteng,	Vogt <i>et al.</i> , 2020
	0.08	Drinking water	Hennops DW 3, Gauteng	Vogt <i>et al.</i> , 2020
RTV	1.95	Drinking water	Olifantsfontein DS1, Gauteng	Vogt <i>et al.</i> , 2020
d4T	0.0009	Drinking water	Durban, Kwa-Zulu Natal	Swanepoel <i>et al.</i> , 2015
DDC	0.008	Drinking water	Hartbeespoort Dam, North West	Wood <i>et al.</i> , 2015
ZDV	0.073	Drinking water	Hartbeespoort Dam, North West	Wood <i>et al.</i> , 2015
	0.0019	Drinking water	Kutsong, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.27	Drinking water	Jukskei DW 1, Gauteng,	Vogt <i>et al.</i> , 2020
	1.18	Drinking water	Jukskei DW 3, Gauteng	Vogt <i>et al.</i> , 2020
	0.52	Drinking water	Jukskei DW 4, Gauteng	Vogt <i>et al.</i> , 2020
	0.45	Drinking water	Hennops DW 3, Gauteng	Vogt <i>et al.</i> , 2020
	0.42	Drinking water	Croc DW 1, Gauteng	Vogt <i>et al.</i> , 2020

ANNEXURES B:

Table 7-4: Quantifiable concentrations of antiretrovirals in wastewater influent from South Africa.

ARVD	Max Con (µg/L)	Matrix	Location	Reference
ABC	3.5	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.4	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.29	WWTP Influent	Newlands Mashu, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	278	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	621	WWTP Influent	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	502	WWTP Influent	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	661	WWTP Influent	Umhlathuzana WWTP (Marrianridge), Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	605	WWTP Influent	Umhlathuzana WWTP (Shallcross), Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	814	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	167.92	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	61.24	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
ATV	0.064	WWTP Influent	DEWATS, eThekwini municipality, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.4	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.21	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	3.5	WWTP Influent	Newlands Mashu, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
DRV	43	WWTP Influent	DEWATS, eThekwini municipality, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.92	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.069	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	17.0	WWTP Influent	Newlands Mashu, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
EFV	17.4	WWTP Influent	Wastewater Treatment Works, Gauteng	Schoeman <i>et al.</i> , 2015
	0.0103	WWTP Influent	Mvudi River, Vhembe District, Limpopo	Robson <i>et al.</i> , 2017
	0.0103	WWTP Influent	Mvudi River, Vhembe District, Limpopo	Robson <i>et al.</i> , 2017
	34	WWTP Influent	DEWATS, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	24	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018

34	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
1.37	WWTP Influent	Amanzimtoti WWTP, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
26.3	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
1.67	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
1.02	WWTP Influent	Umhlathuzana Shallcross, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
2.22	WWTP Influent	Umhlathuzana Marrianridge, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
15.4	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
1.42	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
15.05	WWTP Influent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
10.19	WWTP Influent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.98	WWTP Influent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.05	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
20.42	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.46	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.25	WWTP Influent	Baragwanath WWTP, Gauteng	Horn <i>et al.</i> , 2022
2.186	WWTP Influent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
2.169	WWTP Influent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
0.3453	WWTP Influent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
140.4	WWTP Influent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
96.9	WWTP Influent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
114.4	WWTP Influent	Umbilo WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
89.8	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
13.66	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
14.2	WWTP Influent	Kingsburg WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
4.32	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
6.39	WWTP Influent	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
9.39	WWTP Influent	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
9.6	WWTP Influent	Umhlathuzana WWTP (Marrianridge), Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
1.6	WWTP Influent	Umhlathuzana WWTP (Shallcross), Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
23.3	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023

FTC	10.09	WWTP Influent	King Williams Town WWTP, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
	6.78	WWTP Influent	King Williams Town WWTP1, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
	4.76	WWTP Influent	Mdantsane Township WWTP, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
	4.08	WWTP Influent	Mdantsane Township WWTP, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
	7.13	WWTP Influent	Scenery Park WWTP, East London, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
	28.75	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	48.72	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	3.1	WWTP Influent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.3	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	1.47	WWTP Influent	Umbilo WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	172	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	31.3	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	14.782	WWTP Influent	Plankenburg WWTP, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	101.2	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
IDV	44.65	WWTP Influent	Kingsburg WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	0.26	WWTP Influent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.59	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.3	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
3TC	2.2	WWTP Influent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.84	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.9	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	20.9	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
LPV	3.67	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	1.001	WWTP Influent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
	0.0089	WWTP Influent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	86	WWTP Influent	Newlands Mashu, eThekweni, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	0.81	WWTP Influent	Plankenburg, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	2.5	WWTP Influent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.3	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.2	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018

NVP	24.35	WWTP Influent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	8.55	WWTP Influent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
	1.85	WWTP Influent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
	5.1	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	20.11	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	10.23	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	5.5	WWTP Influent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
	2.1	WWTP Influent	WWTP, Gauteng	Schoeman <i>et al.</i> , 2015
	2.8	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	2.1	WWTP Influent	DEWATS, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.67	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.681	WWTP Influent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	0.41	WWTP Influent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.09	WWTP Influent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.22	WWTP Influent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.08	WWTP Influent	Welgedacht WWTP, Gauteng	Horn <i>et al.</i> , 2022
	1.3	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.3	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.2	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.06	WWTP Influent	Baragwanath WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.1	WWTP Influent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.026	WWTP Influent	Daspoort WWTP, Pretoria	Mhuka <i>et al.</i> , 2020a
	0.0073	WWTP Influent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	0.42	WWTP Influent	Newlands Mashu, eThekweni, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	1.72	WWTP Influent	Pretoria, Gauteng	Akawa <i>et al.</i> , 2021
	261	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	106	WWTP Influent	Amanzimtoti WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	68.4	WWTP Influent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	119.7	WWTP Influent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	145.48	WWTP Influent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024

MVC	0.082	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.32	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.083	WWTP Influent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
RAL	0.81	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.061	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	17.0	WWTP Influent	DEWATS, eThekwini, KwaZulu-Natal,	Abafe <i>et al.</i> , 2018
RTV	6.3	WWTP Influent	Newlands Mashu, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	3.2	WWTP Influent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.6	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.6	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.394	WWTP Influent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
	0.059	WWTP Influent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	1.69	WWTP Influent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.64	WWTP Influent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
SQV	1.36	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.18	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
TDF	0.25	WWTP Influent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.17	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.1	WWTP Influent	Umhlathuzana, Marrianridge, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	32.25	WWTP Influent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	48.4	WWTP Influent	Kingsburg WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
ZDV	53	WWTP Influent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	6.9	WWTP Influent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	11	WWTP Influent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.23	WWTP Influent	WWTP, Pretoria, Gauteng	Akawa <i>et al.</i> , 2021
	3.68	WWTP Influent	Zeekoegat WWTP, Gauteng	Horn <i>et al.</i> , 2022

ANNEXURES C:

Table 7-5: Quantifiable concentrations of antiretrovirals in wastewater effluent from South Africa.

