Titre: Title:	Occurrence and Treatment of Hormones in Drinking Water Sources and Plants
Auteur: Author:	Hadis Yarahmadi
Date:	2017
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	Yarahmadi, H. (2017). Occurrence and Treatment of Hormones in Drinking Water Sources and Plants [Ph.D. thesis, École Polytechnique de Montréal]. PolyPublie. https://publications.polymtl.ca/2938/

UNIVERSITÉ DE MONTRÉAL

OCCURRENCE AND TREATMENT OF HORMONES IN DRINKING WATER SOURCES AND PLANTS

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ÉCOLE POLYTECHNIQUE DE MONTRÉAL

THÈSE PRESENTÉE EN VUE DE L'OBTENTION

DU DIPLÔME DE PHILOSOPIAE DOCTOR

(GÉNIE CIVIL)

DÉCEMBRE 2017

UNIVERSITÉ DE MONTRÉAL

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

Cette thèse intitulée :

OCCURRENCE AND TREATMENT OF HORMONES IN DRINKING WATER SOURCES AND PLANTS

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en vue de l'obtention du diplôme de : Philosophiae Doctor

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برای پدر، مادر، و علی عزیزم

برای نیکان، شادی دل مامان

"Yesterday I was clever, so I wanted to change the world. Today I am wise, so I am changing myself."

Rumi

ACKNOWLEDGEMENTS

I would like to give all my sincere gratitude to my Ph.D. supervisor, Michèle Prévost, for encouraging me from the first beginning days of my Ph.D. to the last weeks of redaction and submission of this thesis. Thank you Michèle, for being always encouraging and supportive. You never cease to amaze me with how dedicated you are to your students. Thank you for being patient with my life challenges, maternity leave, leaving Canada and then coming back. Thank you for your support and compassion during all those hard days. I cannot thank you enough for your generosity in teaching me, mentoring me and advising me.

I would like to thank my co-supervisor, Sébastien Sauvé, for accepting me as a member of his research lab and letting me profit from all the hi-tech analytical instruments in his lab. Thank you Sébastien, for your guidance, your advice, and your patience in coaching me through this project.

I wish to thank Prof. Benoit Barbeau, for his invaluable advice during the realization of the ozonation part of my project. Thank you Benoit for indulging my spur of the moment questions with impromptu meetings or prompt responses through emails.

I wish to thank all other co-authors of my papers: Sung VO DUY, Arash Zamyadi, Sarah Dorner, and Mounia Hachad. Having you as co-authors was a great privilege to me. Thank you for sharing your knowledge and expertise. Thank you Sung for being patient with my ordinary questions on analytical methods and helping me to interpret my results. Thank you for all those off-hour times you stayed in the lab with me to make sure that analysis of my samples are well progressing.

I also would like to express my gratitude to the members of my examination committee, Eric Wert, Yves Comeau, and Francoise Bichai for taking their time to review my thesis.

I wish to thank the National Sciences and Engineering Research Council (NSERC) and the industrial chair partners, John-Meunier Inc., City of Laval, and City of Montréal, who made this Ph.D. project possible.

I would like to give special thanks to the Chair staff; I was privileged to work with such a highly qualified, friendly team during this time. Thank you Jacinthe and Julie for helping me integrate with other group members. Thank you for your expert advice in lab work and also in my life

dealing with the amazing Québec culture. Thank you Yves, for always being ready to help and smiling, even during our sampling from rivers in freezing weather. I also thank Mireille, Mélanie Rivard, Mélanie Bolduc, and Denis Bouchard for their endless help in the lab. I particularly want to thank Shokoufeh Nour, for sharing her experience with me which always helped me to better integrate with our group. I thank Laura for her patience dealing with our countless demands, her availability, help and support.

During my Ph.D. I had the chance to meet some of my best friends, Kim, Evelyne, Fatemeh, and Laleh. I am so happy to have you lovely friends, coming from different cultures. I admire all these differences and learned a lot from you. I always enjoyed our discussions. Thanks Kim, for being so kind and trustful. You taught me that we can always smile and be logic even after big failures in our research. Thank you Evelyne, for being always energetic, enthusiastic and spreading your energy throughout the lab. Thank you for correcting my French spelling faults very delicately during our discussion and emails. All my thanks to: Fatemeh and Laleh, thank you girls for being beside me during all those hard days of work. Thank you for your support and help.

I wish to thank my friend Celso. I will always remember you although you are no longer with us. Thank you for sharing your experience and exciting stories with us. Thank you for helping Sung with the last run of my analysis when I had to leave Montréal.

I also wish to thank my friends, Anne-Sophie, Céline, Émilie, Élise, Émile, Flavia, Félix, Isabelle, Ken, Samira, Mariam, Mounia, Milad, Mathieu, Nargess, Pirouz, Paul, Sanaz, Shifteh, Vanessa and all the students I met during my Ph.D. at Polytechnique and the University of Montréal.

I would like to thank my friends in Montréal and Toronto. Thank you: Ameneh, Donya, Hanifa, Hoda, Hoory, Mahsa, Shifteh, Ali KM, Ahmad, Mohammad Reza, Mohsen, Nima KM. Thank you for all the amazing time we spent together. I hope one day I come back to Montréal again and we make a lot of new memories together in chilly nights of winter. I'm grateful to have you as my friends.

There are no words that can express my gratitude to my family, my mom and dad, my beautiful and kind sisters, Hasti and Forough. Thank you for your unconditional support and love during

my whole life. Thank you for coming to Canada and taking care of Nickan when I had to stay in the lab too late and I was confident that you are taking care of Nickan better than me. I'm so proud of you.

Behind any successful job done, there is always a strong friendship and trust which made that success possible. My best friend and support during my Ph.D. and from 12 years ago is my Ali. Thank you for your patience and endless support during all of the ups and downs of my study. I am always so proud to have you as my best friend and my lovely husband.

RÉSUMÉ

Depuis les dernières décennies, de nombreuses préoccupations scientifiques et publiques ont émergé concernant les effets potentiels des hormones qui sont libérées dans l'environnement, causant des troubles sexuels, nuisant à la reproduction et au développement des organismes aquatiques. Les stations de récupération des ressources de l'eau (StaRRE) sont connues pour être une source majeure d'hormones dans l'environnement aquatique. Plusieurs études ont été publiées à l'échelle internationale au cours des dernières décennies, afin de documenter la présence d'hormones naturelles et synthétiques dans les eaux usées brutes, les effluents StaRRE et à des concentrations plus faibles dans les eaux de surface/ souterraines. La plupart de ces études se sont concentrées sur la quantification des hormones dans l'eau des rivières (fraction dissoute seulement) et dans les biosolides (hormones totales) des eaux usées ou dans les sédiments. Comme la phase particulaire est probablement dominante, la quantification la plus pertinente pour les usines de traitement de l'eau potable devrait inclure à la fois des mesures des phases dissoutes et particulaires. Les eaux de surface sont les principales sources d'eau potable au Canada en fournissant 89% de l'eau utilisée dans les municipalités. Par conséquent, la libération de stéroïdes par les StaRRE peut affecter la qualité de l'eau potable. Ce problème doit mieux être compris afin d'améliorer l'efficacité de l'élimination des stéroïdes dans les StaRRE et les usines de traitement d'eau potable (UTEP).

Les objectifs de la présente thèse étaient d'abord de générer plus de connaissances sur le sort global d'un groupe d'hormones stéroïdiennes à partir d'affluent et d'effluent d'eaux usées jusqu'à l'eau potable traitée. Les résultats de l'étude apportent des connaissances sur les variations saisonnières de l'occurrence et du devenir des stéroïdes dans les phases dissoutes et particulaires ainsi que dans les particules de boues et les sédiments fluviaux des StaRRE, des eaux de rivières, et les UTEP. Un échantillonnage exhaustif (10 échantillons d'UTEP, 3 de StaRRE, 24 d'eaux de rivière, 36 de sédiments de fond) a été réalisé dans trois campagnes de suivi pour quantifier les hormones naturelles (E1, E2, E3, progestérone et testostérone) et synthétiques (EE2, medroxyprogesetérone, noréthindrone et lévonorgestrel) dans la phase dissoute et particulaire de l'eau de la rivière et ensuite dans les sédiments. Les concentrations de stéroïdes dans la phase dissoute de tous les échantillons étaient inférieures à la limite de détection, sauf pour les

échantillons provenant des affluents à la StaRRE, ce qui montre clairement la prédominance de leur forme particulaire. Des niveaux plus élevés de stéroïdes ont été trouvés dans les matières en suspension dans les rivières durant les périodes les plus froides (total: 677 ng L^{-1}) comparativement aux échantillons prélevés en été (total: 163 ng L^{-1}). Les concentrations de stéroïdes totaux mesurées dans les sédiments variaient de 1651 à 4584 ng g^{-1} en été et en automne, respectivement. Dans les échantillons provenant de l'eau brute des usines de traitement d'eau potable, les particules contenaient des niveaux similaires de testostérone, de noréthindrone, d'estradiol et de 17α-éthinylestradiol comme dans les effluents des StaRRE, indiquant la persistance des hormones adsorbées depuis leur point de rejet dans la rivière jusqu'à la prise d'eau. Dans l'ensemble, cette partie de l'étude confirme la présence d'hormones stéroïdiennes dans les sources d'eau potable sous forme particulaire, ce qui soulève des inquiétudes quant à leur devenir dans les UTEP et leur potentiel dans le lit de boues des décanteurs.

Le deuxième objectif était de quantifier la capacité d'adsorption et la cinétique de sorption de 8 hormones stéroïdiennes sélectionnées sur quatre échantillons de sédiments provenant de la rive contenant une quantité variable de matière organique, à l'échelle du laboratoire pendant 95 heures. Les stéroïdes sélectionnés présentaient une sorption rapide sur les sédiments atteignant un quasi-équilibre en moins d'une heure. La progestérone et l'estrone (E1) présentaient une affinité de sorption plus élevée envers tous les sédiments que la testostérone et l'estradiol (E2). La sorption minimale et maximale dans S4 avec f_{OC} =17% à t = 0 était de 14% pour E2 et de 56% pour la progestérone, respectivement. Alors que la sorption minimale dans S2 avec f_{OC} =73% à t=0 était de 61% pour E2 et de 78% pour la progestérone.

Les constantes de vitesse de sorption de pseudo-second ordre ont été mesurées pour les stéroïdes sélectionnés dans des expériences à l'échelle de laboratoire dans la première heure du processus. La constante cinétique pour les stéroïdes variait entre 1,09E-03 (pour l'estrone) et 7,05E-02 g min μg^{-1} (pour l'estradiol) en S1 et de 5,38E-03 (pour le lévonorgestrel) à 1,94E-03 (pour l'estradiol) pour S2.

La quantité d'hormone adsorbée sur les sédiments à l'équilibre et les coefficients de distribution (K_d) entre l'eau et les sédiments ont été mesurés. Les valeurs de K_d variaient entre 5,05 et 19 L kg^{-1} (échantillon de sédiment ayant la concentration de matière organique la plus basse, S4) et

9,8- 22,2 L kg^{-1} (échantillon de sédiment ayant la concentration de matière organique le plus élevé, S2). Ces résultats indiquent que la disponibilité des stéroïdes en phase solide est directement liée à la teneur en matière organique de l'échantillon de sédiments. Les coefficients de sorption ont été déterminés en utilisant le modèle isotherme linéaire. Le coefficient de sorption linéaire dans le modèle isotherme linéaire représente le coefficient de distribution (K_d). Les valeurs de K_d obtenues expérimentalement étaient compatibles avec les coefficients isothermes linéaires dans un intervalle de confiance de 95-percentile. Une linéarité plus élevée a été observée pour les isothermes de l'échantillon ayant la plus faible teneur en matière organique. Bien que les progestatifs synthétiques aient été reconnus comme étant des perturbateurs endocriniens, il existe encore plusieurs lacunes dans les données sur le devenir de ces composés dans les eaux de rivière. Cette partie de la thèse permet de mieux comprendre la capacité de sorption, les données cinétiques et les modèles isothermes des progestatifs moins étudiés et de la testostérone qui jouent un rôle important dans leur devenir dans l'environnement.

Le troisième objectif était d'évaluer le potentiel de l'ozone pour l'oxydation des hormones stéroïdiennes récalcitrantes à l'oxydation (testostérone, progestérone, médroxyprogestérone, noréthindrone et lévonorgestrel) pendant le traitement de l'eau et à différentes températures. À cette fin, les constantes de vitesse d'ordre de deux pour la réaction des hormones sélectionnées avec l'ozone (k_{O3}) ont été déterminées dans des expériences en laboratoire utilisant de l'eau ultrapure. Les taux d'élimination des composés sélectionnés avec de l'ozone ont été estimés dans l'eau de rivière filtrée et également dans l'effluent de StaRRE dilué en utilisant des constantes de vitesse de second ordre provenant d'expériences dans de l'eau ultrapure. A une température de 21 ° C et un pH de 6 ou 8 et en présence de piège à radicaux, les constantes de vitesse pour la progestérone, la médroxyprogestérone, la testostérone, la noréthindrone et le lévonorgestrel variaient de $590 < K_{O3} < 2300$ $M^{-1}s^{-1}$, démontrant la réactivité de ces composés avec l'ozone. Le lévonorgestrel et la noréthindrone ont montré une plus grande réactivité vis-à-vis de l'ozone comparée aux trois autres composés avec des constantes de vitesse de 2233 et 2292 M^{-1} s^{-1}. Pour tous les composés, une augmentation de la température de 5 à 35 ° C a entraîné une augmentation des constantes de vitesse du deuxième ordre de 3 fois pour la noréthindrone à 5,5 fois pour la progestérone. L'énergie d'activation requise a été estimée pour les cinq stéroïdes sélectionnés et allait de 30 kJ (noréthindrone) à 39 kJ (progestérone). Enfin, cette partie du projet

a montré que les processus d'ozonation à des doses typiques de traitement de l'eau (Ct_{O3} = 2 mg min L^{-1}) étaient seulement capables d'éliminer 77% (progestérone) à 99% (lévonorgestrel) à 21 ° C et même moins (47 % médroxyprogestérone à 96% de noréthindrone) à 5 ° C des composés sélectionnés. Considérant qu'aucune source supplémentaire de radicaux hydroxyles n'a été ajoutée aux expériences d'oxydation, l'exposition aux radicaux hydroxyles (Ct_{OH}) a été trouvée très faible et une réaction directe avec l'ozone a été suggérée comme étant le mécanisme régissant l'élimination des stéroïdes.

Les connaissances issues de cette étude peuvent servir de base à une estimation ultérieure des rejets de stéroïdes des StaRRE dans l'environnement aquatique et / ou être utilisées pour évaluer comment la conception et l'exploitation des StaRRE peuvent être optimisées en termes d'élimination de ces substances et ainsi minimiser la charge rejetée dans l'environnement.

ABSTRACT

During the last few decades, there has been considerable scientific and public concern about potential impacts of hormones that are released to the environment where they cause sexual disorders, impair reproduction and development of aquatic organisms. Wastewater treatment plants have been known to be a major source of hormone release into the aquatic environment. Several studies have been published internationally during the recent decades, to document the presence of natural and synthetic hormones in raw wastewater, WRRFs effluents, and at lower concentrations in surface/ground waters. Most of these studies have focused on hormone quantifications in river water (dissolved only) and in biosolids (total hormones) of wastewater or in sediments. As the particulate phase is likely dominant, the most relevant quantification for drinking water plants should include both dissolved and particulate measurements. Surface waters are the main sources of drinking water in Canada by providing 89% of water used in Canadian municipalities. Therefore, the release of steroids by WRRFSs may affect drinking water quality. This issue is needed to be better understood in order to improve the removal efficiency of steroids at WRRFSs and DWPs.

The objectives of the present thesis were first to generate more knowledge about the overall fate of a group of steroid hormones from their source raw and treated wastewater to treated drinking water. The results of the investigation provide knowledge about the seasonal variations in the occurrence and fate of steroids in the dissolved and particulate phases as well as in sludge particles and river sediments from WRRFSs, river water, and DWPs. Extensive sampling (10 samples from DWP, 6 from WRRFSs, 24 from river water, 36 from bed sediments) was undertaken in three monitoring campaigns to quantify natural (E1, E2, E3, progesterone, and testosterone) and synthetic (EE2, medroxyprogesterone, norethindrone, and levonorgestrel) hormones in the dissolved and particulate phase of the river water and then in sediments. The concentrations of steroids in the dissolved phase of all samples were below the limits of detection (LOD) except for samples from raw sewage showing the clear dominance of their particulate form. Higher levels of steroids were found in river suspended particles in colder periods (total: 677 ng L^{-1}) compared to in samples taken in summer (total: 163 ng L^{-1}). The total steroids measured in sediments ranged from 1651 to 4584 ng g^{-1} in summer and autumn,

respectively. In samples from DWPs source water particles contained similar levels of testosterone, norethindrone, estradiol and 17α-ethinylestradiol as in the WRRFSS effluents, indicating the persistence of adsorbed hormones from their discharge point in river to the water intake. Overall, this part of study confirms the presence of steroid hormones in drinking water sources under the particulate form which raises concerns about their fate in DWPs and their potential accumulation in sludge bed of clarifiers.

The second objective was to quantify the adsorption capacity and investigate the kinetics of the sorption of 8 selected steroid hormones on three samples of shore river sediments containing varying amount of organic matter in bench scale over 95 hours. The selected steroids showed rapid sorption onto sediments reaching quasi-equilibrium after less than an hour. Progesterone and estrone (E1) showed highest sorption affinity toward all sediments than testosterone and estradiol (E2). The minimum and maximum sorption in S4 with f_{OC}= 17% at the t= 0 was 14 % for E2 and 56 % for progesterone, respectively. Whereas the minimum sorption in S2 with f_{OC}= 73% at the t = 0 was 61 % for E2 and the maximum was 78 % for progesterone.

