

Bioassays to screen for estrogenic and anti-estrogenic activity

EDC activity in the samples were determined by bioassays, T47D-Kbluc assay which was described by Wilson *et al.*¹⁰³⁻¹⁰⁴ and the MDA-kb assays described also by Wilson *et al.*¹⁰⁵ in another study. The protocol followed was according to the South African Water Research Commission (WRC) Toolbox project: K5-1816.¹⁰¹ Also, Nitrile (latex free) gloves was worn when preparing the assay components and while doing the assay. Working with latex gloves could have affected the outcome of the assays.¹⁰¹

Preparation of all glassware used

All glassware was prepared by washing in chromic acid a then rinsing in tap water, double distilled EDC free water and HPLC grade ethanol consecutively. The glassware was dried in the oven and covered with foil. The glassware was then sterilized by autoclaving at 121°C for 20 minutes.¹⁰¹

The T47D-KBluc assay

The T47D-Kbluc reporter gene assay was established to be a sensitive and specific assay for samples screening for estrogenic and anti-estrogenic activities. Human breast cancer cells, T47D cells, can naturally express estrogen receptor alpha and beta. These cells were transfected with an estrogen-responsive element luciferase reporter gene construct. Active ligands that bind to the estrogen receptor result in the luciferase reporter gene's activation and dose-dependent production of the luciferase enzyme.

Materials

The materials used included T47D-Kbluc cells, RPMI 1640 powder, sodium bicarbonate (NaHCO₃), glycylglycine, adenosine 5'-triphosphate (ATP), bovine serum albumin (BSA), magnesium chloride (MgCl₂) solution, E2, D(+)-glucose, Ethanol (EtOH), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer solution, sodium pyruvate, antibiotic/antimycotic solution, Hank's buffered salt solution (HBSS), trypsin, phosphate buffered saline (PBS), recovery cell culture freezing media, Fetal bovine serum (FBS), charcoal/dextran treated FBS (c/d FBS),

reporter lysis buffer, beetle luciferin and ICI 182,780. The apparatus used was 25cm² and 75 cm² tissue culture flasks 96-well luminometer plates, cryovials and 50 mL centrifuge tubes and CoolCell freezing containers.

Methods

When testing chemicals using the T47D-Kbluc cells, estrogen is defined as “a chemical that induced a dose-dependent luciferase activity, which could be specifically inhibited by the anti-estrogen ICI 182,780.”¹⁰⁴ T47D-Kbluc cells were maintained in RPMI growth media supplemented with 2.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L NaHCO₃, 10% fetal bovine serum (FBS), 100 µg/mL penicillin, 100 U/mL streptomycin and 0.25 µg/mL amphotericin B. One week before the assay, cells were placed in growth media modified by replacing 10% FBS with 10% dextran-charcoal treated FBS, excluding antibiotic supplements.¹⁰⁴

As per figure 10, cells were then seeded at 5 x 10⁴ cells per well in 96-well luminometer plates which was then allowed to attach overnight. Dosing dilutions was prepared in growth media containing 5% dextran charcoal treated FBS, and vehicle (ethanol) did not exceed 0.2%. Each plate contained a positive agonist control (E₂), negative control (solvent only), antagonist control (E₂ plus ICI) and background control (solvent plus ICI). Each sample was tested separately and also for the presence of 0.1 nM E₂ or ICI. Cells were incubated 24h with 100 µL/well dosing solution at 37°C, with 5% CO₂.

Following the incubation period, cells were washed with phosphate-buffered saline at room temperature and lysed with 25 µL lysis buffer. The microtiter plate luminometer determined Luciferase activity and was quantified as relative light units (RLU). Each well received 2 5µl reaction buffer (25 mM glycylglycine, 15 mM MgCl₂, 5 mM ATP, 0.1 mg/mL BSA, pH 7.8), followed by 2 5µL 1mM D-luciferin 5 s later. RLU were then converted to a fold induction above the vehicle control value.

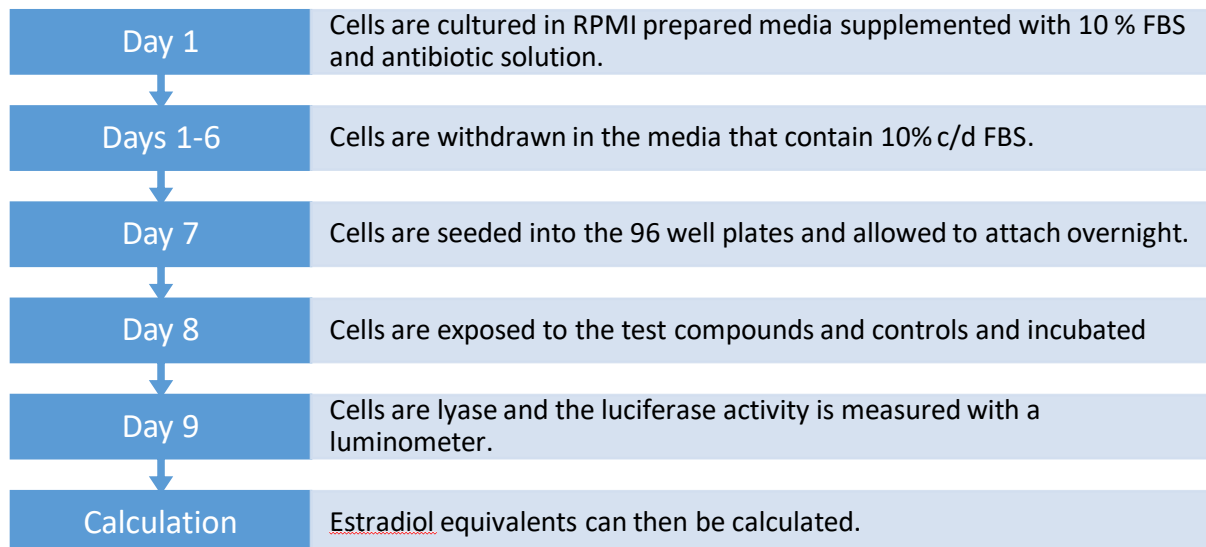


Figure 10: Summary of steps of a The T47D-Kbluc assay.¹⁰⁰⁻¹⁰¹

Bioassay for androgenic and anti-androgenic activity

MDA-kb assay

The MDA-kb2 is a cell line developed to screen androgen agonists and antagonists. This was done to characterize its specificity and sensitivity to endocrine-disrupting chemicals. The breast cancer cell line, MDA-MB-453, was stably transformed with the mouse mammary tumour virus promoter (MMTV). luciferase.neo reporter gene construct. Since both glucocorticoid receptors (GR) and androgen receptors (AR) are present in the MDA-MB-453 cells. These two receptors can act through the MMTV promoter, compounds that act through either AR or GR and thus activate the MMTV luciferase reporter.¹⁰⁵ A Novel Cell Line, MDA-kb2, that Stably Expresses an Androgen- and Glucocorticoid-Responsive Reporter for the detection of hormone receptor agonists and antagonists.¹⁰⁵

Materials

The materials used were MDA-kb2 cells, Lebovitz's L-15 growth media, 10% FBS, Penicillin, streptomycin, amphotericin B. The apparatus used was 25 cm² and 75 cm² tissue culture flasks, 96-well luminometer plates, centrifuge tubes, cryovials and CoolCell freezing containers

Methods

As per figure 11, MDA-kb2 cells were maintained in Lebovitz's L-15 growth media (Cat. No. 41300-021, Gibco, Scientific Group, SA) supplemented with 10% FBS (characterised, Cat. No. SH30071.03, Hyclone, Separations, SA), 100 ug/m; penicillin, 100 U/m; streptomycin and 0.25 ug/m; amphotericin B (Cat. No. 15240-062, Gibco, Scientific Group, SA) as described in Wilson *et al.*¹⁰⁵

Cells were seeded at 5 x 10⁴ cells per well in 96-well luminometer plates and allowed to attach overnight. Dosing solutions were prepared in growth media and vehicle (ethanol), not exceeding 0.2%. Each plate contained a positive agonist control (dihydrotestosterone, DHT), negative control (vehicle only), antagonist control (DHT plus flutamide) and background control (vehicle plus flutamide). Each sample was tested alone as well as in the presence of 0.1 nM DHT or flutamide. Cells were incubated for 24hrs with 100; dosing solution per well at 37°C, without supplemental CO₂. After the incubation period, cells were lysed, and luciferase activity determined as described for the Kbluc assay.

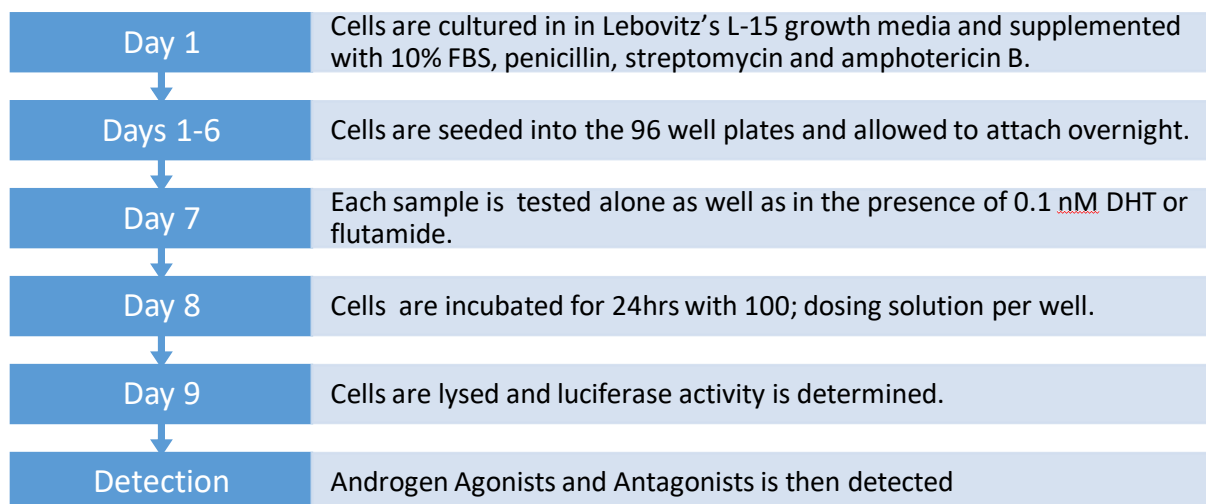


Figure 11: Summary of steps of the MDA-kb2 assay.¹⁰⁰⁻¹⁰¹

3.3.2 Pharmaceutical Screening

Chemical screening of the water extract was conducted using a UPLC (ultra-high-performance liquid chromatography) system consisting of an Agilent 1290 Infinity Binary pump (G4220A); 1290 Infinity Autosampler (G4226A); and 1290 Infinity

Thermostatted Column Compartment (G1316C) coupled to an Agilent 6540 Accurate mass Q-TOF/MS (G6540A) (Agilent Technologies, Santa Clara, CA, USA). Agilent's Personal Compound Database and Library software, 7 500 compound forensic/toxicology database, and 2 500- compound MS/MS Broecker, Herre & Pragts PCDL library enabled identification of a long list of compounds. This list includes human doping drugs, designer drugs, veterinary drugs, pesticides, mycotoxins, cannabinoids, hallucinogens, stimulants, benzodiazepines, hypnotics, neuroleptics, barbiturates, antidepressants, cardiovascular medicine, anti-epileptics, opioids, anabolic agents, pharmaceuticals and personal care products and hormones. The software used was MassHunter's data acquisition (version 10.1), qualitative analysis (version 10.0) and quantitative analysis (version 10.1). The mass axis calibration of the QTOF was performed daily for positive and negative ionisation with a tuning mix (G1969-85000, Agilent). A reference solution with masses of 121.050873 [M+H] and 322.048121 [M+H] was constantly infused to serve as an accurate mass reference.

Liquid chromatography (LC) and mass spectrometry (MS) parameters

The injection volume for analysis was 1 μ L. A Poroshell 120 Bonus-RP column (Agilent, 2.1 x 100 mm, 2.7 μ m) kept at 25°C was used for separation. The mobile phase for positive ionisation consisted of water (solvent A) and acetonitrile (solvent B) both containing 0.1% formic acid.¹⁰⁶ The mobile phases for negative ionisation was water (solvent A) and methanol (solvent B) without modifiers.

Qualitative screening of unknown compounds

The data obtained after the chromatographic analysis were utilised to screen for and identify some of the compounds present in the extracts. Compound possibilities were generated based on molecular features and subjected to the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL). The library includes 9 200 compounds including human doping drugs, designer drugs, veterinary drugs, pesticides, mycotoxins, cannabinoids, hallucinogens, stimulants, benzodiazepines, hypnotics, neuroleptics, barbiturates, antidepressants, cardiovascular medicine, anti-epileptics, opioids, anabolic agents, pharmaceuticals and personal care products and

hormones. This PCDL combined with the accurate mass capabilities of the Q-TOF instrument confirm the presence of compounds based on accurate monoisotopic mass, isotope patterns, fragment confirmations and retention time.

Quantitative analysis of specific target compounds

Matrix-matched-calibration.

The matrix to be analysed was drinking and surface water. To account for matrix effects during quantification an external matrix-matched calibration curve was used. To mimic natural water in the matrix-matched calibrations, deionised water (18.2 mΩ·cm) was supplemented with 0.7 mM NaHCO₃, 2 mM CaCl₂·2H₂O, 0.5 mM MgSO₄·7H₂O, and 75 μM KCl to create artificial freshwater (ISO, 2012). This water was inoculated with 50 mL of the same water after 25 mature specimens of the freshwater snail *Bulinus tropicus* had been living in one litre of the first type for at least 24 hours. This was done to assist to simulate at least some form of organic content.