ARVD	Max Con (µg/L)	Matrix	Location	Reference
ABC	0.57	WWTP Effluent	Newlands Mashu DEWATS, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	40.8	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	85.2	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	124	WWTP Effluent	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	116	WWTP Effluent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	103	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	36.5	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	178.02	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
ATV	0.08	WWTP Effluent	DEWATS, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.74	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.3	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.31	WWTP Effluent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
	0.31	WWTP Effluent	Jukskei River, Northern WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	3.5	WWTP Effluent	Newlands Mashu DEWATS, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
DRV	17	WWTP Effluent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.35	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.13	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	12	WWTP Effluent	Newlands Mashu DEWATS, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
EFV	7.1	WWTP effluent	WWTP, Gauteng	Schoeman <i>et al.</i> , 2015
	4	WWTP effluent	WWTP, Gauteng	Schoeman <i>et al.</i> , 2017
	34	WWTP Effluent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	33	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	20	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	3.63	WWTP Effluent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020

37.3	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
3.27	WWTP Effluent	Umbilo WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
3.64	WWTP Effluent	Umhlathuzana, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
9.15	WWTP Effluent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
18.1	WWTP Effluent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
24.6	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
19.96	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
4.61	WWTP Effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.43	WWTP Effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.17	WWTP Effluent	Waterval WWTP, Gauteng	Horn <i>et al.</i> , 2022
7.28	WWTP Effluent	Welgedacht WWTP, Gauteng	Horn <i>et al.</i> , 2022
1.53	WWTP Effluent	Baragwanath WWTP, Gauteng	Horn <i>et al.</i> , 2022
20.3	WWTP Effluent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
2.04	WWTP Effluent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
0.51	WWTP Effluent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
1.97	WWTP Effluent	Jukskei River, Northern WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
86.9	WWTP Effluent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
78.8	WWTP Effluent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
93.1	WWTP Effluent	Umbilo WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
12.98	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
7.57	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
12	WWTP Effluent	Kingsburg WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
2.47	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
22	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
4.45	WWTP Effluent	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
9.01	WWTP Effluent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
3.13	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
6.0	WWTP Effluent	King Williams Town WWTP, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
4.0	WWTP Effluent	Mdantsane Township WWTP, Eastern Cape, South Africa	Netshithothole <i>et al.</i> , 2024
28.75	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024

FTC	103.32	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	0.22	WWTP Effluent	Amanzimtoti WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.22	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.35	WWTP Effluent	Umbilo WWTP, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	0.11	WWTP Effluent	Umhlathuzana Effluent, Durban, KwaZulu-Natal	Mlunguza <i>et al.</i> , 2020
	41.7	WWTP Effluent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	4.06	WWTP Effluent	WWTP, Western Cape Province	Mosekiemang <i>et al.</i> , 2019
	0.55	WWTP Effluent	Plankenburg, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.53	WWTP Effluent	Veldwagters, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	36.75	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
IDV	39.38	WWTP Effluent	Kingsburg WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	0.03	WWTP Effluent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.04	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.04	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
3TC	0.13	WWTP Effluent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.32	WWTP Effluent	Daspoort WWTP, Pretoria, South-Africa	Mhuka <i>et al.</i> , 2020a
	0.01	WWTP Effluent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	0.11	WWTP Effluent	Juikskei River, Northern WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	47.02	WWTP Effluent	Bellville South Industrial Area, Western Cape	Olabode, 2021
	52.89	WWTP Effluent	Khayelitsha Informal Settlement, Western Cape	Olabode, 2021
	150	WWTP Effluent	Newlands Mashu DEWATS, eThekweni, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	0.01	WWTP Effluent	Plankenburg, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
LPV	0.13	WWTP Effluent	Hartbeesfontein WWTP Outflow, Gauteng	Wood <i>et al.</i> , 2015
	3.8	WWTP Effluent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	3.8	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.9	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	26.85	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	38.45	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	33.64	WWTP Effluent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
	5.9	WWTP Effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022

NVP	0.91	WWTP Effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.8	WWTP Effluent	Waterval WWTP, Gauteng	Horn <i>et al.</i> , 2022
	5.35	WWTP Effluent	Welgedacht WWTP, Gauteng	Horn <i>et al.</i> , 2022
	2.04	WWTP Effluent	Baragwanath, Gauteng	Horn <i>et al.</i> , 2022
	15.3	WWTP Effluent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.0004	WWTP effluent	Boskoppies WWTP, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.35	WWTP effluent	WWTP, Gauteng	Schoeman <i>et al.</i> , 2015
	0.47	WWTP effluent	WWTP, Gauteng	Schoeman <i>et al.</i> , 2017
	1.4	WWTP effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	1.9	WWTP effluent	DEWATS, eThekweni, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.54	WWTP effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.76	WWTP effluent	WWTP, Western Cape	Mosekiemang <i>et al.</i> , 2019
	0.46	WWTP effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.42	WWTP effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.32	WWTP effluent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.23	WWTP effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.15	WWTP effluent	Vlakplaats WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.08	WWTP effluent	Waterval WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.18	WWTP effluent	Welgedacht WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.04	WWTP effluent	Baragwanath WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.4	WWTP effluent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
	0.08	WWTP effluent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
	0.01	WWTP effluent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	0.03	WWTP effluent	Jukskei River, Northern WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	182.33	WWTP effluent	Bellville South Industrial Area, Western Cape	Olabode, 2021
	207.7	WWTP effluent	Khayelitsha Informal Settlement, Western Cape	Olabode, 2021
	0.44	WWTP effluent	Newlands Mashu DEWATS, eThekweni, KwaZulu-Natal	Späth <i>et al.</i> , 2021
	0.87	WWTP effluent	Pretoria WWTP, Gauteng	Akawa <i>et al.</i> , 2021
	0.74	WWTP effluent	Pretoria WWTP, Gauteng	Akawa <i>et al.</i> , 2021
	34.2	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023

	18.8	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	59.1	WWTP Effluent	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	28.6	WWTP Effluent	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	74.7	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	42.07	WWTP Effluent	Darvil WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
	104.98	WWTP Effluent	Umbilo WWTP, KwaZulu-Natal	Xolo and Mahlambi, 2024
MVC	0.04	WWTP effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
RAL	0.09	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	3.5	WWTP Effluent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	4.1	WWTP Effluent	Newlands Mashu DEWATS, eThekwini, KwaZulu-Natal	Späth <i>et al.</i> , 2021
RTV	1.	WWTP effluent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.91	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.46	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.68	WWTP Effluent	Daspoort WWTP, Pretoria, Gauteng	Mhuka <i>et al.</i> , 2020a
	0.05	WWTP Effluent	Apies River, Daspoort WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	0.47	WWTP Effluent	Jukskei River, Northern WWTP, Gauteng	Mhuka <i>et al.</i> , 2020b
	1.95	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	1.9	WWTP Effluent	Olifantsfontein WWTP, Gauteng	Horn <i>et al.</i> , 2022
	1.77	WWTP Effluent	Sunderland WWTP, Gauteng	Horn <i>et al.</i> , 2022
	1.1	WWTP Effluent	Fliphuman WWTP, Gauteng	Horn <i>et al.</i> , 2022
TDF	0.002	WWTPS Effluent	Kutsong WWTP, Gauteng	Swanepoel <i>et al.</i> , 2015
	25.03	WWTP Effluent	Northern WWTP, Durban, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
ZDV	0.45	WWTP Effluent	Hartbeesfontein WWTP Outflow, Gauteng	Wood <i>et al.</i> , 2015
	0.97	WWTP Effluent	Zeekoegat WWTP Outflow, Gauteng	Wood <i>et al.</i> , 2015
	0.0003	WWTP Effluent	Kutsong WWTP, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.5	WWTP Effluent	DEWATS, eThekwini, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.09	WWTP Effluent	Northern WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	0.43	WWTP Effluent	Phoenix WWTP, KwaZulu-Natal	Abafe <i>et al.</i> , 2018
	66.98	WWTP Effluent	Bellville South Industrial Area, Western Cape	Olabode, 2021

9.17	WWTP Effluent	Khayelitsha Informal Settlement, Western Cape	Olabode, 2021
0.78	WWTP Effluent	Pretoria WWTP, Gauteng	Akawa <i>et al.</i> , 2021
0.83	WWTP Effluent	Pretoria WWTP, Gauteng	Akawa <i>et al.</i> , 2021

DEWATS = decentralised wastewater treatment system

ANNEXURES D:

Table 7-6: Quantifiable concentrations of antiretrovirals in surface water from South Africa.

