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ENVIRONMENTAL AND HUMAN HEALTH RISK ASSESSMENT OF PHARMACEUTICALS IN SURFACE AND DRINKING WATER TREATED BY CONVENTIONAL AND MEMBRANE SEPARATION PROCESSES

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ENVIRONMENTAL AND HUMAN HEALTH RISK ASSESSMENT OF PHARMACEUTICALS IN SURFACE AND DRINKING WATER TREATED BY CONVENTIONAL, AND MEMBRANE SEPARATION PROCESSES

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O correr da vida embrulha tudo, a vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem.

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RESUMO

O objetivo deste trabalho foi avaliar a ocorrência de risco toxicológico ambiental e para a saúde humana de compostos farmaceuticamente ativos (PhACs) nas águas brutas e tratadas de quatro sistemas de abastecimento brasileiros e analisar a capacidade de redução de risco dos processos nanofiltração (NF), osmose inversa (OI) e destilação por membranas (DM). Para tanto, 28 PhACs foram selecionados para avaliação. Os coeficientes de risco (CR) foram calculados pela razão entre as maiores concentrações de PhACs medidas (MEC) e a concentração prevista de não efeito (PNEC). A margem de exposição (MOE) humana foi calculada pela razão entre o nível de exposição seguro, estimado pela ingestão diária tolerável (IDT), e a MEC. Resíduos de PhACs foram detectados em todos os mananciais avaliados, em concentrações na ordem de ng/L. Os fármacos betametasona, prednisona e fluconazol apresentaram as maiores frequências e concentrações, tanto nas águas brutas quanto nas tratadas. A ocorrência de PhACs se mostrou relacionada à sazonalidade e às condições sócio-econômicas da região. Menos de 1% dos PhACs avaliados não foram classificados como tóxicos e aproximadamente 60% foram considerados altamente tóxicos. Tanto a água bruta quanto a tratada dos quatro mananciais estavam sujeitas a risco ambiental em algum nível devido a pelo menos um PhAC. A capacidade de redução de risco toxicológico das ETA convencionais foi apenas parcial, fazendo com que a água potável ainda representasse risco para o ambiente. Além disso, a atorvastatina apresentou MOE abaixo de 100, indicando risco significativo para a saúde pública. Os processos NF, OI e DM foram aplicados para o tratamento da água de um dos mananciais avaliados e os resultados confirmaram a alta capacidade de remoção de fármacos dos processos de separação por membranas (PSM). A remoção de PhACs dos processos NF e OI decresceu com o aumento do grau de recuperação de permeado (RR). Já a DM foi capaz de reduzir a concentração dos PhACs para valores abaixo do limite de quantificação do método até um RR de 70%. Devido à alta eficiência de remoção e, consequentemente, à baixa concentração de PhACs na água tratada pelos PSM, não foi observado risco toxicológico ambiental nem para a saúde humana. Além de propiciar a maior remoção de PhACs, a DM não apresentou tendência à incrustação, que foi, por sua vez, a principal causa de declínio de fluxo nos processos OI e NF. Em contrapartida, a DM apresentou o maior custo operacional, que pode, contudo, ser reduzido através do uso de energias de baixo custo, como energia solar e calor residual.

Palavras-chave: compostos farmaceuticamente ativos; avaliação de risco ambiental; avaliação de risco para a saúde humana; nanofiltração; osmose inversa; destilação assistida por membranas.

ABSTRACT

The aim of the present study was to assess environmental and human toxicological risk of pharmaceutically active compounds (PhACs) in raw and treated water of four Brazilian water supply systems and to analyze the risk reduction capacity of nanofiltration (NF), reverse osmosis (RO) and membrane distillation (MD). In order to achieve this, 28 compounds were selected for evaluation. PhACs were quantified by high performance liquid chromatography (HPLC) coupled to mass spectrometer (MS). The hazard quotients (HQ) were calculated by the ratio between the highest measured environmental concentrations (MEC) in each water source and the predicted non-effect concentration (PNEC). The human margin of exposure (MOE) was calculated by the ratio between the safe exposure level, estimated by the tolerable daily intake (TDI), and the MEC. Pharmaceutical compounds residues were detected in all evaluated water supply systems, in concentrations in the order of ng/L. Betamethasone, prednisone and fluconazole were the most frequent and abundant compounds, both in surface and treated water. PhAC occurrence was susceptible to seasonality and to the socio-economic conditions of the region. Regarding toxicity potential, less than 1% of the evaluated PhACs were classified as non-toxic and approximately 60% were considered highly toxic. Both raw and treated water from the four water supply systems were subject to environmental risk at some level owing to at least one PhAC. The toxicological risk reduction capacity of conventional DWTPs was only partial, making potable water still a risk to the environment. Besides, atorvastatin presented MOE below 100, indicating a significant risk to public health. NF, RO and MD processes were applied for the treatment of one of the evaluated water supply systems and the results confirmed the high PhAC removal capacity of the membrane separation processes (MSP). PhAC rejection of NF and RO decreased with the increase of the permeate recovery rate (RR). It was possible to quantify the compounds in permeate stream at 40% and 60% of recovery rate, respectively. MD was able to reduce PhAC concentrations until below the quantification limit up to a RR of 70%. Owing to the high removal efficiency and, consequently, the low concentration of PhACs in the water treated by the MSP, no environmental or human health toxicological risk was observed. Besides presenting the highest PhAC removal, MD showed no tendency to fouling, which was the main cause of flux decline in RO and NF processes. On the other hand, MD presented the highest operating cost, which could be reduced, however, by using low-cost energy, such as solar or residual heat.

Keywords: pharmaceutically active compounds; environmental risk assessment; human health risk assessment; nanofiltration; reverse osmosis; membrane distillation.

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A/P	Amortization factor
A_M	Effective membrane area
AOP	Adverse outcome pathway
BMD	Benchmark dose
CapEx	Capital expenses
C_F	Feed concentration
CONAMA	National Council for the Environment
CP	Concentration polarization
C_P	Permeate concentration
DWEL	Drinking water equivalent level
DWTP	Drinking water treatment plants
EC50	Median effect concentration
EIC	Expected introductory concentration
EMEA	European Medicines Agency
ERA	Environmental risk assessment
F	Fouling
FD	Flux decline
FDA	US Food and Drug Administration
FUNASA	National Health Foundation
GDP	Gross domestic product
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
HDI	Human development index
HPLC	High performance liquid chromatography
HQ	Hazard quotient
HRA	Human health risk assessment
i_c	Investment rate
J_{NF}	Nanofiltration permeate flux

$J_{P(MD)}$	Membrane distillation permeate flux
J_{PC}	Water flux of the physically cleaned membrane
J_{RO}	Reverse osmosis permeate flux
J_{SD}	Effluent permeate flux
J_W	Pure-water permeate flux
K	Membrane water permeability
LC50	Median lethal concentration
LOAEL	Lowest observed adverse effect level
MBR	Membrane bioreactor
MD	Membrane distillation
MEC	Measured environmental concentration
MEEC	Maximum expected environmental concentration
MHQ	Mixture hazard quotients
MOA	Mode of action
MOE	Margin of exposure
MS	Mass spectrometer
MSP	Membrane separation processes
NF	Nanofiltration
NOAEL	No-observed adverse effect level
NOEC	No-observed effect concentration
NSAID	Non-steroidal anti-inflammatory drugs
OpEx	Operational expenses
PEC	Predicted environmental concentration
PhACs	Pharmaceutically active compounds
PNEC	Predicted non-effect concentration
QSAR	Quantitative structure activity relationship
R_F	Fouling resistance
R_{FB}	Feed boundary layer resistance

R_{fir}	Irreversible fouling
R_{fr}	Reversible fouling
R_M	Membrane resistance
RO	Reverse osmosis
R_{PB}	Permeate boundary layer resistance
RR	Permeate recovery rate
R_T	Total resistance
SEC	Specific energy consumption
STEC	Specific thermal energy consumption
SUS	Brazilian health system
TDI	Tolerable daily intake
TMP	Transmembrane pressure
TOC	Total organic carbon
TSS	Total suspended solids
UF	Ultrafiltration
USEPA	US Environmental Protection Agency
V_F	Initial volume of feed
V_P	Volume of permeate
WHO	World Health Organization
WWTP	Wastewater treatment plants
$\Delta P - \Delta \pi$	Effective pressure
μ	Viscosity

1st Chapter

Theme presentation

1 BACKGROUND

Pharmaceutically active compounds (PhACs) are an important tool for human health recovery and maintenance and they are widely used for diseases diagnosis, treatment, minimization and prevention. According to their function and mode of action in the body, the PhACs can be classified into different therapeutic classes, such as anti-inflammatories, lipid regulators, antibiotics, antidepressants, chemotherapeutic agents and endocrine regulators. Owing to the medicine advance in the last decades, the world PhACs consumption has grown exponentially. Antibiotics, for example, had their estimated annual worldwide consumption already between 100,000 and 200,000 t back in 2003 (KÜMMERER, 2003). By 2014, total pharmaceutical revenues in the world exceeded one trillion US dollars. Chemotherapeutic drugs are one of the most widely used therapeutic classes, as are antibiotics and anti-inflammatories. By 2015, cancer drugs have reached nearly $ 79 billion in revenue globally. Other important therapeutic classes are analgesics, antihypertensives and antidiabetics (STATISTA, 2016).

Despite their undeniable importance for human life quality, PhACs characteristics also make them potential agents of pollution. After being consumed, the compounds are only partially metabolized and, thus, are excreted through urine and feces. Within domestic sewage, PhACs reach the wastewater treatment plants (WWTP) and, eventually, reach the receiving water bodies, since conventional treatment methods are not able to remove them completely (VERLICCHI *et al*., 2012). Other important sources of PhACs contamination are effluents from pharmaceutical industries and animal husbandry farms. Several studies indicate PhACs presence in WWTP effluents and in natural surface and groundwater, at concentrations ranging from ng/L to μg/L (HEBERER *et al*., 2007; JELIC *et al*., 2011; PATROLECCO *et al*., 2015; ARCHER *et al*., 2017). Godoy (2014) compiled in his work a group of several authors who discussed pharmaceuticals presence in aqueous matrices, from 2003 to 2011, in the United Kingdom, India, Spain, the United States, France, Brazil, Austria and Sweden. More than 30 different active principles, belonging to 14 different pharmaceutical classes, have been reported.

When in the environment, PhACs can be adsorbed or transported, can bioaccumulate (ZENKER *et al*., 2014) or undergo transformation processes, such as biotic and abiotic degradation or reactivation, adsorption or hydrolysis (BAGNIS *et al*., 2018). Since they are biologically active, these compounds exert pharmacological action even in trace concentrations and may therefore cause various deleterious effects on aquatic organisms. For example, diclofenac affects reproduction rate in aquatic vertebrates (YOKOTA *et al*., 2016); ciprofloxacin may interfere with higher plants photosynthesis pathway, leading to morphological abnormalities or growth

inhibition (ARISTILDE; SPOSITO, 2010). In addition, the antibiotic-resistant bacteria spread, caused by the presence of antibiotics in the environment, is an emerging concern (MARTI *et al.*, 2013). Jacob (2017) evaluated PhACs toxicity potential through ecotoxicological tests, noting that drugs are capable of generating disequilibrium in the ecosystem by promoting acute and chronic effects, as well as provoking individuals avoidance.

According to the World Health Organization (WHO, 1999), risk assessment is defined as the process which allows adverse health effects quantitative characterization and prediction for a given population caused by any substance at a particular concentration. The guidelines for environmental risk assessments (ERA) of new and existing chemicals have been presented by the European Medicines Agency (EMEA) and the US Food and Drug Administration (FDA). Both agencies guidelines are based on the relationship between exposure and effect, which is expressed by the hazard quotient (HQ), given by the ratio between the measured environmental concentration (MEC) and the predicted non-effect concentration (PNEC). The higher the HQ value, the greater the likelihood that the PhAC will pose toxicological effects (FDA, 1998; EMEA, 2006).

Risk assessment methodology is essential to determine if PhACs presence in surface water does, indeed, pose a risk to the aquatic ecosystem and human health and, consequently, to determine whether these compounds should be regulated by environmental agencies and legislation. Despite its importance, studies on PhACs toxicological risk assessment are still scarce in the international literature (GODOY *et al.*, 2015; PETRIE *et al.*, 2015), and limited by the geographic region evaluated and the available toxicological data quality (NETO; SARCINELI, 2009). Some studies that have developed PhACs risk assessments in surface waters are presented in Table 1.1.

Table 1.1 - Papers published in the literature on PhACs ERA.

Authors	Year	Country	Abstract
SCHWAB *et al.*	2005	USA	26 PhACs and/or their metabolites ERA, representing 14 different therapeutic classes for which environmental monitoring data are available in the United States. The HQ values found were very low for all PhACs, indicating that there is no significant toxicological risk owing to the presence of these compounds in surface waters.
HERNANDO *et al.*	2006	Spain	Primary PhACs residues (antibiotics, analgesics/anti-inflammatories, lipid regulators, β-blockers, antiepileptics and steroid hormones) detected in waste water, surface and sediments ERA. The authors identified high risk owing to anti-inflammatories (ibuprofen, aproxeno, diclofenac and ketoprofeno) and antiepileptic (carbamazepine) and medium risk owing to β-blockers (propanolol) in superficial waters.
GARCIA *et al.*	2014	Spain	ERA for 26 high consumption and occurrence in Spanish aquatic environment PhACs. About 65% of the compounds showed high toxicity or were harmful to the environment. Nine PhACs presented risk at some level and the others presented acceptable HQ values.
GAFFNEY *et al*.	2015	Portugal	31 PhACs monitoring in supply water in Lisbon. Out of them,16 were quantified in the analyzed samples, with levels varying from 0.005 to 46 ng/L. ERA showed that toxicological risk occurrence is extremely unlikely, since HQ values found were below 0.001.
LIU *et al*.	2015	China	Lipophilic PhACs such as antibiotics (roxithromycin, erythromycin and ketoconazole), anti-inflammatories (ibuprofen and diclofenac), β-blockers (propranolol), antiepileptics (carbamazepine) and steroid hormones (17α-ethinyl estradiol) ERA in rivers downstream of WWTP in Nanjing, China. The results indicated that diclofenac, ibuprofen and 17α-ethinylestradiol pose chronic risks for high trophic organisms (fish).
PENG *et al*.	2017	China	Monitoring of emerging organic contaminants (3 endocrine disruptors and 17 pharmaceuticals and personal care products) in six urban rivers in Guangzhou, China. ERA showed that 4-nonylphenol (4-NP) and triclosan (TCS) pose potential risks to aquatic organisms at most sampling sites. For individual taxa, 4-NP poses risk for several groups of aquatic organisms, while TCS only poses high risks for algae.

In addition to the effects on the environment, PhACs presence in the supply water can also pose risks to human health. Although the pharmaceutical behavior is well known, the effects caused by chronic exposure to these compounds via drinking water are still mysterious. Drinking water treatment plants (DWTP) may be another barrier to prevent PhACs return to the human body. However, conventional treatment systems (coagulation/flocculation, decantation, filtration and disinfection) do not ensure their complete removal (CARMONA *et al*., 2014; HU *et al*., 2017).

Biodegradation in slow sand filters and sorption in particulates that are removed by coagulation, for example, are phenomena that could assist in PhACs removal. However, bench scale studies using ferric and aluminum chloride as coagulants for natural or synthetic water treatment have shown that coagulation (with or without chemical softening) is largely ineffective in PhACs removal (WESTERHOFF *et al*., 2005). Filtration does not present high PhACs removal efficiencies neither (HUERTA-FONTELA *et al*., 2011; SIMAZAKI *et al*., 2008). Chlorination and ozonation, in turn, can achieve higher removal rates, and their efficacy depends on the PhAC chemical structure and on treatment conditions, such as pH and oxidant dose (SNYDER *et al*., 2007; KIM *et al*., 2007). In some studies, it was found that chlorine oxidized approximately half of the investigated pharmaceuticals. Antibiotics such as sulfamethoxazole, trimethoprim and erythromycin are among the compounds that showed high removal through chlorination (KHIARI, 2007).

Table 1.2 summarizes some studies published in the literature on the conventional water treatment processes efficacy in PhACs removal.

Table 1.2 - PhACs removal efficiencies of conventional drinking water treatment processes in some studies reported in the literature.

Treatment process	Removal (%)	Scale	Country	Number of compounds	Reference
Coagulation Chlorination	24-72 25-75	Bench	EUA	49	WESTERHOFF *et al*. (2005)
Coagulation	<5-30	Bench	Finland	5	VIENO *et al*. (2006)
Chlorination Coagulation	20-100 <15	Bench	Japan	9	SIMAZAKI *et al*. (2008)
Coagulation	0	Industrial	South Korea	6	KIM *et al*. (2007)
Desinfection	2-97	Industrial	France	7	ANSES (2011)
Pre-Chlorination Coagulation/Floculation/ Filtração Chlorination	0->99 <30-100 14-100	Industrial	Spain	35	HUERTA-FONTELA *et al*. (2011)

Once it is possible that treated water is still subject to PhACs contamination, it is essential that, in addition to ERA, risk assessments for human health (HRA) are carried out. The evaluation of such risk methods involve the margin of exposure (MOE) determination. MOE is obtained through the ratio between the safe exposure level and the highest concentration detected in the evaluated medium. The safe exposure level can be estimated by tolerable daily intake (TDI) (derived from the no-observed adverse effect level - NOAEL and safety factors) (WHO, 2011). According to the US Environmental Protection Agency (USEPA), MOE values above 100 indicate a low probability of risk.

The Drinking Water Inspectorate presented a study on the human health risks of 396 PhACs marketed in the United Kingdom (DWI, 2007). The MOE values found were greater than 1000. The AWWA Research Foundation in 2008 investigated 62 compounds, including 20 drugs, in the United States and none of them had MOE below 100. In that study, a conservative approach was adopted, determining the TDI values from NOAEL and severe safety factors, which varied according to the compound type (SNYDER *et al*., 2007). In the Australian Guidelines for Water Reuse (NRMMC, EPHC & NHMRC, 2008[1]), the calculated MOE values were greater than

[1] Natural Resources Management Ministerial Council; Environment Protection and Heritage Consul; and National Health and Medical Research Consul.

1000 for the vast majority of the evaluated PhACs. Therefore, risk assessments in the United Kingdom, the USA and Australia indicate that adverse effects on human health owing to exposure to trace PhACs concentrations found in drinking water are unlikely. Nevertheless, specific circumstances may lead to higher concentrations of these compounds, increasing their risk potential. Besides, the effects of chronic exposure to pharmaceuticals and to the complex mixture in which they are found are still poorly understood (WHO, 2011).

Thus, it is of great importance that advanced water treatment methods more efficient in PhACs removal are studied. Membrane separation processes (MSP) have gained great relevance in the current scientific scenario. These processes use membranes as selective barriers in order to control the chemical species permeation through their structure. The driven force applied can be thermal, electrical or posed by concentration or pressure gradient (HABERT *et al*., 2006). The MSP combines process stability with excellent treatment quality and, therefore, it has been observed in recent years an expressive increase in their use for several purposes, including for the removal of pharmaceutical compounds (DUTTA *et al*., 2014; PARK *et al*., 2017). Taheram *et al*. (2016) reviewed several studies in which the reverse osmosis (RO), nanofiltration (NF) and membrane bioreactor (MBR) processes were applied for different PhACs removal. The authors observed that RO and NF showed removals greater than 75% for all evaluated compounds. BRM presented PhACs removals varying from 0 to 90%, owing to the different effects exerted by the compounds on the microorganisms.

In RO processes the applied driven force is the pressure differential and dense membranes are used, which allows the process to reach retentions greater than 99% for salts and low molecular weigh dissolved molecules (HABERT *et al*., 2006). Since the membranes used are dense, i.e., they do not have pores in contact with the feed solution, higher operating pressures must be applied, making its operational cost higher. In contrast, the permeate quality is usually higher.

NF is characterized by membranes with rejection capacity between ultrafiltration (UF) upper limit and RO lower limit, which makes it possible to obtain higher permeate fluxes than RO keeping a high degree of rejection (BAKER, 2004). Besides that, NF presents lower propensity to fouling (ANDRADE *et al*., 2014). Permeate flux and retention efficiency are related to operational conditions, such as pH and feed concentration, presence of pre-treatment and permeate recovery rate (BAKER, 2004). Driven by the high permeate quality obtained through NF, authors from all over the world have applied this process aiming PhACs removal (LIU, 2014; GARCIA-IVARS *et al*., 2017).

In addition to RO and NF, processes already consolidated in the scientific community, membrane distillation (MD) has been presented as a promising technology and has been gaining prominence in high quality permeates generation.

MD driving force is the temperature difference between two phases separated by a microporous and hydrophobic membrane. The vapor pressure gradient leads to the passage of water vapor from the hot phase (feed) to the cold (permeate) one (QU *et al*., 2009). Thus, since the membrane allows only water vapor passage, the non-volatile solutes theoretical rejection is 100% and, in addition, it is possible to concentrate solutions to their saturation point without significant loss of permeate flux (FRANCIS *et al*., 2014). When compared to conventional MSP, MD present some advantages, such as low operating temperature, which allows the association with alternative energy sources (solar, geothermal and residual heat); low need for pre-treatment; and lower fouling occurrence and intensity on the membrane surface (MANNA, PAL, 2016). However, it is a process associated with high energy demand.

Table 1.3 presents some published works on the application of these MSP aiming PhACs removal. The results reported confirm the high efficiency of NF, RO and MD on removing these compounds.

Table 1.3 - Papers published in the literature on MSP application aiming PhACs removal from aqueous matrices.

Authors	Year	Country	Abstract
YOON *et al*.	2007	South Korea	The study investigated 27 PhACs removal from various drinking water sources by NF and UF. The results showed that hydrophobic adsorption and size exclusion mechanisms are equally determinant in NF membrane retention.
RADJENOVIC *et al.*	2008	Spain	Investigation of several PhACs (analgesics and anti-inflammatories, β-blockers, antiepileptics, antibiotics, lipid regulators and diuretics) removal by NF and RO. Excellent overall performances were observed for both processes, with high rejection percentages (>85%) for almost all investigated PhACs. Low acetaminophen (45-73%), genfibrozil (50-70%) and mefenamic acid (30-50%) retentions were observed.
LIU *et al*.	2014	China	Systematic investigation on antibiotics removal from a municipal WWTP effluent through NF. Four high-frequency detected antibiotics (norfloxacin, ofloxacin, roxithromycin and azithromycin) were selected. High rejections (>98%) were obtained in all sets of experiments.
RODRIGUEZ-MOZAZ *et al*.	2015	Spain	Evaluation of 28 PhACs and 20 pesticides removal from a municipal WWTP effluent. The UF-RO combined treatment was able to considerably remove the micropollutants present in the WWTP effluent to values in the range of a few ng/L or below the quantification limits.
WOLDEMARIAM *et al*.	2016	Sweden	A pilot scale MD system was applied for a WWTP effluent treatment. Most PhACs were removed with high degree, often below the method detection limit. In addition, it was found that the energy required for the process could be supplied by the heating return line.
WANG *et al*.	2018	China	Evaluation of NF membranes rejection capacity in relation to 40 PhACs. The results showed that NF was able to remove most of the compounds satisfactorily (removal efficiencies greater than 80%), presenting low retention capacity for lower molecular weight PhACs, which led the researchers to conclude that the main retention mechanism is the steric effect.