The concentrations used in the calibration curve were determined based on the expected levels of pharmaceuticals present in the sample (after enrichment) and the performance of the instrument. Samples were concentrated 2 000 times during extraction. This should be accounted for when choosing a calibration range (Table 7). Matrix-matched calibration curves were prepared by extracting 1L of the prepared water, evaporating the extract to dryness and resuspending it in 500 μL. This reconstitute was spiked with a mixture of pharmaceuticals and internal standards at the calibration range concentrations. Serially dilutions were not prepared but originated from different stocks. These standards were analysed in triplicate to assess the reportable range (Westgard, 2008). They were injected in order of increasing concentration, with blank injections between batches to prevent carry-over.

Table 7: Concentration range of calibration curves used for pharmaceuticals quantification

Pharmaceutical compound	Calibration curve range (ug/mL)
Ampicillin	0–10 (1.2x serial dilution)
Chloramphenicol	0–10 (1.2x serial dilution)
Ciprofloxacin	0–2 (1.2x serial dilution)

Efavirenz	0–2 (5x serial dilution)
Erythromycin	0–8 (1.2x serial dilution)
Fluconazole	0–8 (5x serial dilution)
Nevirapine	0–10 (5x serial dilution)
Lopinavir	0–10 (5x serial dilution)
Sulfamethoxazole	0–5 (1.2x serial dilution)
Trimethoprim	0–0.5 (1.2x serial dilution)
Tetracycline	0–4 (1.2x serial dilution)
Ritonavir	0–10 (5x serial dilution)
Vancomycin	0–20 (1.2x serial dilution)

Precision and accuracy

Precision (repeatability, in terms of % RSD) and accuracy (percentage recoveries) were estimated by recovery experiments at two spiked levels, each one analysed in triplicate.

Accuracy was determined as the recovery of spiked samples (Table 8).

Precision was calculated using $\% \text{RSD} = (\text{SDEV of QCs}/\text{mean of QCs}) \times 100$ (Table 7).

Linearity

The linearity of the calibration curve was assessed by determining the R^2 value.

Good linearity is indicated with R^2 as close to 1 as possible (at least 0.9).¹⁰⁷ The linearity of the calibration curve for this analysis can be found in table 8.

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

Sensitivity of an analytical method is defined as “the increased response of the analyte linear to the analyte concentration.”¹⁰⁸ A calibration curve and the slope of the calibration curve is used as a display. By using linear regression statistics, the uncertainties of the calibration curve can be used to calculate limit of detection(LOD) and limit of quantification(LOQ) for the method from the external matrix-matched

calibration curve. By use of the $y = m x + c$ model, LOD is calculated by $3 \cdot Sa/b$ and LOQ by $10 \cdot Sa/b$; where Sa is the SD of the intercept (abundance) and b is the slope of the calibration curve.¹⁰⁹

Table 8: Method validation parameters

Pharmaceutical compound	Precursor ion (m/z)	RT (min)	R ²	LOD (ug/mL)	LOQ (ug/mL)	Accuracy (%)	Precision (%)
Ampicillin	350.1500	3.3	0.993	0.96	3.19	83	8
Chloramphenicol	320.9358	0.9	0.995	0.73	2.44	73	7
Ciprofloxacin	332.1446	3.464	0.999	0.09	0.30	93	5
Efavirenz	314.0193	2.0	0.988	3.06	10.23	78	12
Erythromycin	734.7401	8.914	0.996	0.60	2.01	48	10
Fluconazole	307.1111	5.007	0.998	0.44	1.48	71	4
Nevirapine	267.1224	7.58	0.993	1.48	4.93	87	9
Sulfamethoxazole	254.0588	9.2	0.994	0.48	1.61	75	16
Trimethoprim	291.1473	3.181	0.997	0.03	0.11	111	5
Tetracycline	445.1649	3.4	0.988	1.9	6.5	72	15
Vancomycin	1447.1446	3.2	0.998	0.91	3.04	61.8	7

3.3.3 Viral Content

Virus Concentration and Nucleic Acid Extraction

The glass wool adsorption-elution technique was used for viral recovery from water samples.¹¹⁰ The recovered viruses were eluted from glass wool using 100 millilitres (mL) of glycine-beef extract buffer (pH 9.5) (Glycine; Merck KgaA) (BBL™ Beef Extract; Becton, Dickinson and Company, Sparks, MD) and the pH of the eluate was adjusted to pH 7 using 1 M HCl (Merck KgaA). The viruses in 100 mL eluate were further concentrated into a final volume of 10 mL in phosphate-buffered saline (PBS) (pH 7.4) (Sigma-Aldrich Co., St. Louis, MO) by polyethylene glycol 8000/sodium chloride (PEG8000/NaCl) precipitation (PEG8000; Amresco LLC, Solon, OH) (NaCl;

Merck KgaA).¹¹¹⁻¹¹² The extracted nucleic acids will be eluted in a final volume of 100 μ L and stored in 10 μ L aliquots at -70 °C.

Detection and Quantification of Norovirus (NoV) and Adenovirus (AdV)

Commercial real-time reverse transcription-PCR (RT-PCR) kits were used for the detection and quantification of NoV and AdV GI and GII using CeeramTools™ (Ceeram S.A.S, La Chapelle Sur Erdre Cedex, France) and (norovirusGII@ceeramTools™) (Ceeram S.A.S). All kits used contained internal, positive, and negative PCR inhibition controls to monitor the efficiency of target amplification. According to the manufacturer's instructions, NoV and AdV GI and GII were detected and quantified using one-step real-time qRT-PCR. Norovirus and Adenovirus standard curves were generated using plasmid DNA standards (Norovirus and Adenovirus GI Q Standard) (Norovirus and Adenovirus GII Q Standard) (Ceeram S.A.S). Norovirus and Adenovirus concentrations were adjusted to compensate for extraction efficiencies below 100% and expressed as genome copies/litre (gc/L). All NoV and AdV-negative samples were re-tested using a 1:10 dilution of RNA in nuclease-free water to exclude possible false-negative results due to inhibition.

3.4 Data Management and Analysis

3.4.1 Assays to assess endocrine disruption

T47-Kbluc-Assay

The E2 standard curve was fitted (sigmoidal function, variable slope) using GraphPad Prism (version 4), which was then used to calculate the minimum, maximum, slope, EC50 value and 95% confidence limits. The EEq values of extracts with greater than a two-fold induction above the vehicle control was interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

MDA-kb assay

Each well was receive 25; reaction buffer (25 mM glycylglycine, 15 mM MgCl₂, 5 mM ATP, 0.1 mg/m; BSA, pH 7.8), followed by 25mM; 1 mM D-luciferin 5s later. The RLU

was converted to a fold induction above the vehicle control value. The curves for DHT and selected EC samples was fitted (sigmoidal function with variable slope) using GraphPad Prism (version 4), then the minimum, maximum, slope, EC50 value and 95% confidence limits was calculated.

3.4.2 Pharmaceutical screening

Quantitative screening of known compounds

The concentration of target compounds was calculated by using the below formula:

$$X_{\text{compound}} = ((\text{native/stable isotope}) - c) / m \times \text{ISO conc}$$

where:

X_{compound} = calculated analyte concentration
native = native abundance

stable isotope = stable isotope abundance

c = calibration curve is the y-intercept

m = slope of the calibration curve

ISO conc = stable isotope concentration

Qualitative screening of unknown compounds

The data obtained after the chromatographic analysis were utilised to screen for and identify some of the compounds present in the extracts. Compound possibilities were generated based on molecular features and subjected to the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL). This PCDL combined with the accurate mass capabilities of the Q-TOF instrument confirm the presence of compounds based on accurate monoisotopic mass, isotope patterns, fragment confirmations and retention time

3.5 Ethical and legal considerations

Ethical approval was attained from the University of Pretoria's Faculty of Health Sciences Research Ethics Committee with the ethical approval numbers 151/2021 and letter attached at Annexure A.

The risk of the study was negligible since water sampling was used. Principles of Helsinki and Good Clinical Practice was adhered to, and there were no conflicts of interest. No samples or information were obtained from the community or other people and no experimental animals were used. Permission to conduct the study was obtained from the City of Tshwane, please see Annexure B.

CHAPTER 4: RESULTS

4.1 Estrogenic activity: T46D-Kbluc-assay

T46D-Kbluc-assay results indicated that water samples analysed from selected sample points contained compounds with estrogenic activity. Estrogenic activity was detected in thirteen samples with only 2 samples (C3 and E) below the detection limit (dl). The estradiol equivalency (EEq) values ranged from below the dl to 0.216. The samples collected from the houses in samples A1, A2 and A4 were collected from JOJO A (sample A). Samples collected from JOJO tank A's EEq values (average of 3 houses= 0.028 ng/L) were higher than their source (JOJO A). Higher activity was seen in the samples collected from the houses compared to source JOJO was also seen in samples B1, B2 and B3 (average= 0.03 ng/L) from the source, JOJO B. Higher or same EEq values were also seen in the houses' samples (C1, C2 and C4 except sample C3) compared to source JOJO C. The average across all these houses was 0.018 ng/L. As seen in the graph in Figure 10 sample B3's EEq value was 193.65% more than the source JOJO B and the house with the highest EEq value. The average EEq value across all the houses was 0.025 ng/L and the EEq value average of the 3 JOJO tanks (samples A, B and C) were 0.012 ng/L which is almost double that of the houses. The JOJO tank with the highest EEq value was JOJO C.

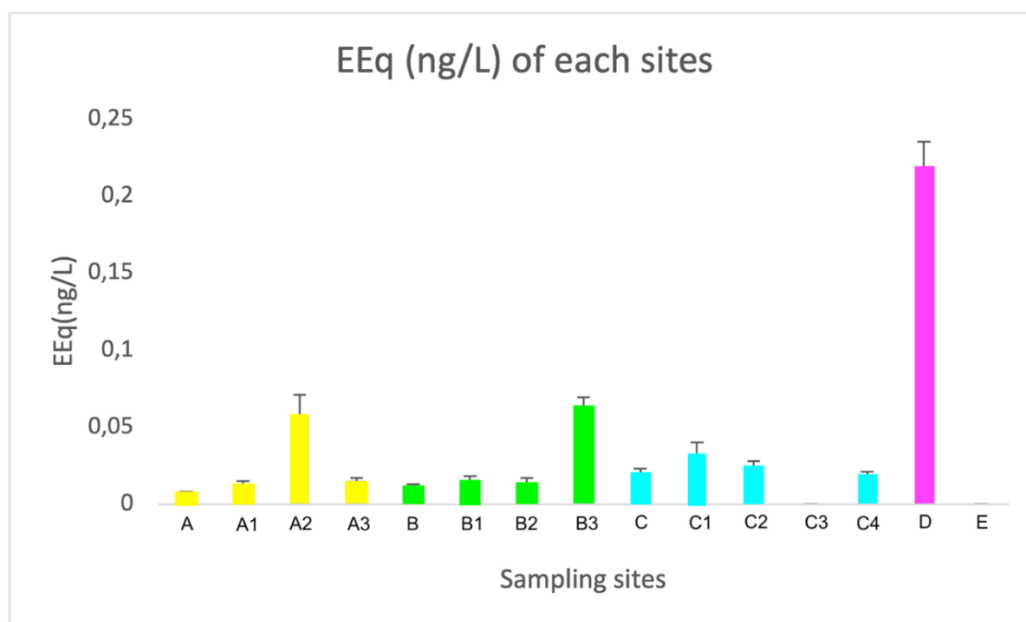


Figure 12: Estradiol equivalency (EEq) across all samples.

The only house with an EEq below the level of quantification of the bioassay was house sample C3. The dam (sample D) had the highest EEq value of 0.216 also seen how high the EEq value is compared to other samples. The community tap (Sample E) which is sourced from a borehole had an EEq value below the level of quantification of the bioassay. No cytotoxicity or anti-estrogenic activity was detected in any of the samples at the concentrations tested (Table 9).

Table 9: Estrogenic and Cytotoxic activity across all samples

Sample code	EEq (ng/L)	Cytotoxicity
A	0.007 ± 0.001	-
A1	0.013 ± 0.002	-
A2	0.057 ± 0.014	-
A3	0.015 ± 0.002	-
B	0.010 ± 0.003	-
B1	0.015 ± 0.003	-
B2	0.013 ± 0.004	-
B3	0.062 ± 0.007	-
C	0.019 ± 0.004	-
C1	0.030 ± 0.010	-
C2	0.024 ± 0.004	-
C3	<loq	-
C4	0.019 ± 0.002	-
D	0.216 ± 0.019	-
E	<loq	-

4.2 Androgenic activity: MDA-kb2 reporter gene assay

No androgenic activity was observed in any of the water samples, as none of the samples had a RLU (Relative light unit) values above the level of quantification (loq) for each plate. Therefore, none of the samples were able to suppress the activity of DHT, which shows none of the samples have anti-androgenic activity at the tested concentrations.

4.3 Emerging contaminant screening and quantification

4.3.1 Emerging contaminant screening

The most predominant compound seen across all samples is Atrazine-desisopropyl a common herbicide. The second most possibly detected compound is also an herbicide, Trietazine. These two herbicides are seen in almost all samples. The most common pharmaceuticals possibly detected was Enprofylline, a Bronchodilator and Valdetamide, a sedative (Table 10).