ARVD	Max Con (µg/L)	Matrix	Location	Reference
ABC	0.0016	Surface water	Central Port Elizabeth, Eastern Cape	Swanepoel <i>et al.</i> , 2015
	0.0001	Surface water	Vaal River, Barkley west, Free State	Swanepoel <i>et al.</i> , 2015
	19	Surface water	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	102	Surface water	Amanzimtoti, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	57.4	Surface water	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	80.7	Surface water	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	7.85	Surface water	Msunduzi River, Camps Drift, Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambi, 2024
	13.15	Surface water	Msunduzi River, Wood House, Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambi, 2024
ddl	0.054	Surface water	Gariep Dam Oviston, Orange River System, Eastern Cape	Wood <i>et al.</i> , 2015
	0.05	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
EFV	0.01	Surface water	Dzindi River, Vhembe District, Limpopo	Robson <i>et al.</i> , 2017
	0.002	Surface water	Nandoni Dam, Vhembe District, Limpopo	Robson <i>et al.</i> , 2017
	0.0016	Surface water	Luvuvhu River, Vhembe District, Limpopo	Robson <i>et al.</i> , 2017
	0.15	Surface water	Otter Bridge, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
	0.0003	Surface water	Rietvlei Spruit, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
	0.08	Surface water	Marais Dam, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
	0.03	Surface water	Marais Dam Channel, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
	0.08	Surface water	Harbour, Hartbeespoort Dam catchment, Gauteng	Rimayi <i>et al.</i> , 2018
	0.3	Surface water	Crocodile River, Hartbeespoort Dam catchment, North West	Rimayi <i>et al.</i> , 2018
	0.01	Surface water	Magalies River, Hartbeespoort Dam catchment, North West	Rimayi <i>et al.</i> , 2018
	0.35	Surface water	Midrand, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.13	Surface water	N14, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018

	0.17	Surface water	Kyalami, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.17	Surface water	Buccleuch, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.3	Surface water	Marlboro, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.14	Surface water	uMngeni River, KwaZulu-Natal	Rimayi <i>et al.</i> , 2018
	2.38	Surface water	Hennops 3, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	4.18	Surface water	Hennops 4, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	3.62	Surface water	Hennops 5, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	2.74	Surface water	Hennops 6, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	1.86	Surface water	Hennops 8, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	1.38	Surface water	Hennops 9, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	1.25	Surface water	Jukskei 1, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	1.5	Surface water	Jukskei 2, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	1.49	Surface water	Jukskei 4, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.83	Surface water	Jukskei 6, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	2.49	Surface water	Jukskei 8, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.83	Surface water	Jukskei 9, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	2.2	Surface water	Jukskei 10, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	1.59	Surface water	Crocodile 1, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.09	Surface water	Muldersdrift se Loop, Johannesburg, Gauteng	Mhuka <i>et al.</i> , 2020b
	2.45	Surface water	Bishopstowe, Msunduzi River, Pietermaritzburg, KwaZulu-Natal	Mtolo <i>et al.</i> , 2019
	9.89	Surface water	Kingsburgh River, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	3.56	Surface water	uMngeni River, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	18.7	Surface water	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	5.67	Surface water	Amanzimtoti WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	8.52	Surface water	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	21.7	Surface water	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambi, 2023
	2.18	Surface water	Msunduzi River, Wood house Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambi, 2024
	1.95	Surface water	Msunduzi River, Bishopstowe, Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambi, 2024
FTC	0.01	Surface water	Marlboro, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.002	Surface water	Buccleuch, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018

3TC	0.001	Surface water	N14, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
	0.01	Surface water	uMngeni River, KwaZulu-Natal	Rimayi <i>et al.</i> , 2018
	0.004	Surface water	Eerste River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	6.16	Surface water	Plankenburg River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	10.03	Surface water	Plankenburg River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	1.31	Surface water	Plankenburg and Krom rivers, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.99	Surface water	Plankenburg, Eerste, and Krom rivers, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.61	Surface water	Eerste River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	12.2	Surface water	Kingsburgh River, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	17.93	Surface water	uMngeni River, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
	0.18	Surface water	S.E. Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.15	Surface water	Rowing Club Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.24	Surface water	Pienaars River Inflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.13	Surface water	Southern Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.95	Surface water	Nouthern Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.0009	Surface water	Orange River – Gariep Dam, Free State	Swanepoel <i>et al.</i> , 2015
	0.0001	Surface water	uMngeni River, KwaZulu-Natal	Rimayi <i>et al.</i> , 2018
	0.29	Surface water	Plankenburg River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.39	Surface water	Plankenburg River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.13	Surface water	Plankenburg and Krom rivers, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.04	Surface water	Plankenburg, Eerste, and Krom rivers, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
LPV	0.04	Surface water	Eerste River, Stellenbosch, Western Cape	Holton <i>et al.</i> , 2022
	0.31	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.28	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.001	Surface water	Kutsong, Gauteng	Swanepoel <i>et al.</i> , 2015
	4.9	Surface water	Hennops 1, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	4.92	Surface water	Hennops 2, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	43.32	Surface water	Hennops 3, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020

	45.52	Surface water	Hennops 4, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	34.12	Surface water	Hennops 5, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	63.69	Surface water	Hennops 6, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	18.44	Surface water	Hennops 7, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	53.15	Surface water	Hennops 8, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	52.31	Surface water	Hennops 9, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	7.73	Surface water	Crocodile 1, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	19.51	Surface water	Crocodile 2, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	16.17	Surface water	Crocodile 3, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	8.38	Surface water	Jukskei 1, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	15.13	Surface water	Jukskei 2, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	3.24	Surface water	Jukskei 3 Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	18.17	Surface water	Jukskei 4, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	2.97	Surface water	Jukskei 5, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	4.23	Surface water	Jukskei 6, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	4.88	Surface water	Jukskei 7, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	17.1	Surface water	Jukskei 8, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	6.26	Surface water	Jukskei 9, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	23.12	Surface water	Jukskei 10, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
NFV	0.0008	Surface water	Mooi River – Potchefstroom, North-West	Swanepoel <i>et al.</i> , 2015
NVP	0.24	Surface water	Angling area, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.34	Surface water	S.E. Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.32	Surface water	Motorboat Launch, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.29	Surface water	Rowing Club, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.35	Surface water	Roodeplaat Outflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.18	Surface water	Nothern Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.14	Surface water	Ditholo, Gauteng	Wood <i>et al.</i> , 2015
	0.13	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.14	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	1.48	Surface water	Pienaars River Inflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015

0.004	Surface water	Kutsong, Gauteng	Swanepoel <i>et al.</i> , 2015
0.002	Surface water	Fochville, North-West	Swanepoel <i>et al.</i> , 2015
0.001	Surface water	Pongola River – Pongola, KwaZulu-Natal	Swanepoel <i>et al.</i> , 2015
0.001	Surface water	Vaal River – Potchefstroom, North-West	Swanepoel <i>et al.</i> , 2015
0.007	Surface water	Mooi River – upstream from Potchefstroom, North-West	Swanepoel <i>et al.</i> , 2015
0.001	Surface water	Mooi River – Boskop dam, North-West	Swanepoel <i>et al.</i> , 2015
0.07	Surface water	Otter Bridge, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.02	Surface water	STR Fam, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.07	Surface water	Rietvlei Spruit, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.02	Surface water	Bird Hide Stream, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.02	Surface water	Marais Dam, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.23	Surface water	Marais Dam Channel, Rietvlei Nature Reserve, Gauteng	Wooding <i>et al.</i> , 2017
0.02	Surface water	Albasini, Rietvlei Nature Reserve, Limpopo	Wooding <i>et al.</i> , 2017
0.04	Surface water	Dam wall 30m Hartbeespoort Dam catchment, North-West	Rimayi <i>et al.</i> , 2018
0.04	Surface water	Harbour, Hartbeespoort Dam catchment, North-West	Rimayi <i>et al.</i> , 2018
0.04	Surface water	Magalies River, Hartbeespoort Dam catchment, North-West	Rimayi <i>et al.</i> , 2018
0.07	Surface water	Crocodile River, Hartbeespoort Dam catchment, North-West	Rimayi <i>et al.</i> , 2018
0.05	Surface water	Midrand, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
0.02	Surface water	Kyalami, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
0.06	Surface water	Buccleuch, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
0.02	Surface water	N14, Jukskei River, Gauteng	Rimayi <i>et al.</i> , 2018
0.07	Surface water	uMngeni River, KwaZulu-Natal	Rimayi <i>et al.</i> , 2018
0.18	Surface water	Hennops 3, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.62	Surface water	Hennops 4, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.54	Surface water	Hennops 5, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.64	Surface water	Hennops 6, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.53	Surface water	Hennops 7, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.34	Surface water	Hennops 8, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.35	Surface water	Hennops 9, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.11	Surface water	Jukskei 1, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020