Pseudo second-order sorption rate constants were measured for the selected steroids in batch mode experiments within the first hour of process. Smaller rate constants were obtained for samples with higher organic content. The kinetic constant for steroids ranged between 1.09E-03 (for estrone) and 7.05E-02 g min μg^{-1} (for estradiol) in S1 and from 5.38E-03 (for levonorgestrel) to 1.94E-03 (for estradiol) for S2.

The amount of hormone adsorbed onto sediments at equilibrium time and the distribution coefficients (K_d) between water and sediment were measured. The K_d values varied in the range 5.05- 19 L kg^{-1} (lowest organic matter sediment sample, S4) and 9.8-61 L kg^{-1} (highest organic matter sediment sample, S2). These results indicate that availability of steroids in solid phase is directly related to the organic content of sediment sample. Sorption coefficients were determined using the linear isotherm model. The linear sorption coefficient in linear isotherm model represents distribution coefficient (K_d). The experimentally obtained K_d values were well compatible with linear isotherm coefficients within the 95^{th} confidence intervals. Higher linearity was observed for isotherms from sample with lowest organic content. Although synthetic progestogens have been found as endocrine disruptors, there are still several data gaps on fate of

these compounds in river waters. This part of thesis provides better understanding the sorption capacity, kinetics data and isotherm models of less studied progestogens and testosterone which play an important role in their fate in the environment.

The third objective was to assess the potential of ozone for the removal of oxidation recalcitrant steroid hormones (testosterone, progesterone, medroxyprogesterone, norethindrone, and levonorgestrel) during water treatment and also for the first time to evaluate the effect of temperature on reaction of steroids and ozone. For this purpose, second-order rate constants for the reaction of selected hormones with ozone (k_{O3}) were determined in laboratory scale experiments using buffered ultrapure water. The removal rates of the selected compounds with ozone were estimated in filtered river water and also in diluted WRRFS effluent using second order rate constants from experiments in ultrapure water. At a temperature of 21 °C and pH of 6 or 8 and in presence of radical scavenger, the rate constants for progesterone, medroxyprogesterone, testosterone, norethindrone, and levonorgestrel ranged from $590 < K_{O3} < 2292$ $M^{-1}s^{-1}$, demonstrating the moderate reactivity of these compounds with ozone. Levonorgestrel and norethindrone showed higher reactivity towards ozone compare to three other compounds with rate constants of 2233 and 2292 M^{-1} s^{-1}. For all compounds temperature increase from 5 to 35 °C resulted in the second-order rate constants increase from 3 folds for norethindrone to 5.5 folds for progesterone. The required activation energy was estimated for the five selected steroids and ranged from 30 kJ (norethindrone) to 39 kJ (progesterone). Finally, this part of project showed that ozonation processes at typical water treatment dosages (Ct_{O3}= 2 mg min L^{-1}) were only capable of removing 77% (progesterone) to 99% (levonorgestrel) at 21 °C and even less (47% (medroxyprogesterone to 96% norethindrone) at 5 °C of the selected compounds. Considering that no extra source of hydroxyl radicals was added to any of the oxidation experiments, the hydroxyl radical exposure (Ct_{OH}) was found very low and direct reaction with ozone was suggested to be the governing mechanism for steroid removal.

The knowledge from this study can form the basis for later estimation of the discharges of steroids from the WRRFSs to the aquatic environment and/or be used to assess how the design and operation of WRRFSs can be optimised in terms of removal of these substances and thereby minimisation of the load on the environment.

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AC	Activated Carbon
AOPs	Advanced Oxidation Processes
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photoionization
A	Frequency factor (M^{-1} s^{-1})
BOD	Biological Oxygen Demand
CCL	Contaminant Candidate List
CI	Chemical Ionization
COD	Chemical oxygen demand
CSOs	Combined Sewage Overflows
CSTR	Continuous Stir-tank Reactor
Ct	Residual concentration of oxidant multiples time of contact
DES	Diethylstilbistrol
Diss.	Dissolved
DOC	Dissolved Organic Carbon
DRM	Distributed Reactions Model
DW	Drinking Water
DWTP	Drinking water treatment plant
E_{act}	Activation energy ($J.mol^{-1}$)
E1	Estrone
E2	17β-Estradiol
EE2	17α-ethinylestradiol
EDCs	Endocrine disrupting compounds
EI	Electron Ionization
ER	Estrogen Receptor
ESI	Electrospray Ionization
eV	Electron volt
EV	β-estradiol 17-valerate

$FeCl_3$	Ferric Chloride
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
GC	Gas Chromatography
HOCl	Chloric Acid
HPLC	High performance liquid chromatography
HRT	Hydraulic Retention Time
k_{O_3}	Second-order rate constant with ozone ($M^{-1}s^{-1}$)
k´	Pseudo-first-order ozone decay rate (min^{-1})
K_{ow}	Octanol-Water Partition Coefficient
K_d	Sediment-Water Partitioning Coefficient
K_H	Henry's Constant (atm m^3 mol^{-1})
K_{bio}	Biodegradation Rate Constant
K_{oxy}	Oxidation Rate Constant
LC-MS-MS	Liquid Chromatography-Tandem Mass spectrometry
LDTD	Laser Diode Thermal Desorption
LLD	Liquid-Liquid Extraction
LOD	Limit of Detection
Levo	Levonorgestrel
MDRXY-Prog	Medroxyprogesterone
Milli-Q	Ultra-pure Water
Nore	Norethindrone
NOM	Natural Organic Matter
NPD	Nitrogen Phosphorous Detector
% OC	Organic Carbon content
OCl^-	Hypochlorite Ion
OECD	Organisation for Economic Co-operation and Development
OH°	Hydroxyl Radical
Part.	Particulate
P.E.	Population Equivalent

pK_a/pK_b	Acid/Base Dissociation Constant
Prog	Progesterone
q_e	Sorption Capacity
R_{CT}	Ratio of ozone exposure (Ct_{O3}) and hydroxyl radicals exposure (Ct_{OH})
SPE	Solid phase extraction
SRT	Sludge Retention Time (day)
tertBuOH	Tert-butyl alcohol
Testo	Testosterone
THM	Trihalomethane
TOC	Total Organic Carbon
TS	Total Solids
U.K.	United Kingdom
USA	United States of Amercia
USE	Ultrasonic Extracion
UV	Ultra violet
VS	Volatile Solids
WWT	Wastewater Treatment
WWTP	Wastewater Treatment Plant
WRRF	Water Resource Recovery Facilities
ε_{HOMO}	Highest Occupied Molecular Orbital
ε_{LUMO}	Lowest Unoccupied Molecular Orbital
$\Delta\varepsilon$	Energy difference between the HOMO of the nucleophile reactant and the LUMO of the electrophile oxidant
ρCBA	Para-chlorobenzoic acid

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CHAPTER 1 INTRODUCTION

Steroid hormones are divided in two general categories, natural hormones which are excreted by humans and livestock; synthetic hormones which are extensively used as contraceptives and growth promoters. Both natural and synthetic steroids have been widely detected in aquatic environment. Pharmaceuticals containing synthetic hormones and also natural hormones excreted by human body undergo variety of transformations before their excretion in municipal sewage through urine or excrements and have to pass through wastewater treatment plant before entering water sources [1]. Steroid hormones have been detected in wastewater treatment plant effluents all over the world with concentrations ranging between few ng L^{-1} to µg L^{-1} [2, 3]. Contamination of water may be caused by incomplete removal of receiving compounds after sewage treatment. Additionally, from their physicochemical properties, they are expected to sorb on soil/ sediments or on sludge particles of clarification tanks and end up in soil and ground water by using sludge for agricultural uses. Estrone and estradiol have been found in WRRFS sludge at levels ranging between 1 ng g^{-1} and 48.9 ng g^{-1} [4]. Several studies were also reported the occurrence of steroid hormones in sediments at concentrations in range of 3-111 ng g^{-1} (progestogens), 86-149 (estrogens) [5, 6].

Despite their very low concentrations in the environment, natural and synthetic hormones are pollutants of high concerns because they are physiologically active and highly stable in aqueous media [7]. They may interfere with the reproduction of aquatic lives, livestock, and human [8] [9]. A complete feminization was observed between the fathead minnow when exposed to 4 ng L^{-1} of 17α-ethinylestradiol [10]. Progesterone and other synthetic progestogens, including levonorgestrel and medroxyprogesterone were found to make transcriptional effect in fish [11].

Knowing the ubiquitous presence of natural and synthetic hormones in the WRRFS effluents and their negative effect on aquatic life, advanced treatment processes would be essential for effective removal of such compounds. Quantification of hormones under different forms, including dissolved phase and attached to the suspended particles or sediments proved a perspective on their overall presence in the aquatic environment and the fate they are expected to follow during their journey from raw sewage to drinking water.

Since concentrations of hormones at highest levels in influent of WRRFSs are as low as ng L^{-1}, specific analytical techniques are required for the detection and quantification of these

compounds in aqueous and solid matrices. Especially in the case of real water samples which contain several types of impurities and detection of such low concentrations becomes more difficult. Recently, modern analytical methods are in use to reduce the duration of analysis by liquid chromatography and off-line extraction processes. Online SPE coupled with LC/MS-MS and Laser Diode Thermal desorption tandem MS-MS (LDTD/MS-MS) have been used for quantification and identification of steroid hormones [12, 13].

1.1 Problem under study

Despite the fact that surface waters provide large part of drinking water in Canada (89%)[1], few studies have been conducted on the occurrence of steroid hormones in different environmental systems [14-16]. Data are even more scarce on the effects of environmental conditions such as temperature variation and rainfall on steroid levels and the contribution of WRRFSs and CSOs in total steroids (dissolved and particulate phases) found in source and treated drinking water. Quantification of steroids in water systems without taking into account their fraction attached to the suspended particles would underestimate the total concentration of compounds. Therefore, investigation of the steroids profile in aquatic environments considering their concentration in both dissolved and particulate phases and also in sediments is necessary to provide realistic information on the occurrence and fate of these compounds. According to the physicochemical properties of steroid hormones, sorption to suspended particles in water or onto the sediments is fairly expected. Sorption onto solids and biological degradation are two main pathways for removal of steroids from aqueous phase. Steroid hormones are non-polar hydrophobic compounds that can be easily adsorbed onto river sediments or sludge particles. Hence, sorption to sludge particles might be an important way for steroid removal during wastewater treatment. Adsorption of steroids on aquatic sediments can directly affect their mobility, transformation, bioavailability and fate in the whole natural water systems. Studies of the sorption of steroids onto sediment and sludge are important, since these compounds have low solubility in water and high solid-water distribution coefficients which increase the chance of their removal through

[1] https://www.ec.gc.ca/eau-water/default.asp?lang=En&n=0BBD794B-1

wastewater treatment by sorption on sludge particles or from the other side their presence in suspended particles in wastewater effluent and their subsequent deposition in river sediments. Sorption of steroid estrogens (E1, E2, and EE2) onto sludge and sediment are comprehensively reviewed [17-19]. However, more investigations are still required on sorption kinetics of estrogens and also of the other groups of steroids such as progestogens and androgens.

From WRRFS effluent to receiving waters, steroids can reach drinking water treatment plants where many current drinking water treatment systems, such as ozonation, are expected to effectively reduce the trace concentrations of EDCs in drinking water to the below the detection levels. Ozonation is a multiuse advanced technology for water treatment and is applied in several countries such as the USA, Canada, Germany, and Switzerland. Beside the fact that ozonation appears among the most effective processes for micropollutant removal, it must be considered that there is limited information on the oxidation of recalcitrant compounds to oxidation such as progestogens and androgens, or the effect of different operationally relevant parameters such as pH and temperature on the removal efficiencies of such compounds.

1.2 Structure of dissertation

This dissertation consists of 8 chapters. Chapter 1 introduces the studied problem and explains the framework of research on occurrence and fate of steroid hormones in water treatment plants and receiving waters. Chapter 2 provides the literature review on the occurrence, functions, and environmental importance of hormones. The research objectives along with the methodology are presented in Chapter 3. Chapter 4 through 6 present research results in the form of two submitted scientific publications and also a chapter (chapter 5) on the adsorption kinetics of steroids in sediments. Chapter 4 covers the seasonal variations in general profile of steroid hormones in WRRFSs effluent, receiving river waters, and drinking water treatment plant intakes. The nature of sorption of steroid hormones to various sediments, the sorption isotherm and sorption kinetics of steroid hormones are determined in Chapter 5. Chapter 6 focuses on the ozone oxidation of 5 steroid hormones in natural water aimed to produce drinking water. Chapters 7 and 8 provide general discussion, conclusion, and suggestions for future work.

CHAPTER 2 LITERATURE REVIEW

2.1 General characteristics of steroid hormones

Natural hormones are secreted in human body and include progestogens, glucocorticoids (cortisol), androgens and estrogens [8]. A common structure for steroids is three hexagonal rings (A, B, and C) and one pentagonal ring (D). One of these rings is usually phenolic ring such as in estrogens [20]. Androgens and progesterones are less active than estrogens because these groups of steroids do not contain phenolic group. The phenolic group is usually linked to estrogenic activity and compounds with a phenolic group attached with OH group have strong estrogenic activities. 17β-estradiol (E2) is one of the most active estrogens with two OH groups at each end of its structure which are responsible of estrogen receptor (ER) binding. Any modification or substitution in these two OH groups can strongly affect E2 activity. Table 2-1 lists naturally produced and synthetic hormonal compounds used in hormonal therapy.

2.2 Specific properties of steroid hormones related to their environmental fate

After their release in the environment, different fates such as photodegradation, vaporization and in higher extent biological degradation and sorption are expected for steroid hormones [21]. Their specific characteristics and physicochemical properties affect their pathway through the aqueous and solid environments. This part presents physicochemical properties of hormones determining their fate in the environment. Parameters which are used for different steps of this research project are discussed in more detail in the following sections. Properties of mostly detected natural and synthetic hormones in different environmental matrices are summarized in Table 2-3.

Table 2-1: Natural and synthetic hormones produced in body or in hormonal therapy medications.

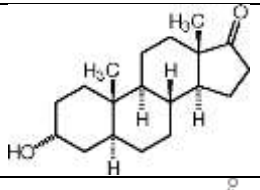
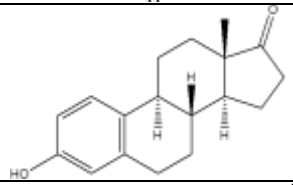
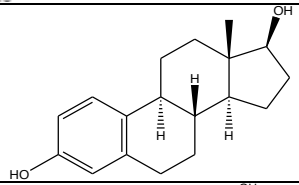
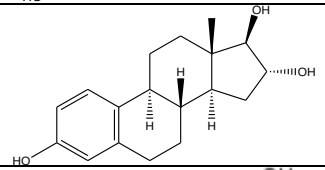
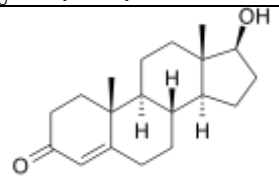
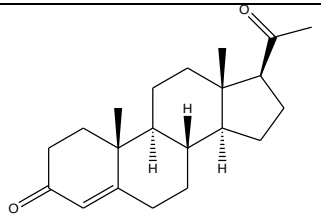
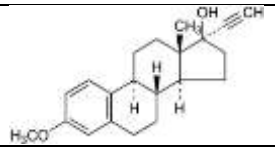
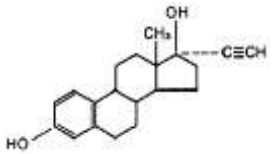
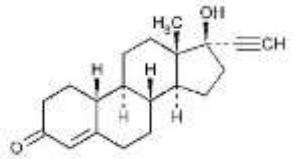
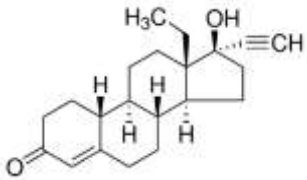
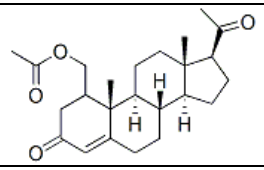
Hormones	**Structure**	**Molecular Weight [g mol^{-1}]**	**Application**
Androsterone		290.44	Weak androgen produced from metabolism of testosterone in liver
Estrone (E1)		270.37	Reproductive female estrogen
Estradiol (E2)		272.39	Predominant sex hormone present in females. Also present in males, being produced as an active metabolic product of testosterone
Estriol (E3)		288.39	One of the three main estrogens produced by the human body. Levels in female are not significantly different from levels in men
Testosterone		288.39	Responsible of male reproductive tissues as well as developing muscles, bone mass and body-hair
Progesterone		314.47	Steroid hormone involved in the female menstrual cycle and pregnancy of human and animals

Table 2-2(Continued): Natural and synthetic hormones produced in body or in hormonal therapy medications.

Hormones	**Structure**	**Molecular Weight [g mol^{-1}]**	**Application**
Mestranol		310.44	Derivative of ethinylestradiol used in firstly produced contraceptives
Ethinylestradiol (EE2)		296.4	Biologically active estrogen used in almost all of oral contraceptives
Norethindrone		298.43	The first orally active progestin synthesized to treat premenstrual and menopausal syndrome. It is used in some combined contraceptives
Levonorgestrel		312.46	Progestin compound used in hormonal contraceptives. A mixture of two isomers which only one of them is biologically active
Medroxyprogesterone		344.5	Medroxyprogesterone or its derivative Medroxyprogesterone acetate is progestin used to treat menstrual disorders

2.2.1 Water solubility and Octanol-Water partitioning coefficient (K_{ow})

Water solubility is the maximum amount of a dissolved substance in water at equilibrium (saturation) condition and at a given temperature and pressure. Water solubility is a good predictor of the mobility of the chemicals. The more soluble compound in water means, the more mobile in the environment is likely to be. Chemical compounds are divided in two groups according to their solubility in the water: polar compounds which are soluble and hydrophilic and non- polar compounds which are moderately soluble in water and hydrophobic. The solubility of steroids in water varies over a wide range from 1.13 mg L^{-1} for mestranol to 30 mg L^{-1} for E1.