Table 10: Frequently screened compounds across all the samples

Ranking	Samples containing compounds in top 5	Name	Used as a
1	A, A1, A3, B1, B2, B3, C, C1, C3, C4	Atrazine-desisopropyl	Herbicide
2	A1, A2, B2, B3, C, C3, E	Trietazine	Herbicide
3	A, A3, B3, C1. E	Enprofylline	Bronchodilator
3	A1, B1, C, C2, D	Valdetamide	Hypnotic; Sedative
3	A, B3, C, C3, E	Propisochlor	Herbicide
4	A, A1, A3, C1	Dimazole	Antimycotic
5	C3, C4, E	Atrazine	Herbicide
5	A3, B2, D	Nonoxinol 9	Spermicide
6	A, C3	Atraton	Herbicide

There is one pharmaceutical which may be prevalent in JOJO A and all the corresponding houses, A1, A2 and A3, namely Dimazole which is known to be an Antimycotic (An antifungal medication). In addition, Atrazine-desisopropyl (herbicide

metabolite) is also seen in JOJO A as well as houses A1, A3. Another pharmaceutical, Enprofylline (Brochodilator) which is seen in JOJO A as well as house A3. All the top 5 compounds seen in JOJO A is also seen in all the other samples' top 5 compounds. For example, Enprofylline is seen in 4 other samples, Atraton seen in 1 other sample, Atrazine-desisopropyl is seen in 9 other samples, Propisochlor is seen in 4 other samples and Dimazole is seen in 3 other samples. In other words, there is no compound unique only in JOJO A. (Table 11)

Table 11: Compounds with highest screening scores from samples A,A1,A2 and A3

Sample A CAS		Notes	Mass (DB)	Score (DB)	Formula	Height
41078-02-8	Enprofylline 5	Bronchodilator	194.0804	97.48	C8 H10 N4 O2	135982
1610-17-9	Atraton 2	Herbicide	211.1433	96.39	C9 H17 N5 O	61908
1007-28-9	Atrazine-desisopropyl 10	Herbicide metabolite	173.0468	95.72	C5 H8 Cl N5	80764
86763-47-5	Propisochlor 5	Herbicide	283.1339	94.56	C15 H22 Cl N O2	73747
95-27-2	Dimazole 4	Antimycotic	293.1562	93.88	C15 H23 N3 O S	176583
Sample A1						
1007-28-9	Atrazine-desisopropyl 10	Herbicide metabolite	173.0468	94.95	C5 H8 Cl N5	50459
95-27-2	Dimazole 4	Antimycotic (skin infections)	293.1562	93.12	C15 H23 N3 O S	89286
1912-26-1	Trietazine 7	Herbicide	229.1094	88.53	C9 H16 Cl N5	89902
15534-92-6	Terbuficin	Antilipidemic (cholesterol medication)	468.324	87.81	C30 H44 O4	10821
512-48-1	Valdetamide 5	Hypnotic; Sedative	155.131	87.28	C9 H17 N O	10000
Sample A2						
15793-40-5	Terodiline 2	Coronary Dilator	281.2143	98.02	C20 H27 N	28521
511-96-6	Gitogenin	Biomolecule	432.324	93.49	C27 H44 O4	75369
	Nonoxinol 15	Evidence of developmental/endocrine/reproductive	880.5759	93.08	C45 H84 O16	5000

		effects; chronic aquatic toxicity; acute aquatic toxicity				
1912-26-1	Trietazine	Herbicide	229.1094	90.34	C9 H16 Cl N5	22404
56695-65-9	Rosaprostol	Anticancerative	298.2508	88.8	C18 H34 O3	11259
Sample A3						
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	96.52	C5 H8 Cl N5	26845
95-27-2	Dimazole	Antimycotic	293.1562	96.39	C15 H23 N3 O S	52505
9016-45-9	Nonoxinol 9- 3	Spermicide	616.4186	95.15	C33 H60 O10	37821
41078-02-8	Enprofylline	Bronchodilator	194.0804	93.24	C8 H10 N4 O2	53701
23790-08-1	Moxipraquine	Antiamoebic (parasites)	414.2995	86.6	C24 H38 N4 O2	14097

House A1 also may contain Atrazine-desisopropyl and Dimazole which may also be in JOJO A. House A1 may also have Trietazine (an herbicide) which is seen in 6 other samples as well especially sample A2. Furthermore, House A1 may also contain Valdetamide (Hypnotic; a sedative) which is seen in 4 other samples. House A1 may contain a unique compound, Terbuficin, which is only seen in house A1's top 5 compounds and not in another sample's top 5. Terbuficin is known to be an Antilipidemic which is used as a cholesterol medication.

House A2 may have 3 unique compounds namely, Gitogenin, Nonoxinol 15, Rosaprostol. Gitogenin is known to be a biomolecule and Rosaprostol is known to be an anticulcerative. Interestingly Nonoxinol 15, is known to show evidence of developmental/endocrine/reproductive effects, chronic aquatic toxicity, and acute aquatic toxicity.

House A3 may contain Nonoxinol 9, a spermicide, which is also seen in 2 other sample's top 5 possible compounds. In Sample A4 there is also one unique possible compound, namely Moxipraquine which is an antiamebic.

JOJO B shares no possible compounds in its top 5 compounds with any other sample. Propentofylline (Vasodilator), Cholesta-3,5-dien-7-one (biomolecule found in high concentrations in fatty/cirrhotic alcoholic liver), delta8-Tetrahydrocannabinol (Psychedelic), Hetramine (Antihistamine), Phenamidine (Chemotherapeutic) are all unique to sample B (Table 12).

Sample B1, B2 and B3 all share a possible compound, Atrazine-desisopropyl. Sample B1 has 3 unique compounds, namely Tacrolimus (immunosuppressant), Norephedrine (Phenylpropanolamine) and Tolyethanol.

Table 12: Compounds with highest screening scores from samples B, B1, B2 and B3

Sample B CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
55242-55-2	Propentofylline	Vasodilator	306.1692	92.61	C15 H22 N4 O3	11094
567-72-6	Cholesta-3,5-dien-7-one	High concentrations in fatty/cirrhotic alcoholic liver	382.3236	89.03	C27 H42 O	191958
28646-40-4	delta8-Tetrahydrocannabinol	Psychedelic	330.2195	85.56	C21 H30 O3	16367
531-08-8	Hetramine	Antihistamine	256.1688	84.76	C15 H20 N4	5337
101-62-2	Phenamidine	Chemotherapeutic	254.1168	82.58	C14 H14 N4 O	3528
Sample B1						
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	92.09	C5 H8 Cl N5	11816
104987-11-3	Tacrolimus	Immunosuppressant	803.482	91.02	C44 H69 N O12	37646
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	87.35	C9 H17 N O	45758
14838-15-4	Norephedrine (Phenylpropanolamine)	Anorectic, sympathomimetic; synonym = Phenylpropanolamin with Pragst ID = P158	151.0997	87.33	C9 H13 N O	38868
536-50-5	Tolyethanol		136.0888	87.13	C9 H12 O	15066
Sample B2						
142-91-6	Isopropyl palmitate	Dermatic	298.2872	94.49	C19 H38 O2	16805
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	93.73	C5 H8 Cl N5	13342
1912-26-1	Trietazine	Herbicide	229.1094	91.21	C9 H16 Cl N5	8938

9016-45-9	Nonoxinol 9	Spermicide	616.4186	89.29	C33 H60 O10	235240
120-80-9	Pyrocatechol	Insecticide	110.0368	87.78	C6 H6 O2	10405
Sample B3						
86763-47-5	Propisochlor	Herbicide	283.1339	92.92	C15 H22 Cl N O2	76134
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	92.27	C5 H8 Cl N5	53431
1912-26-1	Trietazine	Herbicide	229.1094	90.61	C9 H16 Cl N5	96920
60762-57-4	Pirlindole	Antidepressant	226.147	85.7	C15 H18 N2	5320
835-31-4	Naphazoline	Vasoconstrictor	210.1157	85.18	C14 H14 N2	8703

Sample B2 and B3 share 2 possible herbicide compounds, Atrazine-desisopropyl and Trietazine. Sample B2 has 2 unique possible compounds, Isopropyl palmitate and Pyrocatechol which are a dermatic and an insecticide, respectively. Sample B3 and 2 other samples share the possibility of containing Pirlindole, an anti-depressant. Sample B# has 1 unique possible compound Naphazoline, which is a vasoconstrictor.

Sample C, which is one of the JOJO tanks may possibly have 5 compounds which is seen across all corresponding houses' samples. Atrazine-desisopropyl is seen in sample C1, C3 and C4. Propisochlor is also seen in sample C3 and Valdetamide is possibly seen in sample C2 (Table 13). Sample C1 shows to possibly have 2 unique compounds namely N-Desalkyl-pentazozin an opioid medication and Hydracarbazine, a diuretic. Sample C2 possibly has 2 unique compounds one an anabolic and another an antimycotic, which is Zearalenone and N-(2-hydroxyethyl)-10-Undecenamide, respectively. Sample C3's top 5 possible compounds are Herbicides, namely trazine, Propisochlor, Atrazine-desisopropyl, Trietazine, Atraton. There are also no unique compounds only seen in sample C3's top 5 compounds. Atrazine is also seen in 2 other samples, sample C4 and the community tap sample E Sample C4 shows to possibly have 3 unique compounds. Th first unique compound in this sample's top 5 possible compounds is Leucomalachite green, which is a dye used as a dye for materials such as silk, leather, and paper and controversially as an antimicrobial in aquaculture. In addition, 2 another unique compound possibly in sample 3 is Octamylamine which is a Parasympatholytic and Cassaidine which is used as a Cardiotonic.

Table 13: Compounds with highest screening scores from samples C, C1, C2, C3 and C4

Sample C						
CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	97.44	C5 H8 Cl N5	17823
86763-47-5	Propisochlor	Herbicide	283.1339	89.1	C15 H22 Cl N O2	19968
1912-26-1	Trietazine	Herbicide	229.1094	88.67	C9 H16 Cl N5	30555
60762-57-4	Pirlindole	Antidepressant	226.147	86.69	C15 H18 N2	33572
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	85.91	C9 H17 N O	32861
Sample C1						
	N-Desalkyl-pentazozin	Opioid pain medication	217.1467	98.93	C14 H19 N O	50724
41078-02-8	Enprofylline	Bronchodilator	194.0804	96.19	C8 H10 N4 O2	78072
3614-47-9	Hydracarbazine	Diuretic	153.0651	92.84	C5 H7 N5 O	55862
95-27-2	Dimazole	Antimycotic	293.1562	88.72	C15 H23 N3 O S	92153
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	88.22	C5 H8 Cl N5	37083
Sample C2						
15793-40-5	Terodiline	Coronary Dilator	281.2143	95.32	C20 H27 N	54998
17924-92-4	Zearalenone	Anabolic	318.1467	89.27	C18 H22 O5	20912
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	86.62	C9 H17 N O	16097
60762-57-4	Pirlindole 3	Antidepressant	226.147	86.58	C15 H18 N2	23884
20545-92-0	N-(2-hydroxyethyl)-10-Undecenamide	Antimycotic	227.1885	84.34	C13 H25 N O2	7223
Sample C3						

1912-24-9	Atrazine	Herbicide	215.0938	96.45	C8 H14 Cl N5	23895
86763-47-5	Propisochlor	Herbicide	283.1339	96.28	C15 H22 Cl N O2	57211
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	93.77	C5 H8 Cl N5	41379
1912-26-1	Trietazine	Herbicide	229.1094	89.62	C9 H16 Cl N5	78236
1610-17-9	Atraton	Herbicide	211.1433	86.15	C9 H17 N5 O	8082
Sample C4						
	Leucomalachite green	Dye unique	330.2096	99.62	C23 H26 N2	96731
502-59-0	Octamylamine	Parasympatholytic	199.23	98.99	C13 H29 N	76941
26296-41-3	Cassaidine	Cardiotonic	407.3036	97.54	C24 H41 N O4	17452
1007-28-9	Atrazine-desisopropyl	Bronchodilator	173.0468	96.72	C5 H8 Cl N5	29585
1912-24-9	Atrazine 3	Herbicide	215.0938	94.65	C8 H14 Cl N5	11836

Sample D shares the possibility to have Nonoxinol 9 present in the sample with samples A3 and B2 as well as Valdetamide with 4 other samples. Simvastatin a cholesterol synthesis inhibitor is a unique to the dam as well as Leptacline (Stimulant) and Enprazepine (antidepressant). Sample D contain no Herbicides (Table 14).

Table 14: Compounds with highest screening scores from sample D

Sample D CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
79902-63-9	Simvastatin	Cholesterol synthesis inhibitor	418.2719	96.26	C ₂₅ H ₃₈ O ₅	26105
9016-45-9	Nonoxinol 9	Spermicide	616.4186	89.49	C ₃₃ H ₆₀ O ₁₀	203820
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	86.59	C ₉ H ₁₇ N O	29045
5005-72-1	Leptacline	Stimulant	181.183	86.16	C ₁₂ H ₂₃ N	7513
47206-15-5	Enprazepine	Antidepressant	292.1939	85.52	C ₂₀ H ₂₄ N ₂	8060

Sample E, which is the community, tap at the clinic, contains 3 herbicides, 1 insecticide and 1 pharmaceutical, with only 1 unique compound in its top 5 possible compounds, namely Fenazaquin, which is an insecticide (Table 15).

Table 15: Compounds with highest screening scores from sample E

Sample E						
CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
1912-24-9	Atrazine	Herbicide	215.0938	93.12	C ₈ H ₁₄ Cl N ₅	14287
86763-47-5	Propisochlor	Herbicide	283.1339	89.23	C ₁₅ H ₂₂ Cl N O ₂	42812
1912-26-1	Trietazine	Herbicide	229.1094	89.09	C ₉ H ₁₆ Cl N ₅	66791
41078-02-8	Enprofylline	Bronchodilator	194.0804	86.36	C ₈ H ₁₀ N ₄ O ₂	10117
N/A	Fenazaquin	Insecticide	306.1732	82.9	C ₂₀ H ₂₂ N ₂ O	14050

4.3.2 Quantification of Pharmaceuticals

The UPLC analysis showed the presence of the 3 antibiotics, Ciproflaxin, Sulfamethoxazole and Vancomycin (Table 16). Samples A1, A3, B, C1, C3, and E showed the presence of Ciproflaxin ranging between 0.2 ug/L - 0.3 ug/L. Sulfamethoxazole was quantified in 2 samples namely sample A3 (0.6 ug/L) and B (1ug/L). Only sample A3 was quantifiable for Vancomycin at a concentration of 8 ug/L. In addition, sample A3 was the only sample where all three antibiotics, Ciproflaxin (0.3 ug/L), Sulfamethoxazole (0.6 ug/L) and Vancomycin (8 ug/L) was quantified. JOJO tank B was the only source JOJO to show the presence of a pharmaceutical, namely Sulfamethoxazole. Interestingly, the community tap sample showed the presence of Ciproflaxin at a concentration of 0.2 ug/L. It is important to note that sample B3 was lost through analysis.