RTV	0.15	Surface water	Jukskei 2, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.24	Surface water	Jukskei 3 Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.15	Surface water	Jukskei 4, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.01	Surface water	Jukskei 5, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.19	Surface water	Jukskei 8, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.13	Surface water	Jukskei 10, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.15	Surface water	Crocodile 2, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.22	Surface water	Crocodile 3, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.001	Surface water	Muldersdrift se Loop, JHB, Gauteng	Mhuka <i>et al.</i> , 2020b
	0.5	Surface water	S1 (Tembisa), Hennops River, Gauteng	Khulu <i>et al.</i> , 2022
	1.64	Surface water	S2 (Irene), Hennops River, Gauteng	Khulu <i>et al.</i> , 2022
	0.62	Surface water	S3 (Centurion Mall), Hennops River, Gauteng	Khulu <i>et al.</i> , 2022
	0.58	Surface water	S4 (Blackwood Road Bridge), Hennops River, Gauteng	Khulu <i>et al.</i> , 2022
	0.83	Surface water	S5 (Witstinkhout Road), Hennops River, Gauteng	Khulu <i>et al.</i> , 2022
	0.95	Surface water	S1 (US Hazelmere Dam), Umdloti River, KwaZulu-Natal	Khulu <i>et al.</i> , 2022
	0.75	Surface water	S2 (DS Hazelmere Dam wall), Umdloti River, KwaZulu-Natal	Khulu <i>et al.</i> , 2022
	0.78	Surface water	S3 (US Verulam WWTP), Umdloti River, KwaZulu-Natal	Khulu <i>et al.</i> , 2022
	0.58	Surface water	S4 (DS Verulam WWTP), Umdloti River, KwaZulu-Natal	Khulu <i>et al.</i> , 2022
	1	Surface water	S5 (Estuary), Umdloti River, KwaZulu-Natal	Khulu <i>et al.</i> , 2022
	28.5	Surface water	Northern WWTP, KwaZulu-Natal	Ngwenya and Mahlambe, 2023
	35.6	Surface water	Amanzimtoti WWTP, KwaZulu-Natal	Ngwenya and Mahlambe, 2023
	36	Surface water	Umhlathuzana WWTP, Durban, KwaZulu-Natal	Ngwenya and Mahlambe, 2023
	52.3	Surface water	Umbilo WWTP, KwaZulu-Natal	Ngwenya and Mahlambe, 2023
	2.33	Surface water	Msunduzi River, Camps Drift, Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambe, 2024
	6.38	Surface water	Msunduzi River, Bishopstowe, Pietermaritzburg, KwaZulu-Natal	Xolo and Mahlambe, 2024
	1.15	Surface water	Jukskei 4, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	2.25	Surface water	Jukskei 8, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	3.06	Surface water	Jukskei 10, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	3.51	Surface water	Klip 1, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	4.52	Surface water	Klip 2, Klip River, Gauteng	Vogt <i>et al.</i> , 2020

	4.51	Surface water	Klip 3, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	5.04	Surface water	Klip 4, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	4.84	Surface water	Klip 5, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	3.04	Surface water	Klip 6, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	3.11	Surface water	Klip 7, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	3.81	Surface water	Klip 8, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	1.93	Surface water	Klip 9, Klip River, Gauteng	Vogt <i>et al.</i> , 2020
	1.99	Surface water	Crocodile 2, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	2	Surface water	Crocodile 3, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	3.1	Surface water	Hennops 3, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	4.34	Surface water	Hennops 4, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	3.4	Surface water	Hennops 5, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	5.14	Surface water	Hennops 6, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	1.4	Surface water	Hennops 7, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	4.3	Surface water	Hennops 8, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	4.5	Surface water	Hennops 9, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	0.02	Surface water	Muldersdrift se Loop, JHB, Gauteng	Mhuka <i>et al.</i> , 2020b
d4T	0.78	Surface water	Pienaars River inflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.1	Surface water	Angling area, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.41	Surface water	Roodeplaat outflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.0007	Surface water	Kutsong, Gauteng	Swanepoel <i>et al.</i> , 2015
	0.0011	Surface water	Stormberg spruit, Eastern Cape	Swanepoel <i>et al.</i> , 2015
TDF	0.24	Surface water	Pienaars River inflow, Roodeplaat Dam, Gauteng	Wood <i>et al.</i> , 2015
	0.19	Surface water	Orange River (Bethulie) Orange River System, Northern Cape	Wood <i>et al.</i> , 2015
	0.15	Surface water	Gariiep Dam, Orange River System, Free State	Wood <i>et al.</i> , 2015
	0.0006	Surface water	Loopspruit – Potchefstroom, North-West	Swanepoel <i>et al.</i> , 2015
	0.11	Surface water	Hartbeespoort Dam, North-West	Mlunguza <i>et al.</i> , 2020
	2.71	Surface water	uMngeni River, KwaZulu-Natal	Sigonya <i>et al.</i> , 2022
DDC	0.07	Surface water	Orange River (Bethulie), Northern Cape	Wood <i>et al.</i> , 2015

ZDV	0.03	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.34	Surface water	Pienaars River Inflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.63	Surface water	Angling Area, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.3	Surface water	Angling area, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.29	Surface water	Motorboat Launch, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.31	Surface water	Rowing Club, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.22	Surface water	Roodeplaat Outflow, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.16	Surface water	Southern Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.19	Surface water	Nouthern Bank, Roodeplaat Dam System, Gauteng	Wood <i>et al.</i> , 2015
	0.05	Surface water	Vaal Dam Inflow, Free State	Wood <i>et al.</i> , 2015
	0.35	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.14	Surface water	Hartbeespoort Dam, Meerhof, North-West	Wood <i>et al.</i> , 2015
	0.0009	Surface water	Harts River – Spitskop dam, North-West	Swanepoel <i>et al.</i> , 2015
	0.0006	Surface water	Msunduzi river – Pietermaritzburg, KwaZulu-Natal	Swanepoel <i>et al.</i> , 2015
	0.0003	Surface water	Mooi River – Potchefstroom, North-West	Swanepoel <i>et al.</i> , 2015
	0.87	Surface water	Jukskei 1, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	1.28	Surface water	Jukskei 2, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.31	Surface water	Jukskei 3 Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	2.74	Surface water	Jukskei 4, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	1.14	Surface water	Jukskei 5, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.58	Surface water	Jukskei 6, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.86	Surface water	Jukskei 7, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.84	Surface water	Jukskei 8, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.75	Surface water	Jukskei 9, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.95	Surface water	Jukskei 10, Jukskei River, Gauteng	Vogt <i>et al.</i> , 2020
	0.34	Surface water	Crocodile 1, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.57	Surface water	Crocodile 2, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.52	Surface water	Crocodile 3, Crocodile River, Gauteng	Vogt <i>et al.</i> , 2020
	0.6	Surface water	Hennops 1, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
	0.34	Surface water	Hennops 2, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020

1.91	Surface water	Hennops 3, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
2.04	Surface water	Hennops 4, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
2.22	Surface water	Hennops 5, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
1.34	Surface water	Hennops 6, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
0.75	Surface water	Hennops 7, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
1.61	Surface water	Hennops 8, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020
1.04	Surface water	Hennops 9, Hennops River, Gauteng	Vogt <i>et al.</i> , 2020

Detail on the precision of the analytical methods used in this review

Holton *et al.* (2022), Horn *et al.* (2022), and Swanepoel *et al.* (2015) report a %RSD range of >20%. This suggests high variability for certain analytes, which may require method optimisation to reduce inconsistency for critical measurements. Such variability could arise from differences in the chemical properties of analytes (solubility, stability) or challenges in the extraction/detection process for specific compounds. While the majority of the remaining authors reported low %RSD ($\leq 10\%$). This narrower range of %RSD suggests better overall precision, making it a potentially more reliable method for similar ARVD monitoring applications.