Following the international interest in natural waters PhACs contamination, Brazilian scientific community recently also began to pay attention to this subject and some national studies can already be found in the literature, as exemplified in Table 1.4. Regarding the toxicological risk assessment in Brazilian surface waters, the only work found was Pusceddu *et al*. (2015). The authors developed the ERA for the PhACs ibuprofen, 17α-ethynylestradiol and triclosan in sediment samples from Santos Bay, São Paulo. Ibuprofen (49.0 ng/g) and triclosan were detected (15.14 ng/g) in all samples and their HQ values were higher than unity, indicating high environmental risks.

Table 1.4 - Published works on PhACs occurrence in Brazilian natural waters.

Authors	Year	State	Abstract
SODRÉ *et al*.	2010	São Paulo	Tap water samples were analyzed for 11 contaminants. Six (stigmasterol, cholesterol, bisphenol A, caffeine, estrone and 17β-estradiol) were detected. The concentrations found were higher than the average values reported in the literature, which was explained by the intense discharge of sewage in the water courses.
FERREIRA	2014	Rio de Janeiro	Gandu River contamination by psychiatric pharmaceuticals was investigated. The study revealed benzodiazepine derivatives presence in all samples at concentrations of 42 ng/L, 198 ng/L and 335 ng/L for bromazepam, clonazepam and diazepam, respectively.
BERETTA *et al*.	2014	Bahia	Atenolol, caffeine, carbamazepine, diazepam, diclofenac, erythromycin, ibuprofen and personal care products monitoring. The PhACs examined were present in all sediment samples in parts per billion dry sediment. The highest concentrations were: 23.4 ng/g of caffeine; 14.3 ng/g of ibuprofen and 9.84 ng/g of atenolol.
THOMAS *et al*.	2014	Amazonas	Evaluation of PhACs occurrence in Negro river Propranolol, diclofenac, amitriptyline, citalopram, carbamazepine, carbamazepine epoxide, metoprolol, carisoprolol and sertraline were detected in concentrations of ng/L. The concentrations in the Negro river were lower than the detection limits owing to the high dilution level.
MONTAGNER *et al*.	2014	São Paulo	Six rivers in São Paulo were monitored for triclosan (TCS) and caffeine. Out of 71 samples analyzed, 32 contained TCS at concentrations above the quantification limit, ranging from 2.2 to 66 ng/L, which corresponds to a 86% PNEC exceedance frequency (six in seven sites). No correlation was observed between CSC and caffeine and one of the reasons pointed out for this was the local population different consumption pattern.
TORRES *et al*.	2015	São Paulo	This study monitored PhACs presence in surface and treated water samples. The results showed that the samples were contaminated by estriol, estrone, progesterone, 17β-estradiol and 17α-ethinylestradiol hormones, with median concentrations of 90, 28, 26, 137 and 194 ng/L, respectively. Ecotoxicological tests indicated little estriol hormone toxicity for *D. magna*.
PEREIRA *et al*.	2016	São Paulo	PhACs environmental concentrations in Santos Bay investigation. Five sampling points under strong influence of sewage discharge were chosen. 33 compounds were investigated. Seven PhACs (atenolol, acetaminophen, caffeine, losartan, valsartan, diclofenac and ibuprofen), were detected in all samples, in concentrations ranging from ng/L to µg/L.

The few studies found in the literature on the occurrence and, mainly, on PhACs toxicological risk in Brazilian natural waters indicate how this issue is recent in national surveys and demonstrate the relevance of conducting new research on the subject.

Thus, this work, which is part of a large project funded by the National Health Foundation (FUNASA), entitled "PhACs OCCURRENCE IN WATER FOR HUMAN CONSUMPTION: SUBSIDIES FOR INSERTION IN THE BRAZILIAN POTABILITY STANDARD", was intended to verify PhACs toxicological risk occurrence, both for the environment and human health, in Brazilian natural waters. Four water supply systems located in different regions of the country were evaluated and the water was treated by conventional process, NF, RO and MD in order to analyze the capacity of conventional and membrane separation processes to reduce such risk. Previous works linked to the same project have developed the analytical methodology of identification and quantification of the PhACs that will be applied in this work; analyzed the occurrence of these compounds in other water sources and in the water treated by different DWTPs, analyzing the removal capacity of each of them comparatively; and initiated the application of the PSM for water treatment in order to remove the PhACs present.

2 JUSTIFICATION

The significant increase in PhACs world consumption combined with analytical techniques improvement that allow the detection of contaminants in increasingly lower concentrations has encouraged the scientific community to pay special attention to pharmaceutical compounds, called emerging concern contaminants. However, even in countries where surveys related to water bodies PhACs contamination are more advanced, it is recognized that little information is available regarding to water quality and risk management.

Many studies have been published on PhACs identification and quantification in surface waters, as well as on the performance of different processes in their removal. However, knowing the PhACs contamination extent alone is not sufficient to determine whether its presence in supply waters, at the concentrations in which they are found, poses a risk to the environment and to human health. For this, toxicological risk assessments must be carried out. Contrary to its great importance, PhACs toxicological risk assessments are still scarce in the literature, highlighting this work relevance.

If it is recognized that there is not much information available on PhACs toxicological risks in the international literature, in Brazil the situation is even more embryonic. The country's scientific community has begun to pay attention to this subject recently and, therefore, little has been published on the PhACs contamination issue in national waters. Thus, evaluating PhACs occurrence in water supply systems in different Brazilian regions and verifying if they pose environmental and human health toxicological risk is an important step towards national knowledge construction.

This work is justified, therefore, by allowing a better understanding of the toxicological risks both to the environment and to human health owing to PhACs presence in surface waters. In addition, it is hoped to contribute with national knowledge about PhACs occurrence and toxicological risk in Brazilian water supply systems, providing the sanitary authorities with new information on the issue.

3 OBJECTIVES

3.1 General objective

This work aims to develop PhACs environmental and human health risk assessment for surface and treated water from Brazilian water supply systems and to analyse the risk reduction capacity of conventional and membrane separation processes applied in water treatment.

3.2 Specific objectives

i. Develop PhACs ERA and HRA for surface and drinking water treated by conventional DWTPs from four Brazilian water supply systems, representing three regions of the country, and to identify the PhACs that pose risks to the aquatic ecosystem and to human health;

ii. Develop PhACs ERA and HRA for the water of one of the supply systems treated by NF, RO and MD and evaluate the performance of these three processes in PhACs removal, identifying the predominant separation mechanisms and the compounds physical-chemical properties that influence their removal.

4 DOCUMENT STRUCTURE

To better organize the work developed, this dissertation was divided into five chapters, named: Chapter I) Theme presentation; Chapter II) PhACs environmental and human health risk assessment: guidelines, limitations and recent approaches; Chapter III) PhACs ERA and HRA in surface waters and risk reduction by conventional treatment processes; Chapter IV) PhACs toxicological risk reduction by membrane separation processes; Chapter V) Final considerations.

Within this organization, Chapter I aims to introduce the theme and contextualize the dissertation, presenting the PhACs contamination and the associated risk assessment issue. Also, it presents the desired objectives and demonstrates the relevance of the work developed. Chapter II aims to seek in the international literature the main concepts, guidelines, limitations and new approaches to risk assessment methodology. Chapters III and IV refer to the specific objectives presented for the dissertation. In the first one, PhAC toxicological risk assessment for raw water and water treated by conventional DWTPs from four Brazilian water supply systems was presented. PhACs were classified according to their toxicological potential and the risk they pose, and the conventional water treatment processes ability to remove PhACs from surface water was evaluated. Chapter IV, in turn, is intended to present PhACs toxicological risk assessment for the water of one of the evaluated water supply systems treated by NF, RO and MD. The PhAC removal and risk reduction capacity of these processes were analyzed and operational and economical evaluations were performed for all three processes. Finally, in Chapter V, the conclusions of the previous chapters were discussed in an integrative approach, in order to present this research final conclusions and recommendations.

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2nd Chapter

PhAC environmental and human health risk assessment: guidelines, limitations and recent approaches

1 INTRODUCTION

The global consumption and production of pharmaceuticals is increasing concomitantly with concern regarding their environmental fate and effects. Improvements in analytical technologies made it possible to detect pharmaceutically active compounds (PhACs) in water matrices at very low concentrations (order of ng/L) and a great number of studies have been demonstrating PhACs presence in surface and groundwater worldwide (HEBERER, 2007; KIM *et al*., 2007; JELIC *et al*., 2011; PATROLECCO *et al*., 2015; ARCHER *et al*., 2017).

PhACs are mainly released into aquatic environment through wastewater treatment plants (WWTP) effluent discharge (ARCHER *et al.*, 2017). Other sources of contamination include effluent from the pharmaceutical industry and hospitals untreated or incompletely treated; incorrect disposal of medicines; animal husbandry farms residues and effluents; and untreated sewage disposal (LIMA *et al*., 2017). Once in the environment, PhACs can undergo natural attenuation processes, i.e. dilution, sorption or chemical transformation, depending on the compound physical-chemical properties, such as water solubility, lipophilicity and vapor pressure, and environmental conditions, such as pH, temperature and ionic strength (GURR; REINHARD, 2006; LIN *et al*., 2010; TAPPIN *et al*., 2012, 2014; WEST; ROWLAND, 2012). The adverse effects caused by these compounds have been reported in the international literature since the 1990s, when it was discovered that these substances are capable of causing negative effects on ecosystems in concentrations as low as nanograms per liter (PURDOM *et al*., 1994; DESBROW *et al*., 1998; ROUTLEDGE *et al*., 1998). Drinking water treatment plants (DWTP) may impose a barrier to prevent PhACs return to human body, however the literature indicates that these processes are not always enough to ensure PhACs removal (HUERTA-FONTELA *et al.*, 2011; SIMAZAKI *et al.*, 2015).

Although clinical testing ensures that human biological effects are well known (ÅGERSTRAND *et al.*, 2015), uncertainties exist concerning the environmental risk posed by PhACs, once the knowledge concerning their fate and behavior in the environment, like their uptake, metabolism and excretion rates (pharmacokinetics) and their target affinity and functional effects (pharmacodynamics), is limited (ARNOLD *et al*., 2014; VERBRUGGEN *et al*., 2018). Moreover, the effects caused by indirect exposure through drinking water over a long time are also poorly known.

Thus, PhACs contamination should be assessed in relation to possible environmental and human health risks. Both human health and environmental risk assessment (HRA and ERA)

deal with the interaction of toxic substances with living organisms, considering various physiological processes (respiration, transport, signalling) and basic building blocks (DNA, proteins, membranes, cells etc.) (DORNE *et al*., 2007). Toxicity measures (e.g. no observed effect levels or concentrations) provides the basis for toxicity data extrapolation, accounting for interindividual variability, interspecies variability, differences in exposure time, differences in endpoints, potential synergistic effects, systematic errors, assumptions and random errors (DORNE *et al*., 2006; MONTFORTS, 2006).

The development of specific ecological risk assessment for pharmaceuticals began in the late 1960s, when the US Food and Drugs Administration (FDA) required the basic assessment of new drugs as part of the license application process in the USA. During the years, the process has undergone some revisions until it reaches the guidelines published in 'Guidance for industry: Environmental assessment of human drugs and biologics applications' (FDA, 1998), which are in force to this day. Along with FDA, the European Medicines Agency (EMEA) also presents directives for the development of risk assessments. Originally based on Directive 65/65/EEC and later refined in 93/39/EEC, the process was continuously improved until the "Guideline on the environmental risk assessment of medicinal products for human use' was published (EMEA, 2006) (Doc. Ref. EMEA/CHMP/SWP/4447/00 corr. 2).

For HRA, the first guidelines were presented by US Environment Protection Agency (USEPA), in 1986, when 'Guidelines for the Health Risk Assessment of Chemical Mixtures' was published. Since then, revisions and improvements have been made and new documents have been published by the agency. In 2010, the World Health Organization (WHO) launched the 'WHO Human Health Risk Assessment Toolkit: Chemical Hazards', aiming to provide users with guidance to identify, acquire and use the information needed to assess chemical hazards, exposures and the corresponding health risks.

Both HRA and ERA objective is to protect human population and the environment from potential chemical harm and they are the backbone of an effective and successful environmental policy. To achieve this, the assessment procedure has to be based on best available knowledge. With the advancement of analytical technologies and as knowledge about PhACs occurrence and effects in natural waters increases, the enhancement of risk assessment methodologies is a growing need and represents one of the greatest challenges for the scientific community. In order to improve it, reducing the effect of uncertainties and leading to a more effective and reliable system, several techniques and new approaches have been studied, such as new points of departure (PoD) (JOHNSON *et al*., 2014; KLAMINDER *et al*., 2014), the development of

mathematical models (BESSEMS *et al*., 2014), use of different methodologies (DIAMOND *et al*., 2017; KOKANGÜL *et al*., 2017) and the application of *in vitro* toxicological tests (JUDSON *et al*., 2014).

Therefore, this issue aims to provide an overview of environmental and human health risk assessment methodologies, presenting the current concepts, guidelines and limitations and proposing recommendations for its improvement. In addition, this review focuses on presenting what is new about this subject, presenting new techniques and approaches developed.

2 PhAC OCCURRENCE IN WATER MATRICES: BRIEF OVERVIEW

Owing to the medicine advance in the last decades, the world PhACs consumption of all therapeutic classes has grown considerably and the production of these compounds already reaches high values. Antibiotics, for example, had their estimated annual worldwide consumption already between 100,000 and 200,000 t still in 2003 (KÜMMERER, 2003). Until last decade, only in the United States, more than 20,000 t of antibiotics were produced per year (BROWN *et al*., 2006) and in the European Union about 3,000 different pharmaceutical compounds were used (CHRISTEN *et al*., 2010). By 2014, total pharmaceutical revenues in the world exceeded one trillion US dollars and, in 2016, only one anti-inflammatory drug (humira) generated more than 16 billion dollars of revenue (STATISTA, 2016).

After consumption, PhACs are metabolized and excreted in its original form and as metabolites. Removal in WWTP and in DWTP varies broadly owing to their different physicochemical properties and degradability (TADKAEW *et al*., 2011; PETRIE *et al*., 2015). Incomplete removal results in PhACs detection in surface and drinking water. Several recent studies indicate PhACs presence in water matrices at concentrations ranging from ng/L to µg/L. In 2011, the German Federal Environment Agency (UBA) published a review reporting data from environmental monitoring in Germany. The study confirmed a total of 156 pharmaceuticals detected in surface water, groundwater and drinking water (BERGMANN *et al*., 2011). On a global scale, several review papers have highlighted aquatic monitoring of PhACs in the European Union (LOOS *et al*., 2013), the USA (KOSTICH *et al*., 2014), China (LIU; WONG, 2013) and United Kingdom (PETRIE *et al*., 2015) and demonstrated how often these compounds are detected. Godoy (2014) compiled in his work data from 2003 to 2011, in the United Kingdom, India, Spain, the United States, France, Brazil, Austria and Sweden and proved that there is effective PhACs contamination in natural waters. According to Petrie *et al*. (2015), the most studied therapeutic classes are non-steroidal anti-inflammatory drugs (NSAIDs), b-blockers, antidepressants and antibiotics.

Owing to uncertainties of the existing sampling methods, there is lack of understanding on spatial and temporal variations in PhACs concentrations (PETRIE *et al*., 2015). However, some studies have been conducted with this goal and the results indicate that PhAcs occurrence are subject to seasonal distribution, since this factor impact directly in pharmaceuticals consumption patterns (CAMACHO-MUNOZ *et al.*, 2014; KOSTICH *et al.*, 2014). For example, monthly prescription information for the UK showed that prescriptions for anti-histamines were 100% greater in summer compared to winter months because pollen production

is greatest (NATIONAL HEALTH SERVICE, 2012). Coutu *et al*. (2013) investigated temporal variations of antibiotics within influent wastewater sampling every month for a one-year period and they observed that the ciprofloxacin mass fluxes were higher in winter and spring months, which was explained by seasonal therapeutic use, since ciprofloxacin is used to treat airway infections, more common when temperatures are lower. Assessing PhACs spatial distribution is notoriously more difficult, since collation and interpretation of literature data from a variety of sources has limitations owing to each local particularity (PETRIE *et al*., 2015).

Once in the environment, PhACs concentrations may be reduced by physical, biological or chemical processes, including dilution; transformation reactions such as photo-degradation, biodegradation and hydrolysis; and sorption (GURR; REINHARD, 2006). Natural attenuation may reduce PhACs concentrations and therefore their toxicity potential (TAPPIN *et al.*, 2012; 2014) and it determines the contaminants fate in the environment, i.e., occurrence, distribution, and bioavailability (LIN *et al*., 2010). Pharmaceuticals which are persistent, bioavailable, toxic and that present high solubility are of greatest concern (BAGNIS *et al*., 2018). Besides, metabolites generation during sewage treatment (AQUINO *et al*., 2013) or in the environment (ESCHER; FENNER, 2011) creates further uncertainty regarding toxicological exposure and effects, since they can be found at concentrations much greater than the corresponding parent PhAC and can also be pharmacologically active, sometimes even more toxic than the original compound (KASPRZYK-HORDERN *et al*., 2008; BOXALL *et al*., 2012). For example, Huerta-Fontela *et al*. (2010) found a major carbamazepine metabolite in influent wastewater at concentrations ranging from 880 to 4026 ng/L while the parent compound was found at <1.5-113 ng/L.

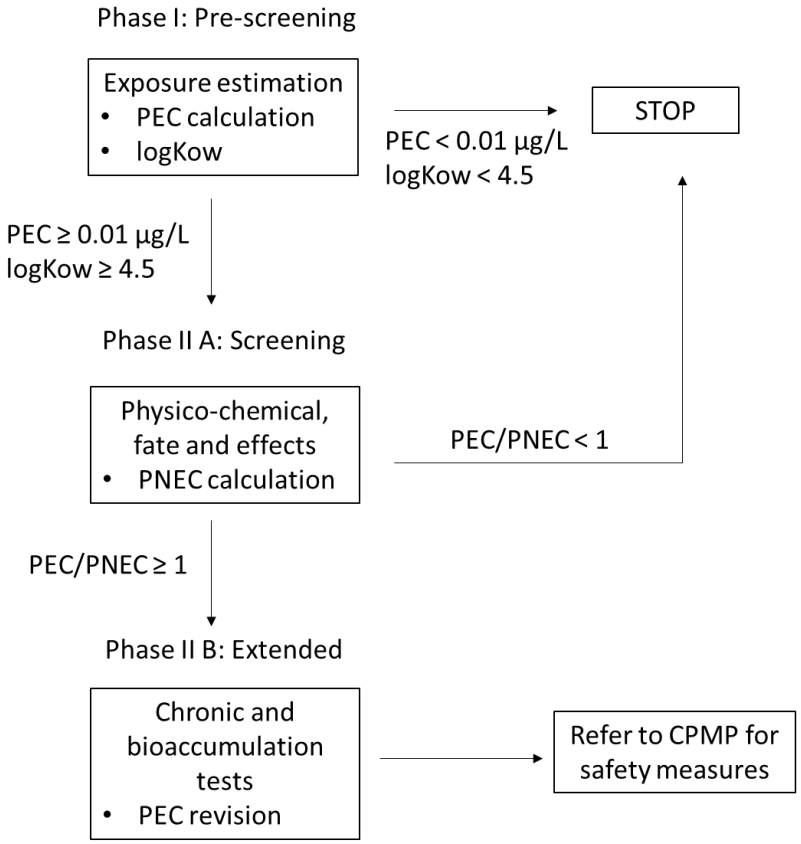
Since PhACs are designed to be biologically active, even at trace levels they can elicit physiological change (KÜSTER; ADLER, 2014) and impose undesired effects on target and non-target organisms (ZHOU *et al.*, 2016). For instance, estrogenic substance ethinylestradiol causes impaired reproduction in fish and leads to fish feminization (JOBLING *et al.*, 1998; DESBROWN *et al.*, 1998); negative reproductive and survival effects of organisms on account of exposure to propanolol, diclofenac, gemfibrozil, ibuprofen and fluoxetine (LARRSON *et al*., 2007); diclofenac has high antiovulatory effects on aquatic vertebrates (YOKOTA *et al.*, 2016); ciprofloxacin interfere with the photosynthesis pathway of higher plants, leading to morphological abnormalities or growth inhibition (ARISTILDE; SPOSITO, 2010). Moreover, dissemination of antibiotic resistant bacteria in the environment caused by the presence of antibiotic is an emerging concern (MARTI *et al.*, 2014). The pollutant potential of these compounds is further aggravated by its ease of biological barriers transposition and by its high

capacity of bioaccumulation (FENT *et al*., 2010; ZENKER *et al*., 2014). The main criteria for accumulation tendency is the partition coefficient octanol/water, expressed by a logKow>3 (ZENKER *et al*., 2014). The authors Howard and Muir (2011) rated 92 out of 275 PhACs detected in the environment as potentially bioaccumulative using quantitative structure property relationships (QSPR). Besides, PhACs presence in the environment is even more concerning considering that they do not appear individually, but as a complex mixture, which could lead to unwanted synergistic effects (CLEVEURS, 2004; 2005).

Given the certainty that PhACs presence causes deleterious effects on the aquatic environment and concerning about these effects on human health, environmental and human health risk assessments should not be neglected.

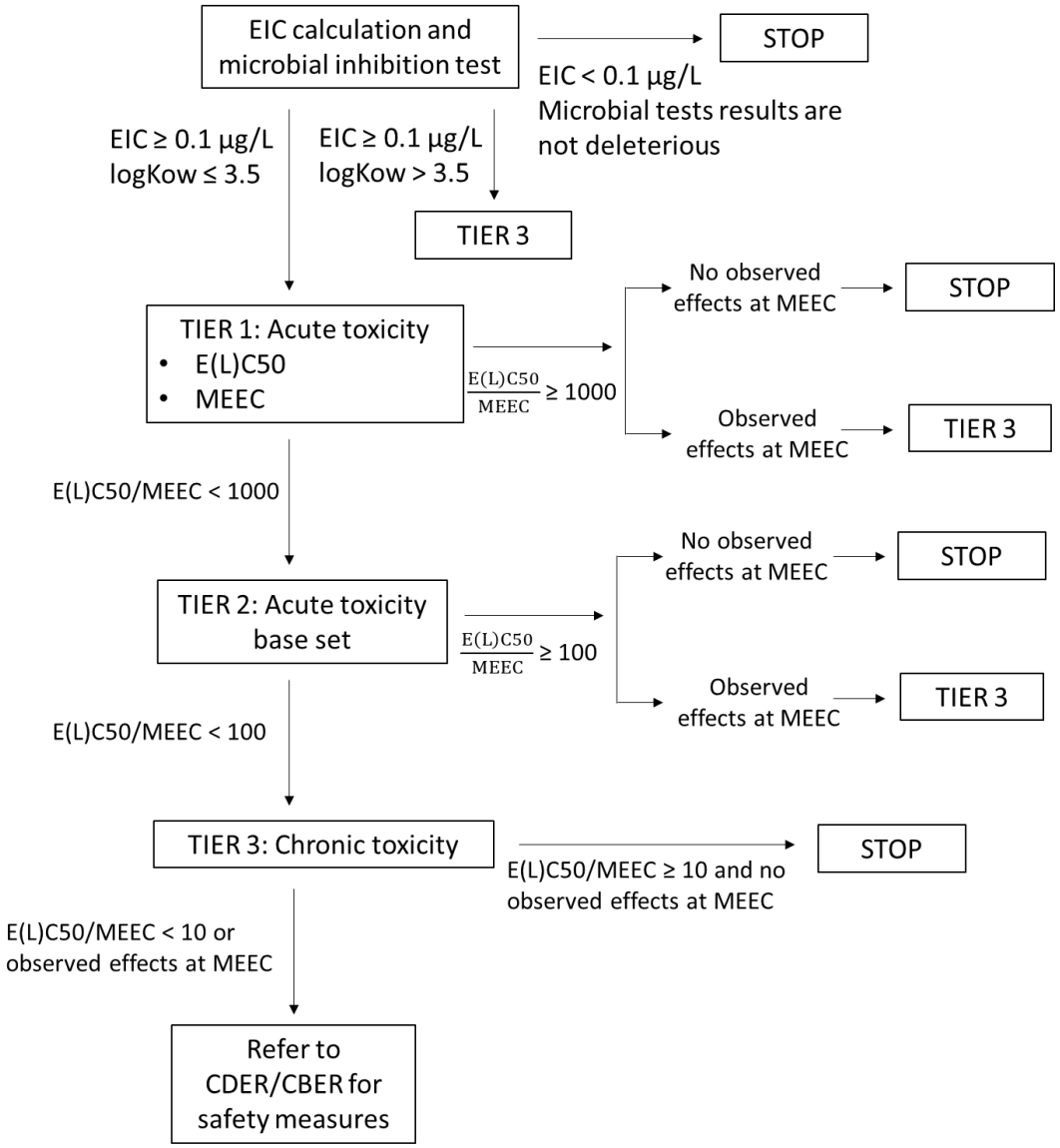
3 ERA AND HRA GUIDELINES

For new PhACs release, FDA and EMEA risk assessment approaches employ a similar tiered system that uses predicted or expected environmental concentrations as an indicator of the dose-response relationship and relate it to predicted non-effect concentrations or safe exposure levels, estimated from standard toxicity assays, to evaluate which compounds are more likely to cause toxicological risks (FDA, 1998; EMEA, 2006). EMEA and FDA risk assessment decision making processes are shown in Figure 2.1 and Figure 2.2, respectively.