Table 16: Pharmaceuticals quantification (ug/mL) using UPLC/Q-TOF/MS

Sample	Ciprofloxacin	Erythromycin	Fluconazole	Sulfamethoxazole	Trimethoprim	Vancomycin	Nevirapine	Tenofovir	Tetracycline	Efavirenz	Chloramphenicol
A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
A1	0.2±0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
A2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
A3	0.3±0.1	<LOD	<LOQ	0.6±0.5	<LOD	8±6	<LOD	<LOD	<LOD	<LOD	<LOD
B	<LOD	<LOD	<LOD	1±0.08	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B2	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1	0.3±0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3	0.3±0.4	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C4	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
D	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
E	0.2±0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

4.4 Screening and quantification of Viruses: RT-PCR for viruses

Two samples were taken from the dam for RT-PCR to detect the presence of Norovirus and Adenovirus. Norovirus GI and GII were undetected in both samples. Both samples exhibited inhibition of the internal control when testing the concentrated nucleic acids. Thus, both samples were also tested at 1 in 10 dilutions of the nucleic acids. The inhibition was resolved when the nucleic acids were diluted, but norovirus GI and GII were still not detected.

However, Adenoviruses were detected in the concentrated screen, 1 and 2. Sample 1 was detected at a concentration of 36.8 and 2 at a concentration of 40, which is the cut-off for positive samples. Both samples showed weak, but clear positives. (Table 17)

Table 17. Noro GI, GII and Adenovirus detection in dam water in Melusi informal settlement

Sample ID	Nucleic acid concentration	IC	Noro GI	IC	Noro GII	IC	Adenovirus
1	Concentrated	34,5	ND	ND	ND	31,5	36,8
2	Concentrated	34,5	ND	ND	ND	31,5	40,0

CHAPTER 5: DISCUSSION

This study sought to determine the estrogenic, anti-estrogenic, androgenic and anti-androgenic activity in drinking water sources using a battery of in vitro bioassays. Additionally, the occurrence of selected pharmaceuticals and viruses will be determined to assess the water quality and propose possible strategies to minimise adverse health effects and health risk for the community of Melusi, Pretoria.

There is a paucity of evidence in South Africa regarding personal exposure to multi-pollutants. Thus, our aim with screening and quantifying multi-pollutants, has the potential to reveal multi-pollutant exposure patterns that could inform future toxicology and epidemiology research. Findings will contribute to a transdisciplinary strategy to reduce exposure to multi-pollutants (SDG 3 – indicator 3.9) in water sources (SDG6 – 6.3, 6b) by using novel technology through partnerships (SDG 17 – 17.6) and educational tools (SDG 4) to achieve resilient and sustainable environments (SDG 11 – 11a) to ensure health and wellbeing (SDG 3) for communities like the community of Melusi, Pretoria.

Table 18 below summarizes all the results obtained through our investigation across all samples tested.

Table 18: Comprehensive summary of all results across all samples

Sample	Appearance and storage	EEQ	Pharmaceuticals	Shared compounds	Unique compounds	Viruses
A	Clear, JOJO tank	0.007	-	<ul style="list-style-type: none"> • Enprofylline • Atraton • Atrazine-desisopropyl • Propisochlor • Dimazole 		Not Applicable
A1	Clear, paint container, shade	0.013	Ciprofloxacin	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Dimazole • Trietazine • Valdetamide 	<ul style="list-style-type: none"> • Terbuficin 	Not Applicable

A2	Clear, paint container, sun	0.057	-	<ul style="list-style-type: none"> • Terodiline • Trietazine 	<ul style="list-style-type: none"> • Gitogenin • Nonoxinol 15 • Rosaprostol 	Not Applicable
A3	Green with algae, white container, shade	0.015	Ciprofloxacin Sulfamethoxazole Vancomycin	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Dimazole • Nonoxinol 9 • Enprofylline 	<ul style="list-style-type: none"> • Moxipraquine 	Not Applicable
B	Clear, JOJO tank	0.010	Sulfamethoxazole		<ul style="list-style-type: none"> • Moxipraquine • Propentofylline • Cholesta-3,5-dien-7-one delta8- • Tetrahydrocannabinol • Hetramine • Phenamidine 	Not Applicable
B1	Clear, white container, shade	0.015	-	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Valdetamide 	<ul style="list-style-type: none"> • Tacrolimus • Norephedrine • Tolyethanol 	Not Applicable
B2	Clear, paint container, shade	0.013	-	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Trietazine • Nonoxinol 9 	<ul style="list-style-type: none"> • Isopropyl palmitate • Pyrocatechol 	Not Applicable
B3	Clear, blue container, sun	0.062	N/A	<ul style="list-style-type: none"> • Propisochlor • Atrazine-desisopropyl • Trietazine • Pirlindole 	<ul style="list-style-type: none"> • Naphazoline 	Not Applicable
C	Clear, JOJO tank	0.019	-	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Propisochlor • Trietazine • Pirlindole • Valdetamide 		Not Applicable
C1	Clear, paint container, sun	0.030	Ciprofloxacin	<ul style="list-style-type: none"> • Enprofylline • Dimazole • Atrazine-desisopropyl 	<ul style="list-style-type: none"> • N-Desalkyl-pentazozin • Hydracarbazine 	Not Applicable
C2	Clear, paint container, sun	0.024	-	<ul style="list-style-type: none"> • Terodiline • Valdetamide • Pirlindole 	<ul style="list-style-type: none"> • Zearalenone • N-(2-hydroxyethyl)-10-Undecenamide 	Not Applicable

C3	Clear, white container, shade	<loq*	Ciprofloxacin	<ul style="list-style-type: none"> • Atrazine • Propisochlor • Atrazine-desisopropyl • Trietazine • Atraton 		Not Applicable
C4	Clear, white container, shade	0.019	-	<ul style="list-style-type: none"> • Atrazine-desisopropyl • Atrazine 	<ul style="list-style-type: none"> • Leucomalachite green • Octamylamine • Cassaidine 	Not Applicable
D	Pungent smell and cloudy	0.216	-	<ul style="list-style-type: none"> • Nonoxinol 9 • Valdetamide 	<ul style="list-style-type: none"> • Simvastatin • Leptacline • Enprazepine 	Adenovirus
E	Little cloudy but clear	<loq*	Ciprofloxacin	<ul style="list-style-type: none"> • Atrazine • Propisochlor • Trietazine • Enprofylline 	<ul style="list-style-type: none"> • Fenazaquin 	Not Applicable

*<loq is below limit of detection.

Table 18 shows that estrogenic activity was detected in most samples (13 out of 15 samples) in this study using the T47D-KBluc bioassay, with the EEq values ranging from below limit of detection (<loq) to 0.216 ng/L. However, none of the samples exceeded the trigger value of 0.7 ng/l for estrogenic activity in drinking water.¹¹³ If the trigger value is exceeded, possible adverse health effects are implicated and warrants further investigation and continued testing of the water.¹¹⁴ Estrogens (estradiol, estrone, estriol) are female hormones responsible for the maintenance and development of reproductive tissues and secondary sex characteristics in females.¹¹⁵ In addition, estrone is also the main metabolite of 17 β estradiol (C₁₈H₂₄O₂, a natural estrogen) and reaches the environment via the sewer system or animal excretion.¹¹⁶ It should also be kept in mind that estradiol equivalents are only rough estimates, as the intricacy of the sample, the pH of the water, extraction procedure used and the nature of the assay (i.e. a biological system) might all have an influence on the results. As the trigger value, these ranges were determined by assuming long-term continuous use (lifelong exposure) and incorporating a margin of safety. In conjunction with the South African National Standards (SANS), the water-quality guidelines do not take into consideration the very sensitive receptors among humans which EDCs are known to effect, especially infants and children. Also the latest edition of the SANS has a limited

requirement in terms of known EDC which needs to be considered more thoroughly in the following editions which will ultimately contribute to SDG 6 which focuses on ensuring a clean and stable water supply and effective water sanitation for all people by the year 2030.

There was no cytotoxicity detected in the samples. Additionally, no anti-estrogenic activity was detected in any of the water samples in this study. It should also be kept in mind that a water sample consists of a complex mixture of chemicals with possible (anti)-androgenic and (anti)-estrogenic activity, as well as other chemicals not measured, that could affect the outcome of the assay. In a Dutch study, by Van der Linden et al.¹¹⁷ no antagonist activity was reported in various water sources and this was ascribed to the complex mixture of agonist and antagonist interaction which could be masking the contribution of each individual compound. The androgenic activity measured in the MDA-kb2 assay was absent and may be attributed to the complexity of the samples. This could be that possible androgens present were also below the limit of detection of the MDA-kb2 assay. Furthermore, Blake *et al.*¹¹⁸ concluded in their study that the steroidal estrogen estradiol possibly could bind to the androgen receptor in MDA-kb2 cells. Agonism and antagonism is showed by Estradiol in MDA-kb2 cell line, however only at high concentrations. Steroidal estrogens therefore potentially interfere with the response of the cells to androgens.¹¹⁸ So, in future, methods such as effect-based monitoring should be explored to identify the activity of these complex environmental mixtures.¹¹⁹

It is interesting to note that the estrogenic activity is more in the samples taken from the homes than in the sourced JOJO tank. This can be attributed to the way the water is stored in the homes, either in blue or white containers or paint containers and predominantly stored outside in the sun. These containers are made from Base polyethylene with pigment antioxidants and UV stabilizers to ensure extended service life. Studies have shown that High-Density Poly Ethylene (HDPE), is a thermoplastic polymer made from petroleum¹²⁰ is currently considered an EDC, as contains nonylphenol, which has been found to be dangerous to aquatic life.¹²¹⁻¹²² It is suggested that HDPE is UV resistant, however, according to a study done by Said et al.¹²³, polypropylene fibres can only withstand approximately 6 days of exposure to high-intensity UV light before losing 70% of their strength to resist UV rays. These

containers are used not only for six days but for several years. Ultimately this enclosed environment, coupled with potentially poor water quality may lead to a source for estrogenic contamination.

JOJO A had the lowest estrogenic activity of all the JOJO tanks and the third lowest of all the samples, this may be because it was noted from the community that this JOJO tank is used by the most people and is regularly filled by the municipality. Therefore, the water does stay for a long period of time in the JOJO tank and limited leaching from the JOJO tank can occur. When focusing on the homes, houses A2 and B3 had the highest EEq value compared to all the other houses. When screening water from House A2, the presence of Nonoxinol 15 was identified in the samples. Nonoxinol 15 is part of the Nonoxinols group which is produced by ethoxylation of alkylphenols and vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups. Nonoxynols have been used as detergents, emulsifiers, and wetting agents in cosmetics, including hair products, and defoaming agents.¹²⁴ Only nonoxynol-9 with 9 repeating ethoxy groups, has been used as a spermicide, which is seen in samples A3, B2 and D.

House B3 had an EEq value almost twice that of its source JOJO B. It is interesting to note that House B3 with its high EEq value was also the only house to store water in a blue container. This high EEq value may be from phthalates used to produce this plastic container, or from the blue dye, or another unidentifiable source. Phthalates are known EDCs and have an estrogenic effect.¹²⁵ Phthalates may induce alterations in puberty, the development of testicular dysgenesis syndrome, cancer, and fertility disorders in both males and females.¹²⁵⁻¹²⁶ However, there was no possible Phthalate found in the Qualitative screening of unknown compounds, only herbicides and pharmaceuticals which can also contribute to estrogenic activity.

JOJO C had the highest estrogenic activity compared to JOJO A and B. It is important to note that according to the community, this JOJO tank was filled for the first time in 4 months. This may contribute to the EEq value observed for the JOJO tank heated up to a higher degree for there was no water inside to cool it down and this could cause leaching from the JOJO tank to the water. In addition temperature shows to affect the rate of chemical reactions in a stored water body and it plays an important role in the survival of microorganisms.¹²⁷⁻¹²⁸ The highest noted temperature in water storage tanks was reported to be 23.1°C which exceeds the WHO permissible limit of

drinking water guidelines of 15°C. Furthermore, high temperature favours the re-growth of bacteria.¹²⁸ The storage tank material and colour can affect the temperature of the water in the JOJO tanks. Additionally placing the JOJO tank in a shaded location can reduce temperature meanwhile microbial contamination.¹²⁹

House C3 had the lowest estrogenic activity seen across all houses. This is quite interesting for the owners of this house, who pertinently stated that they wash their white container every time before refilling it and never leave it in the sun.