Horn *et al.* (2022) reported %RSD values as high as 196% for ARVDs in surface and wastewater, emphasising the inconsistencies associated with matrix composition. Matrix-specific calibrations or surrogate standards may be necessary to address these issues. Wastewater often contain high concentrations of organic and inorganic matter, as well as a complex mixture of chemicals (Bingul *et al.*, 2023). This complexity increases the likelihood of matrix effects, such as ion suppression or enhancement in mass spectrometry, which can reduce detection sensitivity and accuracy. Studies like Mosekiemang *et al.* (2019) analysed wastewater and reported a broader LOD and LOQ range, likely reflecting these interferences. To mitigate matrix effects, methods such as SPE with advanced cartridges (Oasis HLB) are commonly employed to improve analyte recovery (Rimanyi *et al.*, 2018; Xolo and Mahlambi, 2024).

Swanepoel *et al.* (2015) and Vogt *et al.* (2020) used UHPLC/MS QTOF to detect ARVDs in drinking water, both reporting broad LOQ and LOD ranges. Drinking water often comes from diverse sources and undergoes different treatment processes leading to variation in composition. This variability could potentially influence method performance and contribute to broader LOD and LOQ ranges.

ANNEXURES E:

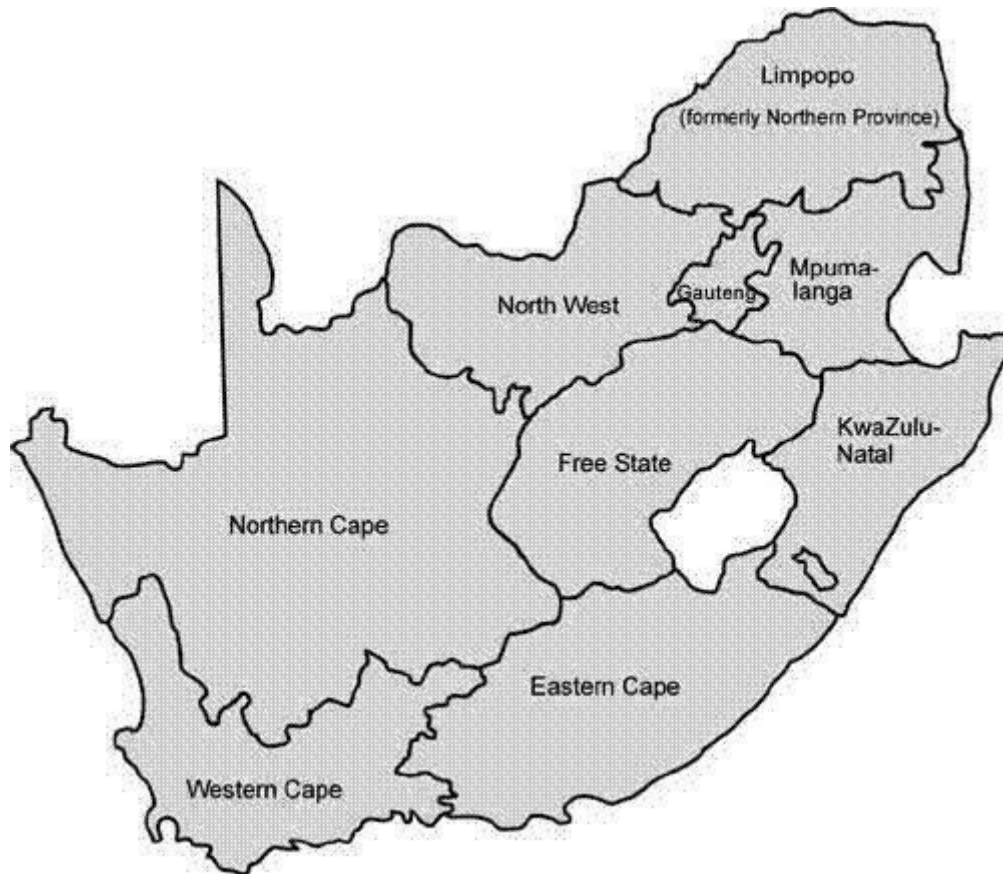


Figure 7-1: A map showing the nine provinces of South Africa.

ANNEXURES F:

Table 7-7: Mean concentrations of selected ARVDs in surface water samples from the Klip River system over several months.

Sample month	Sample Site	Mean concentration (µg/L)				
		EFV	LPV	NVP	RTV	ZDV
March	Klip 1	<LOD	29.22	0.41	<LOQ	<LOD
	Klip 2	<LOD	22.91	<LOD	<LOQ	<LOD
	Klip 3	<LOD	26.19	<LOD	2.44	<LOQ
	Klip 4	<LOD	8.93	<LOD	<LOD	<LOD
	Klip 5	<LOQ	41.27	<LOD	4.84	<LOQ
	Klip 6	<LOD	16.48	<LOD	<LOQ	<LOD
	Klip 7	<LOD	13.40	<LOD	<LOQ	<LOQ
	Klip 8	<LOD	13.57	<LOD	<LOQ	<LOD
	Klip 9	<LOD	5.58	<LOD	<LOD	<LOD
April	Klip 1	<LOD	5.40	<LOD	<LOD	<LOD
	Klip 2	<LOD	<LOD	<LOD	<LOD	<LOD
	Klip 3	<LOD	3.56	<LOD	<LOD	<LOQ
	Klip 4	<LOD	3.59	<LOD	<LOD	<LOQ
	Klip 5	1.55	16.22	<LOD	<LOQ	<LOQ
	Klip 6	<LOD	3.54	<LOD	<LOD	<LOQ
	Klip 7	<LOD	2.74	<LOD	<LOD	<LOQ
	Klip 8	<LOD	<LOQ	<LOD	<LOD	<LOQ
	Klip 9	<LOD	<LOQ	<LOD	<LOD	<LOD
May	Klip 1	<LOD	0.96	<LOD	<LOD	<LOD
	Klip 2	<LOD	<LOD	<LOD	<LOD	<LOD
	Klip 3	1.81	<LOD	<LOD	<LOD	<LOQ
	Klip 4	<LOQ	3.27	<LOD	<LOD	<LOD
	Klip 5	<LOQ	4.06	<LOD	<LOD	<LOD

	Klip 6	<LOD	2.96	<LOD	<LOD	<LOD
	Klip 7	<LOD	5.07	<LOD	<LOD	<LOQ
	Klip 8	<LOD	1.66	<LOD	<LOD	<LOQ
	Klip 9	<LOD	5.55	<LOD	<LOD	<LOD
August	Klip 1	<LOQ	39.49	<LOD	3.51	<LOD
	Klip 2	<LOQ	39.62	<LOD	4.52	<LOD
	Klip 3	1.76	46.75	<LOD	4.51	<LOD
	Klip 4	<LOQ	39.84	<LOD	5.04	<LOQ
	Klip 5	<LOQ	30.69	<LOD	3.38	<LOQ
	Klip 6	<LOQ	26.44	<LOD	3.04	<LOD
	Klip 7	<LOQ	24.34	<LOD	3.11	<LOQ
	Klip 8	<LOQ	24.45	<LOD	3.81	<LOQ
	Klip 9	<LOQ	21.37	<LOD	1.93	<LOQ
October	Klip 1	<LOD	2.02	<LOD	<LOD	<LOD
	Klip 2	<LOD	<LOQ	<LOD	<LOD	<LOQ
	Klip 3	<LOD	2.12	<LOD	<LOD	<LOD
	Klip 4	<LOD	<LOQ	<LOD	<LOD	<LOD
	Klip 5	<LOD	4.96	<LOD	<LOD	<LOD
	Klip 6	<LOD	<LOQ	<LOD	<LOD	<LOD
	Klip 7	<LOD	<LOQ	<LOD	<LOD	<LOD
	Klip 8	<LOD	<LOD	<LOD	<LOD	<LOD
	Klip 9	<LOD	<LOQ	<LOD	<LOD	<LOD

LOD = limited of detection; LOQ = limited of quantification; EFV = efavirenz; LPV = lopinavir; NVP = nevirapine; RTV = ritonavir; ZDV = zidovudine.

Table 7-8: Mean concentrations of selected ARVDs in surface water samples from the Hennops River system over several months.