CPMP: Committee For Proprietary Medicinal Products

Figure 2.1 - EMEA risk assessment guidelines.



CDER: Centre for Drug Evaluation and Research; CBER: Centre for Biologics Evaluation and Research.

Figure 2.2 - FDA risk assessment guidelines.

In EMEA approach, PEC for surface water is calculated in Phase I accordingly to Eq. 2.1 and in Phase II B it is reviewed considering the amount of drug excreted by the patient and the amount removed from the system by WWTP processes (Eq. 2.2). PNEC is calculated dividing the toxicity indicators median effective or lethal concentration (EC50 and LC50) or the no-observed effect concentration (NOEC) by an assessment factor of 1000 or 10, respectively.

$$PEC_{surface\ water}(\mu g/L) = \frac{DOSE_{ai} * F_{pen}}{WASTE_{nhab} * dilution} \quad (2.1)$$

$DOSE_{ai}$ is the PhAC maximum daily dose consumed per inhabitant (mg/inhab.day); F_{pen} is the PhAC market penetration (%); $WASTE_{nhab}$ is the wastewater volume per person per day (default = 200 L/inhab.day); and dilution is the factor from WWTWs effluent to surface water (= 10) (EMEA, 2006).

$$PEC_{surface\ water}\left(\mu\frac{g}{L}\right) = \frac{Elocal_{water} * F_{WWTPs}}{WASTE_{inhab} * CAPACITY_{WWTPs} * factor * dilution} \quad (2.2)$$

F_{WWTPs} is the compound fraction that goes to the surface water; factor is the adsorption degree to suspended matter; $CAPACITY_{WWTPs}$ is the local WWTWs capacity (inhab^{-1}); $Elocal_{water}$ is the local emission to waste water and is given by Eq. 2.3:

$$Elocal_{water} = \frac{DOSE_{ai} * F_{excreta} * F_{pen} * CAPACITY_{WWTPs}}{100} \quad (2.3)$$

$F_{excreta}$ is the PhAC excreted fraction.

In FDA approach, the Expected Introductory Concentration (EIC) is calculated accordingly to Eq. 2.4.

$$EIC\ aquatic\ (ppb) = A * B * C * D \quad (2.4)$$

A = kg/year of the PhAC produced for direct use; B = 1/liters per day entering WWTPs (1.214E-11 liters per day entering publicly owned treatment works); C = year/365 days; and D = 10^9 µg/kg (conversion factor). The maximum expected environmental concentration (MEEC) is estimated either by the EIC or the expected environmental concentration (EEC; concentration in surface water), whichever is greater.

Both sets of guidelines indicate a threshold level for the predicted environmental concentrations of a compound, which if exceeded triggers further investigation via a tiered assessment framework. Both of these threshold figures (EMEA 0.01 µg/L and FDA 0.1 µg/L in surface water) are flexible if there is any evidence of ecological threat. However, the environmental risk is not included in the human pharmaceuticals risk–benefit analysis, so a new PhAC may not be prevented from being marketed because it is possible to pose risk for the environment (STRAUB, 2002; EMEA, 2006; KUSTER; ADLER, 2014).

The guidelines defined by the EMEA and FDA agencies for risk assessment for new drugs are also used to assess the occurrence of toxicological risk owing to existing pharmaceuticals that are present in environment. In this case, instead of using PEC, the measured environmental concentration (MEC) in the evaluated medium is used. The risk is assessed by the hazard quotient (HQ), estimated by the ratio between MEC and PNEC (WORLD HEALTH ORGANIZATION, 2011). According to the HQ value, the toxicological risk is classified as: high risk (HQ > 1), medium risk (0.1 ≤ HQ ≤ 1), low risk (0.01 ≤ HQ < 0.1) and negligible risk (HQ < 0.01) (EUROPEAN COMMISSION, 1996).

Similarly, for human PhACs indirect exposure by drinking water owing to natural waters contamination, HRA is set using thresholds below which no toxicity is predicted to relate them to human oral exposure and determine the margin of exposure (MOE). According to USEPA, MOE values above 100 indicate a low probability of risk. The surrogate for the threshold is commonly the no-observed or the lowest observed adverse effect level (NOAEL or LOAEL) or the benchmark dose (BMD), which are determined from chronic animal studies and then divided by an uncertainty factor of 100 (interspecies differences tenfold and human variability tenfold) to derive the tolerable daily intake (TDI) or the reference dose (RfD) (WHO, 2011). From TDI values, drinking water equivalent level (DWEL) is derived, using daily water consumption, the fraction of the tolerable daily intake allocated to water consumption, and subjects body weight, as in Eq. 2.5 (WHO, 2011).

$$DWEL = \frac{(TDI * bm * f)}{C} \quad (2.5)$$

Where *bm* is the body mass; *f* is the relative contribution of water to exposure, which can be considered 100% since PhACS exposure from other sources is insignificant; and *C* is the daily water consumption (WHO, 2011). MOE is so obtained by the ratio between DWEL and MEC.

The main difference between ERA and HRA is that the former aims to protect the whole ecosystem while the latter focuses on the individual. Hence, HRA pharmacological and toxicological studies look at all potential adverse effects, whereas only relevant endpoints for the population level are taken into account in ERA (e.g. growth rate, reproduction and lethality). This explains the special interest of ecotoxicology in pharmaceuticals that are potentially endocrine disrupters since they may influence parameters relevant to population survival, such as reproduction rates (DORNE *et al*., 2006).

4 LIMITATIONS AND RECOMMENDATIONS

According to WHO (2011), the main challenges in assessing risks include the still limited occurrence data available for pharmaceuticals, the diverse range of pharmaceuticals in use, the wide variation in the use of individual pharmaceuticals between countries, the limited number of data in the public domain about toxicological tests and assays and technical limitations relating to assessing risks from chronic exposure to low-dose of pharmaceuticals and mixtures.

The limited toxicity data available in the public domain is even aggravated by the low ampleness of standard toxicological tests. Testing PhACs aquatic ecotoxicity is usually undertaken at controlled laboratory conditions and it often involves determining a single compound acute toxicity to a specific indicator species. The most common taxon is the crustacean *Daphnia magna*, with standard methods available to measure EC50 based on their mobility (OECD, 2006). These methods are conducted in an exposure medium consisting of clean laboratory water and are not representative of real environment conditions. Furthermore, toxicity data collation from different literature sources has limitations owing to the range of test species used, as well as the variety of toxicological endpoints applied. This weakness can be minimized by novel approaches and techniques to derivate toxicity data, as mathematical models and *in vitro* tests, which are going to be forward discussed. Modeling approaches that make use of the available ecotoxicological information are indicated for prospective purposes and they also lower the need for biotesting, for example, for the classification and labeling of chemical products (CLP). Besides, the use of all available ecotoxicity studies, of sufficient reliability and relevance, in the decision process instead of only standard assays would make better use of the available knowledge and could thereby add important information to the environmental risk assessment (ÅGERSTRAND *et al*., 2015).

One great limitation to risk assessment is the clear lack of information on PhACs mixture interfaith impact, particularly at low concentration over longer exposure times. Some studies have shown that pharmaceuticals mixtures exhibit greater effect than the compounds individually (BACKHAUS; FAUST, 2012). Cleveurs (2004) observed that the mixture of antiepileptic carbamazepine and the lipid regulator clofibric acid (which belong to very different therapeutic classes presenting distinct modes of action) exhibited stronger effects to *D. magna* during immobilization tests than the single compounds at the same concentration. Furthermore, Cleveurs (2005) reported considerable acute toxicity for a mixture of non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac, ibuprofen, naproxen and aspirin) where little or no effect was observed for the chemicals individually at the same concentration.

On the other hand, Dietrich *et al*. (2010) investigated single compound and mixture toxicity of carbamazepine (500 ng/L), diclofenac (360 ng/L) and metoprolol (1200 ng/L) to *D. magna* over six generations and found out that the influence of the pharmaceutical mixture was inconsistent and unpredictable. This underpins the need to assess chronic impact of PhACs mixtures at environmentally relevant concentrations, as well as undertaking whole life cycle determinations. So, to further increase ERA and HRA processes, one recommendation is to assess the cumulative risk for PhACs groups with similar modes of action. Such approach could give important insights regarding actual environmental risks. Some theoretical models have been developed and applied to predict mixture toxicity and they largely base on two principles referred to as concentration addition (CA) and independent action (IA), which were proposed to describe mixture effect of components having similar and dissimilar modes of action, respectively (ÅGERSTRAND *et al*., 2015).

The presence of PhACs metabolites in environment is another great concern. Metabolites determination and consideration in risk assessment is essential as they can be persistent during secondary wastewater treatment and may pose greater risk than the parent compound (PETRIE *et al*., 2015). Han and Lee (2017) demonstrated the metabolites significance to PhACs risk assessment estimating the PEC in surface water for 24 selected PhACs and their metabolites using a life cycle based emission estimation model. With the toxicity data, the metabolites HQ were compared with those of individual parent compounds and the results showed that a total of 18 metabolites (from 12 parents) had greater HQ than their parents. This result clearly demonstrated that metabolites should be taken into account when assessing toxicological risks in the preliminary exposure assessment. According to EMEA guidelines, metabolites are only considered if the parent PhAC PEC in surface water is above 0.01 μg/L in Phase I or when Phase II B is required, however, including pharmaceuticals metabolites in the early stage of ERA may provide results that are closer to reality and that alter the concept of risk occurrence related to that compound.

These limitations are valid both for new pharmaceuticals risk assessment regarding environmental licensing and for risk assessment of existing compounds found in natural waters, in order to control pollution. In addition to these specific limitations, there are other points that can be worked out to provide a more efficient risk assessment. Ågerstrand *et al*. (2015) and Küster and Adler (2014) compiled in their work some recommendations for improvement of the guidelines adopted today. The recommendations concern: expanding the scope of the current guideline; refinement of toxicological tests; mandatory reviews; increasing transparency; improving the availability of ERA data; and risk management better options.

The main recommendation for both authors is to include ERA in the risk-benefit analysis when a product is considered for market authorization. Today, as it does not constitute a refusal criterion of a marketing authorization, it is not a priority for pharmaceutical companies, who fail to deliver data. A recent study shows that 37% of the ERAs performed during 2011−2012 were submitted after the deadline and that 83% were missing or of unsatisfactory quality (CANEVA *et al*., 2014). According to Küster and Adler (2014), until recent years many of the ERA still omit relevant studies that are requested according to the guidelines. German Federal Agency (UBA) has reviewed approximately 650 human pharmaceutical products and found out that complete (phase I and phase II) and valid ERA are available for only 120 medicinal products. The evaluation of these substances resulted in the conclusion that approximately 10% are notable regarding their potential environmental risk.

Requiring environmental risk assessment also for products put on the market before 2006 is another strong recommendation, once there is insufficient or no ERA data available for these PhACs and still they are detected in the environment and can be relevant to risk assessment. Besides, according to German PhACs consumption data in 2012 (IMS Health), many existing substances are still produced in high amounts, such as metformin (1.200 t), ibuprofen (975 t), metamizole (615 t), acetaminophen (458 t), iomeprol (255 t) and metoprolol (157 t) and the consumption of these compounds may still heavily increase. As an example, between 2002 and 2012 the consumption of metformin and ibuprofen in Germany increased from 390 to 1200 t and from 250 to 975 t, respectively.

Others recommendations to improve risk assessment guidelines are: perform only one environmental risk assessment per active pharmaceutical ingredient in order to centralize the information and avoid multiple and controversial conclusions; refine the tiered approach to include pharmacological and toxicological data from the drug discovery process, as well as bioconcentration data; and require review of the environmental risk assessments at regular intervals aiming to be updated with significant new environmental information (ÅGERSTRAND *et al*., 2015).

5 RECENT TECHNIQUES AND APPROACHES

Standardized ecotoxicological tests still constitute the fundamental tools when doing risk assessment of aquatic contaminants. However, such tests are not always adequate to predict the actual effects of PhACs on the ecosystem, often underestimating them. ERA regulatory concepts are commonly based on a short-term ecotoxicological studies set in three different species (OECD, 1998; USEPA, 2000; HERNANDO *et al*., 2006) focused on representative organisms of the chain food defined by OECD guidelines for testing chemicals (OECD, 2007). In order to determine the suitability of the standardized toxicity tests, Aguirre-Martinez *et al*. (2015) selected four frequently found pharmaceuticals: caffeine (stimulant), ibuprofen (anti-inflammatory), novobiocin (antibiotic), and carbamazepine (anticonvulsant) and carried acute bioassays with organisms representing different trophic levels. Results indicated that the selected PhACs were harmless for aquatic environment, except when applying the embryo-larval development endpoint. Thus, this study showed the necessity of using more sensitive responses, when assessing PhACs risk in aquatic environments, since endpoints applied in current guidelines may not be suitable. It is of great concern that current application of these guidelines may underestimate the effect of some PhACs, exposing the aquatic biota to unknown chronic effects. Studies also indicate that longer exposition periods are necessary to observe effects when testing pharmaceuticals at concentrations found in environment (GAGNÉ *et al*., 2007; MARTÍN-DÍAZ *et al*., 2009; AGUIRRE-MARTÍNEZ *et al*., 2013a; 2013b; MATOZZO *et al*., 2014).

Aiming to overcome these problems and to improve toxicological tests, new techniques and novel approaches have been developed. For example, toxicological tests designed to detect PhACs therapeutic effects have been standing out. The tests used for risk assessment are designed to measure harmful effects, but pharmacological effects occur at concentrations much lower than concentrations that may be toxic and they can also cause ecological consequences. For example, Klaminder *et al*. (2014) demonstrated how Oxazepan (a common contaminant in surface waters) therapeutic effect lower the mortality rates among exposed *Eurasian perch* from wild populations. Fry hatched from roe that had been exposed to dilute concentrations (1.1 ± 0.3 μg/L) of Oxazepam for 24h 3–6 days prior to hatching showed lower mortality rates and increased activity 30 days after hatching. Thus, the authors concluded that PhACs therapeutic effects need to be considered in risk assessment assays to avoid that important ecological effects from aquatic contaminants are systematically missed. The need for using new approaches that

focus on the pharmacological effects for risk assessment has been also highlighted in other papers (CHRISTEN *et al*., 2010, RAND-WEAVER *et al*., 2013).

Another important point is that the number of PhACs for which environmental regulatory decisions are required far exceeds the current capacity for toxicity testing. High-throughput screening has the potential to increase this capacity. The adverse outcome pathway (AOP) concept has emerged as a framework for connecting high-throughput toxicity testing (HTT) and other results to potential impacts on human and wildlife populations. It aims to increase the depth and breadth of toxicological information and, concomitantly, to reduce cost, increase efficiency and reduce the use of animals. The AOP concept emerged from the field of ecotoxicology as a means to enhance the utility of the quantitative structure activity relationship (QSAR), biomarkers and other types of mechanistic data for both understanding and predicting potential adverse effects of chemical exposure in wildlife populations (ANKLEY *et al*., 2010). These include PhACs mode of action (MOA), pharmacokinetics, and pharmacodynamics, following the basic premise that toxicity is the result of generalizable motifs of biologic failure initiated by the interaction of a chemical with some biomolecule in the body (VILLENEUVE *et al*., 2014). Thus, AOP approach bases on scanning toxicological tests, as mathematical modelling and in vitro assays, to identify PhACs MOA and relate them to provide mechanistic information (MEEK *et al*., 2008). Specific AOP applications include chemical grouping for read-across, design of efficient testing strategies, prioritization for testing, and quantitative risk assessment (MEEK *et al*., 2014; PERKINS *et al*., 2015), with an emphasis on the replacement, refinement and reduction of animal-based testing (BURDEN *et al*., 2015; EDWARDS *et al*., 2016).

Following this tendency, USEPA, through ToxCast programme, is developing an overall approach that can be broken into seven tasks: (i) identifying biological pathways that, when perturbed, can lead to toxicity; (ii) developing high-throughput *in vitro* assays to test chemical perturbations of these pathways; (iii) identifying the universe of chemicals with likely human or ecological exposure; (iv) testing as many of these chemicals as possible in the relevant *in vitro* assays; (v) developing hazard models that take the results of these tests and identify chemicals as being potential toxicants; (vi) generating toxicokinetics data on these chemicals to predict the doses at which these hazard pathways would be activated; and (vii) developing exposure models to identify chemicals for which these hazardous dose levels could be achieved (JUDSON *et al*., 2014). The programme has recently ended his first phase and a large set of environmentally relevant chemicals have been screened in a diverse battery of *in vitro* assays. Toxicity predictive models have been developed using these data and *in vitro* pharmacokinetic

data have been integrated with the *in vitro* assay data, enabling the initial quantitative comparison with *in vivo* rodent toxicity data (USEPA, 2018).

Furthermore, another recent approach aiming to improve risk assessment methodology is the application of the new tools of experimental and computational Systems Toxicology, the integration of classical toxicology with quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization. The increasing need for more predictive and accurate risk-assessment approaches requires a detailed mechanistic understanding of the ways in which xenobiotic substances perturb biological systems and lead to adverse outcomes. Thus, Systems Toxicology approaches offer modern strategies for gaining such mechanistic knowledge by combining advanced analytical and computational tools. In Systems Toxicology, quantitative systems-wide molecular changes caused by some exposure are measured and a causal chain of molecular events linking exposures with adverse outcomes (i.e., functional endpoints) is deciphered. Mathematical models are then built to describe these processes in a quantitative manner. The integrated data analysis leads to the identification of how biological networks are perturbed by the exposure and enables the development of predictive mathematical models of toxicological processes (HARTUNG *et al*., 2012; WATERS; FOSTEL, 2014; STURLA *et al*., 2014).

6 CONCLUSIONS

With the knowledge advancement about natural waters contamination by PhACs, an accurate execution of risk assessments becomes mandatory in the control of the pollution caused by these substances. The current risk assessment guidelines present in their concepts, scope and spread a series of limitations that restrict their applicability and often underestimate the real effects that PhACs can cause to the aquatic ecosystem and even to human health.

Improvements can and should be made and several studies already point to new trends that may improve the effectiveness of risk characterization, increase the predictive accuracy of PhACs adverse effects in aquatic organisms and increase the range of toxicological tests, considering the enormous variability of compounds present in daily life.

The incorporation of these new techniques and approaches to current guidelines is necessary so that regulatory bodies are up to date with the latest technological advances and according to recent knowledge on PhACs environmental behavior.

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3rd Chapter

PhACs ERA and HRA in surface waters and risk reduction by conventional treatment processes

1 INTRODUCTION

Pharmaceutically active compounds (PhACs) presence in surface, ground and drinking water and wastewater has been detected in many different places around the world in concentrations ranging from ng/L to µg/L (SARMAH *et al.,* 2006; KÜMMERER, 2009; WATKINSON *et al.*, 2009; SARAVANAN *et al.*, 2014; NET *et al.*, 2015). PhACs reach superficial water bodies mainly by human excretion (sewage) owing to the incomplete removal of PhACs in wastewater treatment plant facilities (WWTP) (ARCHER *et al.*, 2017). WWTP are the major barrier imposed in order to prevent the contamination of the environment by PhACs. However, the conventional WWTP usually employed for the sewage treatment are designed aiming the removal of easily or moderately biodegradable carbon, nitrogen and phosphorus compounds and microbiological organisms (VERLICCHI *et al*., 2012; GARCIA-IVARS *et al*., 2017). As PhACs have a very stable structure, low volatility, different hydrophobicity, complex structures and extremely low concentration, their removal is challenger.

Drinking water treatment plants (DWTP) may impose another barrier that can prevent the return of these PhACs to human body. Many studies have been carried out in order to detect PhACs in treated water (VULLIET *et al.*, 2011; BOLEDA *et al.*, 2011; CARMONA *et al.*, 2014). The results point that conventional treatment processes like coagulation, flocculation, filtration and chlorination have, in general, poor removal efficiencies (HUERTA-FONTELA *et al.*, 2011; SIMAZAKI *et al.*, 2015), while advanced treatment technologies, such as ozonation, activated carbon adsorption and membrane separation processes (MSP) are successefully applied to PhACs removal (KIMURA *et al*., 2005; HUERTA-FONTELA *et al*., 2011; MESTANKOVA *et al*., 2012; TAHERAM *et al*., 2016; WANG *et al*., 2018). Thus, the conjugation of conventional and advanced methods is highly indicated for efficient PhACs removal.

In general, pharmaceutical compounds levels are water source dependent, which, in turn, depends on the location, popular habits, wastewater treatment type, PhACs consumption patterns, physicochemical properties and stability as well as the weather (CAMACHO-MUNOZ *et al.*, 2014).

The contamination by PhACs may be more delicate when developing countries, like Brazil, are concerned, especially owing to the lack or small coverage of sewage treatment. Brazil has the largest population in Latin America, as well as the largest territorial area, and is one of the countries with the greatest availability of water per capita around the world. However, according to the Brazilian National Health Interview Survey (SISTEMA NACIONAL DE

INFORMAÇÕES SOBRE SANEAMENTO, 2015), 50.3% of the Brazilian population has access to sewage collection, and only 42.67% of the country's sewage is treated. Still, only 10 cities in the 100 largest Brazilian cities treat more than 80% of the sewage generated. Besides, the wastewater treatment facilities for hospital wastewater and pharmaceutical industry are very limited and the adoption of co-treatment of these effluents with sewage is the most common practice. Furthermore, as others developing countries, owing to the insufficient health services coverage, people generally use drugs without a medical prescription resulting in a higher consumption compared to developed countries (BOECKEL *et al*., 2014).