The Melusi community lies adjacent to a dam, which is a decommissioned quarry. The dam had a very pungent smell and was cloudy in appearance with a very high EEq value compared to the other samples. It is evident that this water is polluted and not safe to drink. Water pollution causes 1.8 million deaths worldwide, according to a study published in 2022.¹³⁰ Together with that Contaminated water sickens about 1 billion people worldwide.¹³⁰ And low-income communities like Melusi are disproportionately at risk because their homes are often closest to the most polluting industries, like mines and are exposed to the results such as this decommission quarry hole.¹³⁰

While residents reported that they do not usually use the dam water for drinking, they do regularly use the community tap. The community tap had an EEq value below the detection limit, however almost all the top 5 possible compounds were Herbicides. This may be attributed to the community tap being near a nursery where a high concentration of Herbicides is potentially used. These herbicides may leach into the underground water and ultimately end up in the borehole from which the water is extracted.¹³¹ Additionally, In this study, herbicides, were 5 out of the 10 most frequently possibly detected compounds across all the samples. This can be because herbicides are one of the most major contaminants in the environment as they are widely use in agriculture and are transported into the environment after their application¹³² contributing to water quality degradation.¹³³

When analysing the data from all the sample sites, the most predominant compound across all samples was Atrazine, and its environmental transformation product atrazine-desisopropyl. Atrazine has been shown to increase the conversion of testosterone and other androgens into estrogens, especially estradiol, by increasing the activity of the enzyme aromatase, which is responsible for the conversion.¹³⁴ Resent research, which compared women in Illinois to women in Vermont in the United

States showed that women who drink water contaminated with low levels of atrazine may be more likely to have irregular menstruation and low estrogen levels.¹³⁵

Ciprofloxacin is an antibiotic drug that targets Gram-negative and Gram-positive bacteria and is prescribed to millions of individuals annually.¹³⁶ Low-income countries like South Africa has a higher rate of infectious diseases and generally higher rate of over-the-counter self-medication.⁵⁷ Ciprofloxacin which is widely used in South Africa, it is used to treat microbial infections, and belongs to the fluoroquinolones group. It is widely prescribed and frequently found in sewage due to its incomplete uptake and metabolism in patients.¹³⁷ Studies have shown the presence of ciprofloxacin in sewage treatment effluents, wastewaters, and domestic waters, which ranged from 0.03 µg/L to 5.6 µg/L.¹³⁸⁻¹³⁹ In this study, ciprofloxacin was detected in five samples with concentrations ranging from 0.2-0.3 ug/L. In other studies, ciprofloxacin has been detected in South African river systems at 0.71 µg/L observed concentration¹⁴⁰, and in other countries, the presence of ciprofloxacin has been confirmed in their municipal wastewaters and drinking water.¹⁴¹⁻¹⁴³ The estimate of at which concentration Studies negative health effects or estrogenic effects are associated with ciprofloxacin require investigation. This is concerning as ciprofloxacin has been shown to have endocrine-disrupting effects.^{138,144} This may possibly contribute to the estrogenic activity seen in samples A1,A3 and C1 which contain ciprofloxacin.

Another antibiotic drug screened for and detected was Sulfamethoxazole. This regularly prescribed antibiotic is known to upregulate CYP17 and CYP19 gene expression in the human adenocarcinoma cell line (H295R) and also increase the estradiol hormone levels and aromatase enzyme activity in male fish.¹⁴⁴⁻¹⁴⁵ Sulfamethoxazole was also found in the Surface water and WWTW influent⁷ in KwaZulu-Natal¹⁴⁰, WWTW influent and WWTW effluent in Gauteng¹⁴⁶ and the WWTW influent and sewage treatment works effluent in the Western Cape.¹⁴⁷

Vancomycin is seen in only one sample, sample A3 at a concentration of 8ug/L, which is the highest concentration of any pharmaceutical screened for across all samples. This is significant because sample A3 is also the only sample with no clear appearance but rather a green appearance with algae. In a study done by Cheng et al.¹⁴⁸ that even at trace concentrations of (0.01–2 mg L⁻¹) antibiotics may enhance the growth of microalgae, which may be the reason for the algae seen in the sample taken from

house A3. Vancomycin is also prone to antimicrobial resistance¹⁴⁹ and is shown to cause a reduction in spermatozoa integrity, hormonal levels and sperm morphology that contribute to male infertility.¹⁵⁰

Antibiotics such as the ones detected, Ciprofloxacin, Sulfamethoxazole and Vancomycin are biologically active and can cause non-target toxicity to aquatic organisms. They are increasing concern due to their continuous exposure threatening human health through diet and environmental ecosystems. Even at low concentrations they can produce antibiotic resistant bacteria, which has been detected in sludge, ultimately used as a fertilizer on agricultural fields.¹⁵¹ Antibiotic resistant bacteria is generated resistance which occurs through mutations in their genes or by acquisition of foreign DNA coding through horizontal gene transfer, and the bacteria can survive, and even grow, in the presence of antibiotic drugs, leading to a condition under which the drugs become noneffective on the patient, which aggravates with time and, at the end, may lead to death. Moreover, it has been reported that microplastics can increase the accumulation of these analytes in fish and algae.¹⁵² However it would be advised to perform an Environmental and Human Health risk assessment to understand and evaluate the risk of these antibiotics in the waters of the Melusi community.

All of the above-mentioned possible contaminants have an impact on the homeostatic systems of the body, especially the immune system.¹⁵³ Homeostatic control is affected by the fact that most EDCs tend to bind to steroid hormone receptors including the estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR). As EDCs disrupt the actions of endogenous hormones, they may induce abnormal reproduction, stimulation of cancer growth, dysfunction of neuronal and immune system.¹⁵⁴ In general, sex hormones such as testosterone help stimulate the immune system.¹⁵⁴ This logically implies that immune system is sensitive to EDCs in a manner similar to that of endogenous hormones. However, synthetic non-steroidal compounds such as DES are potent suppressors of thymus-dependent cellular immune responses *via* gene expression alterations in animal.¹⁵⁴ Susceptibility of the immune system to toxic chemicals is increased during the perinatal period as shown by *in vivo* studies of various compounds such as dioxin.¹⁵⁴

Given the wide range of emerging contaminants present in the JoJo tanks and the household water, the presence of Nonoxinol 9, Valdetamide, Simvastatin, Leptacline and Enprazepine in the dam water, is of concern as the presence of a DNA virus was also present. Human adenovirus was the only virus to present in the viral screening and is ubiquitous in the environment and humans are the only reservoir for them.¹⁵⁵ The virus is excreted in large numbers in human faeces and although adenoviruses have been reported to infect a variety of animals, they are more reported in humans to be highly specific to them.¹⁵⁶ The viruses persist wherever the environment has been polluted by human faeces or sewage¹⁵⁷⁻¹⁵⁹

Therefore, in natural aquatic environments, the incidence of human adenovirus is probably attributable to contamination with untreated or inefficiently treated sewage.¹⁵⁹ Various variants of adenovirus have been identified, and over 50 serotypes are known throughout the world.¹⁶⁰⁻¹⁶¹ The burden of disease is caused by adenoviruses manifests as pneumonia, bronchiolitis, otitis media, conjunctivitis, and tonsillitis. The public health implications of these viruses depend upon the physiological status of the wastewater microbial community. The presence of Adenovirus signifies the imminent danger posed to public health by the discharge of poorly treated effluent into the environment because the adenoviral species have been implicated in clinical illnesses. The complex mixture of emerging contaminants and adenovirus in the water from the dam is concerning and an effect-based monitoring approach should be explored to assess the extent of the endocrine disrupting activity the water poses. These are in line with SDG 3 and 6 about health and wellbeing and access to clean and safe drinking water.

CHAPTER 6: CONCLUSION

The exposure to EDCs in our environment through; water, air, soil, food, personal care products and medical devices, are unavoidable. Due to the ubiquity of EDCs in the environment and endocrine disruptive activity, the potential impact of EDCs on public health is a great reason for concern.

There is limited information available on estrogenic activity and pharmaceutical and viral content in drinking and dam water in South Africa. This study has shown the presence of herbicides, pharmaceuticals, and adenovirus in various water sources in the Melusi Community.

The objectives of this study were to:

- 1. Determine the estrogenic and anti-estrogenic activity (T47D-Kblassay), and androgenic and anti-androgenic activity (MDA-kb assay) in drinking water and wastewater samples using a battery of *in vitro* bioassays.**

Findings: Androgenic or anti-androgenic activity together with no anti-estrogenic activity was observed. However estrogenic activity was seen in the tested samples ranging from <loq to 0.216 ng/L. Additionally, estrogenic activity was higher in the samples taken from the homes compared to samples taken from the JOJO tanks. This can be attributed to the manner in which the water is collected and stored in the homes. Understanding the water collection and storage practices would aid in interventions to improve potable water. An interesting observation was that JOJO A (which is frequently re-filled) had the lowest estrogenic activity compared to JOJO C (which was left empty for 4 months) which had the highest estrogenic activity. This high EEq difference observed between the samples from these two tanks requires further investigation. In addition, cleaning containers and removing them from UV exposure may also impact estrogenic activity and require investigation. House C3 has the lowest estrogenic activity seen across all houses and the water storage containers are washed regularly and left in the shade. Investigating community water collection and storage practices may aid in improving access to safe and clean water in the community. Exposure to a large

range of environmental contaminants may n also impact estrogenic activity as seen in the estrogenic activity seen in the sample from the dam and warrants further investigation.

2. Determine the occurrence of selected pharmaceuticals in water samples using ultra-high-performance liquid chromatography (UPLC)

Findings: Quantitative screening showed that three different antibiotics could be detected, Ciprofloxacin, Sulfamethoxazole and Vancomycin in six out of the 15 samples. The qualitative screening showed that the most predominant possible compound across all samples was Atrazine and its metabolite atrazine-desisopropyl. The community tap, near a nursery, is interesting to note as it contains herbicides and requires further investigation.

3. Determine the occurrence of viral contaminants in drinking water and wastewater samples using real-time reverse transcription-polymerase chain reaction (RT-PCR).

Findings: None of the 2 samples taken from the showed to contain any Norovirus GI or GII, however Adenovirus was detected in varying concentrations in both samples and further investigation is needed.

It is evident that water is a potential source of human exposure to EDCs and pharmaceuticals and viruses, for the drinking water was found to be oestrogenic and can contain pharmaceuticals and adenovirus. Also, poverty-stricken rural communities such as Melusi will be at higher risk since they lack proper water services in the area. The findings suggest that community knowledge, attitudes and practices to water pollution and water storage practices would be essential. This information, coupled with the findings from this study, would enable community information sessions and workshops to be held to improve the safe handling of waste, and practices that improve the safe storage of water.

CHAPTER 7: RECOMMENDATIONS

Low levels of estrogenic activity were frequently detected in most samples a monitoring strategy is therefore recommended for the municipalities filling the JOJO tanks. Continued monitoring and constant refilling of JOJO tanks is vital to reduce any potential estrogenic activity of the water in the JOJO tanks (for example the high EEq concentration seen at JOJO C after being left empty) to identify the source and take remedial action as soon as possible. Also, more focus on EDC in water quality guidelines will contribute to better monitoring of water quality regarding EDCs.

This study used a grab sample approach, and a follow up study should be conducted to monitor the changes in concentrations of the emerging contaminants over time with an increased sample size. Effect-based monitoring of water sources would need to be explored to fully elucidate the effects of the complex mixtures seen in the water sources in Melusi.

Based on the estrogenic activity and presence of a wide range of emerging contaminants in the water, future studies should explore a health risk assessment, to understand the risks exposure to these aquatic pollutants may have on the health of the Melusi community.

More awareness can be created of the harm that endocrine disruptors and pharmaceuticals can cause. This research project was presented at a United Nations Children's Fund One Health for Change (UNICEF-OHC) Symposium and more people need to be made aware of the impact that these contaminants have on human health.

The community must be more empowered to better equip them to the harm that these contaminants can cause. This research will contribute to a Community Resilience Planning Guide and the Community Resilience Development Framework that will be developed specifically for the South African context and will address the community's short term and long-term needs. Further research needs to develop strategies to improve community health and well-being, especially through an integrative approach.

This study shows that water is a potential source of human exposure to EDCs and pharmaceuticals, for the drinking water was found to be oestrogenic and contain pharmaceuticals and viruses. Also, poverty-stricken rural communities such as Melusi will be at higher risk since they lack proper water services in the area. Together with other sources of exposure, the potential for health risks needs further investigation. With regards to sustainability going forward our country aims to move toward establishing sustainable cities (SDG 11), access to water and sanitation (SDG 6), and an environment free from chemicals and other pollutants (SDG 3), this study contributed to attaining health and well-being for all.

REFERENCES

1. Edokpayi JN, Rogawski ET, Kahler DM, Hill CL, Reynolds C, Nyathi E, et al. Challenges to sustainable safe drinking water: A case study of water quality and use across seasons in rural communities in Limpopo Province, South Africa. *Water*. 2018; 10(2) doi:10.3390/w10020159
2. Bornman MS, Aneck-Hahn NH, de Jager C, Wagenaar GM, Bouwman H, Barnhoorn IEJ, et al. Endocrine disruptors and health effects in africa: A call for action. *Environmental Health Perspect*. 2017; 125(8):085005. doi:10.1289/EHP1774
3. World Health Organization and United Nations Environment Programme. State of the science of endocrine disrupting chemicals - 2012. Geneva: World Health Organization and United Nations Environment Programme; 2013.
4. Kumar M, Sarma DK, Shubham S, Kumawat M, Verma V, Prakash A, et al. Environmental endocrine-disrupting chemical exposure: Role in non-communicable diseases. *Front Public Health*. 2020; 8:553850. doi:10.3389/fpubh.2020.553850
5. Anekwe Jennifer E, Mohamed Abou-Elwafa A, Stuart H. Pharmaceuticals and personal care products (ppcps) in the freshwater aquatic environment. *Emerg. Contam*. 3(1):1-16. doi: 10.1016/j.emcon.2016.12.004
6. Hull KL, Harvey S. Growth hormone and reproduction: A review of endocrine and autocrine/paracrine interactions. *Int J Endocrinol*. 2014; 2014:234014. doi:10.1155/2014/234014
7. Patrick SM, Bornman MS, Joubert AM, Pitts N, Naidoo V, de Jager C. Effects of environmental endocrine disruptors, including insecticides used for malaria vector control on reproductive parameters of male rats. *Reprod toxicol*. 2016; 61:19-27. doi:10.1016/j.reprotox.2016.02.015
8. Mandaric L, Diamantini E, Stella E, Cano-Paoli K, Valle-Sistac J, Molins-Delgado D, et al. Contamination sources and distribution patterns of pharmaceuticals and

personal care products in alpine rivers strongly affected by tourism. *Sci. Total Environ.* 2017; 590-591:484-94. doi:10.1016/j.scitotenv.2017.02.185

9. Styszko K, Proctor K, Castrignanò E, Kasprzyk-Hordern B. Occurrence of pharmaceutical residues, personal care products, lifestyle chemicals, illicit drugs and metabolites in wastewater and receiving surface waters of Krakow agglomeration in South Poland. *Sci. Total Environ.* 2021; 768 doi:10.1016/j.scitotenv.2020.144360

10. Sinclair RG, Choi CY, Riley MR, Gerba CP. Pathogen surveillance through monitoring of sewer systems. *Adv Appl Microbiol.* 2008; 65:249-69. doi:10.1016/S0065-2164(08)00609-6

11. Cisneros BJ. Safe sanitation in low economic development areas. *Treat Water Sci* 2011;147-200. doi:10.1016/B978-0-444-53199-5.00082-8

12. Farkas K, Walker DI, Adriaenssens EM, McDonald JE, Hillary LS, Malham SK, et al. Viral indicators for tracking domestic wastewater contamination in the aquatic environment. *Water Res.* 2020; 181:115926. doi:10.1016/j.watres.2020.115926

13. Weimann A, Oni T. A systematised review of the health impact of urban informal settlements and implications for upgrading interventions in south africa, a rapidly urbanising middle-income country. *Int J Environ Res Public Health.* 2019; 16(19) doi:10.3390/ijerph16193608

14. Gastañaduy AS, Bégué RE. Acute gastroenteritis viruses: *Infectious Diseases.* 2017:1390-1398.e3. doi: 10.1016/B978-0-7020-6285-8.00162-3.