Sample month	Sample Site	Mean concentration (µg/L)				
		EFV	LPV	NVP	RTV	ZDV
March	Hennops 1	<LOD	4.90	<LOD	<LOD	<LOD
	Hennops 2	<LOD	4.92	<LOD	<LOD	<LOD
	Hennops 3	<LOD	43.32	<LOD	3.10	<LOQ
	Hennops 4	<LOD	45.52	<LOQ	2.81	<LOQ
	Hennops 5	<LOD	34.12	<LOQ	2.48	<LOQ
	Hennops 6	<LOQ	63.69	<LOQ	5.14	<LOD
	Hennops 7	<LOD	18.44	<LOD	1.40	<LOD
	Hennops 8	<LOD	53.15	<LOQ	4.30	<LOQ
	Hennops 9	<LOD	52.31	<LOQ	4.50	<LOD
April	Hennops 1	<LOD	<LOD	<LOD	<LOD	<LOD
	Hennops 2	<LOD	0.57	<LOD	<LOD	<LOD
	Hennops 3	<LOQ	5.81	<LOD	<LOD	1.91
	Hennops 4	<LOD	3.17	<LOD	<LOD	2.04
	Hennops 5	<LOD	3.65	<LOD	<LOD	<LOQ
	Hennops 6	<LOD	2.62	<LOD	<LOD	<LOQ
	Hennops 7	<LOD	1.21	<LOD	<LOD	<LOQ
	Hennops 8	<LOD	3.44	<LOD	<LOD	<LOQ
	Hennops 9	<LOD	3.85	<LOD	<LOD	<LOQ
May	Hennops 1	<LOD	4.11	<LOD	<LOD	<LOD
	Hennops 2	<LOD	0.72	<LOD	<LOD	<LOD
	Hennops 3	<LOD	0.98	<LOD	<LOD	1.12
	Hennops 4	<LOQ	<LOD	<LOD	<LOD	1.17
	Hennops 5	<LOD	1.08	<LOD	<LOD	1.08
	Hennops 6	<LOD	<LOD	<LOD	<LOD	<LOQ
	Hennops 7	<LOD	0.69	<LOD	<LOD	<LOD
	Hennops 8	<LOD	<LOD	<LOD	<LOD	<LOQ

August	Hennops 9	<LOD	3.62	<LOD	<LOD	<LOQ
	Hennops 1	<LOD	2.32	<LOD	<LOD	<LOD
	Hennops 2	<LOD	2.24	<LOD	<LOD	<LOD
	Hennops 3	2.17	18.01	<LOD	2.23	1.17
	Hennops 4	4.18	35.35	<LOD	4.34	<LOQ
	Hennops 5	3.62	31.31	<LOD	3.40	1.41
	Hennops 6	2.74	32.03	<LOQ	3.48	<LOQ
	Hennops 7	<LOD	7.77	<LOQ	<LOD	<LOQ
	Hennops 8	1.86	30.88	<LOD	3.04	<LOQ
October	Hennops 9	1.38	15.87	<LOD	1.58	<LOQ
	Hennops 1	<LOD	0.86	<LOD	<LOD	<LOQ
	Hennops 2	<LOD	0.76	<LOD	<LOD	<LOQ
	Hennops 3	2.38	3.66	<LOD	<LOD	1.77
	Hennops 4	3.20	5.15	<LOQ	<LOD	1.35
	Hennops 5	2.65	11.68	<LOQ	1.33	2.22
	Hennops 6	1.68	8.61	<LOD	<LOD	1.34
	Hennops 7	<LOD	2.40	<LOD	<LOD	<LOQ
	Hennops 8	1.40	7.72	<LOD	<LOD	1.61
	Hennops 9	<LOQ	9.99	<LOD	<LOD	<LOQ

LOD = limited of detection; LOQ = limited of quantification; EFV = efavirenz; LPV = lopinavir; NVP = nevirapine; RTV = ritonavir; ZDV = zidovudine.

Table 7-9: Mean concentrations of selected ARVDs in surface water samples from the Jukskei River system over several months.

Sample month	Sample Site	Mean concentration (µg/L)				
		EFV	LPV	NVP	RTV	ZDV
March	Jukskei 1	<LOD	8.38	<LOD	<LOD	<LOD
	Jukskei 2	<LOD	15.13	<LOD	<LOD	<LOQ
	Jukskei 3	<LOD	3.24	<LOD	<LOD	<LOD
	Jukskei 4	<LOD	18.17	<LOD	<LOQ	<LOQ
	Jukskei 5	<LOD	2.97	<LOD	<LOD	<LOD
	Jukskei 6	<LOD	4.19	<LOD	<LOD	<LOD
	Jukskei 7	<LOD	2.26	<LOD	<LOD	<LOD
	Jukskei 8	<LOD	15.83	<LOD	<LOQ	<LOQ
	Jukskei 9	<LOD	3.54	<LOD	<LOD	<LOD
	Jukskei 10	<LOD	14.56	<LOD	<LOD	<LOQ
April	Jukskei 1	<LOD	0.71	<LOD	<LOD	<LOQ
	Jukskei 2	<LOD	1.81	<LOD	<LOD	<LOQ
	Jukskei 3	<LOD	<LOD	<LOD	<LOD	<LOD
	Jukskei 4	<LOD	1.93	<LOD	<LOD	1.18
	Jukskei 5	<LOD	<LOD	<LOD	<LOD	<LOD
	Jukskei 6	<LOD	2.78	<LOD	<LOD	<LOD
	Jukskei 7	<LOD	2.53	<LOD	<LOD	<LOQ
	Jukskei 8	<LOD	2.86	<LOD	<LOD	<LOQ
	Jukskei 9	<LOD	1.38	<LOD	<LOD	<LOQ
	Jukskei 10	<LOD	6.83	<LOD	<LOD	<LOQ
May	Jukskei 1	<LOD	0.62	<LOD	<LOD	<LOQ
	Jukskei 2	<LOD	3.22	<LOD	<LOD	<LOQ
	Jukskei 3	<LOD	1.81	<LOD	<LOD	<LOD
	Jukskei 4	<LOD	2.20	<LOD	<LOD	<LOQ
	Jukskei 5	<LOD	0.58	<LOD	<LOD	<LOD
	Jukskei 6	<LOD	3.17	<LOD	<LOD	<LOD

	Jukskei 7	<LOD	2.20	<LOD	<LOD	<LOQ
	Jukskei 8	<LOD	1.98	<LOD	<LOD	<LOQ
	Jukskei 9	<LOD	1.77	<LOD	<LOD	<LOD
	Jukskei 10	<LOD	6.61	<LOD	<LOD	<LOQ
August	Jukskei 1	<LOQ	2.17	<LOD	<LOD	<LOQ
	Jukskei 2	1.50	6.09	<LOD	<LOD	1.26
	Jukskei 3	<LOD	0.80	<LOD	<LOD	<LOD
	Jukskei 4	1.49	4.39	<LOD	<LOD	<LOQ
	Jukskei 5	<LOD	0.74	<LOD	<LOD	<LOD
	Jukskei 6	<LOQ	4.23	<LOD	<LOD	<LOQ
	Jukskei 7	<LOD	4.88	<LOD	<LOD	<LOQ
	Jukskei 8	2.23	17.10	<LOD	2.25	<LOQ
	Jukskei 9	<LOQ	6.26	<LOD	<LOD	<LOQ
	Jukskei 10	2.20	23.12	<LOD	3.06	<LOQ
October	Jukskei 1	<LOD	<LOD	<LOD	<LOD	<LOD
	Jukskei 2	<LOD	0.95	<LOD	<LOD	1.28
	Jukskei 3	<LOD	<LOD	<LOD	<LOD	<LOD
	Jukskei 4	<LOQ	1.40	<LOD	<LOD	2.74
	Jukskei 5	<LOD	<LOD	<LOD	<LOD	1.14
	Jukskei 6	<LOD	0.51	<LOD	<LOD	<LOD
	Jukskei 7	<LOD	0.62	<LOD	<LOD	<LOQ
	Jukskei 8	2.49	3.67	<LOD	<LOD	<LOQ
	Jukskei 9	<LOD	0.89	<LOD	<LOD	<LOD
	Jukskei 10	1.99	3.60	<LOD	<LOD	<LOQ

LOD = limited of detection; LOQ = limited of quantification; EFV = efavirenz; LPV = lopinavir; NVP = nevirapine; RTV = ritonavir; ZDV = zidovudine.