Thus, PhACs presence in natural water causes great concern, especially because they can pose toxicological risk to the environment and to public health. Since PhACs are designed to be biologically active, even at trace levels, PhACs may impose undesired effects on target and non-target organisms (ZHOU *et al*., 2016). PhACs presence in the environment is even more concerning considering that they do not appear individually, but as a complex mixture, which could lead to unwanted synergistic effects (CLEVEURS, 2004; 2005). Considering the toxic effects and especially owing to the inadvertent exposure to pharmaceuticals via drinking water, it is important that PhACs human health risk is also assessed.

Thus, this study aims to identify, quantify and qualify PhACs in four Brazilian drinking water treatment systems and to assess the environmental and human health toxicological risk posed by these PhACs. The DWTP analysed present different capacities, raw water quality and treatment processes and are localized in three different Brazilian regions (Northeast, Southeast and South). Each region presents specific characteristics, such as climate, population habits and social-economic condition. So far it is known by the authors, no other study had been carried out in these regions including PhACs identification, quantification and environmental and human health risk assessment.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

The 28 PhACs (Annex 1) analysed were selected crossing the list of pharmaceuticals distributed free of charge by the Brazilian health system (SUS) with the list of the most consumed in the country in 2016 (ANVISA, 2017), in order to represent the Brazilian consumption pattern. In addition, we tried to select representatives of the most varied therapeutic classes, in order to broaden the scope of the research. The physicochemical properties, including molecular weight, geometry, hydrophobicity, polarity and charge of the selected PhACs are shown in Annex 1. The analytical standards of the selected PhACs were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-grade formic acid and solvents were purchased from Dikma (USA). Ultrapure water (18.2 $M\Omega cm^{-1}$) was produced by a Milli-Q unit (Millipore, USA).

2.2 Study area and sample collection

Raw and treated water were collected from four Brazilian DWTP including three differents regions. The basic information of the four study areas is presented in Table 3.1. Different scenarios were contemplated. The rivers that supply water to DWTP 1 and 3 basin cover urban and rural areas, industrial districts, agricultural areas, hospitals and pharmaceutical industries, and receives discharged treated and untreated sewage. The river which fed the dam of the DWTP 2 covers mostly rural areas with subsistence agriculture and monoculture mainly of manioc and receive discharged treated and untreated sewage. The dam is used for multiple usage such as power generation, natural fishing and fish farming, sail and recreation. The lake that supplies DWTP 4 is located in an urban area and is a touristic attraction with recreational use.

The sampling campaign was conducted in April, July and November 2016 and January and April 2017, according to the technical specification requirements for monitoring of surface water and wastewater of the Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

Table 3.1 - Capacity and treatment type of each studied DWTP

DWTP	Region	Capacity (m^3/s)	Water body main characteristics	Climate	Attended Population	Treatment type
1	Southeast	0.04	River with waste disposal nearby the water adduction point. Average water flow: 174 m^3/s	AW – Tropical Winter min. temperature: 15 ºC Summer max. temperature: 33 ºC Annual rainfall: 1060 mm	276,995	Coagulation, flocculation, sedimentation, sand filtration, disinfection (chlorination) and fluoridation
2	Northeast	8.5	Dam Capacity: 4,630 m^3	AF – Equatorial Winter min. temperature: 21 ºC Summer max. temperature: 30 ºC Annual rainfall: 2145 mm	2,331,864	Coagulation, flocculation, sedimentation, sand filtration, disinfection (chlorination) and fluoridation
3	Southeast	6.5	River Average water flow: $25m^3/s$	CAw – Temperate and warm Winter min. temperature: 13 ºC Summer max. temperature: 29 ºC Annual rainfall: 1465 mm	1,514,276	Coagulation, flocculation, sedimentation, sand filtration, disinfection (chlorination) and fluoridation
4	South	0.2	Lake located in a municipal park and doesn't have direct wastewater releases. Volume: 21,2 m^3	CFa – Temperate and warm Winter min. temperature: 13 ºC Summer max. temperature: 28 ºC Annual rainfall: 1519 mm	113,000	Fast coagulation, pebble filtration, disinfection (chlorination) and fluoridation

2.3 Sample preparation and instrumental analysis

PhACs were analysed using HPLC (DGU/20A3 Prominence, Shimadzu, Japan) coupled to micrOTOF-QII mass spectrometer (Bruker) with an electrospray ionization source (ESI). The quantification limit for each PhAC was around 8 ng/L. The uncertainty of estimate was of 1% according to a validation method of the analysis protocol. Recoveries were between 86 and 100% but were compensated by the calibration, which is processed the same way as the samples. Water samples were previously filtered in 0.45 μm hydrophilic PVDF filter. Analytes were isolated from water samples (1 L) in two steps, firstly without pH adjustment (pH 7) and then with pH adjustment to 2 by adding 0.002 mol/L H_2SO_4 solution, using a polymeric C18/18% cartridge (500 mg/6 mL – Applied Separations) preconditioned with 5 mL of methanol and 5 mL of ultra pure water, and then eluted with methanol using Aspec Gilson GX-271 Liquid Handler. Separation was achieved on a Shim-pack XR-ODS C18 column (2.0 mm; 50 mm and 2.0 μm; Shimadzu, Japan) with the mixture of 0.1% of formic acid water and methanol as the mobile phase. The flow rate and injection volume were 0.1 mL/min and 10 μL, respectively. The mobile phase gradient followed an isocratic method using 95% of methanol for 15 minutes.

2.4 Water quality parameters

Colour (2120 C), TSS (2540 B E), conductivity (2510 B) and pH (4500 H B) were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). TOC was analysed using TOC Shimadzu TOC-V CNP. The concentrations of Cl^-, SO_4^{2-}, PO_4^{3-}, F^-, NO_3^-, NO_2^- were measured by ion chromatography (ICS-1000 ion chromatograph equipped with the Dionex AS-22 column and ICS 12a). The metals concentrations K^+, Mg^{2+}, Ca^{2+} and Na^+ were quantified by atomic absorption spectrometry (Atomic Absorption Spectrophotometer - GBC - AVANTA).

2.5 Environmental and human health risk assessment

The potential environmental risks posed by individual compounds were evaluated based on a hazard quotient (HQ). HQ values were calculated based on both measured environmental concentration (MEC) and predicted no effect concentration (PNEC), as shown in Eq. 3.1.

$$HQ = \frac{MEC}{PNEC} \quad (3.1)$$

PNEC was determined for both acute and chronic effects (Eq. 3.2 and 3.3), based on the mean effect or lethal concentration (EC50 or LC50) or on the non-observed effect concentration (NOEC), respectively. In both cases, the toxicity endpoint was divided by safety factors, as recommend in literature (1000 for acute toxicity and 10 for chronic) (WORLD HEALTH ORGANIZATION, 2011).

$$PNEC_a = \frac{E(L)C50}{1000} \quad (3.2)$$

$$PNEC_c = \frac{NOEC}{10} \quad (3.3)$$

For HQ calculation were considered the lowest PNEC values and the highest PhACs concentration in the evaluated waters in order to obtain a worst-case scenario. The risk was classified into the following categories: high risk (HQ > 1), medium risk (0.1 ≤ HQ ≤ 1), low risk (0.01 ≤ HQ < 0.1) and negligible risk (HQ < 0.01) (EUROPEAN COMMISSION, 1996).

E(L)C50 and NOEC values of each PhAC were collected in literature for three trophic levels (algae, crustacean and fish, whenever possible) and considering only values obtained with standard tests, as recommended by the guidelines of the Water Framework Directive (EUROPEAN COMMISSION, 2000). According to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), the compounds were classified as: (i) highly toxic: E(L)C50 ≤ 1 mg/L; (ii) toxic: 1 mg/L < E(L)C50 ≤ 10 mg/L; (iii) harmful to the aquatic ecosystem: 10 mg/L < E(L)C50 ≤ 100 mg/L (UNITED NATIONS, 2011). Some regulatory systems also include a fourth category, non-toxic compounds: E(L)C50 > 100 mg/L. These levels of toxicity have been used in previous studies (CLEUVERS, 2004; HAN *et al.*, 2006; GARCIA *et al.*, 2014).

In order to evaluate the mixture toxicity, the mixture hazard quotient (MHQ) for each water sample was estimated by using the classical concentration addition model, which consists in adding the individual values, as Eq. 3.4.

$$MHQ = HQ_{PhAC1} + HQ_{PhAC2} + \cdots + HQ_{PhACn} \quad (3.4)$$

Where n is the number of PhACs quantified in that sample.

Concerning public health, the risk was estimated through the margin of exposure (MOE), which is the ratio between a concentration below which the probability of adverse effects is negligible and the measured concentrations. The concentration free of risk was based on the tolerable daily

intake (TDI), which values for each PhAC were found in literature or derived from the non-observed adverse effects level (NOAEL), as shown in Eq. 3.5 (WHO, 2011).

$$TDI = \frac{NOAEL}{100} \quad (3.5)$$

Where 100 is the safety factor recommend in literature. For PhACs whose NOAEL value was not found, LOAEL (lowest observed adverse effect level) was used and an additional safety factor of 10 was applied (Eq. 3.6) (DWI, 2007).

$$TDI = \frac{LOAEL}{1000} \quad (3.6)$$

In order to possibilite the comparison, TDI was converted to drinking water equivalent level (DWEL) in mg/L, according to Eq. 3.7.

$$DWEL = \frac{(TDI * bm * f)}{C} \quad (3.7)$$

Where *bm* is the body mass (60 kg); *f* is the relative contribution of water to exposure, which can be considered 100%, since PhACS exposure from other sources is insignificant; and *C* is the daily water consumption (2 L) (WHO, 2011). Finally, the MOE was obtained by the ratio between DWEL and MEC (Eq. 3.8).

$$MOE = \frac{DWEL}{MEC} \quad (3.8)$$

3 RESULTS AND DISCUSSION

3.1 Water quality

The monitored water quality parameters are shown in Table 3.2. All water sources evaluated are classified as class 2, which requires conventional drinking water treatment, according to National Council for the Environment (CONAMA 357/2005). The detected drinking water quality parameters satisfied the standard limit values according to Brazilian legislation (Ministerial Order Nº 2914, 2011).

Water source 1 presents the highest values of turbidity, aparent color, TOC, total nitrogen (TN) and solids (TS). The city doesn't count with a WWTP and the sewer is discarded nearby the water adduction point, which explains the lowest water quality. Turbidity and color are parameteres with close relation to organic matter and therefore can have great influence in PhACs presence in water, since the compounds can bind into them either by hydrogen bonds or adsorption (SADMANI *et al.*, 2014).

Table 3.2 - Main characteristics of the assessed water matrixes (average, standard deviation, n=4).

Characteristic	Water source 1		Water source 2		Water source 3		Water source 4		Legal limit for drinking water
	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated	
pH	7.09±0.03	7.51±0.20	7.16±0.04	6.14±0.24	7.45±0.08	8.40±0.52	6.79±0.09	7.31±1.17	6.0-9.5
EC (µs/cm)	127±28	289±31	254±134	348±87	356±96	498±167	48±11	164±70	-
Color (Hz)	131±53	<2	22±15	<2	51±29	5±1	64±40	<2	15
Turbidity (uT)	22.56±19.15	0.34±0.73	1.55±1.80	0.25±0.13	4.98±1.30	0.08±1.50	6.63±1.20	0.27±0.02	1
TOC (mg/L)	1.59±0.82	<0.10	0.85±0.78	<0.10	0.35±0.13	<0.10	<0.10	<0.10	-
TN (mg/L)	0.78±0.24	<0.10	0.28±0.25	0.33±0.30	0.16±0.14	<0.10	0.14±0.28	0.13±0.26	-
Cl residual (mg/L)	-	3.2±0.5	-	2.6±0.2	-	2.9±0.4	-	3.1±0.7	>2
Alkalinity (mg/L)	17.42±10.11	6.42±1.97	32.75±2.63	10.75±3.30	15.73±1.58	5.86±1.36	7.75±1.50	4.50±1.29	-
TS (g/L)	98±61	0.32±0.09	0.21±0.09	0.14±0.06	0.11±0.15	0.09±0.53	0.15±0.06	0.08±0.02	-

3.2 PhACs occurrence and concentration

Among the 28 investigated compounds, 12 of them were detected during the sampling campaigns. Atenolol, erythromycin, scopolamine, phenazone, fenofibrate, ranitidine, paroxetine, amoxicillin, ampicillin, enoxacin, clarithromycin, danofloxacin, trimethoprim, ketoprofen, ibuprofen, caffeine and genfibrozil were not observed in any of the samples evaluated. Possibly these compounds have concentrations lower than the detection limit, which might be associated with the population consumption habits or greater propensity of these drugs to be hydrolysed under aerobic conditions or adsorbed (RADJENOVIC *et al.*, 2009; LUO *et al.*, 2011). Same behavior can be observed by other studies around the world (CARMONA *et al.*, 2014; SIMAZAKI *et al.*, 2015). The compounds which were not detected or concentrations below the MDL in all samples are not discussed in this study.

The mean concentrations of PhACs observed in raw water range from 11 ng/L (omeprazole in the water source 1) to 4,215 ng/L (fluconazole in water source 3) (Table 3.3). Betamethasone, fluconazole, atorvastatin and prednisone were the most abundant compounds. Moreover, betamethasone, fluconazole and prednisone were detected with high frequency in all water supply systems. The prevalence of these PhACs can be explained by their low degradability and hydrophilic characteristics (VERLICCHI *et al*., 2012; GARCIA-IVARS *et al*., 2017).

Sample concentrations observed in this study were compared with those reported in the literature. Fluconazole concentrations found in this study were significantly higher than that found in rivers from Spain (28.5 ng/L), China (22.8 ng/L) and South Korea (46.2 ng/L) (CASADO *et al*., 2014; HUANG *et al*., 2013; KIM *et al*., 2009). Despite of being one of the most common PhACs in this study, prednisone was not detected in any sample of the United States surface waters (BATT *et al*., 2015). The authors also did not detected atorvastatin in any sample, in accordance with the low detection frequency of this PhAC in this study. Betamethasone concentration is also lower in the US and in German than the ones found here. According to Vestel *et al*. (2016), the Pharmaceutical Assessment and Transport Evaluation model estimated betamethasone concentrations to be <0.6 ng/L in 95% of all U.S. surface waters and in German the concentrations were found to be between 0.07 and 2.8 ng/L (WEIZEL *et al*., 2018). The differences between the concentrations ranges indicate the variation in the consumption pattern among different countries and highlight the high usage of these PhACs in Brazil.

Loratadine, betamethasone, prednisone, fluconazole, atorvastatin and genfibrozil were the only PhACs quatified in treated water, in concentrations ranging from 8 ng/L (genfibrozil in water source 4) to 2,811 ng/L (prednisone in water source 2) (Table 3.3).

Table 3.3 - Minimum, maximum, average and median concentrations of PhACs (ng /L) in raw and treated water from four Brazilian water supply systems.

Pharmaceutical compounds	Raw water															
	1				2				3				4			
	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median
Betamethasone	3	20-701	295	165	4	34-3225	1106	559	2	622-888	755	755	3	326-878	419	473
Cimetidine	-	-	-	-	-	-	-	-	-	-	-	-	1	116	116	116
Fluconazole	3	227-573	356	266	3	83-332	206	204	2	35-4215	2125	2125	4	90-986	382	225
Omeprazole	1	11	11	11	-	-	-	-	-	-	-	-	-	-	-	-
Phenylbutazone	1	132	132	132	-	-	-	-	-	-	-	-	-	-	-	-
Loratadine	-	-	-	-	-	-	-	-	-	-	-	-	1	2481	2481	2481
Prednisone	1	233	233	233	4	2032-3556	2502	2210	2	34-883	458	458	4	327-1509	853	788
Enrofloxacin	-	-	-	-	1	14	14	14	-	-	-	-	-	-	-	-
Norfloxacin	-	-	-	-	-	-	-	-	1	134	134	134	-	-	-	-
Metformin	1	36	36	36	-	-	-	-	-	-	-	-	-	-	-	-
Atorvastatin	-	-	-	-	2	299-506	402	402	-	-	-	-	-	-	-	-
Genfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	1	17	17	17

Pharmaceutical compounds	Treated water															
	1				2				3				4			
	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median	DF[a] (N=5)	C (ng/L) min-max	C (ng/L) average	C (ng/L) median
Loratadine	-	-	-	-	-	-	-	-	-	-	-	-	1	17	17	17
Betamethasone	-	-	-	-	1	34	34	34	-	-	-	-	1	180	180	180
Prednisone	-	-	-	-	3	1650-2811	2105	1853	2	29-84	57	57	3	241-572	370	296
Fluconazole	1	151	151	151	2	349-586	468	468	1	1189	1189	1189	3	91-196	147	154
Atorvastatin	-	-	-	-	1	477	477	477	-	-	-	-	-	-	-	-
Genfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	1	8	8	8

[a] *Detection frequency*

PhACs presence in natural water is susceptible to seasonality (Figure 3.1) owing to their consumption pattern and also the microbial activity that is higher in the warmer months. In addition, the region socio-economic conditions also influence the consumption pattern and the contamination extent (Figure 3.2).

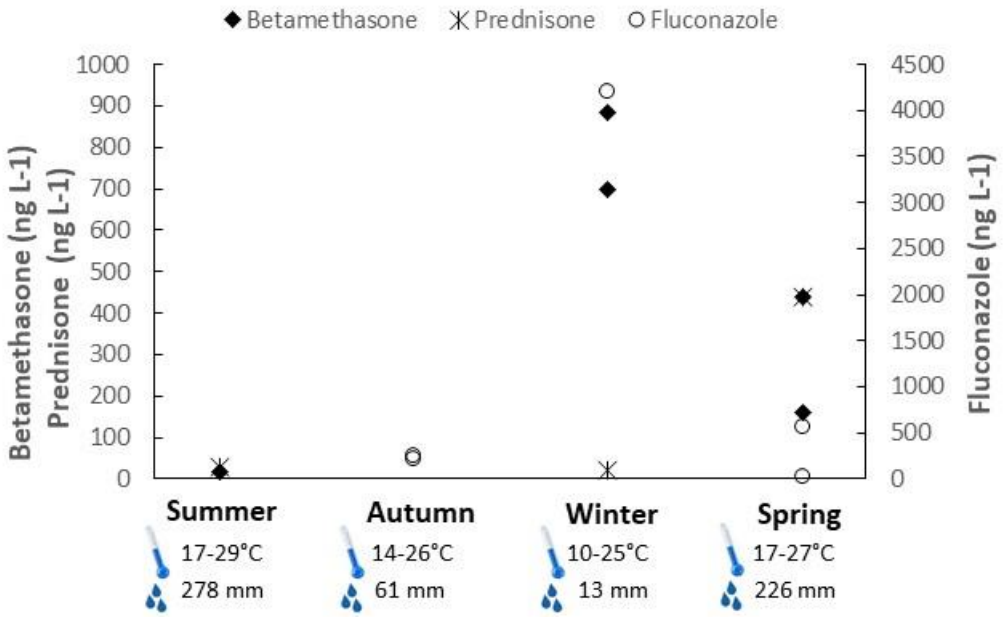

Figure 3.1 - Betamethasone, prednisone and fluconazole concentrations in water source 1 during different seasons.

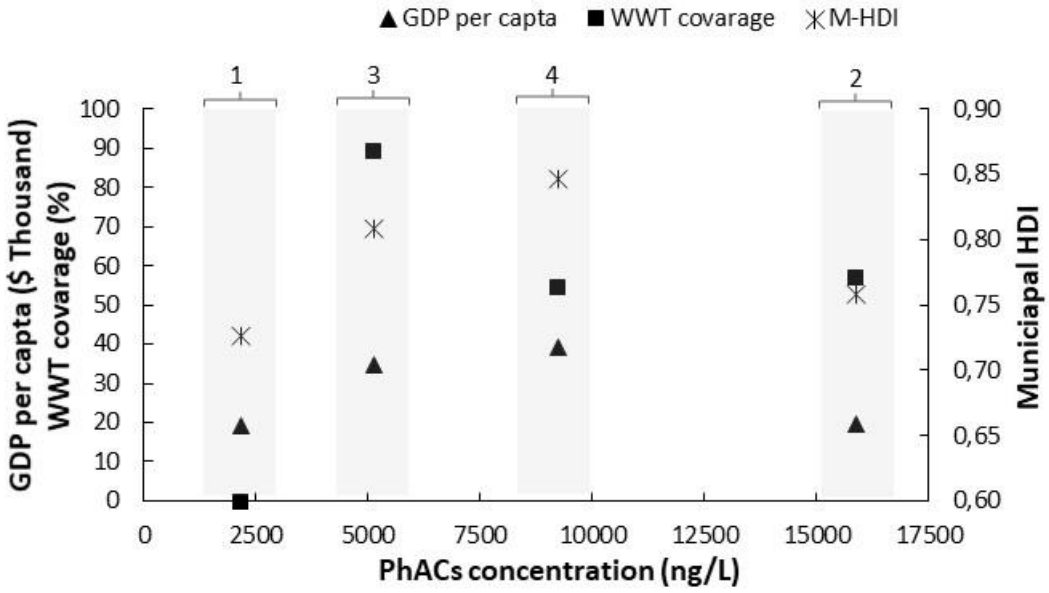

Figure 3.2 - Correlation between accumulated PhACs concentration and wastewater treatment (WWT) coverage index, municipal human developent index (HDI) and gross domestic product (GPD) per capita.

It is possible to observe from Figure 3.1 a seasonal pattern in PhACs concentration in natural water sources. Winter season presented the greatest pick of PhACs, mostly owing to the low

rainfall in the period, which causes a reduction in the river flow and therefore concentrate these pollutants. Also, the low temperatures of this season propitiates the increase in infectious diseases and thus it is observed a higher PhACs consumption. With spring arrival, PhACs concentration begin to reduce and achive its lowest values in summer season, which is characterized by great rainfall index, increasing the dilution, and and high temperatures that may accelerate biodegradation of pharmaceuticals owing to higher microbial activity (LUO *et al*., 2011)

Besides the climate factors, social-economic aspects can also be related to the higher PhACs concentration. As can be see from Figure 3.2, for water supply systems 1, 3 and 4, higher values of gross domestic product per capita (GDP per capita) and human development index (HDI) are associated to higher PhAC concentration. These factors reflect the consumption capacity of the population and so it is expected that the higher the family income, the greater the health care, which impact directly in PhACs consumption. On the other hand, it was not observed a direct relationsheep between PhAC concentration and WWT coverage. For exemple, although water source 1 presented the lowest WWT coverage, it presented the lowest PhACs concentration, which may be related to the low values of GDP per capita and HDI. In turn, water source 4 presented higher PhACs concentration owing to the high GDP per capita and HDI, despite presenting a intermediate WWT coverage. Water source 2 presented the higher concentration of PhACs. This may be owing to different biological and physical mechanisms of degradation of pollutants that occur in a dam. For example, the low flow velocity of these systems limits the aeration and, consequently, the aerobic processes are impaired and may reduce the compounds biodegradation rate. Photodegradation processes also occur to a lesser extent, owing to the lower surface area and volume relation, which limits the availability of sunlight. Furthermore, PhAC accumulation may be caused by their adsorption on colloidal or suspended materials, since the hydraulic retention time in dams is higher.

Besides the seasonality and the socio-economic conditions, other factors may play important role, such as water body preservation and wastewater treatment system.

3.3 Removal of pharmaceuticals in the DWTP

Enrofloxacin, norfloxacin, metformin, cimetidine, phenylbutazone and omeprazole were not quantified in any of the treated water samples. The mechanisms involved in their removal may have been size retention, biodegradation in the filtration step (especially of the antibiotics that

are more easily degraded) (HUERTA-FONTELA *et al.*, 2011; SIMAZAKI *et al.*, 2015), adsorption and chlorine oxidation (Figure 3.3).