15. Andrä Jr, Beyer F, Cornelissen G, Einfeldt Jr, Heseding J, Kümmerer K, et al. Pharmcycle: A holistic approach to reduce the contamination of the aquatic environment with antibiotics by developing sustainable antibiotics, improving the environmental risk assessment of antibiotics, and reducing the discharges of antibiotics in the wastewater outlet. *Environ Sci Eur.* 2018; 30(1):1-7. doi:10.1186/s12302-018-0156-y

16. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee D-H, et al. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 2012; 33(3):378-455. doi:10.1210/er.2011-1050
17. Lecomte S, Habauzit D, Charlier TD, Pakdel F, Sánchez L. Emerging estrogenic pollutants in the aquatic environment and breast cancer. *Genes.* 2017; 8(9) doi:10.3390/genes8090229
18. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, et al. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 2012; 33(3):378-455. doi:10.1210/er.2011-1050
19. Haigh C, Southall J. Endocrine society released scientific, position statements on endocrine-disrupting chemicals. *Endocrine Today.* 2009; 7(9). doi:10.1210/er.2009-0002
20. Tahar A, Tiedeken EJ, Rowan NJ. Occurrence and geodatabase mapping of three contaminants of emerging concern in receiving water and at effluent from waste water treatment plants - a first overview of the situation in the republic of ireland. *Sci. Total Environ.* 2018; 616-617:187-97. doi:10.1016/j.scitotenv.2017.11.021
21. Sacdal R, Madriaga J, Espino MP. Overview of the analysis, occurrence and ecological effects of hormones in lake waters in asia. *Environ. Res.* 2020; 182 doi:10.1016/j.envres.2019.109091
22. Junqueira de Castro F, Rafael Alves dos Santos D, Roberto Picolomini Buongermino C, Sanzi Cortez F, Dias Seabra Pereira C, Brasil Choeri R, et al. Ecotoxicological assessment of four pharmaceuticals compounds through acute toxicity tests. *Drug Chem Toxicol.* 2014: 51-5. doi:10.15343/0104-7809.20143801051055
23. Miege C, Choubert JM, Ribeiro L, Eusebe M, Coquery M. Fate of pharmaceuticals and personal care products in wastewater treatment plants. Conception of a database

and first results. *Environ. Pollut.* 2009; 157(5) :721-1726.
doi:10.1016/j.envpol.2008.11.045

24. Lu G-H, Piao H-T, Gai N, Shao P-W, Zheng Y, Jiao X-C, et al. Pharmaceutical and personal care products in surface waters from the inner city of Beijing, China: Influence of hospitals and reclaimed water irrigation. *Arch. Environ. Contam. Toxicol.* 2019; 76(2):255-64. doi:10.1007/s00244-018-0578-y

25. Sze Yee W, Ahmad Zaharin A. Endocrine disrupting compounds in drinking water supply system and human health risk implication. *Environ Int.* 2017; 106:207-33. doi.org/10.1016/j.envint.2017.05.004

26. Phillips PJ, Smith SG, Zaugg SD, Furlong ET, Kolpin DW, Buxton HT, et al. Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. *Environ. Sci. Technol.* 2010; 44(13):4910-6. doi:10.1021/es100356f

27. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr. Rev.* 2009; 30(4):293-342. doi:10.1210/er.2009-0002

28. Rochman CM, Teh SJ, Lewison RL, Eriksen M, Allen H, Cook AM. Polybrominated diphenyl ethers (pbdes) in fish tissue may be an indicator of plastic contamination in marine habitats. *Sci. Total Environ.* 2014; 476-477:622-33. doi:10.1016/j.scitotenv.2014.01.058

29. Rudel RA, Perovich LJ. Endocrine disrupting chemicals in indoor and outdoor air. *Atmos. Environ.* 2009; 43(1):170-81. doi:10.1016/j.atmosenv.2008.09.025

30. Mir SH, Baddar AH. Association between urinary bisphenol a concentration and obesity prevalence in children and adolescents. *Handbook of research on environmental and human health impacts of plastic pollution: Engineering Science Reference*; 2020. p. 214-45.

31. Saal FS, Coe BL, Angle BM, Taylor JA, Nagel SC. The estrogenic endocrine disrupting chemical bisphenol a (bpa) and obesity. *Mol. Cell. Endocrinol.* 2012; 354(1-2):74-84. doi:10.1016/j.mce.2012.01.001
32. Bazilio FS, Bomfim MVJ, Almeida RJ, Abrantes SMP. Intralaboratory validation of an analytical method for determining the migration of bis(2-ethylhexyl) adipate from packaging to fat foods. *J. Accred. Qual.* 2014; 19(3):195-204. doi:10.1007/s00769-014-1055-6
33. Alamri MS, Qasem AAA, Mohamed AA, Hussain S, Ibraheem MA, Shamlan G, et al. Food packaging's materials: A food safety perspective. *Saudi J. Biol. Sci* 2021; 28(8):4490-9. doi:10.1016/j.sjbs.2021.04.047
34. Xu L, Guang-Guo Y, Hao-Chang S, Xiao-Bing Y, Li W. Simultaneous determination and assessment of 4-nonylphenol, bisphenol a and triclosan in tap water, bottled water and baby bottles. *Environ Int.* 2010; 36(6):557-62. doi: 10.1016/j.envint.2010.04.009
35. Mahai G, Wan Y, Xia W, Yang S, He Z, Xu S. Neonicotinoid insecticides in surface water from the central yangtze river, china. *Chemosphere.* 2019; 229 doi:10.1016/j.chemosphere.2019.05.040
36. Barber LB, Loyo-Rosales JE, Rice CP, Minarik TA, Oskouie AK. Endocrine disrupting alkylphenolic chemicals and other contaminants in wastewater treatment plant effluents, urban streams, and fish in the great lakes and upper mississippi river regions. *Sci Total Environ.* 2015; 517:195-206. doi:10.1016/j.scitotenv.2015.02.035
37. Fasano E, Bono-Blay F, Cirillo T, Montuori P, Lacorte S. Migration of phthalates, alkylphenols, bisphenol a and di(2-ethylhexyl)adipate from food packaging. *Food Contr.* 2012; 27:132-8. doi:10.1016/j.foodcont.2012.03.005
38. D Débora de AA, Silvia L, Paula V, Damià B. Occurrence of nonylphenol and bisphenol-a in surface waters from Portugal. *J Brazil Chem Soc.* 2001; 12(4):532-7. doi.org/10.1590/S0103-50532001000400015

39. Inam EJ, Nwoke IB, Udosen ED, Offiong N-AO. Ecological risks of phenolic endocrine disrupting compounds in an urban tropical river. *Environ Sci Poll Res.* 2019; 26(21):21589-97. doi:10.1007/s11356-019-05458-7
40. Fierens T, Servaes K, Van Holderbeke M, Geerts L, De Henauw S, Sioen I, et al. Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food Chem. Toxicol.* 2012; 50(7):2575-83. doi:10.1016/j.fct.2012.04.029
41. Koch HM, Lorber M, Christensen KL, Pälme C, Koslitz S, Brüning T. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. *Int J Hyg Environ Health* 2013; 216(6):672-81. doi:10.1016/j.ijheh.2012.12.002
42. Bjorklund K, Stromvall AM, Malmqvist PA, Cousins AP. Phthalates and nonylphenols in urban runoff: Occurrence, distribution and area emission factors. *Sci Total Environ.* 2009; 407(16):4665-72. doi:10.1016/j.scitotenv.2009.04.040
43. Pascoli Mn, Lopes-Oliveira PcJ, Fraceto LF, Seabra AB, Oliveira HC. State of the art of polymeric nanoparticles as carrier systems with agricultural applications: A minireview. *Ener Ecol Environ.* 2018; 3(3):137-48. doi:10.1007/s40974-018-0090-2
44. Guo W, Pan B, Sakkiah S, Yavas G, Ge W, Zou W, et al. Persistent organic pollutants in food: Contamination sources, health effects and detection methods. *Int J Environ Res Public Health.* 2019; 16(22) doi:10.3390/ijerph16224361
45. Dabrowski JM. Development of pesticide use maps for South Africa: Research article. *South Afr J Sci.* 2015; 111(1):52-8. doi:10.17159/sajs.2015/20140091
46. Bornman M, Barnhoorn IEJ, Genthe B, van Vuren J, Wagenaar GM, Aneck-Hahn N, et al. Ddt for malaria control: Effects in indicators and health risk. 2010. Pretoria: University of Pretoria: Report No.: 1674/1/09.

47. Aris AZ, Shamsuddin AS, Praveena SM. Occurrence of 17 α -ethynylestradiol (ee2) in the environment and effect on exposed biota: A review. *Environ Int.* 2014; 69:104-19. doi:10.1016/j.envint.2014.04.011
48. Rearick DC, Fleischhacker NT, Kelly MM, Arnold WA, Novak PJ, Schoenfuss HL. Phytoestrogens in the environment, i: Occurrence and exposure effects on fathead minnows. *Environ Tox Chem.* 2014; 33(3):553-9. doi:10.1002/etc.2461
49. A Aris AZ, Shamsuddin AS, Praveena S. Occurrence of 17 α -ethynylestradiol (ee2) in the environment and effect on exposed biota: A review. *Environ Int.* 2014; 69:104–19.
50. Wojnarowski K, Podobiński P, Cholewińska P, Smoliński J, Dorobisz K. Impact of estrogens present in environment on health and welfare of animals. *Animals.* 2021; 11(7) doi:10.3390/ani11072152
51. S Sánchez-Garrido MA, García-Galiano D, Tena-Sempere M. Early programming of reproductive health and fertility: Novel neuroendocrine mechanisms and implications in reproductive medicine. *Hum Reprod Update.* 2022; 28(3):346-75. doi:10.1093/humupd/dmac005
52. Naidu R, Arias Espana VA, Liu Y, Jit J. Emerging contaminants in the environment: Risk-based analysis for better management. *Chemosphere.* 2016; 154:350-7. doi:10.1016/j.chemosphere.2016.03.068
53. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in US. Streams, 1999-2000: A national reconnaissance. *Environ Sci Technol.* 2002; 36(6):1202-11. doi.org/10.1021/es011055j
54. Li S, Wen J, He B, Wang J, Hu X, Liu J. Occurrence of caffeine in the freshwater environment: Implications for ecopharmacovigilance. *Environ Poll: Part B.* 2020; 263(Part B) doi:10.1016/j.envpol.2020.114371

55. Fekadu S, Alemayehu E, Dewil R, Van der Bruggen B. Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. *Science Total Environ.* 2019; 654:324-37. doi:10.1016/j.scitotenv.2018.11.072
56. Boxall ABA, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, et al. Pharmaceuticals and personal care products in the environment: What are the big questions? *Environ Health Perspect.* 2012; 120(9):1221-9. DOI: 10.1289/ehp.1104477
57. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrob Resist Infect Control.* 2017; 6:47. doi:10.1186/s13756-017-0208-x
58. Rivas Chen F, Chefetz B, Thompson ML. Comparison of adsorption behaviors of selected endocrine-disrupting compounds in soil. *J Environ Qual.* 2021; 50(3):756-67. doi:10.1002/jeq2.20221
59. Yu W, Du B, Yang L, Zhang Z, Yang C, Yuan S, et al. Occurrence, sorption, and transformation of free and conjugated natural steroid estrogens in the environment. *Environ Sci Poll Res.* 2019; 26(10):9443-68. doi:10.1007/s11356-019-04402-z
60. Quinn L, Pieters R, Nieuwoudt C, Borgen AR, Kylin H, Bouwman H. Distribution profiles of selected organic pollutants in soils and sediments of industrial, residential and agricultural areas of South Africa. *J Environ Monit.* 2009; 11(9):1647-57. doi:10.1039/b905585a
61. Richardson SD. Water analysis: Emerging contaminants and current issues. *Analyt Chem.* 2009; 81(12) doi.org/10.1021/acs.analchem.7b04577
62. Tijani JO, Fatoba OO, Petrik LF. A review of pharmaceuticals and endocrine-disrupting compounds: Sources, effects, removal, and detections. *Wat Air and Soil Poll.* 2013; 224(11) doi:10.1007/s11270-013-1770-3