Table 7-10: Mean concentrations of selected ARVDs in surface water samples from the Crocodile River system over several months.

Sample month	Sample Site	Mean concentration (µg/L)				
		EFV	LPV	NVP	RTV	ZDV
March	Crocodile 1	<LOD	7.73	<LOD	<LOD	<LOD
	Crocodile 2	<LOD	9.20	<LOD	<LOD	<LOD
	Crocodile 3	<LOD	1.20	<LOD	<LOD	<LOD
April	Crocodile 1	<LOD	1.68	<LOD	<LOD	<LOD
	Crocodile 2	<LOQ	1.44	<LOD	<LOD	<LOQ
	Crocodile 3	<LOD	1.54	<LOD	<LOD	<LOQ
May	Crocodile 1	<LOD	1.53	<LOD	<LOD	<LOD
	Crocodile 2	<LOD	1.03	<LOD	<LOD	<LOQ
	Crocodile 3	<LOD	1.69	<LOD	<LOD	<LOQ
August	Crocodile 1	<LOD	3.86	<LOD	<LOD	<LOD
	Crocodile 2	1.59	19.51	<LOD	1.99	<LOD
	Crocodile 3	<LOD	16.17	<LOD	1.83	<LOQ
October	Crocodile 1	<LOD	1.50	<LOD	<LOD	<LOQ
	Crocodile 2	<LOD	4.64	<LOD	<LOD	<LOD
	Crocodile 3	<LOD	13.03	<LOD	2.00	<LOQ

LOD = limited of detection; LOQ = limited of quantification; EFV = efavirenz; LPV = lopinavir; NVP = nevirapine; RTV = ritonavir; ZDV = zidovudine.

ANNEXURES G:

Table 7-11: Coordinates of samples collected in the Gauteng Province between March and October 2018.

River and sample site	Latitude	Longitude
Crocodile 1	-25.895168	27.91374
Crocodile 2	-25.863174	27.93408
Crocodile 3	-25.797273	27.893952
Hennops 1	-25.878533	28.256195
Hennops 2	-25.888751	28.225992
Hennops 3	-25.951437	28.207384
Hennops 4	-25.922381	28.227477
Hennops 5	-25.88954	28.224148
Hennops 6	-25.829178	28.115976
Hennops 7	-25.840507	28.110298
Hennops 8	-25.822378	28.082272
Hennops 9	-25.793756	28.003707
Jukskei 1	-26.154441	28.130995
Jukskei 2	-26.084225	28.108553
Jukskei 3	-26.056121	28.118883
Jukskei 4	-26.032128	28.111185
Jukskei 5	-26.036721	28.055483
Jukskei 6	-26.009621	28.037822
Jukskei 7	-25.985702	28.002141
Jukskei 8	-25.955545	27.964965
Jukskei 9	-25.977999	27.962469
Jukskei 10	-25.955241	27.962657
Klip 1	-26.325093	27.987896
Klip 2	-26.328337	27.936846
Klip 3	-26.32011	27.941017
Klip 4	-26.306795	27.940254
Klip 5	-26.311805	27.918583
Klip 6	-26.336841	27.902994
Klip 7	-26.289923	27.885756
Klip 8	-26.239979	27.912726
Klip 9	-26.294204	27.836259

The two interpolation techniques that are most frequently employed are IDW and Kriging. IDW is simpler than Kriging, yet some studies showed that it was preferred over the latter (Hodam *et al.*, 2017; Shukla *et al.*, 2020). Also, IDW may handle parameters that are not normally distributed, whereas the Kriging technique is limited to normal distributions and should be used when transformation methods (e.g., log transformation) are applied (Chen and Liu, 2012). The attribute of IDW is that nearby locations are more likely to have similar values and the linear interpolator weights the interpolated data $\hat{z}(x_0)$, at unsampling location x_0 , as follows (Mantzafleri *et al.*, 2009):

$$\hat{z}(x_0) = \sum_{j=1}^m w_j \times z(x_j) \quad (7-1)$$

Where:

$z(x_j)$ = value of drug concentration in water (z) at sampling location (j)

m = number of neighbouring sample sites

w_j = weights according to the distance between the unsampling locations (x_0)

x_j = sampling locations

x_0 = unsampling locations

$\hat{z}(x_0)$ = interpolated data

Therefore, it can be written as follows:

$$\sum_{j=1}^m w_j = 1 \quad (7-2)$$

The final formula for the IDW method is derived as follows (Zisou and Psilovikos, 2012):

$$\hat{z}(x_0) = \frac{\sum_{j=1}^m z(x_j) \times d_{ij}^{-k}}{\sum_{j=1}^m d_{ij}^{-k}} \quad (7-3)$$

Where:

k = distance influence coefficient (power parameter)

d_{ij} = distance between the unsampling location ($i(x_0)$) and the sampling locations $j(x_j)$

In IDW, weights can be raised to the power of k (e.g., squared, linear, or cubed), causing weights to decrease more rapidly as distance increases (Eq. (7-3)). The power k can also be optimised. IDW presumes that the simulated surface is affected by local variations, which can be depicted through the neighbourhood. Adjusting neighbourhood parameters such as sectors, shape, and the number of neighbours can improve the model (Charizopoulos *et al.*, 2018).

Although IDW parameters may be optimized, the following assumptions were made to simplify the method: the extent was set to the bounding box of the study area to avoid unnecessary computation; pixel size was set to 0.01; distance weighting was set to two since it balances local and distant influences; the neighbourhood type was standard (N - nearest neighbours) since data was irregularly distributed. Given that QGIS does not have built-in anisotropy settings, the following default behaviour was assumed: isotropic (circular) influence, which means the anisotropy factor was also set to default 1.0 (no directional bias); the major and

minor semi-axes are equal; the anisotropy angle was set to default 0° (no rotation). All parameter selections were implemented within the IDW interpolation processing tool in QGIS.

ANNEXURES H:

Table 7-12: Ecotoxicity data for antiretroviral drugs (ARVDs) to determine the predicted no effect concentration (PNEC), detailing test species, toxicity endpoints, measured concentrations, and justification for assessment factors (AF) selection.

ARVD	Species	Test	Result (ug/L)	Result (mg/L)	AF	PNEC (mg/L)	AF Reasoning
ABC	Green Algae (<i>Selenastrum capricornutum</i>)	NOEC	3×10 ⁴	30	10	3	Long-term results (NOECs) from 3 trophic levels
	Crustacean (<i>Ceriodaphnia dubia</i>)	NOEC	5600	5.6	10	0.56	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>Pimephales promelas</i>)	NOEC	9.5×10 ⁴	95	10	9.5	Long-term results (NOECs) from 3 trophic levels
ATV	Green algae (<i>Pseudokirchneriella subcapitata</i>)	NOEC	4100	4.1	10	0.41	Long-term results (NOECs) from 3 trophic levels
	Crustacean (<i>Daphnia magna</i>)	NOEC	5100	5.1	10	0.51	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>P. promelas</i>)	NOEC	8600	8.6	10	0.86	Long-term results (NOECs) from 3 trophic levels
ddl	Fish	ChV	2113	2.11	10	0.21	Lowest value of classes
	Daphnid	ChV	8095	8.10	10	0.81	Lowest value of classes
	Green Algae	ChV	5840	5.84	10	0.58	Lowest value of classes
DRV	Green algae (<i>P. subcapitata</i>)	NOEC	4.3×10 ⁴	43	10	4.3	Long-term results (NOECs) from 3 trophic levels
	Crustacean (<i>D. magna</i>)	NOEC	2600	2.6	10	0.26	Lowest long-term results (NOECs) from 3 trophic levels
	Fish (<i>Oncorhynchus mykiss</i>)	NOEC 28d	9400	9.4	10	0.94	Long-term results (NOECs) from 3 trophic levels
EFV	Green Algae (<i>S. capricornutum</i>)	NOEC 12d (growth rate)	26	0.026	10	0.0026	Long-term results (NOECs) from 3 trophic levels