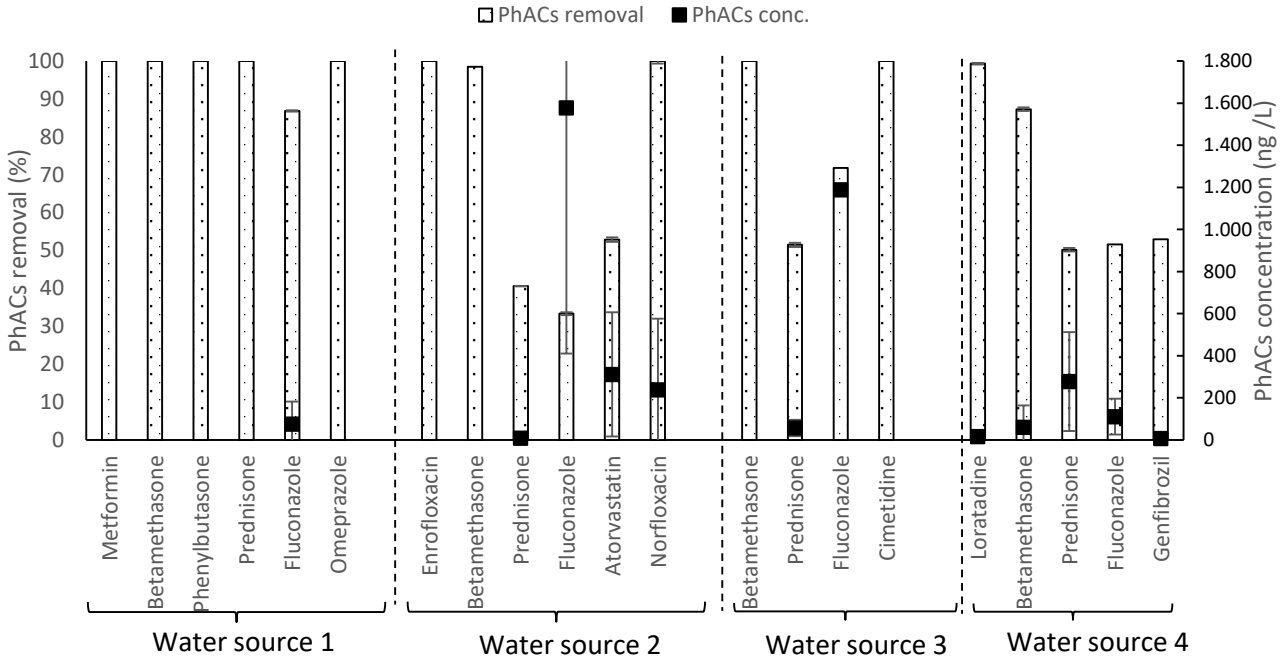

Figure 3.3 - PhACs concentration in treated water and removal efficiency of each evaluated DWTP.

According to Stackelberg *et al.* (2007) the process of clarification (which consists of coagulation, flocculation, sedimentation and filtration) is generally not a primary route by which PhACs in filtered-water samples are degraded or removed, mostly owing to the intrinsic characteristics of the compounds. The low concentration of PhACs in superficial water and the hydropholic behaviour of the PhACs with low log K_{ow} (<3.0) can explain the lower removal efficiencies of fluconazole (log K_{ow}=0.40) and prednisone (log K_{ow}=1.46), since these compounds are not expected to be adsorbed to the particles but to dissociate in the aqueous phase (WANG *et al.*, 2014) and evades the adsorption process. These two PhACs were the most frequent ones in treated water (fluconazole was present in all water sources).

Chlorination is found to be very efficient in some PhACs removal owing to the high reactivity of chlorine with primary and secondary amines (WESTERHOFF *et al.*, 2005; CHAMBERLAIN; ADAMS, 2006). According to Huerta-Fontela *et al.* (2011), the efficiency of chlorination increases for compounds that do not have the imidazole group, since the absence of this group favors the deactivation of the aromatic ring and potentiates the reaction with chlorine. The presence of a bromide instead of chlorine in one of the aromatic rings and the substitution of a benzene ring by a pyridine one blocks the reactivity of this compound through

chlorine attack (KIM *et al.*, 2007). This may explain the higher removal of enrofloxacin, betamethasone and loratadine.

3.4 Environmental and Human health risk assessment

E(L)C50, NOEC, NOAEL and TDI values for each PhAC are presented in Annex 2. The predominant susceptibility order to acute toxicity effects, accounting for 46% of the PhACs, was algae> crustacean> fish, which is in accordance with the results found by Sanderson *et al.* (2003) and Garcia *et al.* (2014). In fact, 62.5% of the drugs for which acute toxicity data were found for fish trophic level were classified as non-toxic, according to GHS. For algae, non-toxic drugs account for only 26% of total data found, while 53% are highly toxic. As for chronic effects, fish are the trophic level most susceptible to 62.5% of the drugs, which may be related to PhACs bioaccumulation tendency. Atorvastatin, for example, has logKow equal to 5.04 (Annex 1) and has one of the lowest NOEC values found. Toxicity indicators reveal the seriousness of PhAcs toxicological potential, since less than 1% was considered non-toxic for all trophic levels and approximately 60% were classified as highly toxic for at least one. The drugs erythromycin, norfloxacin, fenofibrate, loratadine and genfibrozil stand out owing to their high toxicological potential.

The few NOAEL/LOAEL values found make clear the gap regarding toxicological effects of PhACs in literature. Of the 28 drugs selected, there had been found NOAEL values for only seven compounds and LOAEL for only four, which corresponds to less than 30% for both indicators (25% and 14%, respectively). For other six drugs, it was possible to find in literature the TDI values directly (erythromycin, trimethoprim, ibuprofen, atorvastatin, genfibrozil and atenolol), so it was only possible to obtain TDI values for approximately 60% of the selected PhACs.

All the evaluated water sources were subject to toxicological risk, both acute and chronic, owing to at least one PhAC (Table 3.4). Only source 1 is not subject to high toxicological risk. The PhACs related to the highest acute toxicity risks were loratadine in 4 (HQ=124) and norfloxacin in 3 (HQ=3.53). Regarding chronic toxicity, the highest risk was posed by atorvastatin in 2 (HQ=389). In contrast, all these PhACs had low detection frequency, which may be explain by their seasonal consumption pattern or degradability rates. Loratadine and norfloxacin were only detected in winter season, when the consumption of antiallergics is higher and the microbial activity is lower, decreasing the biological degradation of antibiotics. Atorvastatin was only quantified in Northeast region, possibly because despite being an expensive pharmaceutical in

Brazil, one of this region biggest cities government provides it to population. On the other hand, compounds with high detection frequency were related to milder toxicological risks and none of them poses high risk in any of the water sources. Fluconazole and betamethasone posed low risks in all water sources, as summarized by Chen and Ying (2015) for China surface waters and by Vestel *et al*. (2016) for US surface waters. Regarding the human health risk assessment, it was not possible to calculate MOE for three PhACs (omeprazole, metformin and norfloxacin). Among the others, 11 presented MOE values higher than 1000 and six had values higher than 100, indicating a low probability of risk to public health even before the water passes through the treatment system for 94% of the PhACs quantified in the evaluated water sources.

Conventional DWTP were able to promote some reduction in PhACs toxicological risk potential (Table 3.4), however, water source 1 is the only one which treated water is not subject to acute or chronic risk. The others presented toxicological environmental risk owing to at least one PhAC. Prednisone, betamethasone and fluconazole posed low or negligible risks, both for acute and chronic effects. Despite the low detection frequency, atorvastatin highlights for its high toxicity potential. This PhAC posed high chronic risk (HQ=367) and it is also related to one of the highest acute toxicity (HQ=0,183), only lower than loratadine (HQ=0,838) in water source 4. Regarding human health, atorvastatin was the only drug that did not present MOE above 1000 in treated water. As its value was below 100 (MOE=34), this PhAC can pose human health risk. Considering the high hydrophobia of this PhAC (logKow>3), one recommendation to minimize atorvastatin toxicological risk is to add adsorption steps in the water treatment.

Table 3.4 - PhACs environmental and human health risk assessment for raw and treated water from all sources evaluated.

Water source	Raw water						Treated water					
	PhAC	Acute toxicity		Chronic toxicity		Human health	PhAC	Acute toxicity		Chronic toxicity		Human health
		HQ	Classification	HQ	Classification	MOE		HQ	Classification	HQ	Classification	MOE
1	Metformin	0.001	Low risk	-	-	-	Fluconazole	0.002	Negligible risk	0.000	Negligible risk	9934
	Betamethasone	0.022	Low risk	0.001	Negligible risk	2675						
	Phenylbutasone	-	-	0.000	Negligible risk	315789						
	Prednisone	0.004	Negligible risk	-	-	25751						
	Fluconazole	0.006	Negligible risk	0.002	Negligible risk	2613						
	Omeprazole	0.579	Medium risk	0.024	Low risk	-						
2	Enrofloxacin	0.293	Medium risk	0.000	Negligible risk	104466	Betamethasone	0.001	Negligible risk	0.000	Negligible risk	54446
	Betamethasone	0.101	Medium risk	0.003	Negligible risk	581	Prednisone	0.052	Low risk	-	-	2135
	Prednisone	0.065	Low risk	-	-	1687	Fluconazole	0.006	Negligible risk	0.002	Negligible risk	2560
	Fluconazole	0.003	Negligible risk	0.001	Negligible risk	4520	Atorvastatin	0.183	Medium risk	367	High risk	34
	Atorvastatin	0.195	Medium risk	389	High risk	32						
3	Norfloxacin	3.526	High risk	0.838	Medium risk	-	Fluconazole	0.012	Low risk	0.004	Negligible risk	1261
	Betamethasone	0.028	Low risk	0.001	Negligible risk	2112	Prednisone	0.002	Negligible risk	-	-	71429
	Fluconazole	0.042	Low risk	0.014	Low risk	356						
	Prednisone	0.016	Low risk	-	-	371						
4	Cimetidine	0.001	Negligible risk	0.017	Low risk	753	Loratadine	0.838	Medium risk	-	-	53687
	Loratadine	124	High risk	-	-	363	Betamethasone	0.006	Negligible risk	0.000	Negligible risk	10411
	Betamethasone	0.027	Low risk	0.001	Negligible risk	2135	Prednisone	0.010	Low risk	-	-	10497
	Prednisone	0.028	Low risk	-	-	3976	Fluconazole	0.002	Negligible risk	0.001	Negligible risk	7657
	Fluconazole	0.010	Low risk	0.003	Negligible risk	1521	Genfibrozil	0.015	Low risk	0.006	Negligible risk	2100
	Genfibrozil	0.032	Low risk	0.012	Low risk	988						

A significant point concerning PhACs toxicological risk assessment is that, since the pattern of consumption of these compounds varies widely between different regions, depending on several socioeconomic factors (OLIVEIRA *et al.*, 2012; GODOY *et al.*, 2015), HQ and MOE values obtained for a PhAC in a specific region do not necessarily reflect the risks in other regions (CARLSSON *et al.*, 2006). Another important point is the possible contribution of each PhAC to the global risk potential of the complex mixture of compounds found in the environment, even if its individual potential is low (CLEVEURS, 2005). The mixture hazard quotients (MHQ) for each of the evaluated water sources are shown in Figure 3.4. All evaluated sources are subjected to both acute and chronic significant risks. Water sources 2 and 4 presented the greatest risks and water source 1 the milder ones.

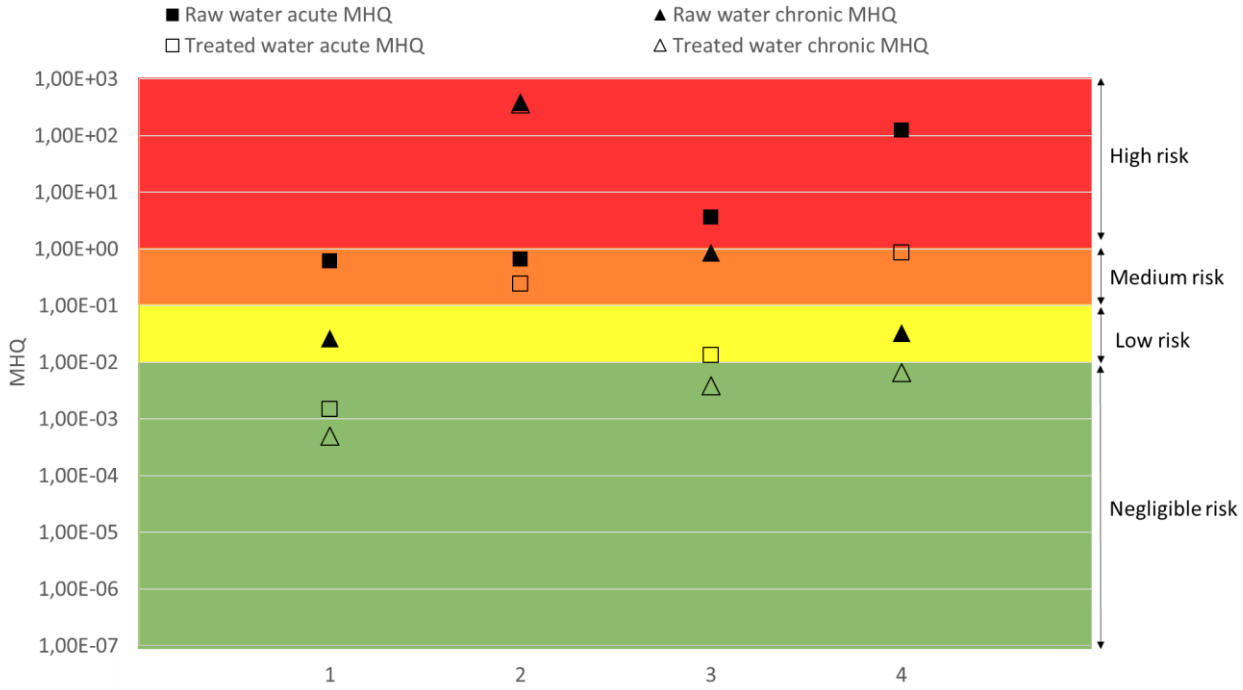

Figure 3.4 - Raw and treated water mixture hazard quotient (MHQ) of all evaluated water sources

As for the PhACs occurrence, the toxicological risk is also subject to seasonality. In water source 3 it is possible to observe peaks of both acute and chronic risk in winter, following the highest PhACs concentrations observed in this season (Figure 3.5). In water source 1, the highest MHQ is observed in autumn. Winter temperatures are milder in this region, which may have caused the highest peaks in autumn season, when temperatures begin to decrease and rainfall decreases considerably, increasing PhACs consumption and reducing the dilution factor. In both sources, the milder risks occur in summer months, as expected.

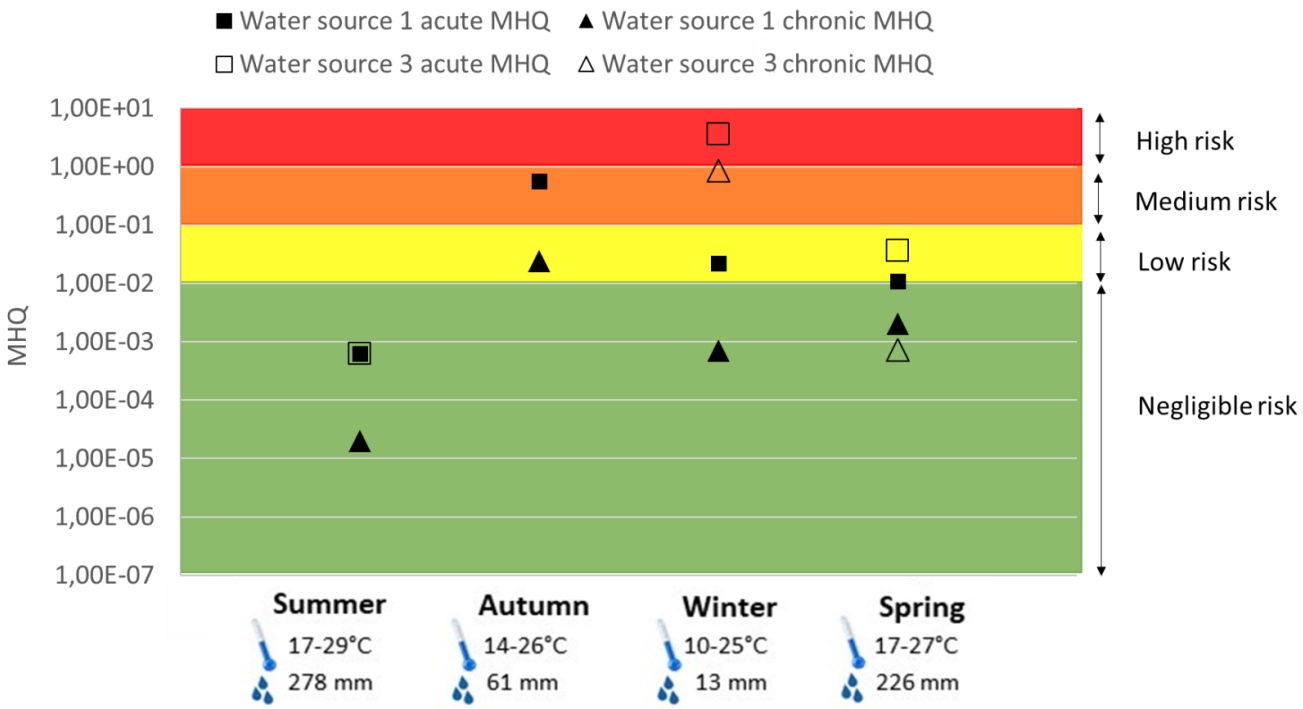

Figure 3.5 - PhACs mixture toxicity in different seasons in water source 1 and 3.

4 CONCLUSION

PhAC contamination is a reality in Brazilian natural waters as trace levels of pharmaceuticals were detected in superficial and drinking water in all assessed water sources. PhACs presence and concentration are subject to seasonality and to regional socio-economic aspects.

The toxicity potential confirms the concern regarding these compounds, since only one of the evaluated PhACs was non-toxic to any trophic level and approximately 60% were highly toxic to at least one level. Both raw and treated water from the four evaluated water sources were subject to toxicological environmental risk at some level owing to at least one drug. In treated water, atorvastatin posed a significant human health risk; therefore, requiring special attention. Toxicological risk is also susceptible to seasonality and mixed PhAC toxicity is higher than that of individual compounds. Since the removal and risk reduction of PhACs using conventional DWTPs are only partial, the application of more efficient technologies must be considered.

Therefore, the results reported here are important as they provide comparative insight about PhAC concentration and risk assessment in water supply systems around Brazil. Besides, owing to the possibility of increased consumption of pharmaceuticals in the future, it is also important to highlight the importance of continual PhACs monitoring, to observe any increase in their concentration, which could pose even higher risks to aquatic environmental and public health.

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ANNEX

Annex 1 - Selected PhACs and their physicochemical properties

PhACs	Structure	Molecular weight (g/mol)	Molar volume (cm^3)	log Kow	pKa	KH ($atm.m^3/mol$)	Vapor pressure (mmHg)
Amoxicillin	$C_{16}H_{19}N_3O_5S$	365	236	0.87	3.23	1.88E-11	1.43E-08
Ampicillin	$C_{16}H_{19}N_3O_4S$	349	239	1.35	3.24	1.52E-11	5.36E-11
Clarithromycin	$C_{38}H_{69}NO_{13}$	748	632	1.70	8.99	1.01E-10	2.12E-11
Danofloxacin	$C_{19}H_{20}FN_3O_3$	357	241	0.51	4.12	1.53E-09	1.53E-09
Enoxacin	$C_{15}H_{17}FN_4O_3$	320	231	-0.23	5.50	7.63E-12	4.89E-10
Enrofloxacin	$C_{19}H_{22}FN_3O_3$	359	259	0.80	5.15	7.18E-09	3.83E-08
Erythromycin	$C_{37}H_{67}NO_{13}$	734	607	3.06	8.90	1.28E-11	1.08E-10
Norfloxacin	$C_{16}H_{18}FN_3O_3$	320	237	-0.30	5.77	1.00E-11	8.88E-10
Trimethoprim	$C_{14}H_{18}N_4O_3$	290	232	0.981	6.6	9.94E-08	5.69E-09
Scopolamine	$C_{17}H_{21}NO_4$	303	231	0.98	7.75	4.86E-10	2.12E-08
Paroxetine	$C_{19}H_{20}FNO_3$	329	272	3.60	9.77	4.64E-07	1.66E-05
Metformin	$C_4H_{11}N_5$	130	101	-1.37	12.40	3.46E-09	4.42E-01
Cimetidine	$C_{10}H_{16}N_6S$	252	198	0.40	6.80	6.43E-10	3.95E-07
Loratadine	$C_{22}H_{23}ClN_2O_2$	383	304	5.20	4.33	1.60E-08	5.34E-09
Ranitidine	$C_{13}H_{22}N_4O_3S$	314	265	0.27	8.08	7.29E-09	2.65E-08
Betamethasone	$C_{22}H_{29}FO_5$	392	296	3.38	13.40	7.36E-11	3.49E-10
Ketoprofen	$C_{16}H_{14}O_3$	254	212	3.12	4.45	1.45E-09	1.58E-08
Phenazone	$C_{11}H_{12}N_2O$	188	163	0.38	1.40	2.66E-06	9.09E-04
Phenylbutazone	$C_{19}H_{20}N_2O_2$	308	263	3.16	4.50	6.22E-08	6.79E-06
Ibuprofen	$C_{13}H_{18}O_2$	206	200	3.97	4.91	7.83E-08	1.33E-04
Prednisone	$C_{21}H_{26}O_5$	358	274	1.46	12.58	1.24E-09	7.40E-10
Fluconazole	$C_{13}H_{12}F_2N_6O$	306	205	0.40	11.01	7.12E-09	1.02E-06
Omeprazole	$C_{17}H_{19}N_3O_3S$	345	252	2.23	9.29	3.62E-06	6.64E-08
Caffeine	$C_8H_{10}N_4O_2$	194	133	-0.07	10.40	1.59E-06	1.22E-06
Atorvastatin	$C_{33}H_{35}FN_2O_5$	558	452	5.04	4.33	1.08E-11	1.67E-10
Fenofibrate	$C_{20}H_{21}ClO_4$	361	306	5.28	-4.90	4.11E-09	1.93E-07
Gemfibrozil	$C_{15}H_{22}O_3$	250	240	4.28	4.42	8.62E-09	2.93E-06
Atenolol	$C_{14}H_{22}N_2O_3$	266	237	−0.03	N.A.	4.35E-10	7.25E-09

logKow: octanol–water partition coefficient; KH: Henry law constant; pka: acidity constant.
Source: U.S. EPA (2017); DRUGBANK.

Annex 2

Annex 2.1 - Selected PhACs E(L)C50 values.