63. Hester RE, Harrison RM Pharmaceuticals in the environment. [Cambridge]: Royal Society of Chemistry; 2015. doi.org/10.1039/9781782622345.
64. Thompson KC, Gray J Water contamination emergencies :Enhancing our response. Cambridge: Royal Society of Chemistry; 2006 <https://doi.org/10.1039/9781847552426>.
65. Kabir ER, Rahman MS, Rahman I. A review on endocrine disruptors and their possible impacts on human health. *Environ Toxicol Pharmacol*. 2015; 40(1):241-58. doi:10.1016/j.etap.2015.06.009
66. Reiter LW, Falk H, Groat CG, Coussens C, Institute of Medicine . Roundtable on Environmental Health Sciences R, Medicine, et al. From source water to drinking water : Workshop summary. [Internet] Washington, D.C.: National Academies Press; 2004 <http://public.eblib.com/choice/publicfullrecord.aspx?p=3377818>
67. Forte M, Mita L, Mita DG, De Falco M, Laforgia V, Crispi S, et al. Triclosan and bisphenol a affect decidualization of human endometrial stromal cells. *Mol Cell Endocrinol*. 2016; 422:74-83. doi:10.1016/j.mce.2015.11.017
68. Säfholm M, Ribbenstedt A, Fick J, Berg C. Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens. *Philosoph Trans Royal Soc. Series B, Biol Sci*. 2014; 369(1656) doi:10.1098/rstb.2013.0577
69. Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O, Roig B. Effect of endocrine disruptor pesticides: A review. *Int J Environ Res Public Health*. 2011; 8(6):2265-303. doi:10.3390/ijerph8062265
70. Thomas BP, Tarumi T, Sheng M, Tseng B, Womack KB, Cullum CM, et al. Brain perfusion change in patients with mild cognitive impairment after 12 months of aerobic exercise training. *J Alzheimers Dis*. 2020; 75(2):617-31. doi:10.3233/JAD-190977

71. Özen S, Darcan Suk. Effects of environmental endocrine disruptors on pubertal development. *J Clin Res Pediat Endocrinol*. 2011; 3(1):1-6. doi:10.4274/jcrpe.v3i1.01
72. Capolupo M, Valbonesi P, Kiwan A, Buratti S, Franzellitti S, Fabbri E. Use of an integrated biomarker-based strategy to evaluate physiological stress responses induced by environmental concentrations of caffeine in the Mediterranean mussel *mytilus galloprovincialis*. *Sci Total Environ*. 2016; 563:538-48. DOI: 10.1016/j.scitotenv.2016.04.125
73. Cohen PG. Aromatase, adiposity, aging and disease. The hypogonadal-metabolic-atherogenic-disease and aging connection. *Med Hypotheses*. 2001; 56(6):702-8. doi:10.1054/mehy.2000.1169
74. Clayton EMR, Todd M, Dowd JB, Aiello AE. The impact of bisphenol a and triclosan on immune parameters in the U.S. Population, NHANES 2003-2006. *Environ Health Perspect*. 2011; 119(3):390-6. doi: 10.1289/ehp.1002883
75. Hileman B. Antiseptic soaps under scrutiny FDA to assess whether household use leads to bacterial resistance. *Chem Eng News*. 2005; 83(43):14. doi:10.1021/cen-v083n043.p014a
76. Haji Harunarashid NZI, Lim LH, Harunsani MH. Phthalate sample preparation methods and analysis in food and food packaging: A review. *Food Analyt Meth* 2017; 10(12):3790-814. doi:10.1007/s12161-017-0938-7
77. Pironti C, Ricciardi M, Proto A, Bianco PM, Montano L, Motta O. Endocrine-disrupting compounds: An overview on their occurrence in the aquatic environment and human exposure. *Water*. 2021; 13(10):1347. doi:10.3390/w13101347
78. Marlatt VL, Bayen S, Castaneda-Cortès D, Delbès G, Grigorova P, Langlois VS, et al. Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans. *Environ Res*. 2022; 208 doi:10.1016/j.envres.2021.112584

79. Simazaki D, Kubota R, Suzuki T, Akiba M, Nishimura T, Kunikane S. Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Res.* 2015; 76:187-200. doi:10.1016/j.watres.2015.02.059
80. Sharpe RS. Male reproductive health disorders and the potential role of exposure to environmental chemicals. *Chemtrust.* 2009; <http://www.chemtrust.org/wp-content/uploads/ProfRSHARPE-MaleReproductiveHealth-CHEMTrust09-1.pdf>
81. Paciência IS, Cavaleiro Rufo Jo, Silva D, Martins C, Mendes F, Farraia M, et al. Exposure to indoor endocrine-disrupting chemicals and childhood asthma and obesity. *Allergy.* 2019; 74(7):1277-91. doi:10.1111/all.13740
82. Bouwman H, Kylin H, Sereda B, Bornman R. High levels of DDT in breast milk: Intake, risk, lactation duration, and involvement of gender. *Environ Poll.* 2012; 170:63-70. doi:10.1016/j.envpol.2012.06.009
83. Maqbool F, Mostafalou S, Bahadar H, Abdollahi M. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. *Life Sci.* 2016; 145:265-73. doi:10.1016/j.lfs.2015.10.022
84. Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, Jager C. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. *J Androl.* 2007; 28(3):423-34. doi:10.2164/jandrol.106.001701
85. Patrick SM, Aneck-Hahn NH, Van Wyk S, Van Zijl MC, Huma M, de Jager C. Veterinary growth promoters in cattle feedlot runoff: Estrogenic activity and potential effects on the rat male reproductive system. *Environ Sci Poll Res Int.* 2020; 27(12):13939-48. doi:10.1007/s11356-020-07966-3
86. R Rajasärkkä J, Pernica M, Kuta J, Lašňák Js, Šimek Zk, Bláha Lk. Drinking water contaminants from epoxy resin-coated pipes: A field study. *Water Res.* 2016; 103:133-40. doi:10.1016/j.watres.2016.07.027

87. Kamat S, Mategaonkar M, Gharat P. Flow and transport of phenol in groundwater using PGWT equation. *Heliyon*. 2020; 6(2):e03413. doi.org/10.1016/j.heliyon.2020.e03413
88. Kwak JI, Moon J, Kim D, An YJ. Soil ecotoxicity of seven endocrine-disrupting chemicals: A review. *Euro J Soil Sci*. 2017; 68(5):621-49. doi:10.1111/ejss.12467
89. Careghini A, Mastorgio AF, Saponaro S, Sezenna E. Bisphenol a, nonylphenols, benzophenones, and benzotriazoles in soils, groundwater, surface water, sediments, and food: A review. *Environ Sci Poll Res*. 2015; 22(8):5711-41. doi:10.1007/s11356-014-3974-5
90. Theron J, Cloete TE. Emerging waterborne infections: Contributing factors, agents, and detection tools. *Crit Rev Microbiol*. 2002; 28(1):1-26. doi: 10.1080/1040-840291046669.
91. Shaheen MNF. Burden of adenovirus, astrovirus, norovirus and rotavirus gastroenteritis in Egyptian children during 2000-2017. *J Me Microbiol Diag*. 2018; 07(03) doi:10.4172/2161-0703.1000283
92. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *Lancet*. 2012; 379(9832):2151-61. doi:10.1016/S0140-6736(12)60560-1
93. Matthijssens J, Ciarlet M, McDonald SM, Attoui H, Bányai Kn, Brister JR, et al. Uniformity of rotavirus strain nomenclature proposed by the rotavirus classification working group (RCWG). *Arch Virol*. 2011; 156(8):1397-413. doi:10.1007/s00705-011-1006-z
94. 3rd Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) congress, 2009: Adult infectious diseases: Abstracts. *Southern African Journal of*

Epidemiology and Infection. 2009; 24(3):16-52.
<https://doi.org/10.1080/10158782.2009.11441352>

95. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD, World Health Organization-Coordinated Global Rotavirus Surveillance N. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000-2013. *Clin Infect Dis.* 2016; 62(Suppl 2):S96-S105. doi:10.1093/cid/civ1013

96. Mapaseka SL, Dewar JB, van der Merwe L, Geyer A, Tumbo J, Zweygarth M, et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West Province of South Africa during 2003-2005. *J Infect Dis.* 2010; 202. Suppl:S131-138. doi: 10.1086/653558.

97. McCarthy K, Quan V. Communicable diseases surveillance and outbreak investigation in south africa. *S. Afr. Health Rev.* 2018; 2018(1):87-98. doi: 10.1186/s12889-021-12283-6

98. Page NA, Groome MJ, Nadan S, Netshikweta R, Keddy KH, Poonsamy B, et al. Norovirus epidemiology in south african children <5 years hospitalised for diarrhoeal illness between 2009 and 2013. *Epidemiol Infect.* 2017; 145(9):1942-52. doi:10.1017/S0950268817000668

99. Smit TK, Bos P, Peenze I, Jiang X, Estes MK, Steele AD. Seroepidemiological study of genogroup i and ii calicivirus infections in south and southern Africa. *Journal of Med Virol.* 1999; 59(2):227-31. doi:10.1002/(SICI)1096-9071(199910)59:2<227::AID-JMV17>3.0.CO;2-8

100. Oasis glass cartridges care and use manual. Milford, USA: Waters Corporation; <https://www.waters.com/waters/support.htm?lid=10103462&type=USRM>. (Accessed: April 15, 2022).

101. de Jager C, Aneck-Hahn N, Barnhoorn IEJ, Bornman M, Pieters R, Van Wyk J, et al. The compilation of a toolbox of bio-assays for detection of estrogenic activity in water. 2011. WRC Report No.1816/1/10. Pretoria: Water Research Commission
102. Supelco. Solid phase extraction products. Sigma-Aldrich. Available from: <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/581/355/t402150.pdf>. (Accessed: April 16, 2022).
103. K Katso R, Parham J, Caivano M, Clay W, Condreay J, Gray D, et al. Evaluation of cell-based assays for steroid nuclear receptors delivered by recombinant baculoviruses. *J Biomol Screen* 2005; 10(7):715-24. doi: 10.1177/1087057105278873.
104. Wilson VS, Bobseine K, Gray LE, Jr. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. *Toxicol Sci.* 2004; 81(1):69-77. doi: 10.1093/toxsci/kfh180
105. Wilson VS, Bobseine K, Lambright CR, Gray LE. A novel cell line, MDA-kb2, that stably expresses an androgen- and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists. *Toxicol Sci.* 2002; 66(1):69. doi: 10.1093/toxsci/66.1.69
106. Wood TP, Duvenage CSJ, Rohwer E. The occurrence of anti-retroviral compounds used for HIV treatment in south African surface water. *Environ Poll.* 2015; 199:235-43. doi:10.1016/j.envpol.2015.01.030
107. Miller JNM, J.C. *Statistics and chemometrics for analytical chemistry sixth ed.* Harlow, England: Prentice Hall; 2010.
108. Whitmire M, Ammerman J, Lisio Pd. 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. *J Analyt Bioanalyt Tech.* 2011; 01(01) doi:10.4172/2155-9872.S4-001

109. Mashiane M SC. Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. *J Chromatogr Sep Tech.* 2015; 06(04) doi:10.4172/2157-7064.1000272
110. Vilaginès P, Sarrette B, Husson G, Vilaginès R. Glass wool for virus concentration at ambient water ph level. *Water Sci. Technol.* 1993; 27(3-4):299-306. doi: 10.3791/3930
111. Falman JC, Fagnant-Sperati CS, Kossik AL, Boyle DS, Meschke JS. Evaluation of secondary concentration methods for poliovirus detection in wastewater. *Food Environ Virol.* 2019; 11(1):20-31. doi:10.1007/s12560-018-09364-y
112. Manor Y, Shulman LM, Kaliner E, Hindiyeh M, Ram D, Sofer D, et al. Intensified environmental surveillance supporting the response to wild poliovirus type 1 silent circulation in Israel, 2013. *Euro Surveill.* 2014; 19(7):20708. doi: 10.2807/1560-7917.es2014.19.7.20708.
113. Genthe BaMS. Health risk assessment protocol for endocrine disrupting chemicals (edc). WRC Report No.: Water Research Commission of South Africa; 2008. p. 26.
114. Genthe B, Steyn M, South Africa. Water Research C. Health risk assessment protocol for endocrine disrupting chemicals. [Gezina, South Africa]: [Water Research Commission]; 2008.
115. Martini F, Nath JL, Bartholomew EF, Ober WC, Ober CE, Welch K, et al. *Fundamentals of anatomy & physiology.* Eleventh edition. ed. New York: Pearson Education, Inc.; 2018.
116. Ying G-G, Kookana RS, Ru Y-J. Occurrence and fate of hormone steroids in the environment. *Environ Int.* 2002; 28(6):545-51. doi:10.1016/S0160-4120(02)00075-2

117. Van der Linden SC, Heringa MB, Man H-Y, Sonneveld E, Puijker LM, Brouwer A, et al. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor calux bioassays. *Environ Sci Technol.* 2008; 42(15):5814-20. doi: 10.1021/es702897y.