	Crustacean (<i>D. magna</i>)	NOEC 21d (survival)	160	0.16	10	0.016	Lowest long-term results (NOECs) from 3 trophic levels
	Fish (<i>P. promelas</i>)	NOEC 33d (growth)	70	0.07	10	0.007	Long-term results (NOECs) from 3 trophic levels
FTC	Fish (<i>P. promelas</i>)	NOEC 28d	6100	6.1	100	0.061	One long-term NOEC from one trophic level
IDV	Fish	ChV	1923	1.9	50	0.038	Two long-term results (NOEC) from 2 trophic levels
	Crustacean (<i>Mysis diluviana</i>)	ChV	1412	1.4	50	0.028	Two long-term results (NOEC) from 2 trophic levels
	Daphnid	ChV	42.8	0.04	50	0.001	Two long-term results (NOEC) from 2 trophic levels
3TC	Algae	NOEC	9.69×10 ⁴	96.9	100	0.969	One long-term NOEC from one trophic level
LPV	Algae (Green Algae)	EC ₅₀ 96h	1914	1.91	1000	0.0019	One short-term L/EC ₅₀ from 3 trophic levels
	Crustacean (<i>D. magna</i>)	LC ₅₀ 48h	2652	2.65	1000	0.0027	One short-term L/EC ₅₀ from 3 trophic levels
	Fish (<i>Lepomis macrochirus</i>):	LC ₅₀ 96h	2082	2.08	1000	0.0021	One short-term L/EC ₅₀ from 3 trophic levels
MVC	Fish	ChV	74	0.07	10	7.4×10 ⁻³	Lowest long-term results (NOECs) from 3 trophic levels
	Algae	ChV	1.15×10 ⁵	115	10	1.2 ×10 ¹	Lowest long-term results (NOECs) from 3 trophic levels
	Mysid (SW)	ChV	8	0.008	10	7.5 ×10 ⁻⁴	Lowest long-term results (NOECs) from 3 trophic levels
NFV	Green Algae	EC ₅₀	34.2	0.03	1000	0.034	One short-term L/EC ₅₀ from 3 trophic levels
	Mysid	LC ₅₀ 96h	27.3	0.02	1000	0.0	One short-term L/EC ₅₀ from 3 trophic levels
	Fish (SW)	LC ₅₀ 96h	103.7	0.10	1000	0.1	One short-term L/EC ₅₀ from 3 trophic levels
NVP	Green Algae (<i>S. capricornutum</i>)	NOEC 10d	4.3×10 ⁴	43	10	4.3	Long-term results (NOECs) from 3 trophic levels

	Crustacean (<i>D. magna</i>)	NOEC 48h	76.9×10 ⁴	76.9	10	7.69	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>O. mykiss</i>)	NOEC 96h	6.5×10 ⁴	65	10	6.5	Long-term results (NOECs) from 3 trophic levels
RAL	Green Algae (<i>S. capricornutum</i>)	NOEC 96h (growth rate)	3800	3.8	10	0.38	Long-term results (NOECs) from 3 trophic levels
	Crustacean (<i>D. magna</i>)	LC ₅₀ 48h	9500	9.5	10	0.95	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>P. promelas</i>)	LC ₅₀ 96h	9300	9.3	10	0.93	Long-term results (NOECs) from 3 trophic levels
RTV	Crustacean (<i>D. magna</i>)	EC ₅₀ 48h	1500	1.5	50	0.03	Two long-term results (NOEC) from 2 trophic levels
	Fish (<i>L. macrochirus</i>)	LC ₅₀ 24h	1630	1.63	50	0.033	Two long-term results (NOEC) from 2 trophic levels
d4T	Acute crustacean	IC ₅₀ 24h	1.12×10 ⁵	111	1000	0.11	One short-term L/EC ₅₀ from 3 trophic levels
	Green Algae	EC ₅₀	16.10	0.02	1000	2×10 ⁻⁵	One short-term L/EC ₅₀ from 3 trophic levels
	Fish	LC ₅₀	1.07×10 ⁶	1070	1000	1.07	One short-term L/EC ₅₀ from 3 trophic levels
SQV	Green Algae (<i>Raphidocelis subcapitata</i>)	NOEC 72h	1120	1.12	10	0.112	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>O. mykiss</i>)	NOEC 96h	3.88×10 ⁴	38.8	10	3.88	Long-term results (NOECs) from 3 trophic levels
	Fish (<i>Poecilia reticulata</i>)	NOEC 96h	5490	5.49	10	0.549	Long-term results (NOECs) from 3 trophic levels
TDF	Crustacean (<i>Artemia salina</i>)	IC ₅₀	6.18×10 ⁴	61.83	1000	0.062	One short-term L/EC ₅₀ from 3 trophic levels
	Algae (<i>Microcystis novacekii</i>)	EC ₅₀	8.9×10 ⁴	89	1000	0.089	One short-term L/EC ₅₀ from 3 trophic levels
	Fish (<i>P. promelas</i>)	NOEC 32d	9000	9	1000	0.009	One short-term L/EC ₅₀ from 3 trophic levels
DDC	Fish	ChV		2467	10	246.7	Long-term results (NOECs) from 3 trophic levels

	Daphnid	ChV		47.2	10	4.7	Long-term results (NOECs) from 3 trophic levels
	Green Algae	ChV		413	10	41.4	Long-term results (NOECs) from 3 trophic levels
ZDV	Green Algae	ChV		0.01	10	0.001	Long-term results (NOECs) from 3 trophic levels
	Crustacean (<i>D. magna</i>)	NOEC	1.6×10 ⁴	16	10	1.6	Long-term results (NOECs) from 3 trophic levels
	Fish	ChV		2.28	10	0.228	Long-term results (NOECs) from 3 trophic levels

ChV = chronic value (the geometric mean of the NOEC and the LOEC); d = days; h = hours; IC50 = inhibitory concentration 50%; LOEC = lowest observed effect concentration; NOEC = no observed effect concentration.

ANNEXURES I:

Table 7-13: Comparative summary between the two approaches employed to assess the hazard caused by ARVDs in water sources.

Aspect	HA approach 1 (ADD/RfD)	HA approach 2 (CDI/ADI)
Conservativeness in theory (by design)	Less conservative: Focus on worst-case (99 th percentile) HQ values. Used RfD based on strict UF adjustments.	More conservative per unit exposure: Based on ADI derived from TDs with conservative safety factors.
Acute vs. chronic exposure	Emphasises acute exposure via high-exposure scenarios.	Focuses on chronic exposure and general safety trends across lifetime exposure.
Key findings	- Quantitative prioritisation of hazardous ARVDs (e.g., ABC, EFV, LPV, TDF) . - Suggests non-carcinogenic risks at high exposure levels. - Linked population-specific vulnerability with compound-specific toxicity. - Highlighted vulnerable groups (adults and pregnant women) while consistently noting lower HQs for children. - Underlines significant variability across ARVDs, with some drugs (e.g., MRV, IDV) posing negligible risks and others requiring mitigation strategies. - Bridges hazard to risk by incorporating distribution-based exposure (percentiles, 95% CI).	- No toxic potential identified (HQ < 1) for any ARVD or population. - Exposure levels are unlikely to pose non-carcinogenic hazards to any sub-population. - Validated the low toxicity profile of ARVDs in water sources under standard conditions. - Provided baseline for chronic exposure assessments.
Regulatory implications	Calls for targeted mitigation strategies and further risk assessments for hazardous ARVDs.	Suggests no immediate need for intervention.
Regulatory usefulness	Precautionary. Useful for setting limits, prioritising and hotspot analysis.	Best for routine monitoring and chronic risk flagging.

ABC = abacavir; ADI = acceptable daily intake; ADD = average daily dose; ARVD = antiretroviral drug; CDI = chronic daily intake; EFV = efavirenz; HA = hazard assessment; HQ = ; IDV = indinavir; MRV = maraviroc; RfD = reference dose; TD = therapeutic dose; TDF = tenofovir; UF = uncertainty factor.

End