Solubility in water is also correlated to another chemical property, the n-octanol/ water partitioning coefficient (K_{ow}) which is defined as the ratio of the dissolved concentration of a test compound in n-octanol and water at equilibrium. K_{ow} refers to the potential of a chemical compound to partition between water and organic phases such as the bioaccumulation in fatty tissue of microorganisms, or its potential to sorb to soil or sediments [22].

$$K_{ow} = \frac{C_{n-octanol}}{C_{water}} \qquad \text{Equation 1}$$

Where, K_{ow} is the n-octanol/water partitioning coefficient, $C_{n\text{-}octanol}$ is the concentration of test compounds in n-octanol and C_{water} is concentration of test compound in water.

A larger K_{ow} corresponds to a higher tendency of the compound to adsorb onto the soil or sediment's organic phase. Compounds with Log K_{ow} <1 are considered hydrophilic with low tendency to adsorption onto soil and sediment and also bio-concentration. In opposition, compounds with 3 < Log K_{ow} < 6 are highly hydrophobic and bioaccumulative [22]. pH also plays a critical role for partitioning potential affecting the charge of the sorbent and sorbate. At different pH values, the degree of ionization will change then the amount of ionized group which seems more hydrophilic and non-ionized groups which tend to sorb into lipid phase will differ. Ionic groups are more hydrophilic and do not tend to sorb in organic phase. The pH of 7 is usually used for environmental risk assessments. Considering both water solubility and K_{ow} is useful to determine the fate of chemicals in the environment. The value of log K_{ow} for hormones varies between 2.4 to 4.7. Synthetic estrogens have higher log K_{ow} values (Mestranol= 4.68,

EE2= 4.2) than natural estrogens (estrone=3.4, estriol=2.8) which increases their partitioning to the sediment and solid particles [23].

2.2.2 Vapor pressure, Henry's law constant

Vapor pressure of organic compound is a key factor for its distribution in the environment. Tendency of chemical compound to the gas phase affects its partitioning between aqueous or solid phases and gas phase and then its type of removal during treatment [24]. Volatile or semi volatile compounds may exchange between rivers, lakes or any source of water and atmosphere. Henry's constant (K_H), as shown in Table 2-3, is defined as the ratio of the concentration of test compound in gas phase (C_G) and to that in the liquid phase (C_L) at equilibrium.

$$K_H = \frac{C_G}{C_L} \qquad \text{Equation 2}$$

In water treatment applications, Henry's constants are commonly expressed as ratio of the mass of the volatile compound per unit volume of gas to the mass per unit volume of solution [25]. This form of Henry's constant is useful to qualify the solubility and volatility of a compound. During wastewater treatment, aeration stripes volatile or semi volatile chemicals from the water phase into air. The transfer depends on the aeration rate of wastewater and the Henry coefficient. In some cases, using mechanical equipment accelerates the system equilibrium. Also, in membrane bioreactors which use higher amounts of air compare to activated sludge processes, small extents of some pharmaceuticals may be stripped into the air. The higher the constant, the easier the compound is removed by air stripping [26]. For K_H more than $3*10^{-3}$ atm m^3 $mole^{-1}$, air stripping could be observed during aeration of bioreactors, but in the case of steroid hormones which have K_H values less than 10^{-8} no elimination via air stripping is expected. It is obvious, because almost all of these compounds are meant to be effective in aqueous phase (human blood).

2.2.3 Dissociation constant (pK_a)

When a compound is dissolved in a solvent, depending on how strong acid or base it is, it will donate or receive protons to/from solvent. The acid or base dissociation constant (K_a or K_b)

defines solubility and degree of ionization of acids and bases in water and represents the value of pH at which 50% of the compound is ionized in the water [27]. When compound AH is dissociated to its ions H^+ and A^-, the acid dissociation constant (K_a) is generally presented as follow:

$$AH \leftrightarrow H^+ + A^- \ , K_a = \frac{[H^+][A^-]}{[AH]} \ , PK_a = -log[K_a] \qquad \text{Equation 3}$$

pK_a has significant effect on distribution of chemical compound in the environment. At appropriate pH, ionized groups show higher water solubility than non-ionized groups and this will affect their partitioning between water phase and sludge or sediments phase during wastewater treatment. The degree of ionization depends on the pH of the solution. In the case of steroid hormones with different functional groups, the degree of ionization will be different for each group and also behavior of ionized or non-ionized groups is different.

2.2.4 Soil (sediment) - water partitioning coefficient (K_d)

The partitioning coefficient (K_p) or distribution coefficient (K_d) is the concentration of a compound sorbed into the solid phase to its dissolved concentration. Both K_p or K_d values are used for predicting the degree of hydrophobicity of compounds.

Shchwarzenbach [24] proposed an equation according to the ratio of the concentration in aqueous phase and in solid phase to assess the amount of a compound sorb into the solid phase at equilibrium condition.

$$K_d = \frac{C_s}{C_w} \qquad \text{Equation 4}$$

Where, K_d is the compound partitioning coefficient (L g^{-1}), C_s (mg g^{-1}) and C_w (mg L^{-1}) are the concentration of compound in solid (soil/sediment) and water, respectively. The reported K_d values for steroids range between 2.5 L kg^{-1} (E2) [17] to 108 kg^{-1} (E1) [28].

2.2.5 Organic carbon- water partitioning coefficient (K_{oc})

The estimation of K_d is rather difficult in real environmental samples because neutral hydrophobic organic compounds have shown different sorption affinities depending on the carbon content of the sorbent [24]. Therefore, another normalized sorption coefficient, the organic carbon-water partitioning coefficient (K_{oc}) is used for such compounds [29]. K_{oc} is the ratio of the mass of a compound adsorbed in the soil, sludge or sediment per unit mass of organic carbon content of the soil, sludge or sediment per concentration of compound in the solution at equilibrium. K_{oc} values are useful in determining the mobility of contaminants in soil and sediments. The higher K_{oc} values, the less mobile compound and opposite. K_{oc} values are usually used for estimating distribution coefficient (K_d) according to equation below:

$$K_{OC} = \frac{K_d}{f_{oc}} \qquad \text{Equation 5}$$

Where, K_d is the distribution coefficient (L kg^{-1}), K_{oc} is the soil organic carbon- water partitioning coefficient (mg L^{-1}), and f_{oc} is the organic carbon fraction of sludge or sediment.

Organic compounds containing hydroxyl or carboxyl groups dissociate by losing a proton and yield negatively charged groups. Groups with negative charge are more hydrophobic and then more mobile in soil compare to parent compound. Therefore, pH can directly affect the partitioning coefficient of compounds between the solid and liquid phases. K_{oc} value varies with several factors including, type of the soil or sediment, methods that have been used to measure K_{oc}, and analytical method errors. K_{oc} also varies between ionizing and non-ionizing compounds. Since, the pH of soil can affect partitioning of compounds, it can also affect K_{oc} value [22].

Table 2-3. Physicochemical properties of hormones affecting their environmental fate.

Compound	**Water solubility ($mg\ L^{-1}$)**	**Henry's Law constant ($atm\ m^3\ mole^{-1}$)**	**pK_a**	**Log (k_{ow})**	**Log K_d/Log K_{oc}**
Estrone	30	3.8E-010	10.4; 10.5	3.43[a]; 3.1-3.4[b]	2.69-3.1[a]; 2.4-2.9 [b]; 0.531 (sediment)[c]
Estriol	27.34	1.33E-012	10.4[f]	2.45[d]	-
17β-estradiol	3.90	1.41E-012	10.4 ; 10.71	3.94-4.0 [a] ;3.9-4.0 [b]	2.5-3.1 [a]; 2.4-2.8 [b]; 0.551 (sediment)[c]
Progesterone	8.81	6.49E-008	-	3.87[d]	-
Testosterone	23.4	3.53E-009	-	3.32[d]	0.66 (sediment)[c]
17α-ethinylestradiol	11.3	7.94E-012	10.5-10.7	3.9-4.2 [a]; 2.8-4.2 [b]	3.0-3.2 [a] ; 2.5[c] ;2.5-2.8 [b]
Mestranol	1.13	4.51E-009	-	4.68[e]	-
Medroxyprogesterone	2.95	1.34E-008	-	2.69 [f]	-
Norgestrel	2.05	7.7E-010	-	3.08[f]	-
Norethindrone	7.04	7.853E-012	-	2.97[f]	-

(a) [30] ; (b) [31]; (c) [32]; (d) [33]; (e) [23]; (f) [34];

2.3 Occurrence of hormones in different aquatic environments

Natural and synthetic hormones are excreted from human and animal bodies and end up in the environment via discarding animal waste or direct discharge of municipal sewage [1, 8, 21]. Beside natural and synthetic estrogens, occurrence of androgens and progestogens are also of high importance, since they exert from human in higher amounts compare to estrogens [35, 36]. Additionally, synthetic progestogens are extensively used in human and veterinary drugs. Megestrole acetate, Medroxyprogesterone acetate, and Norgestrel are the main continents of contraceptive treatments associated with estrogens. Figure 2-1shows the main routes of hormonal compounds into the environment.

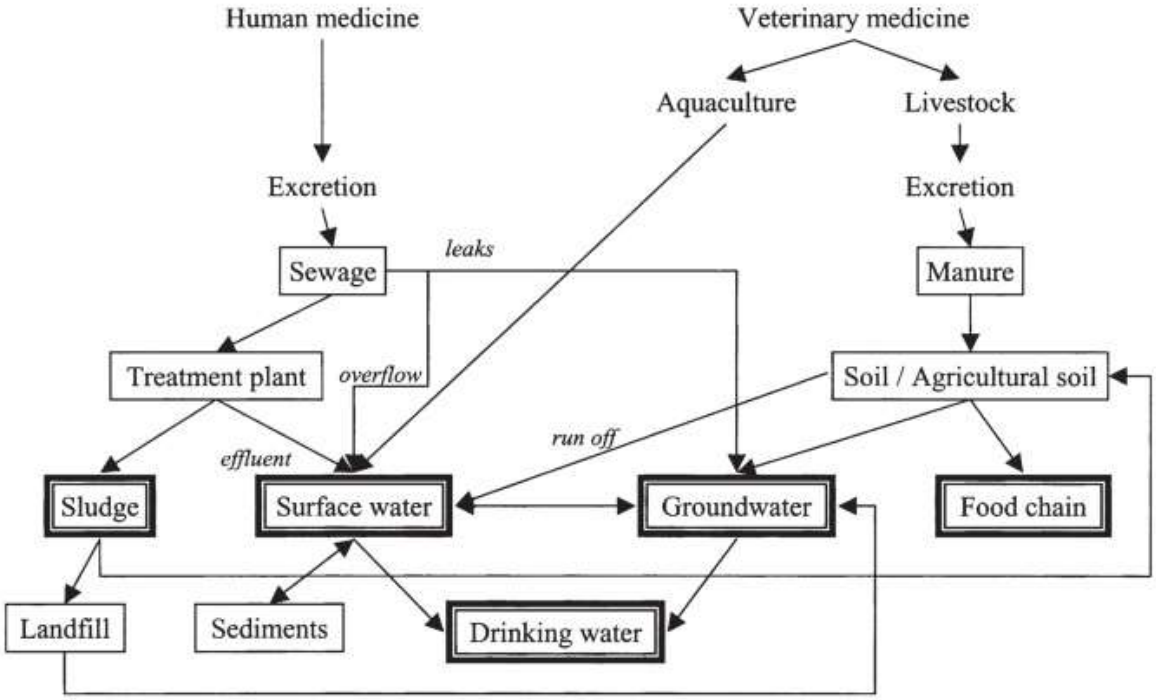


Figure 2-1. Routes of environmental exposure to hormones. Adopted from Kuster et al.2005 [37].

The occurrence of hormonal compounds in the environment is of high environmental relevance because they can interfere with the reproduction systems of human, livestock, fish and wild living animals [38]. Synthetic estrogens are also used for livestock farming and fish farming as growth-promoters and are excreted through manure [39]. The manure is applied to agricultural fields as fertilizers then un-metabolized drugs present in the manure or their biologically active

metabolites can threaten groundwater or reach surface waters through run-off [29, 36]. For this reason, numerous studies have been conducted on the occurrence, fate and health risks of hormonal compounds in the environment [1, 35, 40-43]. Direct correlations have been found between the feminization of male fish and the presence of hormonal compounds in several studies around the world [1, 10, 38, 44].

The following subsections provide a critical review on the recent studies on the occurrence of steroid hormones in different aquatic matrices including wastewater, surface water, ground water, and DW.

2.3.1 Wastewater treatment plants influent and effluent

Many of drugs are not completely metabolized in the human body and usually leave the body in the form of conjugates (e.g. sulfides and glucoronides) [45]. Un-metabolized and conjugated hormones enter sewage and reach WRRFSs, where different treatment processes are applied. These conjugates may be cleaved and reproduce parent compounds during wastewater treatment [46]. It is complex to predict the dynamic transformation of these compounds during wastewater treatment due to their wide range of classification and differences between behavior of parent compounds and their metabolites or conjugates. In the case of more stable compounds (e.g. estrone and 17β-ethinylestradiol), conventional treatment processes are not completely removed and they may reach surface waters through WRRFS effluents [33]. Consequently, in order to accurately evaluate their occurrence and potential risks in the environment, sufficiently sensitive and reliable analytical methods are needed to analyze the different classes of hormones in surface water, wastewater and solid matrices [33].

Steroid hormones have been detected in the influents and effluents of WRRFSs all around the world at concentrations up to hundred micrograms per liter [2, 4, 47, 48]. Table 2-3 presents the range on concentrations of detected steroids in the influents and effluents of WRRFSs by different researchers in Canada. Additionally, Chang et al. [35] analyzed the occurrence of estrogens, androgens and progestogens in seven WRRFSs and in the receiving waters in Beijing, China. Androgens were the most frequently detected hormones in all WRRFS influents and effluents followed by progestogens. Estrogens were found at lower concentrations as compared to two other groups of hormones. In surface water samples, androsterone, a natural androgen,

was the mostly abundant detected compound. Removal efficiency by wastewater treatment of androgens and progestogens were higher as compared to estrogens (91-100% versus 67-80%). A dominance of androgens in the WRRFS effluents and receiving waters has been also reported by Liu et al.[4]. The total concentration of androgens in the WRRFSs effluents was 2 folds greater than concentrations of estrogens and progestogens.

Table 2-4. Occurrence of steroid hormones in the dissolved phase of influents and effluents (ng L^{-1}) of WRRFSs in Canada.

Compound	Applied treatment process	Influent	Effluent	Removal Efficiency (%)	Reference
E1	AS	19-78	1-96	65	Servos et al. (2005)[49]
	AS	29.5	7.6	74	Lishman et al. (2006)[50]
	AS	11	14	-15	Fernandez et al. (2007)[51]
	AS	13.1-104	11.2-370	77- (-340)	Atkinson et al. (2012)[47]
E2	AS	2.4-26	0.2-14.7	88	Servos et al. (2005)[49]
	AS	8.3	ND	100	Lishman et al. (2006)[50]
	AS	ND-66.9	ND-26.7	-	Atkinson et al. (2012)[47]
EE2	AS	ND-5.5	ND-9.8	(-73) - 100	Atkinson et al. (2012)[47]
Testosterone	AS TF Lagoon	42-44 20	0 5	100 76 69	Fernandez et al. (2007)[51]

2.3.2 Surface water

A variety of hormonal compounds have been detected in sewage treatment plant effluents and the surface waters in which they were discharged by researchers in Europe and North America [45, 46, 52-54]. The concentrations of steroid hormones in surface water samples while generally at the low ng L^{-1} level can in certain cases exceed 100 ng L^{-1}. Higher concentrations are reported in samples taken near the discharge of WRRFs effluents. Other factors affect their concentration in surface water including the sampling location (distance from point sources and mixing patterns), and the proportion of wastewater discharges in reference to the flow rate of the receiving river. Local mixing of the wastewater effluent in river flow reduces the concentration of contaminants through dilution, impacting the distribution pattern of micropollutants down flow of WRRFs discharges.

The level of contamination of surface waters by estrogens has been estimated by direct measurement of target compounds using analytical methods for specific compounds such as (E1, E2 and E3 and EE2) [55] or by evaluating the estrogenic activity of water samples [9, 56]. Table 2-1 summarizes findings from selected studies on the occurrence of steroids in surface water from different countries. Vulliet et al. (2011) investigated the occurrence of pharmaceutical compounds including 25 steroids in surface waters across France [43]. Only estrone, the most frequently detected estrogen in surface waters, was detected in 8 samples at concentrations in range 0.08-2 ng L^{-1}. However, testosterone, progesterone, levonorgestrel, and norethindrone were detected in all the samples with mean concentrations of 0.1-15.6 ng L^{-1}. Cargouët et al. 2004 examined samples taken along the Seine River (Paris) to evaluate the presence of estrogens in surface waters [56]. Their results showed that surface waters down stream of WRRFS in the Paris area are contaminated by steroid estrogens (E1, E2, E3 and EE2) with concentrations in range of 0.3- 3.9 ng L^{-1}. Contrary of steroid levels detected in France surface waters, Kolpin et al found considerable amount of estrogens in US streams [3]. The median detected concentration of E1, E2, E3, and EE2 were 27, 160, 19, and 73 ng L^{-1}, respectively. Same levels were found for progesterone (110 ng L^{-1}) and testosterone (116 ng L^{-1}) and norethindrone (48 ng L^{-1}). In another study on the occurrence of steroids in US surface waters, testosterone levels were below 1 ng L^{-1} in all samples, whereas progesterone was detected in range of 14-148 ng L^{-1} [57].