PhACs	Taxon	Specie	E(L)C50	Value (mg/L)	Reference
Amoxicillin	Algae	*Microcystis aeruginosa*	EC50 (Chlorophyll concentration - 7 d)	0.0037	LUTZHOFT *et al.*, 1999
	Crustacean	*Daphnia magna*	EC50 (Immobilization - 48 h)	1000	PARK; CHOI, 2008
	Fish	*Danio rerio*	EC50 (Mortality- 48 h)	132.4	OLIVEIRA *et al.*, 2013
Ampicillin	Algae	*Microcystis aeruginosa*	EC50 (Growth inhibition - 96 h)	0.012	QIN *et al.*, 2012
	Crustacean	*Daphnia magna*	EC50 (Immobilization - 48 h)	1000	PARK; CHOI, 2008
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	1000	PARK; CHOI, 2008
Clarithromycin	Algae	*Pseudokirchneriella subcapitata*	EC50 (Growth inhibition – 72 h)	0.002	ISIDORI *et al.*, 2005
	Crustaceo	*Ceriodaphnia dubia*	EC50 (Growth inhibition - 48 h)	8.16	ISIDORI *et al.*, 2005
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	KIM *et al.*, 2009
Enrofloxacin	Algae	*Microcystis aeruginosa*	EC50 (Chlorophyll concentration - 5 d)	0.049	ROBINSON *et al.*, 2005
	Crustacean	*Litopenaeus vannamei*	EC50 (Immobilization - 48 h)	14.3	WILLIANS *et al.*, 1992
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	PARK; CHOI, 2008
Erythromycin	Algae	*Anabaena sp.*	EC50 (Population growth rate - 72 h)	0.022	GONZALEZ *et al.*, 2013
	Crustacean	*Ceriodaphnia dubia*	EC50 (Abundance - 48 h)	0.22	ISIDORI *et al.*, 2005
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	KIM *et al.*, 2009
Norfloxacin	Algae	*Microcystis wesenbergii*	EC50 (Abundance - 6 d)	0.038	ANDO *et al.*, 2007
	Crustaceo	*Daphnia magna*	EC50 (Food behavior - 48 h)	0.88	LU *et al.*, 2013
Trimethoprim	Algae	*Microcystis aeruginosa*	EC50 (Photosynthesis - 24 h)	6.9	van der GRITEN *et al.*, 2010
	Crustaceo	*Daphnia magna*	EC50 (Immobilization - 48 h)	92	PARK; CHOI, 2008
	Fish	*Oryzias latipes*	LC50 (Mortality- 48 h)	100	KIM *et al.*, 2009
Paroxetine	Crustacean	*Ceriodaphnia dubia*	LC50 (Mortality- 48 h)	0.58	HENRY *et al.*, 2004
Metformin	Algae	*Desmodesmus subspicatus*	EC50 (Population growth rate - 72 h)	320	CLEUVERS, 2004
	Crustacean	*Daphnia magna*	EC50 (Immobilization - 48 h)	64	CLEUVERS, 2004
Cimetidine	Crustacean	*Daphnia magna*	EC50 (Immobilization - 96 h)	271.3	KIM *et al.*, 2009
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	KIM *et al.*, 2009
Loratadine	Algae	-	EC50 (ECOSAR)	0.05	SANDERSON *et al.*, 2004

	Crustacean	-	EC50 (ECOSAR)	0.14	
	Fish	-	EC50 (ECOSAR)	0.02	
Ranitidine	Crustaceo	*Ceriodaphnia dubia*	EC50 (Population growth rate - 7 d)	1.5	ISIDORI *et al.*, 2009
	Algae	-	EC50 (ECOSAR)	41	
Betamethasone	Crustacean	-	EC50 (ECOSAR)	32	SANDERSON *et al.*, 2004
	Fish	-	EC50 (ECOSAR)	37	
	Algae	*Desmodesmus subspicatus*	EC50 (Growth inhibition - 72 h)	315	CLEUVERS, 2004
Ibuprofen	Crustaceo	*Daphnia magna*	LC50 (Mortality- 48 h)	0.032	BRUN *et al.*, 2006
	Fish	*Oryzias latipes*	LC50 (Mortality – 96 h)	100	KIM *et al.*, 2009
Prednisone	Crustacean	*Brachionus calyciflorus*	LC50 (Mortality- 24 h)	54.6	DELLAGRECA *et al.*, 2002
Fluconazole	Crustacean	*Thamnocephalus platyurus*	LC50 (Immobilization - 24 h)	100	KIM *et al.*, 2009
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	KIM *et al.*, 2009
Omeprazole	Fish	*Danio rerio*	LC50 (Mortality - 5 d)	0.021	SELDERSLAGHS *et al.*, 2012
	Algae	*Pseudokirchneriella subcapitata*	EC50 (Population growth rate - 72 h)	150	ZARRELLI *et al.*, 2014
Caffeine	Crustacean	*Daphnia magna*	EC50 (Food behavior - 5 h)	0.44	LU *et al.*, 2013
	Fish	*Pimephales promelas*	EC50 (Growth - 5 d)	70	YOUNG *et al.*, 1996
Atorvastatin	Crustacean	*Amphibalanus amphitrite*	LC50 (Mortality- 96 h)	2.6	AL-AIDAROOS *et al.*, 2017
	Algae	-	EC50 (ECOSAR)	0.1	SANDERSON *et al.*, 2003
Fenofibrate	Crustacean	*Ceriodaphnia dubia*	EC50 (Growth inhibition - 7 d)	0.76	ISIDORI *et al.*, 2007
	Fish	*Poeciliopsis lucida*	EC50 (Cytotoxicity - 24 h)	3.25	LAVILLE *et al.*, 2004
	Algae	*Anabaena sp.*	EC50 (Physiology - 24 h)	4.42	ROSAL *et al.*, 2010
Gemfibrozil	Crustacean	*Ceriodaphnia dubia*	EC50 (Population growth rate - 7 d)	0.53	ISIDORI *et al.*, 2007
	Fish	*Danio rerio*	LC50 (Mortality- 96 h)	0.85	KALASEKAR *et al.*, 2015
	Algae	*Desmodesmus subspicatus*	EC50 (Growth inhibition – 72 h)	620	CLEUVERS, 2005
Atenolol	Crustacean	*Ceriodaphnia dubia*	EC50 (Immobilization – 48 h)	33.4	FRAYSSE; GARRIC, 2005
	Fish	*Oryzias latipes*	LC50 (Mortality- 96 h)	100	KIM *et al.*, 2009

Annex 2.2 - Selected PhACs NOEC values.

PhACs	Taxon	Specie	NOEC	Value (mg/L)	Reference
Amoxicillin	Algae	*Isochrysis galbana*	NOEC (Abundance - 4 d)	250	ORTE *et al.*, 2013
	Fish	*Danio rerio*	NOEC (Enzymatic catalysis - 4 d)	25	OLIVEIRA *et al.*, 2013
Ampicillin	Algae	*Microcystis aeruginosa*	NOEC (Enzymatic catalysis - 4 d)	0.010	QIN *et al.*, 2012
Clarithromycin	Crustacean	*Daphnia magna*	NOEC (Reproduction)	2.1	ISIDORI *et al.*, 2005
Enrofloxacin	Crustacean	*Daphnia magna*	NOEC (Reproduction - 21 d)	5	PARK; CHOI, 2008
	Fish	*Pimephales promelas*	NOEC (Mortality- 7 d)	10	ROBINSON *et al.*, 2005
Erythromycin	Algae	*Synechococcus leopoliensis*	NOEC (Abundance - 6 d)	0.002	ANDO *et al.*, 2007
	Crustacean	*Litopenaeus vannamei*	NOEC (Immobilization - 2 d)	4.9	WILLIANS *et al.*, 1992
	Fish	*Oryzias latipes*	NOEC (Mortality- 100 d)	100	JI *et al.*, 2012
Norfloxacin	Algae	*Microcystis aeruginosa*	NOEC (Abundance - 6 d)	0.0016	ANDO *et al.*, 2007
	Crustacean	*Daphnia magna*	NOEC (Length - 21 d)	0.12	LU *et al.*, 2013
	Fish	*Carassius auratus*	NOEC (Enzymatic activity - 7 d)	0.0027	LIU *et al.*, 2014
Trimethoprim	Algae	*Anabaena variabilis*	NOEC (Abundance - 6 d)	3.1	ANDO *et al.*, 2007
	Crustacean	*Daphnia magna*	NOEC (Reproduction - 21 d)	3.12	LIGUORO *et al.*, 2012
	Fish	*Danio rerio*	NOEC (Morphology - 21 d)	0.157	MADUREIRA *et al.*, 2012
Cimetidine	Fish	*Moina macrocopa*	NOEC (Reproduction - 7 d)	0.07	HOPPE *et al.*, 2012
Ranitidine	Crustacean	*Ceriodaphnia dubia*	NOEC (Population growth rate - 7 d)	0.31	ISIDORI *et al.*, 2009
	Fish	*Danio rerio*	NOEC (DNA concentration - 5 d)	0.0002455	ROCCO *et al.*, 2010
Betamethasone	Fish	*Oryzias latipes*	NOEC	10	VESTEL *et al.*, 2017
Phenylbutazone	Algae	*Scenedesmus subspicatus*	NOEC (Photosynthesis - 1 h)	250	NENDZA; WENZEL, 2006

Ibuprofen	Algae	*Pseudokirchneriella subcapitata*	NOEC (Abundance - 3 d)	0.01	BRUN *et al.*, 2006
	Crustacean	*Daphnia magna*	NOEC (Reproduction - 21 d)	1.23	ERICSON *et al.*, 2010
	Fish	*Danio rerio*	NOEC (Growth - 7 d)	0.001	DAVID; PANCHARATNA, 2009
Fluconazole	Algae	*Pseudokirchneriella subcapitata*	NOEC (Growth inhibition - 72 h)	3.06	CHEN *et al.*, 2014
Omeprazole	Fish	*Danio rerio*	NOEC (Behaviour - 5 d)	0.0050	SELDERSLAGHS *et al.*, 2012
Caffeine	Algae	*Cyanophycota*	NOEC (Abundance - 56 d)	0.005	LAWRENCE *et al.*, 2012
	Crustacean	*Carcinus maenas*	NOEC (Physiology - 28 d)	0.005	AGUIRRE-MARTINEZ *et al.*, 2013
	Fish	*Salmo salar*	NOEC (Growth - 5 d)	0.00001	LOWER, 2008
Atorvastatin	Crustacean	*Daphnia magna*	NOEC (Enzymatic activity - 3 d)	0.001	RICHARDS *et al.*, 2008
	Fish	*Danio rerio*	NOEC (DNA concentration - 14 d)	0.000013	ROCCO *et al.*, 2012
Fenofibrate	Algae	*Pseudokirchneriella subcapitata*	NOEC (Population growth rate - 3 d)	3.12	ISIDORI *et al.*, 2007
	Crustacean	*Ceriodaphnia dubia*	NOEC (Growth inhibition - 7 d)	0.039	ISIDORI *et al.*, 2007
	Fish	*Pimephales promelas*	NOEC (Morphology - 7 d)	0.025	NALLANI, 2010
Gemfibrozil	Algae	*Pseudokirchneriella subcapitata*	NOEC (Population growth rate - 3 d)	3.125	ISIDORI *et al.*, 2007
	Crustacean	*Ceriodaphnia dubia*	NOEC (Population growth rate - 7 d)	0.078	ISIDORI *et al.*, 2007
	Fish	*Pimephales promelas*	NOEC (Genetics - 2 d)	0.014	SKOLNESS *et al.*, 2012
Atenolol	Algae	*Microcystis aeruginosa*	NOEC (Chlorophyll concentration - 4 d)	0.02	CEBALLOS-LAITA *et al.*, 2015
	Crustacean	*Daphnia magna*	NOEC (Reproduction - 21 d)	3.2	KUSTER *et al.*, 2010
	Fish	*Pimephales promelas*	NOEC (Growth - 21 d)	1	WINTER *et al.*, 2008

Annex 2.3 - Selected PhACs NOAEL, LOAEL and TDI values.

PhACs	NOAEL (mg/kg.d)	LOAEL (mg/kg.d)	TDI (mg/kg.d)	Reference
Ampicilline	40	-	0.400	SHARMA *et al.*, 2013
Clarithromycin	-	7,14	0.007	WEBB *et al.*, 2003
Enrofloxacin	5	-	0.050	BARSKI *et al.*, 2011
Erythromycin	-	-	0.040	BROOKS; HUGGETT, 2012
Trimethoprim	-	-	0.100	SNYDER, 2008
Cimetidine	-	2,9	0.003	SCHWAB *et al.*, 2005
Loratadine	3	-	0.030	EMEA, 2004
Betamethasone	6,25	-	0.063	NISHIMURA *et al.*, 1986
Phenylbutazone	140	-	1.400	MIYAGAWA *et al.*, 1995
Ibuprofen	-	-	0.110	BROOKS; HUGGETT, 2012
Prednisone	20	-	0.200	KAVLOCK *et al.*, 1987
Fluconazole	5	-	0.050	PFIZER, 2016
Caffeine	-	20	0.020	ROSSOWSKA *et al.*, 1995
Atorvastatin	-	-	0.001	SNYDER, 2008
Fenofibrate	-	1,43	0.001	WEBB *et al.*, 2003
Gemfibrozil	-	-	0.001	SNYDER, 2008
Atenolol	-	-	0.003	SNYDER, 2008

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4th Chapter

PhACs toxicological risk reduction by membrane separation processes

1 INTRODUCTION

Pharmaceutically active compounds (PhACs) have been detected in concentrations from ng/L to μg/L in surface and ground water, and recognized as potential environment threats (PETRIE *et al*., 2015; TAHERAN *et al*., 2016; CAMACHO-MUNOZ *et al*., 2014). Besides, it has been reported that some PhACs show to be persistent throughout drinking water treatment plants (DWTP) processes, mostly owing to PhACs small size and polarity, which makes them highly soluble in water, very mobile in the environment and difficult to remove by conventional treatment (VERLIEFDE *et al.,* 2009; GABARRON *et al*., 2016).

In face of the limitations associated to conventional treatment processes, the need to achieve PhACs removal have led to explore alternative technologies, among them can be included membrane separation processes (MSP) (NGUYEN *et al*., 2013; SADMANI *et al*., 2014; GARCIA-IVARS *et al,* 2017; PARK *et al*., 2017). MSP such as membrane distillation (MD), reverse osmosis (RO) and nanofiltration (NF) applied at pilot and full-scale installations are being successfully adopted either as a single process or as a combination of different membrane techniques in domestic or industrial wastewater reclamation in order to achieve a high quality permeate by efficiently removing a large spectrum of pollutants, microorganisms, salts, organic micropollutants, proteins, sugars or inorganic ions.

Nanofiltration (NF) and reverse osmosis (RO) processes have been demonstrating promising results on PhACs and other emerging micropollutants rejection (YANGALI-QUINTANILLA *et al*., 2010; SADMANI *et al*., 2014). Despite not promoting the complete removal of ions, NF presents a greater permeate flux and is able to work at lower pressures. It is expected to show effective organic pollutants removal (BRUGGEN *et al*., 2008), including PhACs, since the majority of the PhACs have a molecular weight within 150–500 Da and the molecular weight cut-off (MWCO) for most commercial NF membranes ranges from about 100 to 2000 Da (WANG *et al*., 2014). RO membranes differ from NF membranes mainly in molecular weight cut-off and the thickness of the selective layer. Accordingly, RO membranes usually have a higher desalting ability than NF membranes, but a lower water permeability (GEISE *et al*., 2014). Conventionally, RO membranes have been used for desalination. A number of studies have been conducted to compare RO and NF performances in rejecting different PhACs. Generally, RO membranes perform better than loose NF membranes, especially in the rejection of non-charged and low molecular weight PhACs (DOEDERER *et al*., 2014).

Studies point that steric hindrance effects are the predominant phenomenon in PhACs rejection of these membranes. The electrostatic effect is also significant in charged pharmaceutical compounds rejection, justifying the high rejection of negatively charged PhACs by loose nanofiltration membranes (KONG *et al*., 2015). In addition, PhACs can physically and/or chemically interact with the membrane material, leading to their adsorption onto the membrane and potentially impacting their rejection (ZHAO *et al*., 2017).

Membrane distillation (MD) is a low temperature distillation process that operates transporting water in vapour phase through a microporous and hydrophobic membrane to the distillate side. This process has a theoretical 100% retention of non-volatile components. Owing to the temperature difference between the feed and distillate side, only the most volatile compound (typically water) vaporizes passing through the pore openings at the feed-membrane interface, and then condenses at the distillate-membrane interface. Direct contact membrane distillation (DCMD) is the most widely studied MD system configuration owing to its simple operation (CURCIO; DRIOLI, 2005). MD is less susceptible to membrane incrustation than pressure driven membrane processes, since the later are subject to hydraulic pressure (ALKHUDHIRI *et al*., 2013). Even when a fouling layer forms on the membrane surface, it is expected to be less compact and can be easily removed (ALKHUDHIRI *et al*., 2013). Wijekoon *et al.* (2014) studied the application of MD for removing PhACs during water and wastewater treatment. Results suggested that rejection and fate of the PhACs during MD are governed by their volatility and hydrophobicity. All PhACs with pK_H>9 were completely removed.

Many studies (NGUYEN *et al*., 2013; SADMANI *et al*., 2014; PARK *et al*., 2017; GARCIA-IVARS *et al*., 2017) have evaluated and compared the application of NF and RO. However, only a few studies (HAN *et al*., 2017; ALKHUDHIRI *et al.,* 2013) have focused on the application of MD in removing PhACs from water and wastewater. Besides, most of the studies have been carried out using synthetic or spiked solutions. Therefore, efforts are still needed focusing on the application of MSP to real waters, dealing with real concentration (in order of ng/L to μg/L) and their complex matrices. This allows the improvement of the treatment efficiency, by reducing membrane fouling and energy requirements, as well as understanding the rejection mechanisms and the interactions between the membrane and the PhACs. Therefore, the aim of this study was to compare NF, RO and MD when applied to PhACs removal from a real water matrix in terms of technical, economical and risk assessment.

2 MATERIALS AND METHODS

2.1 Study area and sample collection

The present study was conduct with water sample from Doce river, located at Governador Valadares in Minas Gerais, Brazil. The collecting point was the same one used to supply the Governador Valadares city DWTP (geographic coordinates 18º51'47.83" – latitude and 41º56'47.02" - longitude). The water sample was collected in November 2016, according to the technical specification requirements for monitoring of surface water and wastewater of the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Besides this collection, Doce river's water was monitored for a one-year period, and its main characteristics are shown on Table 4.1. During this monitoring, five pharmaceutical compounds have been quantified in different samples (N=5): betamethasone (295±165 ng/L; quantification frequency (QF)=3), fluconazole (356±266 ng/L; QF=3), phenylbutazone (132 ng/L; QF=1), prednisone (233 ng/L; QF=1), and metformin (36 ng/L; QF=1).

Table 4.1 - Doce river's water main characteristics.

Parameter	Average ± SD	Parameter	Average ± SD
pH	7.09 ± 0.03	Total Coliforms (NMP/100mL[1b])	>2419.2
Conductivity (µS/cm)	127.98 ± 27.96	E. Coli (NMP/100mL[1b])	>5700
Turbidity (NTU)	22.56 ± 19.15	Ca (mg/L)	4.30 ± 0.90
Apparent color (mg Pt-Co/L)	131.40 ± 53.12	Mg (mg/L)	1.59 ± 0.44
Real color (mg Pt-Co/L)	41.20 ± 39.14	Na (mg/L)	2.93 ± 0.96
TS (mg/L)	98.00 ± 60.45	K (mg/kg)	2.45 ± 0.41
TSS (mg/L)	20.40 ± 6.54	Fe (mg/kg)	0.63 ± 0.53
TOC (mg/L)	1.59 ± 0.82	Al (mg/kg)	0.37 ± 0.30
TN (mg/L)	0.78 ± 0.24	As (ppb)	5.20 ± 2.59
Alkalinity (mg CaCO3/L)	17.42 ± 10.11	Pb (ppb)	3.40 ± 1.67
NH_4^+ (mg/L)	<1.25	Si (mg/kg)	6.64 ± 1.77

2.2 Water quality parameters

Colour (2120 C), COD (5220 D) and TSS (2540 B E) were analysed in accordance with the recommendations of Standard Methods for the Examination of Water and Wastewater (APHA, 2012). pH was measured according to the method 4500 H B a digital calibrated pH-meter. TOC was analysed using TOC Shimadzu TOC-V CNP. Conductivity was determined following the method 2510 B with a calibrated conductivity meter (Condutivímetro Hach 44600). The concentration of Cl^-, SO_4^{2-}, PO_4^{3-}, F^-, NO_3^-, NO_2^- was measured by ion chromatography (ICS-1000 ion chromatograph equipped with the Dionex AS-22 column and ICS 12a). The

concentrations of metals K^+, Mg^{2+}, Ca^{2+} and Na^+ were quantified by atomic absorption spectrometry (Atomic Absorption Spectrophotometer - GBC - AVANTA).

2.3 Selected compounds, sample preparation and instrumental analysis

A total of 28 PhACs (Annex 1 – 3rd Chapter) were selected based on the list of pharmaceuticals distributed free of charge by the Brazilian health system (SUS) in order to represent the Brazilian consumption pattern as well as the various classes of micropollutants. The analytical standards of the selected PhACs were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-grade formic acid and solvents were purchased from Dikma (USA). Ultrapure water (18.2 MΩcm−1) was produced by a Milli-Q unit (Millipore, USA).

PhACs were analysed using HPLC (DGU/20A3 Prominence, Shimadzu, Japan) coupled to micrOTOF-QII mass spectrometer (Bruker) with an electrospray ionization source (ESI) in positive mode. The detection limit for each PhAC was around 8 ng/L. The uncertainty of estimate was of 1% according to a validation method of the analysis protocol. Recoveries were between 86 and 100% but were compensated by the calibration, which is processed the same way as the samples. Water samples were previously filtered in 0.45 μm hydrophilic PVDF filter. Analytes were isolated from water samples (1 L) in two steps, firstly without pH adjustment (pH 7) and then with pH adjustment to 2 by adding 0.002 mol/L H_2SO_4 solution, using a polymeric C18/18% cartridge (500 mg/6 mL – Applied Separations) preconditioned with 5 mL of methanol and 5 mL of ultrapure water, and then eluted with methanol using Aspec Gilson GX-271 Liquid Handler. Separation was achieved on a Shim-pack XR-ODS C18 column (2.0 mm; 50 mm and 2.0 μm; Shimadzu, Japan) with the mixture of 0.1% of formic acid water and methanol as the mobile phase. The flow rate and injection volume were 0.1 mL/min and 10 μL, respectively. The mobile phase gradient followed an isocratic method using 95% of methanol for 15 minutes.

2.4 Experimental set-up

Nanofiltration test was carried out with DK nanofiltration membrane and reverse osmosis test was carried out with BW30 membrane. Membranes characteristics are shown on Table 4.2.

Table 4.2 - NF and RO membranes characteristics.

Product	Manufacturer specification						Properties		
	Manufacturer	Membrane chemistry	MWCO (Da)	Salt rejection	Maximum temperature (°C)	Pore size (ºA)	Surface roughness (Ra, nm)	Zeta-potential (mV)	Contact angle (°)
BW30	DOW/Filmtec	Polyamide RO	N/A	99.5% NaCl[b]	45	N/A	68.3[e]	-10.1 (pH = 9.0)[e] -28 (pH = 6.5)[i]	76 ± 7[k] 80[h],
DK	GE Osmonics	Piperazine NF	150 - 300	98% $MgSO_4$[c]	50	0.76[e]	16.4[e]	-18.5 (pH = 9)[e]	40.6 ± 5.2[e]

N/A: not available; MWCO: molecular weight cut-off; test conditions specified by the respective manufacturers: [a]500 ppm NaCl, 25°C, 15% recovery at 10 bar; [b]2,000 ppm NaCl, 25°C, 15% recovery at 15.5 bar; [c]2,000 ppm $MgSO_4$, 25°C, 15% recovery at 7.6 bar; [d]2,000 ppm $MgSO_4$, 25°C, 15% recovery at 4.8 bar; [e]Tang *et al*., 2009; [f]Xu *et al*., 2010; [g]Gryta *et al*., 2012; [h]Yin *et al*., 2017; [i]Widjaya *et al*., 2012; [j]Kaya *et al*., 2006; [k]Pontié *et al*., 2008.