118. Blake LS, Martinović D, Gray LE, Wilson VS, Regal RR, Villeneuve DL, et al. Characterization of the androgen-sensitive MDA-kb2 cell line for assessing complex environmental mixtures. *Environ Toxicol Chem.* 2010; 29(6):1367-76. doi:10.1002/etc.166

119. Brack W, Dulio V, Ågerstrand M, Allan I, Altenburger R, Brinkmann M, et al. Towards the review of the European union water framework directive: Recommendations for more efficient assessment and management of chemical contamination in European surface water resources. *Sci Total Environ.* 2017; 576:720-737. doi.org/10.1016/j.scitotenv.2016.10.104

120. Human and environmental health effects of plastic. *The Specialist Forum.* 2021; 21(7):37-8. doi:10.10520/ejc-nm_specf-v21-n7-a10

121. Fan Y-Y, Zheng J-L, Ren J-H, Luo J, Cui X-Y, Ma LQ. Effects of storage temperature and duration on release of antimony and bisphenol a from polyethylene terephthalate drinking water bottles of china. *Environ Poll.* 2014; 192:113-20. doi:10.1016/j.envpol.2014.05.012

122. Zimmermann L, Dierkes G, Ternes TA, Völker C, Wagner M. Benchmarking the in vitro toxicity and chemical composition of plastic consumer products. *Environ Sci Technol.* 2019; 53(19):11467-77. doi:10.1021/acs.est.9b02293

123. Said MA, Dingwall B, Gupta A, Seyam AM, Mock G, Theyson T. Investigation of ultra violet (UV) resistance for high strength fibers. *Adv Space Res.* 2006; 37(11):2052-8. doi:10.1016/j.asr.2005.04.098

124. Sonnenschein C, Soto AM. An updated review of environmental estrogen and androgen mimics and antagonists. *J Steroid Biochem Mol Biol.* 1998; 65(1-6):143-50. doi:10.1016/s0960-0760(98)00027-2
125. Grindler NM, Vanderlinden L, Karthikraj R, Kannan K, Teal S, Polotsky AJ, et al. Exposure to phthalate, an endocrine disrupting chemical, alters the first trimester placental methylome and transcriptome in women. *Sci Reports.* 2018; 8(1) doi:10.1038/s41598-018-24505-w
126. Hlisníková H, Petrovičová I, Kolena B, Šidlovská M, Sirotkin A. Effects and mechanisms of phthalates' action on reproductive processes and reproductive health: A literature review. *Int J Environ Res Public Health.* 2020; 17(18) doi:10.3390/ijerph17186811
127. Olajire AA, Imeokparia FE. Water quality assessment of Osun river: Studies on inorganic nutrients. *Environ Monit Assess.* 2001; 69(1):17-28. doi: 10.1023/a:1010796410829.
128. Chalchisa D, Megersa M, Beyene A. Assessment of the quality of drinking water in storage tanks and its implication on the safety of urban water supply in developing countries. *Environ System Res.* 2018; 6(1):1-6. doi:10.1186/s40068-017-0089-2
129. Schafer CA, Mihelcic JR. Effect of storage tank material and maintenance on household water quality. *J Am Water Works Assoc.* 2012; 104(9):E521-E9. doi:10.5942/jawwa.2012.104.0125
130. Fuller R, Landrigan PJ, Balakrishnan K, Bathan G, Bose-O'Reilly S, Brauer M, et al. Pollution and health: A progress update. *The Lancet Planetary Health.* 2022; 6(6):e535-e47. doi:10.1016/S2542-5196(22)00090-0
131. Syafrudin M, Ayu R, Yuniarto A, Hadibarata T, Rhee J, Al-Onazi W, et al. Pesticides in drinking water—a review. *Int J Environ Res Public Health.* 2021; 18:468. doi:10.3390/ijerph18020468

132. Li Z. A health-based regulatory chain framework to evaluate international pesticide groundwater regulations integrating soil and drinking water standards. *Environ Int.* 2018; 121(Pt 2):1253-78. doi:10.1016/j.envint.2018.10.047
133. Kribéche MEA, Mechakra H, Sehili T, Brosillon S. Oxidative photodegradation of herbicide fenuron in aqueous solution by natural iron oxide α -Fe₂O₃, influence of polycarboxylic acids. *Environ Technol.* 2016; 37(2):172-82. doi:10.1080/09593330.2015.1065008
134. Fan W, Yanase T, Morinaga H, Gondo S, Okabe T, Nomura M, et al. Atrazine-induced aromatase expression is sf-1 dependent: Implications for endocrine disruption in wildlife and reproductive cancers in humans. *Environ Health Perspect.* 2007; 115(5):720-7. doi: 10.1289/ehp.9758.
135. Cragin LA, Kesner JS, Bachand AM, Barr DB, Meadows JW, Krieg EF, et al. Menstrual cycle characteristics and reproductive hormone levels in women exposed to atrazine in drinking water. *Environ Res.* 2011; 111(8):1293-301. doi:10.1016/j.envres.2011.09.009
136. Zachary CC, Truston JB, Andrew C, Lynn Z. Wicked: The untold story of ciprofloxacin. *PLoS Pathogens.* 14(3):e1006805. doi: 10.1371/journal.ppat.1006805
137. Hom-Diaz A, Norvill ZN, Blánquez P, Vicent T, Guieysse B. Ciprofloxacin removal during secondary domestic wastewater treatment in high rate algal ponds. *Chemosphere.* 2017; 180:33-41. doi:10.1016/j.chemosphere.2017.03.125
138. Fares MM, Al-Rub FAA, Mohammad AR. Ultimate eradication of the ciprofloxacin antibiotic from the ecosystem by nanohybrid GO/O-CNTS. *ACS omega.* 2020; 5(9):4457-68. doi:10.1021/acsomega.9b03636
139. Agunbiade FO, Moodley B. Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa. *Environ Tox Chem.* 2016; 35(1):36-46. doi:10.1002/etc.3144

140. Agunbiade FO, Moodley B. Pharmaceuticals as emerging organic contaminants in Umgeni river water system, KwaZulu-Natal, South Africa. *Environ Monit Assess.* 2014; 186(11):7273-91. doi:10.1007/s10661-014-3926-z
141. Kelly KR, Brooks BW. Global aquatic hazard assessment of ciprofloxacin: Exceedances of antibiotic resistance development and ecotoxicological thresholds. *Prog Mol Biol Transl Sci.* 2018; 159:59-77. doi:10.1016/bs.pmbts.2018.07.004
142. Riemenschneider C, Al-Raggad M, Moeder M, Seiwert B, Salameh E, Reemtsma T. Pharmaceuticals, their metabolites, and other polar pollutants in field-grown vegetables irrigated with treated municipal wastewater. *J Agric Food Chem.* 2016; 64(29):5784-92. doi:10.1021/acs.jafc.6b01696
143. Papageorgiou M, Kosma C, Lambropoulou D. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in central Greece. *Sci Total Environ.* 2016; 543(Pt A):547-69. doi:10.1016/j.scitotenv.2015.11.047
144. Archer E, Wolfaardt GM, van Wyk JH. Pharmaceutical and personal care products (PPCPs) as endocrine disrupting contaminants (EDCs) in South African surface waters. *Water SA.* 2017; 43(4):684-706. doi:10.4314/wsa.v43i4.16
145. Ji K, Choi K, Lee S, Park S, Khim JS, Jo E-H, et al. Effects of sulfathiazole, oxytetracycline and chlortetracycline on steroidogenesis in the human adrenocarcinoma (h295r) cell line and freshwater fish *oryzias latipes*. *J Hazard Mater.* 2010; 182(1-3):494-502. doi:10.1016/j.jhazmat.2010.06.059
146. Archer E, Petrie B, Kasprzyk-Hordern B, Wolfaardt GM. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere.* 2017; 174:437-46. doi:10.1016/j.chemosphere.2017.01.101

147. Hendricks R, Pool EJ. The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues. *J Environ Sci Health A*, 2012; 47(2):289-97. doi:10.1080/10934529.2012.637432
148. Cheng J, Ye Q, Yang Z, Yang W, Zhou J, Cen K. Microstructure and antioxidative capacity of the microalgae mutant *Chlorella py-zu1* during tilmicosin removal from wastewater under 15% CO₂. *J Hazard Mat.* 2017; 324(Pt B):414-9. doi:10.1016/j.jhazmat.2016.11.006
149. Michael CA, Dominey-Howes D, Labbate M. The antimicrobial resistance crisis: Causes, consequences, and management. *Front Public Health.* 2014; 2:145. doi:10.3389/fpubh.2014.00145
150. Naidu ECS, Olojede SO, Lawal SK, Peter AI, Akang EA, Azu OO. Effects of vancomycin linoleic acid nanoparticles on male reproductive indices of sprague-dawley rats. *Artif Cells Nanomed Biotechnol.* 2021; 49(1):587-95. doi:10.1080/21691401.2021.1968883
151. Kortesmäki E, Östman JR, Meierjohann A, Brozinski J-M, Eklund P, Kronberg L. Occurrence of antibiotics in influent and effluent from 3 major wastewater-treatment plants in Finland. *Environ Toxicol Chem.* 2020; 39(9):1774-89. doi:10.1002/etc.4805
152. Yang C, Song G, Lim W. A review of the toxicity in fish exposed to antibiotics. *Compar Biochem Physiol, Part C.* 2020; 237 doi:10.1016/j.cbpc.2020.108840
153. Nowak K, Jabłońska E, Ratajczak-Wrona W. Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. 2019; *Environ Int* 125:350-64. doi: 10.1016/j.envint.2019.01.078.
154. Lee H-R, Jeung E-B, Cho M-H, Kim T-H, Leung PCK, Choi K-C. Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. *J Cell Mol Med.* 2013; 17(1):1-11. doi:10.1111/j.1582-4934.2012.01649.x

155. Osuolale O, Okoh A. Incidence of human adenoviruses and hepatitis a virus in the final effluent of selected wastewater treatment plants in Eastern Cape Province, South Africa. *Virologia*. 2015; 12:98. doi:10.1186/s12985-015-0327-z
156. Medkour H, Amona I, Akiana J, Davoust B, Bitam I, Levasseur A, et al. Adenovirus infections in African humans and wild non-human primates: Great diversity and cross-species transmission. *Viruses*. 2020; 12(6) doi:10.3390/v12060657
157. Pond K. Water recreation and disease: Plausibility of associated infections : Acute effects, sequelae, and mortality. London, IWA Pub.; 2014 <https://doi.org/10.1007/s10669-006-8666-3>.
158. Aminiyan MM, Aitkenhead-Peterson J, Aminiyan FM. Evaluation of multiple water quality indices for drinking and irrigation purposes for the Karoon River, Iran. *Environ Geochem Health*. 2018; 40(6):2707-28. doi:10.1007/s10653-018-0135-7
159. Jiang SC. Human adenoviruses in water: Occurrence and health implications: A critical review. *Environ Sci Technol*. 2006; 40(23):7132-40. doi: 10.1021/es060892o.
160. Blyn LB, Hall TA, Libby B, Ranken R, Sampath R, Rudnick K, et al. Rapid detection and molecular serotyping of adenovirus by use of PCR followed by electrospray ionization mass spectrometry. *J Clin Microbiol*. 2008; 46(2):644-51. doi: 10.1128/JCM.00801-07.
161. Pscheidt VM, Gregianini TS, Martins LG, Veiga ABGd. Epidemiology of human adenovirus associated with respiratory infection in southern Brazil. *Rev Medical Virol*. 2021; 31(4):e2189. doi:10.1002/rmv.2189

APPENDIX

Ethics Approval Letter



Faculty of Health Sciences

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

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

15 June 2022

**Approval Certificate
Annual Renewal**

Dear Miss HJ Swanepoel,

Ethics Reference No.: 151/2021 – Line 1

Title: Endocrine disruptive activity and occurrence of pharmaceuticals and viral content in selected water sources in Melusi, Pretoria

The **Annual Renewal** as supported by documents received between 2022-05-18 and 2022-06-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-06-15 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2023-06-15.
- Please remember to use your protocol number (151/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

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

We wish you the best with your research.

Yours sincerely



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

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

Tshwane Approval Letter



City Strategy and Organisational Performance

Room D2EO01 | 2nd Floor, Block D | Tshwane House | 320 Madiba Street | Pretoria | 0002
PO Box 440 | Pretoria | 0001
Tel: 012 358 4749/0478 | Fax: 086 651 9999
Email: nosiphoh@tshwane.gov.za | www.tshwane.gov.za | www.facebook.com/CityOfTshwane

My Ref:	Research Permission LetterUP	Tel:	(012) 358 4559
Contact Person:	Pearl Maponya	Email:	PearlMap3@tshwane.gov.za
Section/Unit:	Knowledge Management	Date:	28 April 2021

The Principal Investigator
School of Health Systems and Public Health
University of Pretoria
Private Bag x20
Hatfield, Pretoria
0028

Dear Dr Patrick,

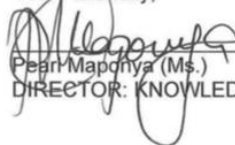
RE: ARCHITECTURE AND PUBLIC HEALTH NEXUS: AN INTERSECTORAL APPROACH TO HEALTH AND WELL-BEING

Permission is hereby granted Dr Sean Mark Patrick, the principal investigator at the University of Pretoria (UP) Architecture; Public Health and Chemical Engineering Departments, to conduct research in the City of Tshwane Metropolitan Municipality.

It is noted that the study aims to investigate the interaction between living spaces, environmental pollution, and diseases in communities to developing a strategy to improve health and well-being. The City of Tshwane further notes that all ethical aspects of the research will be covered within the provisions of the UP Research Ethics Policy. You will be required to sign a confidentiality agreement form with the City of Tshwane prior to conducting research.

Relevant information required for the purpose of the research project will be made available as per applicable laws and regulations. The City of Tshwane is not liable to cover the costs of the research. Upon completion of the research study, it would be appreciated that the findings in the form of a report and or presentation be shared with the City of Tshwane.

Yours faithfully,



Pearl Maponya (Ms.)
DIRECTOR: KNOWLEDGE MANAGEMENT

Date: 26/04/2021

LETTER OF CLEARANCE FROM THE BIOSTATISTICIAN

This letter is to confirm that the student, with the Name(s)

HJ SWANEPOEL (student number 17091315)

Studying at the University of PRETORIA discussed the Project with the title:

“Endocrine disruptive activity and occurrence of pharmaceuticals and viral content in selected water sources in Melusi, Pretoria”

with me.

I hereby confirm that I am aware of the project and also undertake to advise on the Statistical analysis of the data generated from the project. The analytical tool that will be used will be:

T47-KBluc-Assay

The E2 standard curve will be fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4), which calculates the minimum, maximum, slope, EC50 value and 95% confidence limits. The EEq values of extracts with greater than a two-fold induction above the vehicle control will be interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

MDA-kb assay

Each well will receive 25; reaction buffer (25 mM glycylglycine, 15 mM MgCl₂, 5 mM ATP, 0.1 mg/m; BSA, pH 7.8), followed by 25mM; 1 mM D-luciferin 5s later. The RLU will be converted to a fold induction above the vehicle control value. The curves for DHT and selected EC samples will be fitted (sigmoidal function with variable slope) using Graphpad Prism (version 4), then calculates the minimum, maximum, slope, EC50 value and 95% confidence limits.



Name: **Dr Alfred Musekiwa**

Tel: **012 356 3253**

Department or Unit: **SCHOOL OF HEALTH SYSTEMS AND PUBLIC HEALTH**

Letter compiled by:
Dr Alfred Musekiwa
University of Pretoria
SHSPH
Biostatistics section
April 26, 2021