2.3.3 Groundwater

Concerns about contamination of ground water with micropollutants rises when surface water or treated/ untreated wastewater is used to recharge ground water. Contamination of groundwater by steroids can occur during infiltration of contaminated surface water as well as wastewater drains and landfill leakages [58-60]. Because of their lipophilic structure and their resistance to biological degradation, steroids may penetrate through subsurface and reach underground drinking water sources. In one study by Vulliet et al., traces of 52 pharmaceuticals including 26 steroid hormones were investigated in 70 groundwater and 71 surface waters in southeast France [60]. The results of the analyses indicate that 11 out of 26 the steroids were detected in all samples regardless of the origin of water and the sampling season. Estrone was detected in 28

samples with concentrations in range of 0.1- 1 ng L^{-1}. Estradiol and ethinylestradiol were detected in a few samples (10 and 9 % pf samples, respectively), while testosterone and progesterone were present in more than 93% of the samples with mean concentrations of 1.4 and 1.6 ng L^{-1}.

Table 2-5. Occurrence of steroids in the dissolved phase of surface waters in different countries.

Compound	Concentration ($ng\ L^{-1}$)	Location	Reference
E1	<9.7	Canada	Naldi et al. (2016) [61]
	0.5-20.9	Australia	Ying et al. (2009) [62]
	0.017-0.29 2.6-22.9 112(max)	USA	Furlong et al.(2017)[63] Sellin et al. (2009) [9] Kolpin et al. (2002) [3]
	0.8-3.9	France	Cargouet et al. (2004)[56]
	3.6-69.1	Korea	Kim et al. (2009)[64]
E2	<9.5	Canada	Naldi et al. (2016) [61]
	0.3-3.7	Australia	Ying et al. (2009) [62]
	1.9-14.5 200(max)	USA	Sellin et al. (2009) [9] Kolpin et al. (2002) [3]
	0.8-3.6	France	Cargouet et al. (2004)[56]
	1.1-10.1	Korea	Kim et al. (2009)[64]
E3	<10	Canada	Naldi et al. (2016) [61]
	0.6-3.1	France	Cargouet et al. (2004)[56]
EE2	<25	Canada	Naldi et al. (2016) [61]
	n.d-0.5	Australia	Ying et al. (2009) [62]
	831(max)	USA	Kolpin et al. (2002) [3]
	0.6-3.5 1.6	France	Cargouet et al. (2004)[56] Vulliet et al. (2011)[60]
	214(max concentration) <0.3- 1.9	USA	Kolpin et al. (2002) [3] Kolodziej et al. (2004)[65]
	8.6 n.d-0.83	China	Chang et al. (2009) [66] Zhou et al. (2016) [67]
Testosterone	0.15	USA	Furlong et al.(2017)[63]
Progesterone	0.15	USA	Furlong et al.(2017)[63]
Medroxyprogesterone	Trace (<2)	Canada	Viglino et al. (2008([68]
	2.1	China	Chang et al. (2009) [66]
	<0.4-1	USA	Kolodziej et al. (2004)[65]
Levonorgestrel	n.d-4.7 <3.8-5.9	France	Vulliet et al (2011)[60] Labadie et al (2005)[69]
	872 (max)	USA	Kolpin et al. (2002) [3]
Norethindrone	2.7-2.8 2.0	France	Vulliet et al. (2008) [70] Vulliet et al. (2011)[60]

2.3.4 Sludge, sediments and biofilms

The partitioning of hormones between the water and solid phases is critical to their fate in aqueous systems [23]. Knowing the physico-chemical characteristics of steroid hormones, they are expected to sorb onto the solid phase. The majority of studies on the occurrence and fate of hormones in the environment have focused on their presence in the aqueous phase, while ignoring their occurrence in the solid phases. This was in part due to the lack of highly advanced analytical methods for the quantification and qualification of these compounds in solid matrices at low ng L^{-1} concentrations [71]. The few studies conducted on the partitioning of steroids between both dissolved and particulate phases have confirmed the extensive amount of steroids attached to the suspended particles of water or to sediments [5, 17, 48, 72]. According to Octanol-water and water-sediment partitioning coefficients for estrogens ($3.25< \log K_{oc}<3.7$ and $2.81< \log K_{ow}<4.15$), these hydrophobic compounds tend to adsorb onto sediments with high organic carbon contents [8]. Carballa et al (2008) investigated the partitioning of pharmaceuticals and estrogens in WRRFS sludge [73]. Hormones such as E2 and EE2 (log K_d between 2.5-3.0) with a high solid-water partitioning coefficient (K_d) showed higher tendency to bind with solid particles (80-99% sorbed to sludge particles) as compared to pharmaceutical compounds such as ibuprofen and carbamazepine (log K_d between 0.09-1.83). In one study by Huang et al. 2014 [74], testosterone and progesterone were detected in sewage sludge from six WRRFSs in China with concentrations in range of n.d-1.5 and n.d-3.8 ng g^{-1}, respectively. Another study by Wu et al. (2017)[75] reported higher levels of progestogens and testosterone in sewage sludge with concentrations of 0.9-29.3 ng g^{-1} for progesterone and 1.2-2.2 ng g^{-1} for testosterone in thicken sludge. The measured levels in sludge cake were much higher for two compounds with concentrations ranging between 18-238 ng g^{-1} and 2.1-26.1 ng g^{-1} for progesterone and testosterone respectively. Norethindrone was detected in the thicken sludge of one treatment plant at a mean concentration of 75.6 ng g^{-1}. The lowest concentrations were detected in thicken sludge of WRRFS with tertiary treatment processes.

Limited information is available on the occurrence of progestogens and testosterone in river sediments [72, 76, 77]. Table 2-5 presents the occurrence of steroids in river sediments from different locations around the world. Among the 21 progestogens studied by Liu et al. (2014) in sediments of river in China, only progesterone was detected at 3.4 ng g^{-1} while concentrations of

norethindrone and levonorgestrel were below the detection limits (0.02-0.53 ng g^{-1}). In another study by Huang et al. (2015), progesterone and testosterone were detected in sediments at 8.1 and 2.4 ng g^{-1}, respectively. Steroid persistence in solid samples depends on the adsorption capacity of solid particles, biological and photolytic degradation rates, and half-lives in the studied system [29]. Lai and Johnson [23] studied the partitioning of natural (Estradiol, Estrone, Estriol) and synthetic (EE2 and Mestranol) hormones between water and sediments in U.K. rivers. A mixture of 100 ng mL^{-1} of selected estrogens was added to water and sediment mixture (3 g of sediment per 200 mL of aqueous sample). The sorption rate was highest in the first 30 minutes followed by a continuous decrease. The saturation of binding sites and the concentration of available estrogens were identified as the cause of the reduction in sorption rate. The synthetic estrogens with higher K_{ow} values were demonstrated greater tendency to sorb onto sediments as compared to natural estrogens. In another study by Lei et al (2009), the occurrence of estrogens (E1, E2, E3, EE2, DES, and EV) was determined in surface water and sediments from three rivers in Northern China [78]. The mean concentration of total estrogens varied between 13.4-28.5 ng g^{-1}, with the maximum concentrations related to E1 (0.98-021.85 ng g^{-1}) and E2 (n.d.-9.7 ng g^{-1}), respectively.

Another source of steroids in surface water was introduced by Writer et al. 2011 who showed that estrogens were found to accumulate in stream biofilms [79]. Estradiol and ethinylestradiol were readily sorbed to in situ colonized stream biofilm with partitioning coefficients of $10^{2.5-2.9}$ L kg^{-1}. The sorption of estrogens to stream biofilm was linearly correlated with organic content of biofilm indicating the dominance of hydrophobic interactions driving the sorption of estrogens onto biofilm. In another study by same authors, biodegradation of estrogens by stream biofilm and sediments collected from upstream and downstream of WRRFs were investigated. Although biofilms showed high sorption capacity for estrogens, the biodegradation rate of estradiol was higher in river sediments and ethinylestradiol was not degraded at all in biofilm over the time intervals of 70 and 185 d. Consequently, accumulation of estrogens in stream biofilm was suggested to occur following their rapid sorption and lower degradation rates [80].

Table 2-6. Occurrence of steroids in river sediments from different countries.

Compound	Concentration (ng g^{-1})	Location	Reference
E1	6-16	Canada	Viglino et al. (2011) [76]
	n.d-3.5 0.98-21.6	China	Gorga et al. (2015) [52] Lei et al. (2009) [78]
	n.d-3.55	Spain	Lopez de Alda et al (2002) [77]
E2	149 22-70	Canada	Darwano et al. (2014) [5] Viglino et al. (2011) [76]
	n.d-1.6 n.d-9.7	China	Gorga et al. (2015) [52] Lei et al. (2009) [78]
	n.d-1.2	UK	Labadi et al. (2007)
E3	6-18	Canada	Viglino et al. (2011) [76]
	n.d-1.5 n.d-7.29	China	Gorga et al. (2015) [52] Lei et al. (2009) [78]
	n.d-3.37	Sapin	Lopez de Alda et al (2002) [77]
EE2	86 n.d-30	Canada	Darwano et al. (2014) [5] Viglino et al. (2011) [76]
	n.d-22.8	Spain	Lopez de Alda et al (2002) [77]
	n.d-2.1 n.d-9.26	China	Gorga et al. (2015) [52] Lei et al. (2009) [78]
Progesterone	n.d-6.82	Spain	Lopez de Alda et al (2002) [77]
	111 <LOD-12	Canada	Darwano et al. (2014) [5] Viglino et al. (2011) [76]
	8.1 (mean concentration)	China	Huang et al. (2015) [81]
Medroxyprogesterone	n.d-29	Canada	Viglino et al. (2011) [76]
Levonorgestrel	n.d-2.18	Spain	Lopez de Alda et al (2002) [77]
	41 n.d-19	Canada	Darwano et al. (2014) [5] Viglino et al. (2011) [76]
Norethindrone	n.d-1.08	Spain	Lopez de Alda et al (2002) [77]
	45 n.d-90	Canada	Darwano et al. (2014) [5] Viglino et al. (2011) [76]
Testosterone	2.4 (mean concentration)	China	Huang et al. (2015)[81]

2.3.5 Drinking water

Since the occurrence of steroids in effluents of WRRFSs, surface waters and ground waters is confirmed by several studies, concerns have been raised over the presence of these compounds in drinking water sources and the potential associated risk of human exposure. However, human exposure to steroid hormones or in general pharmaceuticals via drinking water is unlikely in

developed countries as a result of the effective treatment processes applied in DWPs. Web et al. (2003) compared the daily therapeutic dosage and potential indirect exposure to pharmaceuticals including EE2 via DW. A minimum 1000 margin was found between the therapeutic dose and daily intake of all studied compounds. In case of EE2, <0.5 ng L^{-1} as maximum detected level in drinking water compare to 25.5 µg L^{-1} as lifetime intake (based on 2 l day^{-1} for 70 years). Adverse health effects of human exposure to high concentrations in contaminated water include allergic reactions, endocrine disrupting effects, breast cancer, and thyroid gland disorders. However, it must be noted that the impacts of chronic human exposure to a mixture of low level pharmaceuticals via drinking water are not fully understood. Although concentration of pharmaceuticals detected in drinking water is few ng L^{-1}, chronic exposure to these compound may have different effects than daily therapeutic doses and there is more concerns about their health effects on human health especially children and fetus which may expose to drugs that have been used by mother [82, 83].

Three factors may affect the amount of hormones in drinking water: i) the location of treatment plant (near wastewater treatment plants or contaminated water sources), ii) the degree of contamination of the source waters, and iii) the water treatment processes used for providing drinking water [84]. Clarification and sand filtration have shown very limited steroid removal efficiencies [85-87]. Filtration with activated carbon is expected to efficiently remove steroids with high log K_{ow} [58, 86, 88]; while oxidation processes such as chlorination, potassium permanganate, ozonation, and advanced oxidation processes have shown reliable removal efficiencies for some steroids [89-93]. The effectiveness of different drinking water treatment processes is more debated in section *2.5. Fate of steroids during drinking water treatment.*

Contrary to surface waters, very limited studies were reported the presence of steroids in treated drinking water. In Canada, Metcalf *et al.* (2014) detected estrone at concentration of 1.5 and 1.6 ng L^{-1} in treated drinking water in two DWPs from 5 measured DWPs of Ontario [94]. Estriol and progesterone were detected in one of three studied DWP in Spain at 11.6 ng L^{-1} and 0.93 ng L^{-1}, respectively [95]. Norethindrone, levonorgestrel, progesterone, and testosterone were found in in treated water of 8 DWPs in France with maximum concentrations of 6.8, 10, 10.7, and 26.4 ng L^{-1}, respectively [43]. While only progesterone was detected at 0.2 ng L^{-1} of one DWP among the 50 studied DWPs over the U.S [63]. A daily exposure of 10 ng L^{-1} of norethindrone has been

reported to cause negative effects on pregnant women and fetus [96] and USEPA has recently added this compound between the contaminant candidate list (CCL4).

2.4 Fate of steroids in natural environmental systems

The possible removal pathways of steroid hormones from different natural environments include volatilization, sorption, photo-transformation, and biological degradation. In the following sections, these pathways are discussed in light of previous studies available in literature.

2.4.1 Volatilization

The extent of volatilization of steroid hormones can be estimated by their vapor pressure or the Henry's law constant (H). Most steroid hormones have high molecular weight with vapor pressures ranging 10^{-10}-10^{-15} mm Hg [8] [97] and tend to remain in the aqueous phase. Therefore, volatilization is not a considerable mechanism for removal of these compounds during their travel upstream to downstream of surface waters.

2.4.2 Phototransformation

Once steroids enter surface waters, photo-transformation can influence the fate of steroids if the surface water is exposed to enough sunlight. Photo-transformation of steroids can affect their degradation products and their estrogenic activity. Estrogens (E1, 2, E3, and EE2) were showed moderate to high degradation in river water when exposed to xenon arc lamp (765W m^{-2}; 290 nm $< \lambda <$700 nm) with half-lives ranging from 2 to 3 h [98]. The intensity of light under xenon arc lamp (765 Wm^{-2}) was reported to be identical to that of midsummer sunlight in California. In another study by Young et al 2013 testosterone was readily degraded under direct sunlight with half-life ranging between 7.6 to 10.8 h at temperatures varying between 19 and 42 °C [99]. Dissolved organic matter (DOM) in surface water was reported to enhance (acting as photosensitizer) or retard the photo-transformation of steroids depending to on the structural properties of DOM (Lin2005 and Young2013). The photo-transformation of steroids in surface waters depends on solar irradiation, suspended solids concentration, and the quantity of DOM acting as photosensitizer [100].

2.4.3 Biological degradation

Biodegradation is reported to act as one of the main mechanisms to reduce the aqueous concentration of steroids in natural environments. Different studies investigated biodegradation of steroids suggest that microorganisms present in raw sewage and in different steps of wastewater treatment can transfer steroids from conjugated form to parent compounds [8, 81, 101-103]. Therefore, steroids are released into surface waters under the conjugated or unconjugated forms [40]. After their release into surface water, microorganisms in water and sediments can degrade steroids. Jürgens et al. 2002 investigated the degradation of estrogens in English river water and sediments. E1 and E2 were degraded with half-lives of 0.2-9 d while synthetic estrogens ethinylestradiol were found to be much more resistant to biodegradation [104]. Degradation rates of E2 remained unchanged in spiked samples throughout the range of 20 ng L^{-1} to 500 μg L^{-1}. Bradley et al. 2016 investigated the potential of estrogens degradation in sediments from surface waters in pristine location (Colorado, USA) [105]. Under aerobic conditions, both natural (estrone and estradiol) and synthetic (ethinylestradiol) estrogens were effectively mineralized to radiolabel CO_2.

Widespread algae present in aquatic environment are reported to affect the fate of micropollutants including steroid hormones via adsorption and biodegradation. Two fresh water microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* were cultivated in laboratory to assess their potential to degrade progesterone and norgestrel [106]. Both progestogens were significantly degraded by microalgae within 5 days following the first order reaction model. The half-lives of norgestrel were 2 times longer than that for progesterone (16 and 39 h for progesterone while 40 and 88h for norgestrel with *S.obliquus* and *C.pyrenoidosa*, respectively). Biodegradation resistance of norgestrel with bacteria from activated sludge was also reported previously with the half-life of 12.5d for norgestrel and 4.3h for progesterone [107].

Biological degradation of steroids in the environment depends on several factors including pH, temperature, organic matter content, redox condition and moisture content of soil and sediment.

2.4.4 Sorption of steroid hormones onto solid phase

Adsorption of a specific compound refers to association of positively charged groups of that compound with negatively charged surfaces of organic fraction of adsorbent (solid or liquid) while absorption refers to interactions of hydrophobic groups of compound with lipophilic part of organic content of adsorbent. In the aquatic environment, the liquid phase could be surface or ground water, WW influent/ effluent, or treated natural water while solid phase could be sludge particles, suspended particles, soil or sediments. Sorption has an important effect on the mobility, bioavailability, and fate of hormones in the aquatic environment. Sorption of steroids onto sediments or sludge may have a dual effect on their fate. It can reduce their levels in natural waters or in treated water and augment their biodegradation via biomass in solid phases. It can also lead to an subsequent increase following their desorption from the solid phase, for example after snow melt or rainfall [18, 19, 108]. Steroids may directly introduce to soil if sludge from treatment plants is applied for agricultural activities. However, information on the sorptive behavior of steroids to environmental solids is quite limited. Most of studies on the sorption of steroids are focused on the determination of solid-liquid distribution coefficient (K_d), sorption rates and usually under uncontrolled biological conditions [18, 19, 32, 109].