Figure 4.1 shows the schematic of the laboratory-scale NF/RO system. The NF/RO unit had a maximum operating pressure of 20 bar, which was provided by a rotary vane pump equipped with a speed controller and maximum flow of 530 L/h. A needle-type valve was used to adjust the feed flow rate and the trans-membrane pressure (TMP). The pressure was measured by a manometer. NF and RO were conducted in a stainless-steel membrane cell with 9 cm diameter and filtration area of 63.6 cm^2. The flat-sheet commercial membranes were properly cut to fit the membrane cell and a feed spacer of 28 mils (25.4 µm) was placed over the membrane to promote flow distribution. The feed temperature was maintained at 20±5ºC by an immersed coil.

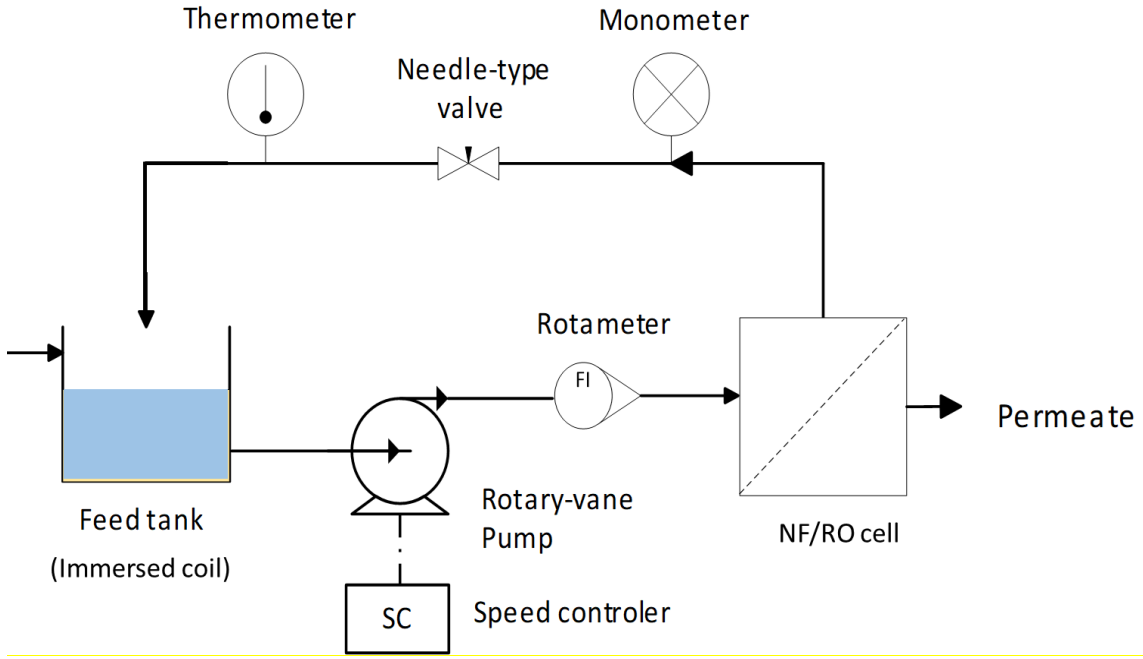


Figure 4.1 - Schematic draw of the NF/RO bench scale unit.

MD tests were conducted using a flat hydrophobic microporous polytetrafloroethylene (PTFE) membrane (Sterlitech). According to the manufacturer, the average pore size and porosity of the MD membrane were 0.22 µm and 70%, respectively. The membrane cell was made of acrylic and a flow channel was engraved in each of the two acrylic blocks that make up the feed and permeate semi cells. The feed solution was circulated from a glass reservoir to the membrane cell and then returned back to the feed reservoir (Figure 4.2). Feed temperature was maintained by a hot plate. The temperature of the distillate was regulated using a chiller (AquaCooler, Australia) equipped with a stainless-steel heat exchanging coil immersed directly in the distillate reservoir. The distillate reservoir was placed directly on an analytical balance (Mettler Toledo, Switzerland) and flux was calculated by the mass increase observed over time.

At the end of each experiment, the solution volume was measured again and the total volume loss was found to be less than 15%.

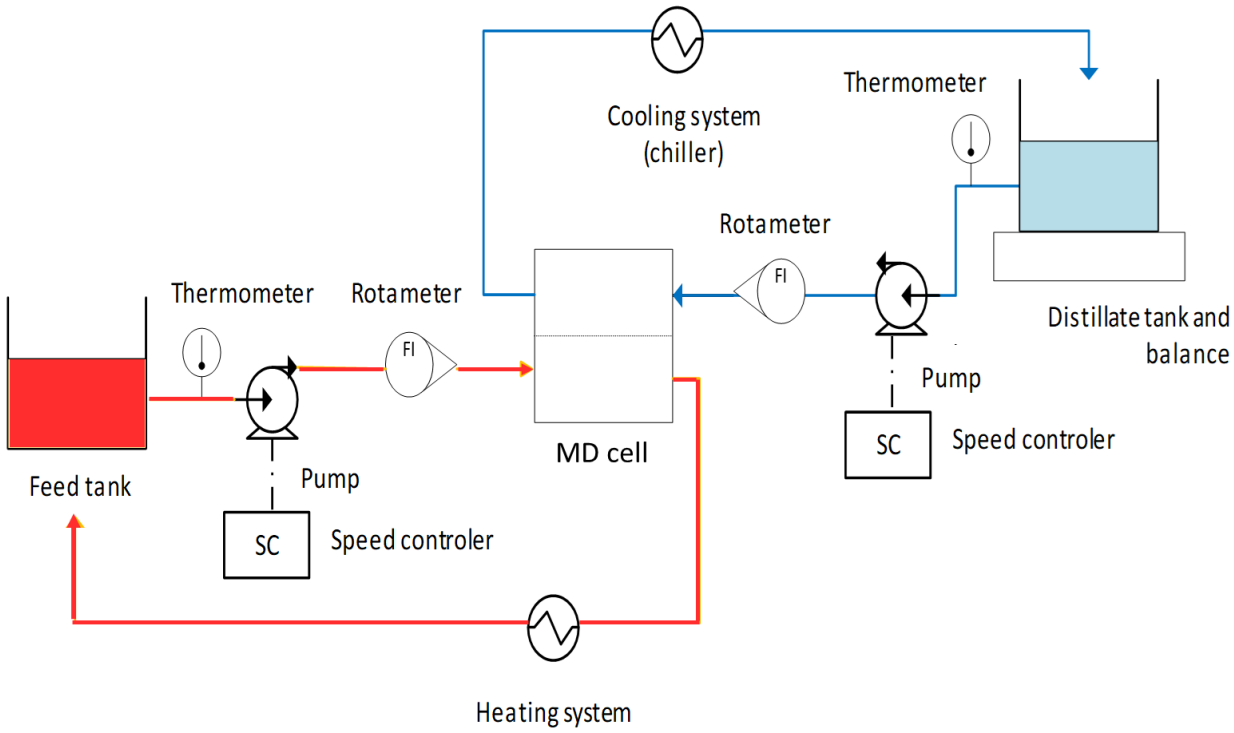


Figure 4.2 - Schematic draw of the MD bench scale unit.

2.5 Experimental procedure

In NF and RO tests, the following procedure was adopted: (i) de-ionized water filtration under three different TMP (10, 8 and 6 bar, values already consolidated in the research group) until a constant flux was obtained to each pressure; (ii) water sample filtration under 10 bar, with concentrated flow rate of 3.2 L min^{-1} and 25°C up to 70% of recovery rate; (iii) washing the fouled membrane module with flowing de-ionized water for 2 minutes with concentrated flow rate of 1.2 L min^{-1} to remove the foulants that loosely deposited on the membrane surface; (iv) de-ionized water filtration under 10 bar for 20 minutes; (v) membrane chemical cleaning (acid citric 2% followed by NaOH 0.4% m/m, as already consolidated in the research group); (vi) de-ionized water filtration under three different TMP (10, 8 and 6 bar) until a constant flux was obtained to each pressure. The flow rate was measured at every 10 minutes throughout the test and permeate samples were collected at every 500 mL for PhACs analysis.

In MD experiments, the feed and distillate temperatures were 60 and 25 °C, respectively, and the cross-flow velocity of the feed and distillate circulation was 11.4 cm/s. The initial feed volume was 2 L and 1L of Milli-Q water was used as the initial distillate. The experiment was

concluded once the water recovery had reached 70%, at which stage the feed and distillate samples were collected for PhACs analysis. PhACs concentration in the distillate was corrected for dilution by taking into account the initial volume of Milli-Q water in the distillate. The duration of each MD experiment was approximately 13 h. After the test, filtration with de-ionized water under the same experimental condition was conducted until a constant flux was obtained.

2.6 Environmental and Human health risk assessment

PhACs potential environmental risks were evaluated based on hazard quotients (HQ). HQ values were calculated for acute and chronic effects dividing measured environmental concentration (MEC) by predicted no effect concentration (PNEC), which was determined dividing the mean effect or lethal concentration (EC50 or LC50) and the non-observed effect concentration (NOEC) by safety factors, whose typical values reported in literature are 1000 and 10, respectively (WORLD HEALTH ORGANIZATION, 2011) (Eqs. 3.1 to 3.3). For HQ calculation were considered the lowest PNEC values in order to obtain a worst-case scenario. The mixture toxicity was estimated by using the classical concentration addition model to calculate mixture hazard quotients (MHQ) (Eq. 3.4). The risk was classified into the following categories: high risk (MHQ > 1), medium risk ($0.1 \leq MHQ \leq 1$), low risk ($0.01 \leq MHQ < 0.1$) and negligible risk (MHQ < 0.01) (EUROPEAN COMMISSION, 1996).

Concerning public health, the concentration of each PhAC in treated water samples was compared with the concentration below which the probability of adverse effects as a result of long-term (lifetime) exposure is negligible to calculate the margin of exposure (MOE). Tolerable daily intake (TDI), which was derived from non-observed adverse effect level (NOAEL) and a safety factor equal to 100, was used to estimate the safe level of exposure (WHO, 2011). Tolerable daily intake (TDI) values for each PhAC were found in literature or derived from NOAEL with, as recommended in literature (DWI, 2007) (Eqs. 3.5 to 3.8).

2.7 Calculations

The volumetric NF (J_{NF}) and RO (J_{RO}) permeate flux (L m^{-2} h^{-1}) were calculated using Eq. 4.1, as follows:

$$J_{NF} = J_{RO} = \frac{\Delta V_P}{A_m \times \Delta t} \quad (4.1)$$

where A_m is the effective membrane area; ΔV_p is the permeate volume collected; and Δt is the collection time. Flux normalization to 25 °C was accomplished by means of a correction factor related to the fluid viscosity, according to Eq. 4.2:

$$J(25°C) = \frac{\Delta V_P}{A_m \times \Delta t} \cdot \frac{\mu(T)}{\mu(25°C)} \tag{4.2}$$

where $J(25°C)$ is the normalized permeate flux at 25 °C; $\mu(T)$ is the water viscosity at the process temperature; and $\mu(25\ °C)$ is the water viscosity at 25 °C. The permeate recovery ratio (RR_{NF} and RR_{RO}) can be defined by Eq. 4.3:

$$RR_{NF} = RR_{RO} = \frac{V_p}{V_f} . 100 \tag{4.3}$$

where V_p corresponds to the accumulated volume of permeate and V_f to the initial volume of the feed.

For the DM system, the permeate flux [$J_{P(MD)}$] was calculated according to Eq. 4.4:

$$J_{P(MD)} = \frac{m_{di} - m_{df}}{A_m . (t_i - t_f)} \tag{4.4}$$

Where m_{di} and m_{df} correspond to the mass (kg) of the initial and final distillate, respectively. A_m is the area of the membrane (in m^2) and t_i and t_f correspond to the initial and final time, respectively.

The recovery rate [RR_{MD}] is calculated by Eq. 4.5:

$$RR_{MD} = \frac{m_{df} - m_{di}}{m_{fi}} . 100 \tag{4.5}$$

Where m_{fi} corresponds to the mass (kg) of the initial feed. The observed rejection was calculated using Eq. 4.6, as follows:

$$Rejection(\%) = \frac{C_f - C_p}{C_f} \times 100 \tag{4.6}$$

where C_f and C_p represent the solute content on the feed and permeate streams, respectively. PhACs losses during the MD experiments were calculated by considering the mass balance of each analysed compound in the feed, concentrate and distillate, as given in Eq. 4.7.

$$C_F x V_F = (C_D x V_D) + (C_C x V_C) + total\ loss \tag{4.7}$$

where C_F, C_D and C_C are concentration in the feed, distillate and concentrate, respectively. Similarly, V_F, V_D and V_C are the feed, distillate and concentrate volume, respectively.

According to the simplified resistance-in-series model, the total filtration resistance (R_T) could be divided into membrane resistance and fouling resistance. The membrane resistance (R_M) was determined from Eq. 4.8:

$$R_M = \frac{1}{K \cdot \mu(25°C)} \tag{4.8}$$

where K is the membrane water permeability for each test. It was obtained from the ratio of normalized permeate flux of pure water (J_w) by applied pressure (ΔP) at 10.0, 8.0, and 6.0 bar linearization. The fouling resistance (R_f) was calculated based on the normalized effluent permeate flux (J_{sd}) obtained near the end of each experiment (Eq. 4.9). This resistance includes concentration polarization, components adsorption on the membrane surface and scaling.

$$R_f = \frac{\Delta P - \Delta\pi}{\mu(25°C) \cdot J_{sd}} - R_M \tag{4.9}$$

where ($\Delta P - \Delta\pi$) is the process effective pressure, i.e., applied pressure minus osmotic pressure. The osmotic pressure difference was calculated using van't Hoff equation (Eq. 4.10):

$$\Delta\pi = \sum_{i=0}^{n} \left(C_r - C_p\right) \cdot R \cdot T \tag{4.10}$$

where R is the universal gas constant; T is the permeation temperature in Kelvin; and the sum of the difference of concentrate (C_c) and permeate (C_p) concentration at each RR.

The fouling resistance (R_f) is a combination of reversible fouling (R_{fr}) and irreversible fouling layer (R_{fir}) (CHEN *et al*., 2015). R_{fir} (Eq. 4.11) is due to adsorption onto membrane surface and into its pores and it can be removed by chemical cleaning. R_{fr} (Eq. 4.11) is mostly due to a cake layer deposition on the membrane surface, which can be removed through physical cleaning, and controlled by adjusting the feed flow conditions.

$$R_{fir} = \frac{1}{K_{ir} \times \mu} - R_M \tag{4.11}$$

$$R_{fr} = R_T - R_M - R_{fir} \tag{4.12}$$

For the MD resistances calculation, i.e., membrane resistance (R_m), feed boundary layer resistance (R_{fb}) and permeate boundary layer resistance (R_{pb}), Eq. 4.13 to 4.15 were used (SRISURICHAN *et al*., 2006).

$$R_m = \frac{P_1 - P_2}{J_{p(MD)}} \tag{4.13}$$

$$R_{fb} = \frac{P_f - P_1}{J_{p(MD)}} \tag{4.14}$$

$$R_{pb} = \frac{P_2 - P_p}{J_{p(MD)}} \tag{4.15}$$

where P_1 and P_2 represent the vapour pressure at feed and permeate membrane surface; and P_f and P_p represent the vapour pressure at the bulk feed and permeate. Pressures were calculated according to Eq. 4.16 and temperatures at the membrane surface were estimated according to Eq. 4.17 and Eq. 4.18 (SRISURICHAN *et al*., 2006).

$$P = exp\left(23.238 - \frac{3841}{T - 45}\right) \tag{4.16}$$

$$T_{w,f} = \frac{h_m\left(T_p + \left({h_f}/{h_p}\right)T_f\right) + h_f T_f - J_{p(MD)}\Delta H_v}{h_m + h_f(1 + \frac{h_m}{h_p})} \tag{4.17}$$

$$T_{w,p} = \frac{h_m\left(T_f + \left({h_p}/{h_f}\right)T_p\right) + h_p T_p - J_{p(MD)}\Delta H_v}{h_m + h_p(1 + \frac{h_m}{h_f})} \tag{4.18}$$

Where $T_{w,f}$, $T_{w,p}$, T_f and T_p represent the temperatures at interface and bulk for feed and permeate, respectively; h_m, h_p, and h_f stand for the convective heat transfer coefficient of the membrane, permeate and feed; and ΔH_v is the vaporization heat.

The total flux decline (FD) was calculated, as follows, for all three processes (Eq. 4.19).

$$FD = \frac{(J_w - J_{sd})}{J_w} \tag{4.19}$$

Flux decline can be attributed to concentration polarization (CP) and fouling (F); thus, the flux decline due to CP was obtained using Eq. 4.20:

$$CP = \frac{(J_{pc} - J_{sd})}{J_w} \tag{4.20}$$

where J_{pc} is the volumetric water flux of the physically cleaned membrane after effluent filtration. The flux decline due to fouling was obtained using Eq. 4.21:

$$F = \frac{(J_w - J_{pc})}{J_w} \tag{4.21}$$

The specific energy consumption (SEC) for NF and RO was calculated from Eq. 4.22 and Eq. 4.23 (ZHU *et al*., 2009):

$$SEC = \frac{W_{pump}}{Q_P} \tag{4.22}$$

$$W_{Pump} = \frac{\Delta p \times Q_F}{\eta} \tag{4.23}$$

where W_{pump} is the pump work rate (kWh/s); ΔP is the difference between the feed pressure at the entrance of the membrane and the pressure of raw water, which is assumed to be equal to atmospheric pressure (N/m²); Q_F and Q_P are the feed and permeate flow rates (m³/s), respectively, and η is the efficiency of the pump, which was considered equal to 0,95. The permeate product water recovery for NF processes (Y) can be defined using Eq. 4.24, as follows:

$$Y = \frac{Q_P}{Q_F} \quad (4.24)$$

By combining Eq. 4.22, Eq. 4.23 and Eq. 4.24, SEC equation can be rewritten as follows (Eq. 4.25):

$$SEC = \frac{\Delta P}{Y} \quad (4.25)$$

Energy consumption for MD system are estimated both for heat/cooling energy and for circulation of the streams. The specific thermal energy consumption, or STEC (kWh/m^3), was calculated according to Qtaishat and Banat (2013) (Eq. 4.26):

$$STEC = \frac{m_f . c_f . (T_{f,in} - T_{f,out})}{J_{sd}} \quad (4.26)$$

Where m_f is the feed flow rate; c_f is the specific heat of the feed (4.18 kJ kg^{-1}K^{-1}); T_f is the temperature of the feed in ($T_{f,in}$) and out ($T_{f,out}$) of the module. The temperature difference represents the thermal energy entering MD process via hot feed cycle.

2.8 Statistic Evaluation

Kruskal Wallis' test was applied in order to check for significant differences between the quality of the water treated by the three different processes. Non-parametric multiple comparisons were investigated among the groups (α = 5%). STATISTICA 8.0 software was used for the statistical analyses.

2.9 Preliminary Investment and Cost Estimate

A preliminary economic evaluation was conducted to estimate the capital and operational expenses (CapEx and OpEx) to treat Doce river's water by NF, RO and MD. The variables membrane unit cost, membrane replacement, chemical cleaning agents, energy consumption and system maintenance were considered.

For NF and RO, the membrane unit capital cost was based on a price provided by a major supplier of commercial membranes in Brazil, of 8,750.00 U$/m^3.h of effluent. For MD, the

membrane unit capital cost was considered to be 7,680.00 U$/m^3.h (SCHWANTES *et al*., 2018). It considered one filtration stage and volumetric flows equal to the designed systems capacity (Q_{des}) of 0.04 m^3/s. To estimate the capital cost per cubic meter of effluent, the capital cost was annualized by means of the amortization factor, as presented in Eq. 4.27 (SETHI; WIESNER, 2000).

$$A/P = \frac{i_c \cdot (1 + i_c)^{DL}}{(1 + i_c)^{DL} - 1} \tag{4.27}$$

where (A/P) is the amortization factor; i_c is the investment rate (in 2018, it was equal to 6.5% in Brazil - SELIC); and DL is the design life of the plant. The membrane systems design life was considered to be 15 years. The capital cost per cubic meter was obtained from Eq. 4.28:

$$C_{cap/m^3} = \frac{C_{cap} \cdot A/P}{Q_{des}} \tag{4.28}$$

where C_{cap/m^3} is the capital cost per cubic meter of effluent and C_{cap} is the system capital cost.

Membrane replacement costs considered an average membrane lifespan of 5 years, which was a consideration to simplify the calculations. The permeate recovery rate was set at the greater value that provided PhAC concentrations below the method quantification limit (MQL) for each assessed treatment. NF, RO and MD membrane costs were provided by a large commercial membrane supplier as 50, 40, and 60 US$/m², respectively.

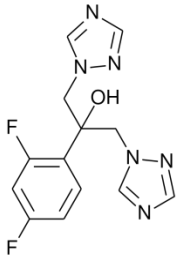
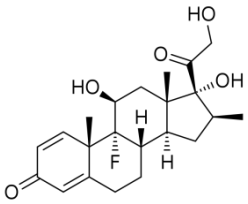
The energy cost estimate comprised the assessed systems feed pump requirement and the energy for heating MD feed solution. A once-through operation process was considered and the power requirement was estimated from Eq. 4.22 to 4.26. The energy tariff paid by the water production company in Brazil is 0.04 US$/kWh (considering an exchange rate of R$1 = US$0.25). The costs of chemicals for membrane cleaning and maintenance costs were estimated at 2 and 5% per year of the initial investment cost, respectively (SHEN *et al*., 2014).

3 RESULTS AND DISCUSSION

3.1 PhACs occurrence in Doce river's water

Out of the 28 PhACs evaluated, only betamethasone (anti-inflammatory) and fluconazole (antifungal) were quantified in the water sample collected in November 2016 (Table 4.3).

Table 4.3 - Betamethasone and fluconazole physical-chemical properties, toxicity indicators and measured concentrations (ng/L) in the water sample collected from Doce river.

Pharmaceutical compound	**Fluconazole**	**Betamethasone**
Therapeutic class	Antifungal	Corticosteroid
Chemical group	Antifungal	Analgesics and anti-inflammatories
Molecular formula	$C_{13}H_{12}F_2N_6O$	$C_{22}H_{29}FO_5$
Structural formula[b]		
Molecular weight (g/mol)	306.1	393.2
Log K_{ow}	0.40	1.94
Dissociation constant	pKa= 12.71	pKa= 12.42
Charge at pH 7	Neutral	Neutral
Molar volume (cm^3/mol) [a]	205	296
Polarizability[a]	26.92	39.70
Molecular radius (Å)[c]	5.49	6.16
KH ($atm\text{-}m^3/mole$)[d]	$7.11x10^{-09}$	$7.36x10^{-11}$
Vapor pressure (mmHg)[d]	$1.02x10^{-06}$	$3.49x10^{-10}$
Acute PNEC (mg/L)	0.100	0.032
Chronic PNEC (mg/L)	0.306	1.000
IDT (mg/kg.d)	0.0500	0.0625
Concentration (ng/L)	573.76	165.12

[a]CHEMICALIZE, 2018; [b]CHEMICALBOOK, 2018; [c]DRUGBANK, 2018; [d]EPA, 2017; logK_{ow} octanol–water partition coefficient; KH Henry law constant; pka: acidity constant

As presented in 3rd Chapter, betamethasone and fluconazole were quantified with high frequency in Doce river's water samples and their occurrence and concentration were subject to seasonality. November is in the beginning of the rainy season in Brazil, which propitiates increased fungal population and may explain the higher fluconazole concentration. This month is also the end of the lower temperatures period, which may explain betamethasone occurrence since diseases treated with anti-inflammatory increase in cold seasons.