Based on the K_{ow} of steroids (typically between 3 and 5), these compounds are expected to sorb onto sludge. Direct correlation was found between the amount of estrogens and organic content of soil and sediment [110], particle size distribution [111], and also salinity in water [97]. Steroid estrogens E1 (61%), E2 (66%), and EE2 (70%) were adsorbed during activated sludge treatment whereas only 0.2% (E1), 0.24% (E2), and 0.29% (EE2), were sorbed to sludge particles in the effluent as the amount of suspended particles in sludge was 800 times more than that in the effluent [19]. Horsing et al measured the fraction of steroids sorbed to the sludge and estimated their K_d values [112]. More than 96 % of estradiol (E2) was adsorbed to the sludge while levonorgestrel and medroxyprogesterone were both adsorbed >98%. A slightly lower sorption was observed for progesterone with 88- 94% sorbed fraction.

Several types of sorption isotherms models have been applied to describe the adsorption of pharmaceuticals and, to a lesser extent, of steroid hormones. The most suitable isotherm models for this purpose are linear, Freundlich, Langmuir, and Distributed Reactivity Model (DRM) [24].

Nonlinear isotherm model is suggested for adsorption of estrogens on sludge (Chen2010 and Lai and Andersen). Lai et al measured sorption coefficients of natural estrogens (E1, E2, E3) and synthetic estrogens (EE2, and MeEE2) onto sediments proposing nonlinear sorption model (Freundlich) with K_f values were 1.71, 1.56, 1.33, 1.72, and 2.26 ($mg^{1-1/n}$ $(m^3)^{1/n}$ g TSS^{-1}), respectively. In another study by Andersen, Freundlich and Linear models equally fitted the isotherm data with K_f values of 89 (E1), 1106 (E2), and 383 (EE2) L^n ng^{1-n} kg^{-1}. Lee et al. also estimated the sorption isotherms for E2, EE2, and Testo in different soils [109]. Linear models fitted for the majority of the studied soils except for one sample, for which the nonlinear Freundlich model fitted because of higher OC% (2.91%) and smaller particle size fraction (21% clay) as compared to other samples.

Despite numerous studies on the sorption behavior of estrogens in solid phases, information is very limited on the sorption kinetics of these compounds. Data is especially scarce for progestogens and androgens as they have received less attention because of their lower estrogenic activity. Results from previous experiments indicate rapid steroid sorption kinetics, approaching equilibrium within a few hours and usually following a pseudo second-order kinetic model [109, 113]. Feng et al investigated the adsorption of EE2 on inactivated sludge [113]. The amount of sorbed EE2 on the sludge during the defined contact time (15 min) increased from 52.9% to 87.9% when the initial concentration of EE2 increased from 0.5 to 5 mg L^{-1}. Among the different kinetic models which applied to kinetic data for EE2, the pseudo second-order model best fit the obtained kinetic data (r^2 >0.99). Another study by Cunha et al. also proposed a pseudo second-order kinetic model for the adsorption of E1, E2, and EE2 to different tropical sediments from Brazil [114]. The amount of estrogens adsorbed to sediments at equilibrium using the kinetic model ranged between 36-140, 81-153, and 49-40 µg g^{-1} for E1, E2, and EE2, respectively. The higher the organic content of the sediment sample, the longer time was taken to achieve equilibrium and also the lower was the calculated sorption kinetic constants. However, the amount of adsorbed estrogen was 10-30% higher in samples with higher OC% (25% as compared to 13%). Also estrogens were competing for interaction with organic content of sediment when the amount of OC is limited while adsorption was similar for all estrogens in sample with highest OC.

To best of our knowledge, there is no report on the comprehensive analysis and quantification of progestogens and androgens in suspended particles of WW and river water. In the study by Andrasi et al., E2 (0.0049-0.032 µg L^{-1} WRRFs effluent) and EE2 (0.35-0.46 ng L^{-1} in river particles) were detected in suspended particles of WRRFs effluent and river water. Their results revealed the important fraction of steroids attached to the suspended particles of WW and surface water with 71% and 64% of the total steroids detected in particulate phase.

Comprehensive discussion on sorption behavior and sorption kinetic of estrogens, progestogens and testosterone is provided in Chapter 5.

2.5 Fate of steroid hormones during wastewater treatment

WRRFSs have an essential role in removal; however steroid hormones, conventional WW treatment processes are not designed to remove such micropollutants and ensure complete or partial removal to very low concentrations (ng L^{-1}). Various mechanisms are expected for steroid removal in WRRFSs including sorption to suspended solids, volatilization, and biodegradation. The effectiveness of various WW treatment processes is discussed as follow considering the recent studies on the mechanism and fate of steroids removal in WRRFs.

2.5.1 Primary treatment

During primary treatment only micropollutants which have highly sorptive characteristics (lipophilic) may attached to solid particles and eliminated. It is expected that conjugates attach together to produce parent compounds, then increase the concentration of target compound in primary effluent or compounds with high log K_d values (> 2.5) sorb into primary sludge [31]. For natural and synthetic hormones it is difficult to conclude if they may remove during primary treatment or not because wide range of K_d values have been reported for these compounds (1-200) [17, 31, 32, 115]. The fate of steroids in primary treatment depends on various parameters such as temperature, pH, HRT, SRT and solid particles characters [31].

2.5.2 Secondary treatment

Secondary treatment processes including activated sludge, aerated lagoon, sequencing batch reactor, oxidation ditch, trickling filters, and membrane bioreactor show varying efficiencies of

steroid removal. Activated sludge processes can be highly efficient for different groups of steroid hormones [20, 21, 40, 74]. In one study, Vymazal et al. (2015) evaluated the removal of estrogens, testosterone and progesterone from three constructed wetlands in Czech Republic [116]. Only estrone was detected in one WRRF's effluent at a level of 5.9 ng L^{-1}. The concentration of progesterone and testosterone were below the detection limits (< 0.5 ng L^{-1}). In another study, Liu et al. compared removal of steroids in two WRRFs with activated sludge (Plant A) or oxidation ditch (Plant B) [4]. Both treatment plants had good removal efficiencies in steroid removal. However, testosterone and progesterone were removed more efficiently in Plant A (102 and 101%) than in Plant B (68 and 75 %). Biodegradation was suggested as the main degradation process for conjugated hormones while progestogens, androgens, and estrogens where removed by both sorption and biodegradation.

Among the secondary treatment processes, trickling filters are reported to be the least efficient process for steroid removal [41, 49]. In trickling filter treatment system, wastewater passes through a fixed layer of plastic or rock media and over the time a biofilm grows on the reactor's circular bed [117]. The low retention time (usually one day) in these systems is suggested as the main reason of low steroid removal efficiency, since a wide range of halve life are reported for biodegradation of steroids, from 4 hours for E2 removal from WRRF biosolids [118] to 12.5 days for norgestrel in activated sludge system [107].

2.5.3 Tertiary treatment

Tertiary treatment processes and advanced wastewater treatments include ozonation, chlorination, UV disinfection, and nitrifying/ denitrifying activities. Chlorination and ozonation removed up to 100% of natural estrogens and synthetic estrogen EE2 [4, 119-121]. Ozonation in wastewater treatment is usually applied for disinfection after secondary biological treatment especially in the case of water reuse or when the WRRFs effluent is used for agriculture irrigation [122]. Ozonation is also used to improve the efficiency of other processes such as coagulation-flocculation-sedimentation or carbon filtration or to improve biological processes by breaking biologically refractory heavy molecules into easily biodegradable compounds. Wastewater disinfection by ozone is not as common as chlorination and UV. However, with increasing indirect potable water reuse of treated urban wastewater, ozone is commonly applied

in countries like Switzerlan, Germany and Canada, because disinfection by ozone ensure significant oxidation of micropollutants and reduces the risk of THM formation [123]. Indeed, testosterone was removed from wastewater (> 44%) with 3.6 mg O_3 L^{-1} and more than 98% with 7.1 mg O_3 L^{-1} [124]. Natural estrogens were also removed efficiently during nitrifying/ denitrifying processes up to 98% [125].

The combination of secondary and tertiary treatment processes can increase steroid removal efficiencies up to 100% and reduce the release of these compounds into the receiving waters.

2.6 Fate of steroid hormones during drinking water treatment

The effect of hormonal compounds on human health effect is not clear as some evidence suggests that hormones at these very low concentrations have no effects on humans. However, their removal is driven by the precautionary rule in absence of complete health information and the acceptance of consumers that calls for high quality drinking water free from any contaminant. Conventional drinking water plants (DWPs) processes usually include of coagulation/flocculation, sedimentation, filtration, and disinfection. To estimate the reduction of the target compounds in drinking water treatment, the following parameters must be considered [126]:

- The dissociation constant (pK_a) of compound, because neutral and ionic forms of compound act differently.
- The second order rate constant (K_{oxy}) of oxidation process.
- The Octanol-water partitioning coefficient (K_{ow}), in the case of adsorption by activated carbon (AC), K_{ow} can indicate the tendency of micropollutants toward AC.
- Molecular weight and surface charge of the membrane as well as molecular weight, shape, and charge of the target compound are determining factor for membrane filtration.

Coagulation/flocculation is not expected to remove polar and hydrophilic hormones because these processes are designed to remove hydrophobic compounds associated with particulate matters. Chang et al. investigated adsorption of estrone (initial concentration of 15 ng L^{-1}) to $FeCl_3$ (5-50 mg Fe L^{-1}) as coagulant through jar test [127]. Despite a good removal of TOC (50%), no remarkable reduction observed in concentration of estrone. Westerhoff et al. studied

the removal of 62 pharmaceutical compounds including 6 hormones from three natural waters using adsorption processes including metal salt coagulation and powdered activated carbon, and oxidation processes including chlorination and ozonation [86]. They concluded that alum sulphate and ferric chloride can only remove less than 20% of hormonal compounds. Activated carbon can be effective to remove pharmaceutical and hormonal compounds [88, 128, 129]. Adsorption with activated carbon is dominated by hydrophobic interactions with organic compounds [128]. Therefore, more removal will be observed for compounds with higher Octanol-water partitioning coefficient (K_{ow}) [129].

Chlorination and ozonation are known as the most effective processes for oxidation ofa majority of pharmaceuticals [91, 124, 130, 131]. For this reason, the effectiveness of these two powerful oxidants in steroid oxidation during drinking water production is more discussed in following sections. Table 2-7 presents the summary of selected literature on removal of steroid hormones selected in this research during drinking water production while Table 2-8 summarizes the available rate constants for the reaction of steroids with ozone, hydroxyl radicals, and chlorine (sodium hypochlorite).

Table 2-7. Summary of studies on applied removal processes on steroid hormones during drinking water production.

Treatment	Estrogens	Progestogens	Androgens
Conventional treatment	Chang et al. 2004[132] Chen et al. 2007[133] Huerta-Fontela et al. 2011[85]	Westerhoff et al. 2005[86]	Westerhoff et al. 2005[86] Kim et al. 2007 [134]
Adsorption	Westerhoff et al. 2005 [86]; Snyder et al.2007[90]	Westerhoff et al. 2005[86]; Snyder et al.2007[90]	Westerhof et al. 2005[86]; Snyder et al.2007[90]
Oxidation (Cl_2)	Chen et al. 2007 [133] Huerta-Fontela et al. 2011[85]	Westerhoff, et al. 2005 [86]	Westerhoff, et al. 2005 [86] Kim et al. 2007 [134]
Oxidation ($KMnO_4$)	Jiang et al. 2012 [135]	Fayad et al. 2013[136]	-
Oxidation ($O_{3)}$	Huber 2004 et al. [137]; Debord et al. 2005 [119]	This study; Broséus et al.2009[130]; Barron et al. 2004 [138]	This study
Advanced oxidation (OH°)	Nakonechny et al.2008 [139]; Rosenfeldt et al. 2004 [140]; Huber 2004 et al. [137]	-	-

2.6.1 Ozonation during drinking water production

Application of ozone in drinking water has two main advantages, disinfecting the treated water and removing micropollutants including steroid hormones. Ozonation is more effective to remove iron and manganese as well as taste and odor, as compared to chlorine and chlorine dioxide [89]. However, at high concentration of bromide (> 50 µg L^{-1}), oxidation by ozone can increase the risk of bromate formation [141]. In conventional drinking water treatment, usually 1-3 mg/L ozone is generally sufficient to achieve disinfection goals [89].

Ozone can react with organic matter existing in water through two pathways: the oxidation of compounds via production of ·OH° radicals or direct oxidation of molecule [129]. The nature of treating water and pH determine which pathway will dominate the oxidation of organic compounds. Advanced oxidation processes (AOPs), including UV/O_3, H_2O_2/O_3, and UV/H_2O_2, can increase the production and concentration of OH° radicals and improve oxidation of trace

organic contaminants. While using ozone for oxidation, disinfection can be reached at the same time. However, when target compounds are resistant to ozonation, it is necessary to convert ozone into OH° radicals (advanced oxidation processes). This transformation step is not in favor of the aim of achieving both oxidation and disinfection together. Therefore, process optimization is required to remove all the pathogens as well as oxidizing micropollutants. On the other hand, although high doses of ozone can effectively remove refractory pathogens but it increases the risk of by-product formation [142]. Aldehydes, ketones, carboxylic acids, alcohols, esters, and bromate are main by-products of ozonation [142]. The majority of these by-products are biodegradable and may be removed by biological filtration[142]. The most important bromide oxidation by-product is bromate BrO_3^- which is of high concern because of its high carcinogenic properties [143]. To keep the balance between oxidant demand and formation of bromate, some factors may be effective. Increase in temperature directly affects pathogen removal and bromate formation. Therefore, an optimum temperature must be selected considering the overall efficiency of treatment. Another controlling option is decreasing pH, the addition of ammonia and the reduction of bromide.

Ozonation of steroid estrogens during drinking water treatment has been investigated in numerous studies [124, 130, 133, 144, 145]. Estrogenic steroids showed high reactivity toward ozone [119, 124], whereas according to the only previous study on ozone oxidation of progestogens, these compounds have low to moderate reactivity with ozone [130]. Complete discussion on reactivity of steroids with ozone is provided in Chapter 6.

2.6.2 Chlorination in water treatment

Chlorine is a strong oxidant, commonly used as disinfectant which is also a selective oxidant and reacts with double bonds in aromatic rings of chemicals [90]. Optimum doses of chlorine for estrogen removal were reported between 1-4 mg L^{-1} [146]. Removal efficiency of estrogens with chlorination depends on their contact time with oxidant. The longer contact time, the greater oxidation [147]. Chlorine can mainly attack ortho and para positions in phenolic ring of hormones and results in cleavage of ring while its reactivity with other functional groups are lower as compared to phenolic groups [146]. In one study by Hu et al. estriol has been completely removed after 10 min rapid reaction with chlorine [148]. Another study with

Westerhoff et al. showed that after applying 3.8 or 3.5 mg Cl_2 L^{-1}, concentration of steroid estrogens were below the detection limits [86].

Although phenolic hormones such as steroid estrogens are rapidly oxidized by chlorine, hormones with ketone groups such as testosterone and progesterone are less reactive with chlorine [86]. The oxidation rate of pharmaceuticals with chlorine or chlorine dioxide is slower than for ozonation [126]. In general, a higher dose of chlorine and longer contact time are necessary for chlorination as compared to ozonation to achieve same efficiencies. For 1 mg/L oxidant concentration, the half-life of 17α-ethinylestradiol has been reported as immediately with ozone, few seconds with chlorine dioxide, and about 30 minutes with chlorine [126].

Table 2-8. Rate constants for the reaction of steroids with ozone, hydroxyl radicals, and chlorine.

Hormone	K_{O3} ($M^{-1}s^{-1}$)	K_{OH} ($M^{-1}s^{-1}$)	K_{Cl2} ($M^{-1}s^{-1}$)
Prog	480^{a} 601^{b}	-	Not reactive[g]
MDRXYProg	558^{b}	-	
Nore	2215^{b}	-	
Levo	1427^{b}	-	
E1	$1.5*10^{5}$- $4.2*10^{9c}$ $6.2*10^{3}$–$2.1*10^{7d}$	- $1.1*10^{9}$–$7*10^{10d}$	$4.15*10^{5g}$
E2	$2.2*10^{5}$- $3.7*10^{9c}$ 10^{6e}	- $1.41*10^{10e}$	$3.64*10^{5g}$
E3	$1.1*10^{5}$- $3.9*10^{9c}$	-	$3.56*10^{5g}$
EE2	$1.8*10^{3}$-$3.7*10^{9c}$ $3*10^{6e}$	$1.08*10^{10f}$ $9.8*10^{9e}$	$3.52*10^{5g}$

Barron 2006[138]; b) Broséus 2009[130]; c) Deborde 2005[119] ; d) Nakonechny 2008 [139] ; e) Huber 2003 [145]; f) Rosenfeldt 2004 [140]; g) Debord 2004 [91]

CHAPTER 3 OBJECTIVES, HYPOTHESES AND RESEARCH APPROACH

3.1 Objectives

The overall objective of this research project is to determine the governing mechanisms of the elimination of hormones in water and river sediments during transport in surface waters and drinking water treatment. The second objective is to identify treatment processes and the conditions under which these compounds can be removed for drinking water production.

We formulated hypotheses and then objectives corresponding to each hypothesis. A summary of hypotheses, applied methodology and expected results are presented in Table 3-1.

The project hypotheses and sub-hypotheses are:

1. Hormonal loadings at drinking water intakes are estimated from available distribution constants and up-flow point source discharges of wastewater.

Originality: this is the first study on the occurrence of hormones in drinking water sources including their partitioning between different phases in river and providing the overall concentration of steroids from raw sewage to drinking water intake.

1.1. Concentration/loadings of (total/dissolved) hormones at water intake (river water) and in raw sewage and in the WRRFs effluent vary seasonally.

Originality: there is no data available on the seasonal variations in total concentration of steroids at water intakes and WRRFs.

This hypothesis will be proven wrong if the total concentration of steroid hormones in dissolved and particulate phase of river water as well as in river sediment and WRRFs remains unchanged between different seasons of sampling (fall, summer, and spring)

1.2. Hormonal loadings estimated from dissolved concentrations following filtration underestimate the total concentration of hormones in surface water and drinking water.

Originality: the majority of studies on the occurrence of micropollutants in water systems report their dissolved concentrations while ignoring the steroids associated to suspended solids. This is the first study on the importance of concentration of steroids attached to suspended solids in river system and WRRFs.

This hypothesis will be proven wrong if the concentration of steroids associated to suspended solids in studied water systems is negligible as compared to their dissolved concentrations.

1.3. Up-flow to down-flow concentration gradients of (total/dissolved) synthetic hormones is more important than those for natural hormones.

Originality: there is no study which compares the partitioning of synthetic and natural steroid hormones between dissolved and suspended phase of river systems.

This hypothesis will be proven wrong if the total/dissolved concentration of natural steroids is equal or higher than the total/dissolved concentration of synthetic steroids in river water and sediments.

2. Hormonal compounds are adsorbed, transformed and accumulated in solids from sludge and sediments after discharge into receiving waters or during drinking water treatment:

Originality: this is the first study evaluating the sorption kinetics of progestogens and testosterone on river sediments. Additionally, there are no information on the accumulation of steroids in sludge of drinking water plants.

2.1. Steroid hormones are attached to the suspended particles in river water, river bed sediments and sludge particles;

Originality: except one study on the occurrence of estrogenic steroids in river suspended solids (Nie et al. 2015), there is no study on the presence of selected steroids in this study (progestogens and testosterone) in river suspended particles and sludge of DWPs.

This hypothesis will be proven wrong if the concentration of steroids in suspended solids of river water, sediment particles, and sludge solids is lower than their limits of detection (LOD: sediments: 5-13 ng g^{-1}; river and sludge particles:18-44 ng g^{-1}) .

2.2. The higher the organic content of the sediment sample, the higher loads of hormones sorbed to the sediments;

Originality: the organic content of sediments is considered as a good sink for organic micropollutants. This study focused on the sorption kinetic of selected steroids, including progestogens and testosterone for the first time, onto different sediments with variety of organic content and particle size.

This hypothesis will be proven wrong if sorption of steroids onto sediment particles is found independent of the organic content of sediment.

3. Oxidation of hormonal compounds using ozonation is influenced by pH, temperature and the amount of organic matter content.

Originality: this is the first study on the effect of temperature on the reaction of steroid hormones and ozone providing the activation energies for these reactions.

3.1. Second order rate constant vary widely between natural and synthetic hormonal compounds.

This hypothesis will be proven wrong if no difference (>20%) is found between the oxidation rate constant of natural (Progesterone and testosterone) and synthetic (Medroxyprogesterone, norethindrone, and levonorgestrel) steroids.

3.2. pH influences the proportion of active oxidation species.

This hypothesis will be proven wrong if changes in pH of water containing steroids make no difference in oxidation rate of steroids and selected steroids show no protonation with pH change.

3.3. Rate constants for OH° and O_3 oxidation are useful to predict compounds removal in natural water.

This hypothesis will be proven wrong if significant difference ($p<0.05$) is found between the observed rate constants of steroids and predicted rate constants in natural waters.

On a more detailed level, the specific objectives of this research work are to:

1) Identify and quantify the sources of hormonal compounds in surface water and during drinking water production (Publication I);

2) Determine the dissolved and particulate fraction of natural and synthetic hormones in water treatment plants, surface waters, and river sediments (Publication I);

3) Evaluate the effect of seasonal variations on the general profile of steroid hormones in surface waters and river sediments (Publication I);

4) Quantify the relative contribution of adsorption to the fate of hormones in river sediments and sludge beds (Chapter 5);

5) Measure the oxidation rate constants (K_{O3}) of hormones by ozonation and evaluate the impact of pH, temperature, and the presence of organic matter (Publication II);

Achieving these objectives will allow us to answer the following questions:

1. Which hormones and under what form do hormones enter the DWP?
2. Where will hormones have more chance to accumulate in the DWP or in river bed?
3. Will hormones sorbed or biodegrade in river water, bed sediment and sludge?
4. Can ozone oxidation efficiently remove hormones under typical operational conditions?

Table 3-1: Experimental approach, and expected results developed to validate (or invalidate) the research hypotheses.

Hypothesis	Scale of Study	Experimental Plan	Expected Results
1) Hormonal loadings at drinking water intakes can be estimated from available distribution constants and up-flow point source discharges of wastewater.	Laboratory scale with field samples	- Identify the most probable sources of steroid hormones in river water against their occurrence in WRRFs effluent - Determine the dissolved and particulate fractions of steroids in surface waters and sediments - Evaluate the seasonal variation of dissolved and particulate fraction of steroid hormones in water and in sediments	- The overall profile and partitioning of steroid hormones in dissolved and solid phases during three sampling surveys - The overall profile of steroid hormones in river bed sediments - Comparison between hormonal loads in dissolved and particulate fractions of hormones during different sampling campaigns
2) Hormonal compounds are adsorbed, transformed and accumulated in sludge and sediments after discharge into receiving waters or during water treatment.	Laboratory scale with field samples	- Determine K_d and K_{OC} values using steroids concentrations in solid/liquid phase at equilibrium and organic content of sediments/sludge - Kinetic experiments for sorption of steroid hormones on sediment/sludge at different initial concentrations of steroids (5, 50, and 100 µg L^{-1}) - Extracting isotherm data for sorption of steroid hormones on sediments/sludge	- Sorption coefficient of hormones onto sediments (K_d) and organic matter (K_{OC}) based on the organic content of sediment. - Pseudo second-order sorption constants for steroid hormones - Isotherm data for adsorption of steroids onto river sediments and sludge

Table 3-1 (Continued): Experimental approach, and expected results developed to validate (or invalidate) the research hypotheses.

Hypothesis	**Scale of Study**	**Experimental Plan**	**Expected Results**
3) Oxidation of hormonal compounds using ozone is influenced by the amount of organic matter, temperature, and pH.	Lab Scale	- Ozonation of steroid hormone in buffered Milli-Q water, natural filtered water, and diluted wastewater spiked with hormones (10 µg L^{-1}) and 2-10 mg L^{-1}. - Ozonation of steroid hormones at different pH and temperatures. - Ozonation of ρ-CBA in parallel to estimate R_{Ct} and Ct_{OH}.	- Comparison of the decay curve of each hormone in natural water and buffered Milli-Q water. - First order reaction rate constant of ozone decay. - The apparent second order oxidation rate constant (K_{O3}) for each compound in natural filtered water, diluted wastewater, and buffered Milli-Q water - Activation energy for the kinetic reaction of steroids with ozone

3.2 Methodology

Specific analytical techniques are required for the detection and quantification of hormones at trace concentrations in complex aqueous and solid matrices. The first part of methodology presents a brief overview of analytical methods used to evaluate trace concentrations of steroid hormones in both the solid and liquid phases. In the second part, the methods most appropriate for our research project are discussed in greater detail. Usually, all the published analytical methods for hormones consist of sampling, extraction of the target compounds from the sample, enrichment of the extracted sample followed by chromatographic separation, and mass spectrometry detection [90].

Sampling. The first step in analyzing a single or complex target compounds is sampling. Some factors must be considered while sampling: i) the time and the period of sampling, as occurrence of contaminants may differ by seasonal, weekly, or even daily or hourly variations. ii) the location of the sampling points taking into consideration the amount and distribution of the contaminants in the area of study. For example, more sampling points have to be placed near the point source or in uneven areas as compared to uniform areas. iii) Sample storage and preparation: stability of sample contents is a determining factor in their storage time prior to analysis and preparation [149]. Correct sample storage is essential to ensure that structural changes do not occur for sensitive compounds.

Aqueous samples are usually filtered before storage. Then, pH is adjusted and samples are kept in amber glass bottles (keep from light and UV degradation) at low temperatures. In some cases, to avoid biodegradation of the analytes, biocides are added.

In the case of solid samples from sludge, sediments and soil, samples must be taken from the aqueous phase at the same time. Sediment samples are sieved, freeze dried at -20 °C [108] and grounded before storage. Some characterization of sediment samples may be required during data analysis including pH, redox potential, total organic carbon and particle size distribution [149]. For sludge sampling, usually grab or more accurate composite samples are taken [108]. Glass fiber filters (2.6 and 0.3 µm) are used to filter sludge samples or the sample is centrifuged to separate the solid fraction from the supernatant then frozen for later analysis [108]. The supernatant should be analyzed as well.

Extraction and Enrichment. Concentration of hormonal compounds detected in different water courses have been reported in the range of ng L^{-1} to µg L^{-1} [7, 41]. Therefore, highly sensitive analytical methods are required for the detection of such compounds in different matrices. Only some analytical apparatus are capable of detecting compounds at these very low levels. Consequently, a preliminary concentration step is needed to increase the concentrations of the target compounds up to detection limits [90]. Liquid-Liquid extraction (LLE), Soxhlet, Steam distillation, and Solid Phase Extraction (SPE) are common extraction methods which have been used to concentrate pharmaceuticals, including steroid estrogens [90, 150]. LLE is an efficient method for separation of non-polar and semi-polar compounds from aqueous samples. Analytes are distributed between two immiscible liquid phases according to their partitioning coefficient. Solvent type, pH, and alkalinity affect the distribution of analytes at equilibrium state [108]. However, SPE is still widely used for sample enrichment. During the extraction process, target compounds from the liquid phase bind to the solid sorbent which is placed into pre-conditioned cartridges. The flow rate through the cartridge should be low enough to allow the analytes to sorb to the sorbent efficiently (2-3 mL min^{-1}, [151]). Extracts are then washed by solvents and for more enrichment dried by nitrogen flow. Finally, targeted compounds are desorbed from the sorbent by washing and will be sent for detection. SPE can be performed both off-line or on-line, where the chromatographic device is directly coupled to the extraction system [152] [153] [72].

Clean-up. Even after an appropriate extraction step, components that can interfere with the recovery may still remain in samples. For this reason, a clean-up step such as passage through a silica gel or aluminum oxide columns is usually applied to separate extracts according to the polarity of its contents. The extract is filtered through the column using a suitable solvent for washing, and the interfering matrix components are captured on the sorption material. Silica gel and aluminum columns can efficiently remove polar interfering compounds such as proteins, humic acids, and fatty acids. Silica gel columns have been widely used for estrogen clean-up in environmental samples [108].

Chromatography. Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the most commonly used methods for the analysis of trace pharmaceuticals in the environmental samples. GC can be used for compounds with higher volatility, while HPLC is more relevant for polar compounds. GC based methods are different in their detectors which provide data to discriminate each compound leaving chromatographic column. Several detectors

can be used such as mass spectrometer (MS), flame photometric (FPD), nitrogen-phosphorous (NPD), and flame–ionization (FID). Mass spectrometry is the most common technique because of its high sensitivity and selectivity. GC based methods have some limitations related to sample purification and difficulties in measurement of target compound in complex samples. Also these methods are time consuming. These disadvantages have led to developing other types of analytical methods, such as liquid chromatography (LC and HPLC). LC and HPLC techniques have been proposed and validated for the analysis of almost all pharmaceutical compounds. When LC is coupled with mass spectrometry (LC-MS), it becomes a method of choice for the analysis of trace contaminants in water. LC/MS is a method of choice for non-volatile moderately polar compounds. LC-MS/MS provides larger range of detection limits as compared to GC-MS/MS. However, there are some problems using this method: For example, large volumes of sample are required for LC while it is not well-matched with high vacuum required for MS analyses. While using liquid samples for LC, it will be difficult to produce gas-phase ions from samples. To overcome these problems, several ionization techniques have been developed. Electrospray ionization (ESI), Atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are other ionization techniques mostly used as LC/MS interfaces for detection of pharmaceuticals.

Mass spectrometry (MS). Mass spectrometry is one of the most common detection techniques with high sensitivity and selectivity for quantification. MS detection is based on mass to charge ratio (m/z) of analytes. Thus, analytes are first ionized using an ionization source. There are two techniques to perform ionization, electron ionization (EI) and chemical ionization (CI). In EI, compounds are exposed to beam of electron and produce negatively or positively charged molecules. Structural properties of compounds are achieved while using EI technique. In CI, compounds are exposed to a radicalized gas (usually methane) and loss or gain hydrogen atom. CI technique provides less information about molecular structures as compared to EI method because it makes less fragmentation than EI. CI is an appropriate method for analyzing trace compounds in complex matrices. After detection, data analysis is required. Mass analyzers include ion trap, magnetic/electric sector, single and triple quadrupole, Fourier-transform and hybrid mass spectrometers. Ion trap and triple quadrupole analyzers can perform tandem mass spectrometry (MS/MS) which provides analysis of complex matrices (e.g. wastewater) with very high selectivity.

Online solid phase extraction coupled with LC-APCI-MS/MS. As mentioned before, offline ordinary SPE methods are time consuming and laborious. Using traditional extraction methods do not let us to measure trace concentrations of steroids in water matrices, especially when other interfering compounds co-exist with target compounds. Online SPE allow to measure large number of samples containing very low concentrations of hormones in a considerably shorter time. Especially when combined with tandem mass spectrometry, online SPE can be efficiently used for analysis of different hormonal compounds [76]. SPE- LC-MS/MS setup consists of following steps: online SPE, liquid chromatography, and mass spectrometry.

Laser Diode Thermal Desorption (LDTD) coupled with APPI-MS/MS. The combination of LDTD source and APCI ionization is a new technology for sample introduction to the mass spectrometer. Complete description of the LDTD method was provided previously [154]. System consists of an infrared diode laser, LazWell 96-well plate, a piston, a stainless steel transfer tube, and a corona discharge needle. The schematic of a LDTD system is shown in Figure 3-1. Samples are deposited into small wells of LazWell plate (1-10µL) and then dried at room temperature or under nitrogen stream. The LazWell plate is made of high density polypropylene with stainless steel inserts. The end part of each small well is hexagonal for better deposition of sample. After drying the samples, the plate is placed in an X-Y stage and infrared laser diode is applied at the back of each small well to produce thermal desorption and convert the deposited sample into the gas phase. The preheated carrier gas (medical grade air) will carry the gaseous sample through the transfer tube, and the corona discharge needle will ionize the sample and transmit it to the mass spectrometer.

The LDTD-APCI system which is used in this research project is produced by Phytronix Technologies company (Quebec, Canada) with the following characteristics: Laser power 980 nm (20W), corona discharge voltage 5500 V, turbo gas temperature 22C, carrier gas flow 2.25 L/min.

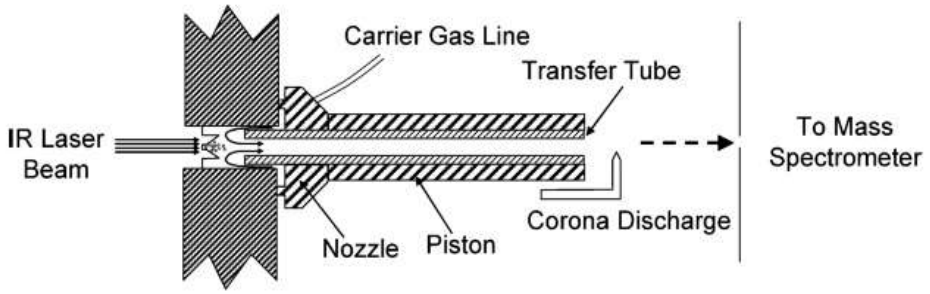


Figure 3-1. Schematic of LDTD system [155].

3.2.1 Selecting the target compounds

Target compounds have been selected according to the following criteria:

- a high occurrence rate in the environment according to other studies on the occurrence of these compounds;
- elevated yearly consumptions: Synthetic hormones are widely used in hormonal therapy and birth control pills;
- treatability during water and wastewater treatment processes which is the direct function of their physico-chemical properties;
- sufficient concentrations in environmental samples to be quantified and identified by existing analytical methods.
- availability of appropriate reference standards.

Site description and sampling locations. The Mille-Îles River is located in the north west of the Island of Laval, Quebec. Fed by Ottawa River through the des Deux Montagnes Lake, the river receives treated wastewater and some industrial discharges from several municipalities in the North Shore and Laval. Several drinking water plants (DWP) intakes are located on the river water at various locations.

Samples were taken from 12 different points along the river and also 3 WRRFs effluent and 5 DWTP. Sampling was performed during wet and dry weather conditions. The three WRRFs receive urban wastewater with capacity to serve (47,450 P.E.), (62,860 P.E.) and (47,683 P.E.) inhabitants respectively. Their treatment is based on biofiltration and UV disinfection or physical-chemical treatment. Treatment processes in these plants is based on chlorination,

filtration, and activated carbon for DWP1 and DWP4; chlorination, filtration, and ozonation for DWP2 and DWP5; and activated carbon and ozonation for DWP3.

Figure 3-2 shows the location of sampling points along the Mille-Îles River, and the drinking water treatment plant intakes and wastewater treatment plant discharge points.