Fluconazole concentrations were found to be significantly higher than the ones found in rivers from Spain (28.5 ng/L), China (22.8 ng/L) and Korea (46.2 ng/L) (CASADO *et al*., 2014; HUANG *et al*., 2013; KIM *et al*., 2009). Betamethasone concentration is also lower in the US and in German than the ones found here. According to Vestel *et al*. (2016), the Pharmaceutical Assessment and Transport Evaluation model estimated betamethasone concentrations to be <0.6 ng/L in 95% of all U.S. surface waters and in German the concentrations were found to be between 0.07 and 2.8 ng/L (WEIZEL *et al*., 2018). The highest concentration observed may be related to untreated sewage discharge, both by the city and by other upstream launches. The city in question does not count with wastewater treatment coverage, and the sewage is released *in natura* in the river. Despite, both fluconazole and betamethasone pose low environmental risks.

3.2 PhACs rejection and toxicological risk reduction

NF and RO membranes PhACs removal capability decreases as the permeate recovery rate increases (Table 4.4). The higher the RR, the higher the compounds accumulation in the feed solution, which results in lower removal because it induces greater passage of the pollutants through the membrane (TAHERAN *et al*., 2016). For NF process, the first PhAC occurrence happened at 40% of permeate recovery; for RO it occurred at 60% of recovery rate. MD showed a removal >99% for both fluconazole and betamethasone up to a 70% RR.

Table 4.4 - Betamethasone and fluconazole permeate concentrations (ng/L) and removal percentages for NF, RO and MD processes according to the permeate recovery rate.

RR	Betamethasone						Fluconazole					
(%)	MD		RO		NF		MD		RO		NF	
Feed	165.12						573.76					
10	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)
20	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)
30	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)
40	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	<MQL	(>99)	9.360	(98)
50	<MQL	(>99)	<MQL	(>99)	8.85	(95)	<MQL	(>99)	<MQL	(>99)	17.25	(97)
60	<MQL	(>99)	21.03	(87)	8.88	(95)	<MQL	(>99)	<MQL	(>99)	76.68	(87)

70	<MQL	(>99)	31.54	(81)	41.14	(75)	<MQL	(>99)	<MQL	(>99)	118.5	(79)

MQL=Method quantification limit

Betamethasone and fluconazole have similar molecular weight, charge and molecular radius; however, they have distinguishing hydrophobicity character. Considering that both PhACs molecular radius are greater than membranes pore radius, the main rejection mechanism involved in both NF and RO appears to be the size exclusion. It also explains the RO higher removal percentages than the NF ones, since the further applies dense membranes. The low NF removal may suggest that other mechanisms also affect PhACs rejection. According to Schäfer *et al*. (2011), interaction factors have more substantial effects on NF rejection capacity, likely due to the lower importance of steric hindrance effects for these membranes.

Since both PhACs are neutral compounds under the experimental conditions, the electrostatic repulsion mechanism did not contribute to their rejection. According to Bellona *et al*. (2004), the hydrophobic interaction between the PhAC and the membrane is also an important rejection factor and the existing interactions between non-ionic solutes and membranes may influence PhACs rejection. In this case, the hydrophobic interactions occurring between the fouled membrane surface and these solutes gain predominance and may explain the decrease in PhAC removal owing to the increased fouling at greater RR (GEANIYU *et al*., 2015). Besides, since betamethasone is more hydrophobic than fluconazole ($\log K_{ow}$ equal to 1.94 and 0.40, respectively), its interaction with the membrane material can explain its limited rejection, especially by RO, since BW30 has a higher contact angle (76.2±7) than DK (40.6±5.2), which indicates that BW30 is a more hydrophobic membrane.

MD process showed a rejection >99% for both fluconazole and betamethasone for a 70% RR. These higher rejection results were expected, since MD rejection processes are mainly governed by volatility and, to a lesser extent, by hydrophobia. Both PhACs present kH values much lower than 10^{-3} $mol/m^3.Pa$ and so they are classified as non-volatile compounds. Since MD membrane only enables volatile compounds permeation, the PhACs are concentrated in the feed solution. Similar results were also observed by Wijekoon *et al.* (2014).

MD high PhACs removal leads to a consequent high toxicological risk reduction. As betamethasone and fluconazole concentrations in the permeate obtained are below the method quantification limit, the water can be considered free of toxicological risk occurrence, both for the environment and for human health. The same is true for NF and RO processes at low permeate recovery rates. Even when these processes reach a 70% recovery rate, the

toxicological risk of the permeates obtained is negligible. Figure 4.3 shows the environmental and human health risk reduction of NF, RO, and MD processes when compared to Doce river's raw water and treated by conventional DWTP water.

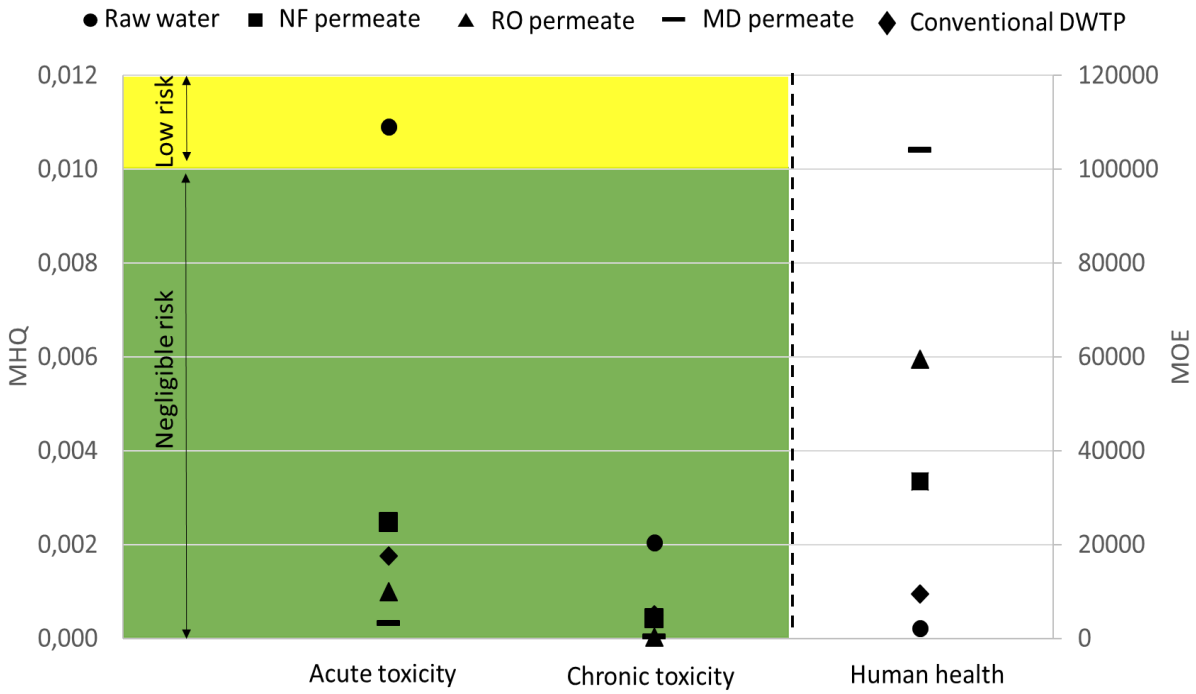


Figure 4.3 - Environmental and human health risk reduction of NF, RO, and MD processes for 70% RR (MHQ=mixture hazard quotient; MOE=margin of exposure).

It is important to state that the concentrate of the three investigated processes are still in need of further treatment in order to degrade the PhACs retained, since these technologies are proven only to concentrate the target compounds and not to eliminate them. Despite, all three concentrates pose low environmental risks and do not pose human health risk even for a 70% RR.

3.3 Membrane desalt ability

In this study, no significant difference was observed in TOC content for the three MSP permeates. Regarding electrical conductivity, a statistical difference was observed for MD and RO permeates (p value = 0.05) (Table 4.5), and a slightly higher quality of MD permeate was noted. These results can be associated to the different permeation mechanisms. On NF and RO membranes, the main separation mechanism is the steric hindrance rejection. In MD process, however, the temperature difference is the driving force and it is not enough to reach the volatile point of ions and organic matter; therefore, only water is capable to pass through the membrane.

These results are in agreement with Han *et al*. (2017) and Meng *et al*. (2014), who applied the same membrane. The author affirmed, however, that the passage of organic matter through the membrane is possibly associated with its amphiphilicity: the hydrophobic part interacts with the membrane matrix, whereas the hydrophilic part can bond to the water molecules (via hydrogen bonds) to diffuse through the membrane (MENG *et al*., 2014).

Table 4.5 - Characteristics of raw water, NF, RO and MD permeates.

Parameter	Doce river's water	NF DK	DK Efficiency (%)	BW30	BW30 Efficiency (%)	MD	MD Efficiency (%)
pH	7.09±0.03	6.9±0.2	-	6.57±0.3	-	6.52±0.2	-
Conductivity (µS/cm)	127±28	69.2±0.9	44.6±10.9	12.75±5.1	90	11.97±0.2	92.56
Turbidity (NTU)	22.56±19.15	0.07±0.01	99.6±0.1	<0.005	99.99	<0.005	>99.99
TSS (mg/L)	20.40±6.54	<0.001	>99.99	<0.001	>99.99	<0.001	>99.99
Apparent color (mg Pt-Co/L)	131±53	<5	>87.9	<5	>87.9	<5	>87.9
TOC (mg/L)	1.59±0.82	0.4	74	0.5	68.5	0.5	68.5
Ca (mg/L)	4.30±0.9	<2.5	>42	<2.5	>42	<2.5	>42
Mg (mg/L)	1.59±0.44	<1.25	>33	<1.25	>33	<1.25	>33
Na (mg/L)	2.93±0.96	<2.5	>37	<2.5	>37	<2.5	>37
K (mg/kg)	2.45±0.41	<2.5	>10	<2.5	>10	<2.5	>10

3.4 Membrane performance: fouling propensity

As expected, RO membrane resistance is much higher than the NF membrane one (Figure 4.4), owing to the dense polymeric structure of the first. This directly impacts the performance of the evaluated membranes. NF has a high initial flux and the final flux is about 70% greater than RO; besides, NF presented much lower flux decline (Table 4.6). The flux decay and increased flux resistance are related to salt precipitation/deposition and pore blocking by organic matter on the membrane surface. Fouling presented a greater contribution than the concentration polarization phenomenon to the flux decline in both analyzed membranes, however, it was much more evident in RO system. Thus, fouling formation seems to be directly related to membrane characteristics such as pore size, hydrophobicity, and surface charge. The greater pore diameter, lesser surface roughness and lower hydrophobicity of the NF membrane (Table 4.2) may have lead to lower fouling potential compared to RO membrane (TU *et al*., 2011).

MD presented constant permeate flux and low conductivity throughout the test, not showing any tendency or indication of critical fouling (Table 4.6). MD is known for the low propensity to scale in comparison with the filtration processes that have the pressure as the driving force (DRIOLI *et al*., 2015). MD performance regarding both flux and water quality highlights this technology as a viable process for surface water treatment.

Table 4.6 - Flux decline and SEC in Doce river's water treatment by NF, RO and MD (20°C; natural pH; flow rate equal to 3.2 L/m; and 10 bar).

Membrane	J_w[a] (L/m².h)	J_{cp}[b] (L/m².h)	J_{fr}[c] (L/m².h)	J_{fir}[d] (L/m².h)	Flux decline type			SEC
					Total	Fouling	CP[e]	
					(%)	(%)	(%)	(kWh.m³.m²)
NF	50.00	47.71	48.63	49.10	4.58	2.74	1.84	0.32
RO	41.50	27.47	30.52	40.00	33.79	26.46	7.33	1.12
MD	17.14	17.14	17.14	17.14	-	-	-	41.63

[a] Initial water effluent permeate flux; [b] Final effluent permeate flux; [c] Water permeate flux after physical cleaning; [d] Water permeate flux after chemical cleaning; [e] Concentration polarization

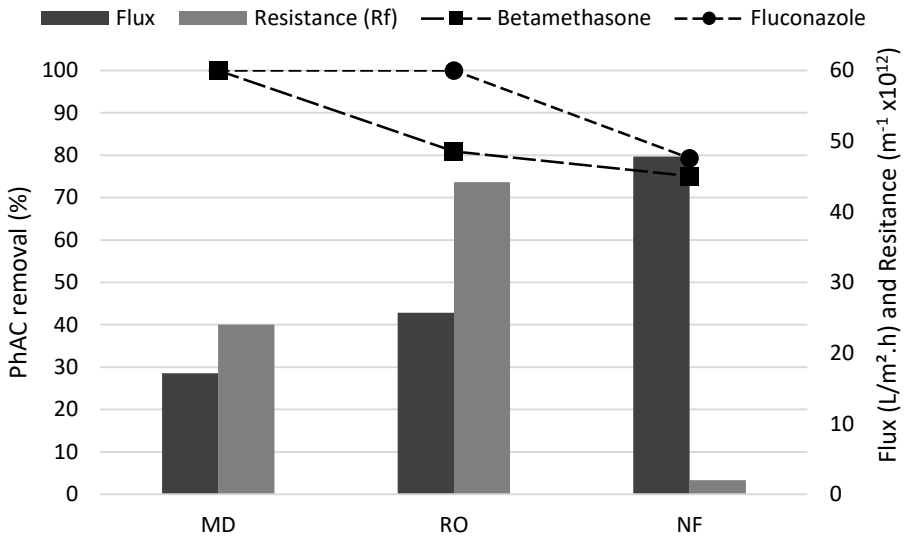


Figure 4.4 - Fluconazole and betamethasone rejection, resistance (Rf) and flux applying NF, RO and MD with 70% of permeate recovery.

The specific energy consumption relates the permeate flux with the required energy. This factor is directly associated with operational costs. Since NF membrane has a less salient hydraulic resistance, it is possible to observe a smaller energy requirement (Table 4.6); the higher energy requirement for RO is due to the higher resistance imposed by the dense membrane. Regarding MD process, two types of energy demand should be considered: pump and heating requirement. Despite the first one was the lowest (0.02 kWh.m³.m²) the heating requirement was significantly higher (41.61 kWh.m³.m²). The values obtaineid here is in accordance with values reported in literature (REIS *et al*., 2018).

3.5 Preliminary cost evaluation

The cost-effectiveness of NF, RO and MD processes for treating surface water was studied in order to supply a medium-sized Brazilian city. In such systems, membrane separation processes are generally employed to ensure the quality of the final product, in this case, removal of organic matter, part of the salts and PhACs. RO was successfully applied in achieving the goals, but this system was more expensive than NF system. The higher cost of RO is owing to its denser membrane and, therefore, higher energy consumption. NF has become popular owing to the also high rejection efficiency, higher permeate flux, and smaller energy consumption, making this treatment the cheapest among the three evaluated systems (Table 4.7).

Table 4.7 - Cost estimation of NF, RO and MD treatment systems for Doce river's water.

	Description	Values			Units
		NF	RO	MD	
System Characteristics	Annual System Capacity	182,500	182,500	182,500	m^3/year
	Average Permeate Flux	0.0305	0.0137	0.0058	$m^3/h.m^2$
	Permeate Recovery Rate	30	50	70	%
	Required Membrane Area	205	760	2514	m^2
	Design Plant Life	15	15	15	years
	Membrane Lifespan	5	5	5	years
	Brazil Investment Rate	6.5	6.5	6.5	%
	Energy Price	0.04	0.04	0.04	US$/kWh
CapEx	Systems	182,292	182,292	160,000	US$
OpEx	Membrane Replacement	0.011	0.033	0.165	US$/$m^3$
	Capital Cost Amortization	0.106	0.106	0.093	US$/$m^3$
	Cleaning Agent	0.002	0.002	0.002	US$/$m^3$
	Energy Requirement	0.013	0.045	1.664	US$/$m^3$
	Maintenance	0.005	0.005	0.005	US$/$m^3$
	Total	**0.14**	**0.19**	**1.93**	**US$/$m^3$**

For the same capacity, MD operational cost was greater than those of RO and NF, which is in accordance with what is found in the literature (BRUGGEN *et al*., 2001; COSTA; PINHO 2006). This is attributed to the fact that the operational cost is dominated by the heating energy requirement (Figure 4.5). The operational cost found here is also higher than the stated in the literature, which reports values between 0.30–1.20 \$/m^3 (ZUO *et al*., 2011). However, the cost may be further reduced if low cost energy, such as solar energy or residual heat, is applied. Considering residual heat use, the operational cost could reach 0.30 \$/m^3 (HAN *et al*., 2017); regarding the use of solar energy, several studies are being developed successfully, minimizing the use of thermal energy and electricity (ASHOOR *et al*., 2016).

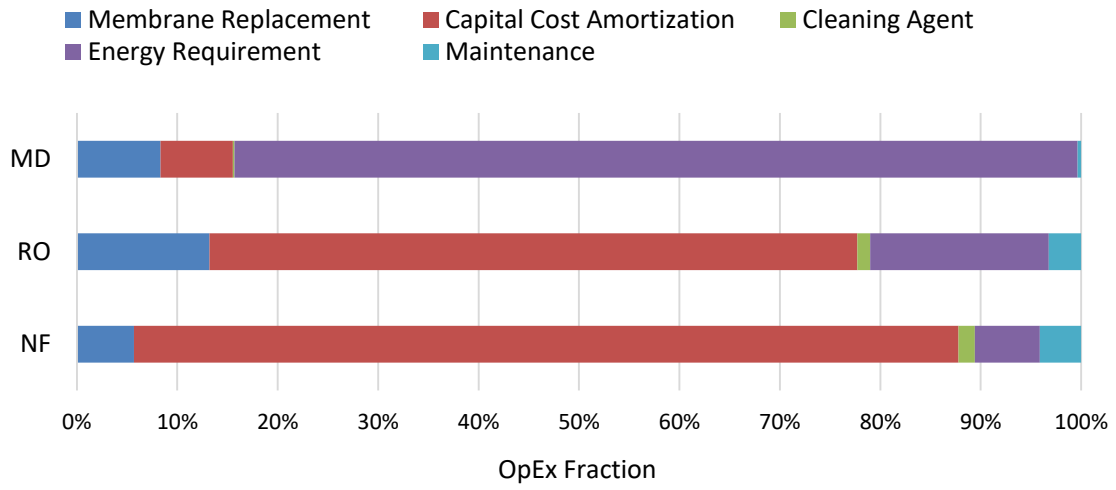


Figure 4.5 - OpEx components distribution.

4 CONCLUSION

NF, RO and MD technologies are efficient as a single step to surface water treatment to achieve drinking water quality and PhAC removal. NF and RO rejection of PhACs is mainly owing to size exclusion and hydrophobic interactions and MD rejection is mainly owing to PhACs low volatile. Besides presenting the highest PhAC removal, MD did not present fouling tendency, whereas it was the principal cause of flux decline for RO and NF processes. This possibilitates the application of higher recovery rates, which leads to lower concentrate generation, reducing environmental impact and cost of disposal.

Despite being the best process from the technical point of view, MD presented the highest operating cost, making NF the best option in relation to economic viability, since this process was able to produce a permeate of good quality and to achieve high PhAC removal for lower RR with low operating cost. However, MD cost can be reduced by applying low cost energy, such as solar energy or residual heat.

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5th Chapter

Final considerations

1 DISSERTATION OVERVIEW AND MAIN RESULTS

This work assessed PhAC environmental and human health toxicological risk in raw and treated waters of four Brazilian water supply systems, covering three regions of the country, subject to different climatic and socio-economic conditions. The evaluated DWTPs presented different capacities and types of treatment. In addition, the performance of three different membrane separation processes (NF, RO and DM) was compared regarding technical and economic feasibility and PhAC removal capacity. The results confirmed the contamination of Brazilian natural waters by PhACs and the occurrence of environmental and human health risks, besides elucidating the mechanisms and properties involved in their removal through conventional and membrane separation processes.

Chapter 2 provided an overview of environmental and human health risk assessment methodologies, presenting the current concepts, guidelines and limitations and proposing recommendations for its improvement. In addition, it presented what is new about this subject, considering new techniques and approaches developed. Some of the points that drew more attention were the large gap in international literature regarding pharmaceuticals toxicity data, mainly concerning chronic toxicity, and the low quality of the available data owing to several limitations, such as low coverage and suitability of standard toxicological tests.

In Chapter 3, PhACs trace levels were detected in superficial and drinking water in all assessed water supply systems and betamethasone, prednisone and fluconazole were the most common PhACs. PhACs presence and concentration were dependent on the population habits and seasons. Surface water from all water sources were subject to environmental risk at some level owing to at least one PhAC and drinking water was subject to human health risk, since DWTPs capacity to remove PhACs and to reduce toxicological risk was only partial.

In contrast to the DWTPs low removal percentages, PhACs were efficiently removed by all PSM evaluated in Chapter 4. PhAC removal of NF and RO processes decreased with the increase of the RR, whereas MD was able to reduce PhAC concentrations until below the MQL up to a recovery rate of 70%. Owing to the high removal efficiency of the MSP, no environmental or human health risk was observed. Although MD presented the highest PhAC removal and did not present tendency to fouling, it also presented the highest operating cost. However, the use of low-cost energy, such as solar or residual heat, could reduce its cost. NF was the cheapest process and it was able to produce a high quality permeate.

2 CONCLUSIONS AND RECOMMENDATIONS

Based on this work, it was possible to confirm that PhACs contamination is a reality in Brazilian natural waters and that they pose toxicological risks for both the environment and public health. Thus, there is no doubt that the presence of PhACs in surface waters should not be neglected. Besides, it is not enough that this issue is of interest only to the academic community; it is necessary that the problem reaches the managing entities, legislatures and companies of water treatment. The results found also confirmed the low efficiency of conventional drinking water treatment processes to remove PhACs, whereas membrane separation processes are highly effective in their removal.

The results presented here are important because they contribute to the construction of national knowledge about the occurrence and toxicological risk of PhACs. However, new researches should be conducted to refine and extend the scope of these results. Some recommendations to continue this research are:

• This dissertation was conducted in three Brazilian regions, each one represented by a city. Owing to the Brazilian population plurality and to cultural and socio-economic diferences, both inter and intra-regions, it is important that other regions not covered in this study are also evaluated and that other cities are included in the evaluation, in order to increase the scope of the research and better match the reality of the country;

• PhACs monitoring was performed during one year; however, continuous monitoring over long periods of time is fundamental to identify patterns of occurrence, consumption and seasonality. In addition, continuous monitoring is also essential to verify if there is any increase in PhACs concentrations, which could lead to higher toxicological risks;

• Considering the high PhAC removal capacity and the low fouling tendency of MD, it is recommended to associate solar energy, widely available in Brazil, with this process. The use of photovoltaic panels, for example, can considerably reduce MD operating cost, making the process also economically viable.