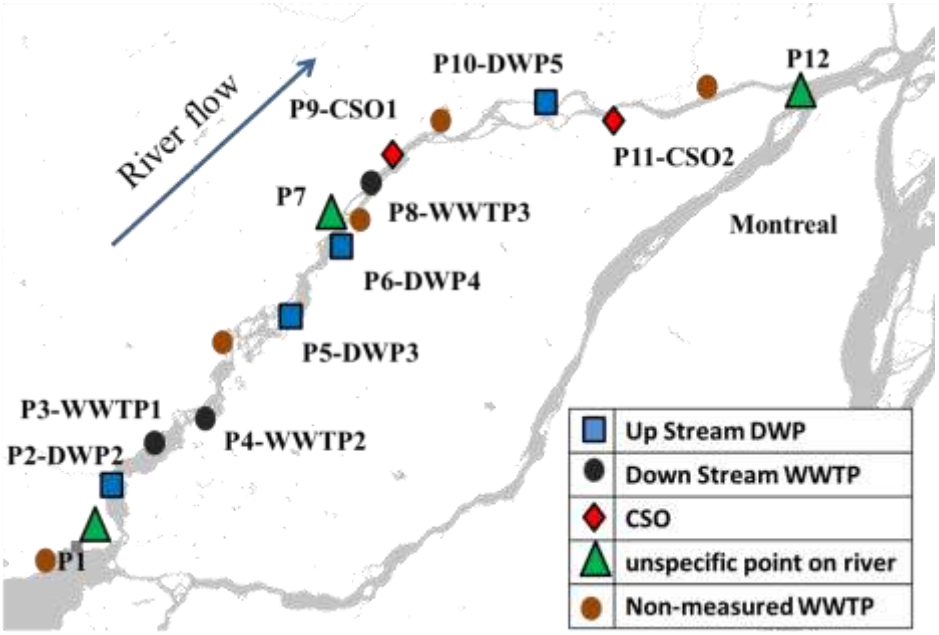


Figure 3-2. Sediment and water sampling points along the Mille-Iles River.

Sample collection and preservation. Our sampling protocol was designed as to allow the: 1) contribution of different sources of trace hormones into the Mille-Îles River; 2) determination of partitioning of hormones between water and sediments and 3) estimation of seasonal variation of hormonal loadings in the river. Water samples (5 L) or sediment samples (500 g) were collected in amber glass bottles and kept in 4 °C until delivery to laboratory. Samples were immediately filtered on 2.6 and 0.3 µm pore size cellulose filters to remove particulate matter and suspended solids. The particulate matter remaining on filters was analyzed to evaluate the partitioning of target compounds between aqueous phase and particulate matter. This sequence of sample analysis is a significant difference between this project and previous researches by Viglino et al 2008 [68]. The pH of filtrate was adjusted by adding formic acid (0.1%) in order to maintain the structure of compounds with acidic properties and improve their retention during SPE [156]. Sample analysis must be done within a day after sampling in order to prevent biological

degradation or transformation of compounds without adding preservatives [68]. The schematic of sample pre-treatment is shown in Figure 3-3.

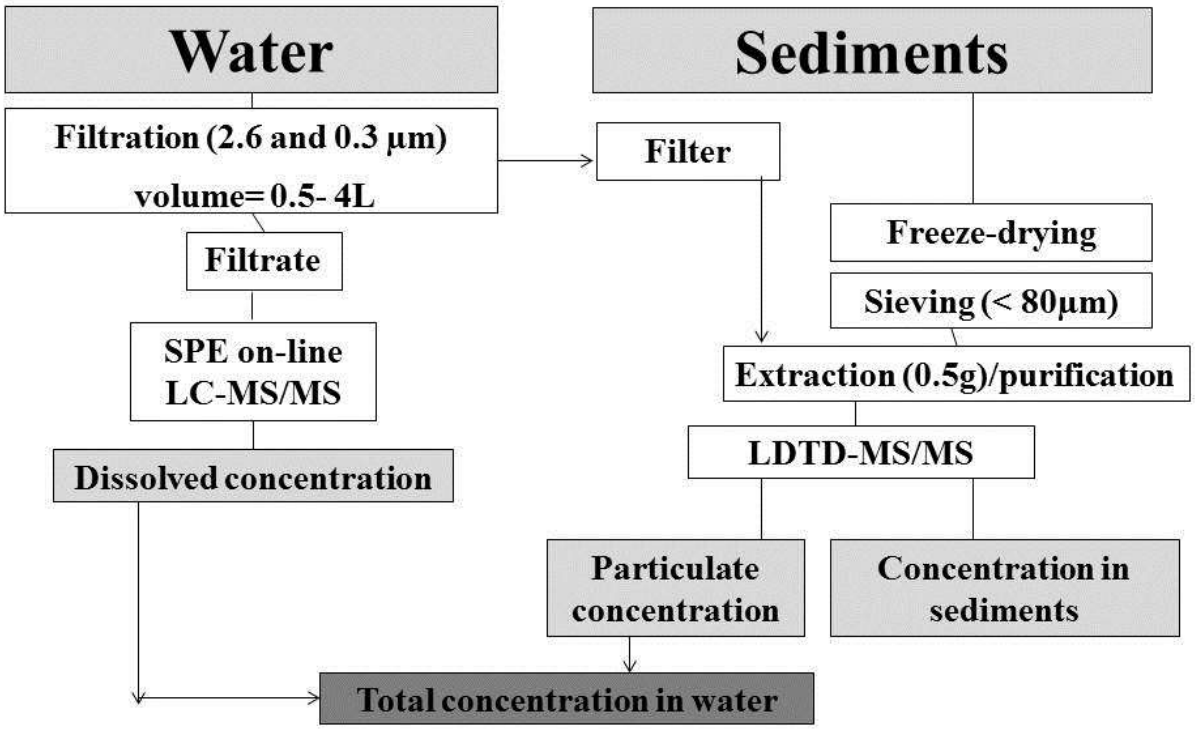


Figure 3-3.Schematically sample pre-treatment procedure.

3.2.2 Identification and quantification experiments

The concentrations of hormones in water and solid samples were measured using following analytical methods:

- On-line SEP-LC-APCI-MS/MS method was used to measure concentration of dissolved hormones.

- Concentration of hormones in the solid phase (sludge, sediment, and wastewater solids) was measured using LDTD method.

Online solid-phase extraction and liquid chromatography. The online SPE consists of tandem liquid chromatography coupled with mass spectrometry, an auto-sampler, and a mass

spectrometer with an APCI source which has already been used for analysis of hormones by Viglino et al. 2008 [68]. Figure 3-4 shows the schematic diagram of the LC-MS/MS system. In loading position, an auto-sampler will inject 1-5 mL of the aqueous sample into the 1-10 mL loop and then LC-pump gradient will concentrate the sample. In injecting position, the concentrated sample will be back flashed into the analytical column and separation will be done through the MS-pump gradient. After complete transfer of analytes, system will back to its initial position. The primary column will be washed and preconditioned for another sampling procedure.

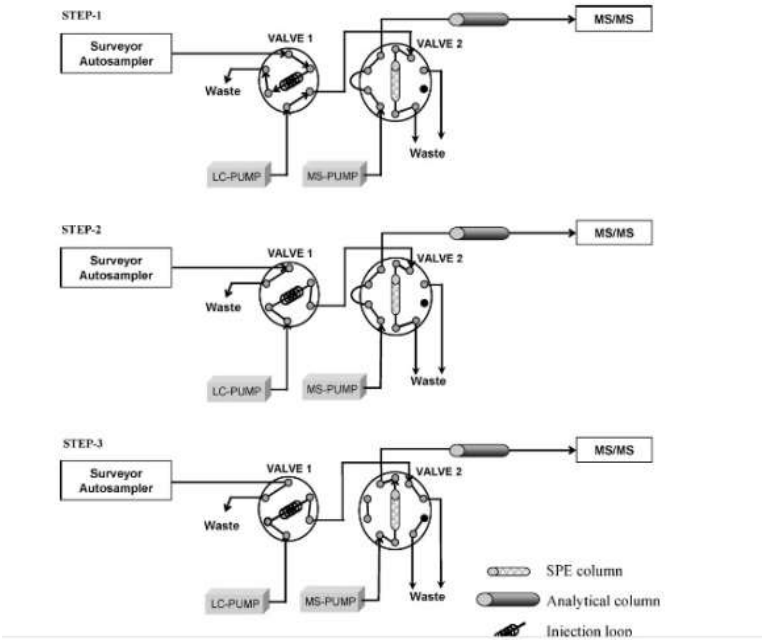


Figure 3-4. On-line Solid-Phase Extraction-LCMS/MS [68].

Sample preparation for solid samples. All the solid samples (sludge bed, wastewater solids, and river sediments) were freeze-dried for minimum of 24 hours and stored at -20°C before analysis. After adding the surrogate standard, the sample was shaken vigorously to become well homogenized. The extraction step was done using sequential ultrasonic extraction (USE) for 30 min at 30°C then stirred for 45 minutes at ambient temperature. Centrifugation was done for 10 minutes to separate the organic phase. In this step internal standards were added to the sample. The volume of sample was reduced to 250 µL under the nitrogen stream and then was sent to further analysis with LDTD-MS/MS with APCI ionization source.

Laser diode thermal desorption method (LDTD). The LDTD/APCI ionization source was used to ionize the hormones before identification with mass spectrometry. Samples are spotted into the 96-well plate with conical shape sample wells containing an appropriate solvent. Samples (1-10µL) are left to dry at ambient temperature then transferred to the housing unit of LDTD. An infrared laser diode thermally desorbs dried samples and converts them into the gas phase. Then purified air was carry desorbed gas phase for ionization by APCI and then to mass spectrometry. Figure 3-5 shows the LDTD system.

Figure 3-5. LDTD-APCI system. www.Phytronix.com.

Mass spectrometry. After on-line or off-line solid-phase extraction of samples and ionization in APCI or LDTD/APCI ionization sources, analytes were sent to mass spectrophotometer for identification. The MS/MS spectrum of each compound was obtained according to their standard compounds and in both negative and positive ionization modes.

3.2.3. Sorption of hormones onto river sediments

Sorption kinetics of selected hormones on river sediments was conducted according to OECD test guideline No. 106 [157]. All the biological activities in water and sediments were stopped by gamma irradiating (30 kGy, 5.2 h). Sediments were wet sieved (< 1.25 mm) with river water from the same sampling point in order to remove debris. Two sediments: water ratios were used for sorption experiments, 1:5 and 1:1 in order to investigate the effect of solid: liquid ratio on amount of sorption and equilibrium time. An appropriate volume of standard and mix solutions of hormones was added to each reactor (15 mL polypropylene conical tubes) in order to obtain

desired concentrations (5, 50, and 100 µg L^{-1}). The reactors were placed on the rotary shaker for maximum of 96 h. To determine the equilibrium time and kinetic parameters, samples were equilibrated for 0, 0.08, 0.25, 0.5, 1, 24, 48, and 96h. Individual reactors in duplicate were assigned to each reaction time. The liquid phase of each reactor was separated by centrifuging at 6000 rpm for 1 min (for reactors at time t=0- 1 h) or for 15 min (for reactors at time 24, 48, and 96 h).

The liquid phase of each reactor was filtered through 0.3 µm pore size glass micro fiber filter to remove any residual particulate matter. The filters were also analysed for any loss of steroids through adsorption on suspended particles on filter. The filtrate was then diluted to 50% by ultrapure water in order to prevent the HPLC column from saturation. In order to avoid any probable biological degradation, the liquid sample was acidified using formic acid (> 95% purity) as 25 µL for each 5 mL of liquid phase prior to LC-MS/MS analysis.

The freeze-dried solids from each reactor were extracted by sonication-assisted solvent extraction using a 3:1; v:v mixture of methanol and acetone. The extraction method is described in detail in previous study by Darwano et al. 2014 [5]. The extract was reconstituted to 5 mL with acidified water (0.1 % formic acid) containing 5 % methanol, sonicated at 30 °C for 10 min, and then centrifuged for 10 min at 6000 rpm. The extract was then filtered as the liquid phase and analysed by LC-MS/MS.

Data Analysis of sorption isotherms. The sorption isotherms are constructed with batch experiments over a wide range of steroids concentrations (5, 10, 25, 50, 75, and 100 µg L^{-1}). The adsorption of steroids onto sediment was verified by the most frequently used isotherm models. Freundlich, Linear, and Langmuir sorption isotherms were generated and fit to determine which isotherms best fits the sorption of steroids on sediments. The Statistica. Ink 13 (Dell Inc., OK, USA) was used for data evaluation, using 95% confidence interval for the best-fit sorption isotherms.

Solid-liquid distribution coefficient calculation. The K_d is defined as the ratio of the concentration of a dissolved substance in aqueous phase and solid phase at the equilibrium condition. The K_d value is identical for each solid phase type. A normalized form of K_d, the organic carbon partitioning coefficient (K_{oc}) is defined for natural systems. The K_{oc} is deducted from total organic carbon content of the adsorbent and K_d.

3.2.4. Ozonation experiments

Ozonation experiments were conducted in bench scale with solutions using buffered ultrapure Milli-Q water and then repeated with natural filtered water samples from treatment plant intakes and diluted wastewater from sedimentation tank of WRRF, in order to assess the effect of water quality on effectiveness of ozone in oxidation of hormones. Ozone decay was quantified by estimating the apparent first order decay constant in both synthetic and natural water test samples. Thereafter, second order ozonation rate constants were determined for each target compound in both synthetic and natural water. Since it is difficult to directly measure the concentration of OH° radicals, a probe compound p-chlorobenzoic acid (ρ-CBA) was used to calculate the exposure rate to OH° (CT_{OH}). The ρ-CBA only reacts with hydroxyl radicals with very low reactivity with ozone molecules (K_{O3}= 0.15 M^{-1} S^{-1}). Then it is possible to measure the CT_{OH} by evaluating the oxidation of ρ-CBA. The overall removal efficiency of target compounds was estimated using R_{CT} ratio which has previously defined by Elovitz et al. 1999 [158].

Experimental setup. Experiments were performed by spiking selected compounds at 10 µg L^{-1} initial concentrations into 1 L continuous stirred tank reactor (CSTR). The pH value of pure water was adjusted by adding phosphate buffer, but for natural water samples no buffer was added. The ozone stock solutions (50- 60 mg/L) were prepared by injecting gaseous ozone in ultrapure chilled water (4 °C). Appropriate contents of stock solutions are added in the reactor in order to have (2-10 mg O_3 L^{-1}) desired ozone doses. The reactor is closed with a floating Teflon lid to keep ozone from leaking. For those experiments concerning estimation of ozone decay rate and ozonation rate constant of compounds, tert-butanol was added as OH° scavenger. To evaluate the effect of OH° on oxidation and to estimate the CT_{OH}, ρ-CBA was added to the reactor containing Milli-Q water free of hormones. Samples were taken from reactor at defined time intervals (0- 15 min contact time) for analysis of ozone residual, ρ-CBA and target compounds. After sampling, ascorbic acid (5 g L^{-1}) was added to quench the residual ozone and prevent further degradation before analysis.

Analysis of hormones, ozone residual and p-CBA. The quantification of hormones in 20 mL samples taken from the oxidation reactors was done using on-line SPE-LC-MS/MS with APCI ionization source. Concentration of ozone in stock solutions and ozone residual was determined using the indigo trisulfonate method (5 mL samples of ozone with 20 mL indigo solution and 15

min contact time)[159, 160]. To analysis ρ-CBA, 2 mL samples were taken from the reactor in parallel with ozone analysis and concentration of ρ-CBA was determined using HPLC.

CHAPTER 4 ARTICLE 1: SEASONAL VARIATIONS OF STEROID HORMONES RELEASED BY WASTEWATER TREATMENT PLANTS TO RIVER WATER AND SEDIMENTS: DISTRIBUTION BETWEEN PARTICULATE AND DISSOLVED PHASES

In evidence of the negative effect of some steroid hormones on aquatic creatures and potential risk of human exposure, investigating their prospective sources in environmental waters is totally important. While studying the occurrence of steroid hormones in natural water matrices, ignoring the fraction of compounds attached to the suspended particles results in underestimation of an important fraction of available steroids. This chapter investigates the occurrence of natural and synthetic steroids in dissolved and particulate phases of water from the inlet of WRRFs to DWP intakes. The steroid patterns along the river and in river bed sediment are assessed and effect of temperature on distribution of these compounds between suspended particles and sediments is considered. The results from this study are presented as a research paper submitted to the Science of the Total Environment. Supplementary information is provided in APPENDIX A.

SEASONAL VARIATIONS OF STEROID HORMONES RELEASED BY WASTEWATER TREATMENT PLANTS TO RIVER WATER AND SEDIMENTS: DISTRIBUTION BETWEEN PARTICULATE AND DISSOLVED PHASES

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ABSTRACT

The occurrence of steroid hormones in the dissolved phase has been widely documented, but data regarding their particulate phase loadings and their overall fate in surface waters are sparse. New data are provided on the temporal and spatial distribution of detected steroids between the dissolved and particulate phases of wastewater treatment plants (WWTPs), receiving river water and sediment, and also in drinking water plant (DWP) intakes. Three extensive sampling campaigns (10 samples from DWP, 6 from WWTPs, 24 from river water, and 36 from bed sediments) were undertaken. The concentrations of steroids in the dissolved phase of river for all sampling times were below the method reporting limits (3-52 ng L^{-1}). Total steroid concentrations found in suspended particles in colder periods in the river were higher compared to samples taken in summer (722 ng L^{-1} vs 178 ng L^{-1}). Total steroids measured in sediments were in the range of 7-1213, 5-25, and 22-226 ng g^{-1} in autumn, spring, and summer, respectively. In DWP intakes, levels of testosterone, norethindrone, estradiol and 17α-ethinylestradiol in the particulate phase (5-94 ng L^{-1}) were similar to those found in the WWTP effluents (4.5-77 ng L^{-1}), indicating their persistence from discharges including untreated sewage effluents.

Our findings confirm the remarkable presence of the mixture of steroid hormones in drinking water sources, demonstrate the effect of temperature on the distribution of steroids between dissolved and particulate phases, illustrate the importance of the fraction of steroids attached to suspended particles, and raise concerns about the fate of steroid hormones in DWPs and their effects on aquatic wildlife.

KEYWORDS

Progestogens, testosterone, temperature, suspended solids, partitioning

4.1 Introduction

Steroid hormones are ubiquitous in aquatic environments at trace concentrations ranging from a few ng L^{-1} to µg L^{-1} [11, 35, 52]. Negative effects of steroid hormones on aquatic organisms such as sexual disorders, feminization, masculinization, and infertility have been confirmed by several

studies [10, 44, 161]. When assessing the occurrence and fate of steroid hormones in drinking water sources, it is important to consider all environmental sources of dissolved and particulate phases of hormones in wastewater discharges, river water and sediments. The dominant fraction for most hormones is likely to be in the particulate phase according to their limited solubility. The environmental significance of the particulate fraction of steroids had been debated, being first judged to be very low on large particles ≥ 20 μm [162] and then found to elicit estrogen receptor (ERα) and Aryl Hydrocarbon Receptor (AhR) response when considering smaller particles (≥ 0.7μm) [163]. The relative loads of dissolved and particulate fractions of steroids have been assessed in raw sewage and treated wastewater [48] and in river water [53, 164]. In Southwestern France, some dissolved estrogens were systematically detected in a wastewater treatment plant (WWTP) effluent (17-71 ngL^{-1} E1, not detected (ND)-4.4 ngL^{-1} E2, ND ngL^{-1} EE2) but without any clear seasonal trends. However, no steroids were detected in the particulate phase (≥ 0.7μm) of the WWTP effluent presumably because of the low suspended particle concentration (50 mg L^{-1}) with 30% carbon content [53].

Based on an overview of recent studies, the final concentration of steroids in WWTP effluent depends on the efficiency of the applied processes that reduce the amount of suspended particles in the effluent. Activated sludge and waste stabilizing ponds provide higher removal efficiencies for estrogenic activity and estrogens removals [165]. In contrast, trickling filters were found to be less effective processes for suspended particles because of short sludge retention times [163]. Degradation accounted for 78-99% and 73-96% of the removal of estrogens and progestogens for total WWTP influent concentrations of up to 102 and 57 ng L^{-1} from aerobic and anaerobic tanks [166].

Water temperature can directly affect the fate of steroids in both dissolved and particulate phases. The fluctuation of estrone and estradiol levels between influent and effluent was found to be highly temperature dependent [165]. The concentrations of both compounds in the effluent in summer at 27 °C were up to 30 fold higher than that in the effluent during spring and winter (12-19 °C). Additionally, biological processes were more effective during the warmer temperatures as microbial degradation of estrogens increases with higher temperatures (75.4% removal for estriol and more than 90% for other estrogens). Lower steroid levels have been detected in sediments during warmer months most likely due to a higher biodegradation rates at higher temperatures [77, 101].

To date, no data are available on the integral hormone loadings from WWTP influent to Drinking Water Plant (DWP) intakes considering their occurrence in the dissolved/particulate phases and in sediment for mixtures of estrogens, progestogens and androgen. Estrogens were detected in the dissolved phase of samples taken downstream of WWTPs in southern Australia with mean concentrations of 4.49, 0.93, and 0.05 ng L^{-1} for E1, E2, and EE2, respectively [62]. Higher levels of estrogens were potentially related to colder weather and higher rainfall that led to increased loads of estrogens form agricultural lands and animal farms. Although estrogens are the most frequently detected steroids in surface waters, relatively higher concentrations of progestogens and androgens have been reported compared to estrogens [3, 35, 66]. Testosterone was found in 42% of samples from four rivers in Beijing, China with a maximum concentration of 8.6 ng L^{-1} Progesterone was found in 93% of samples with a peak concentration of 199 ng L^{-1} [66].

Given the relatively low partitioning coefficients of steroid hormones, with octanol/water partitioning coefficients (log K_{ow}) mostly between 3 and 6, river sediments are likely act as sinks for these compounds. According to previous studies on the fate of estrogens in river beds, between 13% to 92% of estrogens ended up in the river sediment during the first hours of discharge to the river[97, 101]. From the various steroid hormones investigated in several studies, estriol, ethinylestradiol, and norethindrone were the most frequently detected steroids in sediments [77]. The average concentration of steroids in river sediments from Spain was 0.51 ng g^{-1} in summer and 4.43 in winter and spring [77].

The primary objective was to quantify the role of particles from sewage and treated wastewater on the selected steroids loads found at DWP intakes located on an urban river subject to multiple Combined Sewer Overflows (CSO) and WWTP discharges. Specific objectives included: (1) quantifying the partitioning of steroids in the incoming sewage and effluent of three wastewater plants; (2) monitoring the seasonal variation of the particulate associated steroids, including testosterone, in the suspended solids and sediments along a 42-km river; (3) quantifying the accumulation of particle associated steroids in drinking water sludge.

4.2 Materials and reagents

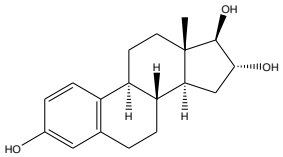
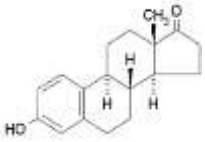
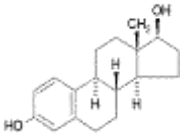
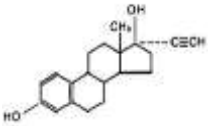
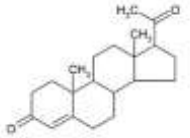
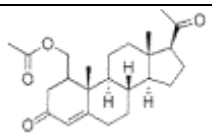
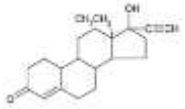
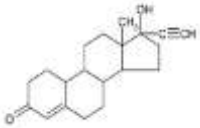
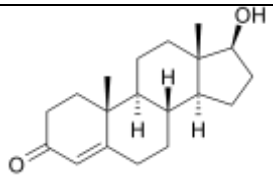
4.2.1 Chemicals and standards

Studied steroids, namely estrogens (estriol (E1), 17ß-estradiol (E2), 17α-ethinylestradiol (EE2)), progestogens (progesterone (Prog), Medroxyprogesterone (MDRXY-Prog), levonorgestrel (Levo), and norethindrone (Nore)) and testosterone (Testo) were all purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Chemical structure and some general characteristics of the selected compounds are summarized in Table 4-1. The internal standard (^{13}C2)-Ethinylestradiol (^{13}C2-EE2) was supplied by ACP Chemical Inc. (Montreal, QC, Canada). All solvents were of HPLC-grade from Fisher Scientific (Whitby, ON, Canada). Individual stock solutions of hormones were prepared at 1g L^{-1} in acetone and stored at -20°C. Subsequent dilutions were made in order to obtain working solution mixtures. The SPE cartridges (6 mL, 500 mg Starta C18-E) were obtained from Phenomenex (Torrance, CA, USA). Glass micro-fiber filters (2.6 µm and 0.3 pore size and 47 mm diameter) were purchased from the Sterlitech Corporation (Kent, WA, USA).

4.2.2 Description of the studied area

Sampling was carried out in a river located in the province of Quebec, Canada. Along its 42-kilometre length, the river supplies drinking water to 5 DWPs serving more than 566000 people in a total area of about 1081 km^2. The land use along the river served is predominantly urban, but several of the creeks draining to the river receive agricultural runoff. The river also receives the effluents of 14 WWTPs and 194 combined sewer overflows (CSOs). River flowrates (44 years of data) vary, on average, from 50 to 550 m^3 s^{-1} with the lowest flows occurring in August (minimum recorded of 15 m^3 s^{-1}) and peak flows in May (maximum of 1500 m^3 s^{-1}). In the present study, the flows of the river during sampling in spring, summer and autumn periods were approximately 350, 135, and 400 m^3 s^{-1}, respectively.

Table 4-1.Structure and properties of the selected compounds.

Compound	MW ($g\ mol^{-1}$)	Log K_{ow}	Use	Chemical Structure
Estriol (E3)	288.4	2.81	Estrogens	
Estrone (E1)	270.4	3.43		
Estradiol (E2)	272.4	3.94		
17α-ethinylestradiol (EE2)	296.4	4.15	Synthetic estrogen	
Progesterone	314.5	3.87	progestogen	
Medroxyprogesterone	344.5	2.69	Synthetic progestogens	
Levonorgestrel	312.4	3.08		
Norethindrone	298.5	2.97		
Testosterone	288.4	3.32	Androgen	

4.2.3 Sample collection and preparation

Sampling campaigns were undertaken in cold and warm weather during the period of November (at 10 °C), July (at 21°C), and May (at 14°C). Sampling locations are depicted in Figure 4-1. The river water and sediment characteristics are reported in Table 4-2.

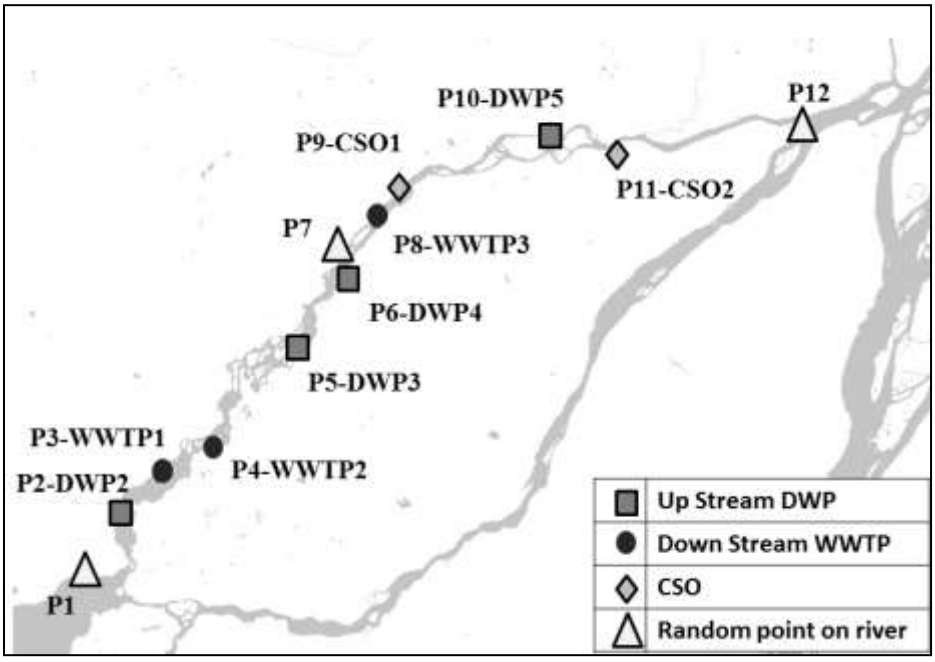


Figure 4-1. Sampling locations along the river. Points are numbered from upstream to downstream of the river and indicate whether sample was taken before DWP intake or after WWTP discharge points.

River sediments. River sediments were sampled at different points along the river near the shore. The top surface of sediments was sampled at a water depth of 1.5 meters using a core sediment sampler. Samples were placed in 100 mL sterilized polypropylene pots, kept on ice in a cooler at 4 °C and were transported to the laboratory the same day. All samples were frozen at -20°C and freeze-dried, ground, and sieved to separate the fraction of particles smaller than 80 µm for further analyses. Sieved samples were stored at -20°C until extraction and analysis.

River water. Water was sampled along the river beginning at the upstream lake as shown on Figure 4-1. Samples were collected in 5L propylene containers, kept on ice while carried to the laboratory and filtered immediately through 2.6 and 0.3 µm filters. Filtrates were kept at 4°C and

analysed within 24 hours to minimize the risk of biodegradation. No preservatives were added because filtered samples were shown to remain unchanged for at least 3 days [167]. Water samples were preconcentrated by Online Solid Phase Extraction (SPE), followed by Liquidchromatography tandem mass spectrometry (LC-MS/MS) for separation and quantification. The filters were dried at 30°C overnight, re-weighed and stored at 4°C before ultrasonic solvent extraction.

Table 4-2. River water and sediment characteristics; Reported values are mean value of 12 samples taken along the river ±STDV and values in parentheses are minimum and maximum values.

TYPE OF SAMPLE	Parameter	Summer	Spring
Water	pH	7.8±0.2	7.5±0.1
	TOC ($mg\ L^{-1}$)	7.9±0.2 (7.6-8.3)	8.07±3.8 (7.8-8.9)
	DOC ($mg\ L^{-1}$)	7.5±0.2 (7.3-7.8)	7.7±3.5 (7.4-8.4)
	Turbidity (NTU)	13.4±5.4 (7-28)	12.36±1 (9.5-12)
	TSS ($mg\ L^{-1}$)	9.7±3.7 (4-16)	20.48±8 (10.3-22.5)
River sediment	Organic matter (% dry wt)	57±21 (28-81)	54±16 (31-82)
	F<80µm (%)	9±9.4 (1-31)	3±2 (0.3-8)
	80µm<f<120µm (%)	85±14 (52-99)	28±24 (1-79)

DWPs intakes and WWTPs effluents. Samples were taken from 5 DWP intakes and 3 WWTP influents and effluents for the quantification of dissolved and particulate phase distribution of steroids. The capacity and treatment processes of the selected treatment plants involved in this study are summarized in Table A-1. 1 and Table A-1. 2. All water samples from treatment plants were filtered then filtrate and filter were processed as previously mentioned.

Ultrasonic solvent extraction. For the quantification of steroids in the particulate phase, filters were used to retain suspended matter (2.6 and 0.3 µm pore size), while for the analysis of river sediments, 0.5 g of freeze-dried sediment sample was used for extraction and analysis. Filters

and sediment samples were transferred in a 15 mL conical polypropylene centrifuge tube and were extracted as described in Darwano et al 2014 [5]. Briefly, samples underwent a solvent-assisted ultrasonic extraction involving two cycles with 5 and 3mL of MeOH/acetone (3:1, v/v), respectively. Each extraction cycle consisted of 20min ultrasonic bath at 30°C, then 30 min rotary shaker, and a 20-min centrifugation step at 6000 rpm. After each extraction cycle, the supernatants were collected in a centrifuge tube and the combined extract was evaporated under a gentle nitrogen stream at ambient temperature.

Sample clean-up. A clean-up step was carried out to remove impurities from extracted filters and sediments. Each sample was reconstituted with 3 mL acetonitrile-water (7:3, v/v) then put in an ultrasonic bath for 10 min. The clean-up procedure was performed using STRATA C18-E SPE cartridges. The cartridges were first conditioned with 2 mL of methanol, then with 2 mL of acetonitrile-water (7:3, v/v). The sample was then added to the cartridge and the clean extract immediately collected in a polypropylene centrifuge tube. The final extracts were evaporated to dryness under a gentle nitrogen stream at 30°C. The clean samples were reconstituted in a 300 µL internal standard solution (^{13}C2-EE2) prior Laser Diode Thermal Desorption tandem Mass Spectrometry (LDTD-MS/MS) analysis.

1.1. Analytical methods

LDTD- MS/MS system. The particulate fraction of steroids in water samples and their partitioning in river sediments were determined with the LDTD-APCI ionization source developed and manufactured by Phytronix Technologies (Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples containing 100 µg L^{-1} IS were spotted (5 µL) into the LazWell 96-well plate and allowed to dry at 40 °C for 10 min. The plate is then placed in the apparatus for analysis of samples and then introduced directly into the mass spectrometer. The detailed analysis procedure is available in [5, 168]. Results of the MS/MS peaks were interpreted using the Interactive Chemical Information System algorithm of Xcalibur 2.2 software from Thermo Fisher Scientific and concentrations were measured from the ratio of the analyte area to that of the internal standard.

On line SPE and LC-MS/MS system. Methods used for the quantification of steroids with LC-MS/MS system were described previously by [169, 170]. Samples were extracted and purified by on-line solid phase extraction (SPE) coupled with liquid chromatography and tandem mass spectrometry (LC-MS/MS). An Atmospheric Pressure Chemical Ionization (APCI) source was used for steroid detection. The on-line SPE was achieved using a Hypersil Gold aQ (20 mm×2.1 mm, 12 µm particle size) column and chromatographic separation was done with a Hypersil Gold (100 mm ×2.1 mm, 1.9 µm particle size) column. Ionization of steroids was achieved by the Ion Max API source mounted on a Quantum Ultra AM triple quadrupole mass spectrometry Thermo Fisher Scientific (Waltham, MA) operated in selected reaction monitoring (SRM) mode for quantification and detection. The sample loading volume varied between 1 mL and 5 mL for wastewater and river water, respectively. Ionization of hormones was achieved with an APCI source in positive (PI) mode. The MS/MS peaks were integrated using the Xcalibur 2.2 software from Thermo Fisher Scientific and concentrations were measured from the ratio of the analyte area to that of the internal standard. The limits of detection (LOD) were determined using a six point calibration curve, analysed in duplicate, in analyte-free water matrixes. The LOD were calculated by multiplying by 3.3 the error on the y-intercept and divided by the slope of the regression line equations.

4.3 Results

4.3.1 Steroids in WWTPs effluent and the receiving river water

Testosterone and progesterone were observed in the dissolved phase of all samples taken from influents of WWTPs (Table 4-3). The concentration of Testo varied between 39.3-146.8 ng L^{-1} in influents and 30.1-116.9 ng L^{-1} in effluents. Progesterone was detected at lower concentrations with a mean concentration of 23±3 ng L^{-1} in influents. The progesterone level was below the detection limit (10 ng L^{-1}) in the effluent of WWTP1. Its levels remained almost constant between influent and effluent of WWTP2 and WWTP3. E2 and E3 stand out as the compounds with the highest mean concentrations in both influents (246±40 and 336±58 ng L^{-1} respectively) and effluents (152±5 and 257±10 ng L^{-1} respectively), whereas MDRXY-Prog was the compound presenting the lowest mean concentration (4 ng L^{-1} influent and 2 ng L^{-1} in effluents) (Figure A-1. 1).

In wastewater, the levels of steroids associated with suspended particles were higher than those in the dissolved phase with concentrations ranging from 4.5-198 ng L^{-1}. Figure 4-2 demonstrates the total steroids measured in the particulate phase of WWTPs influent and effluents. Between 9 studied compounds in spring samples; E2, EE2, Nore, and Testo were found in the particulate phase of all influent/effluent samples, whereas Levo was only detected in WWTP3 with 27 and 4.5 ng L^{-1} in the influent and effluent, respectively. MDRXY-Prog was detected in WWTP2 (11.5-10.4 ng L^{-1}) and WWTP3 (31-5.8 ng L^{-1}).

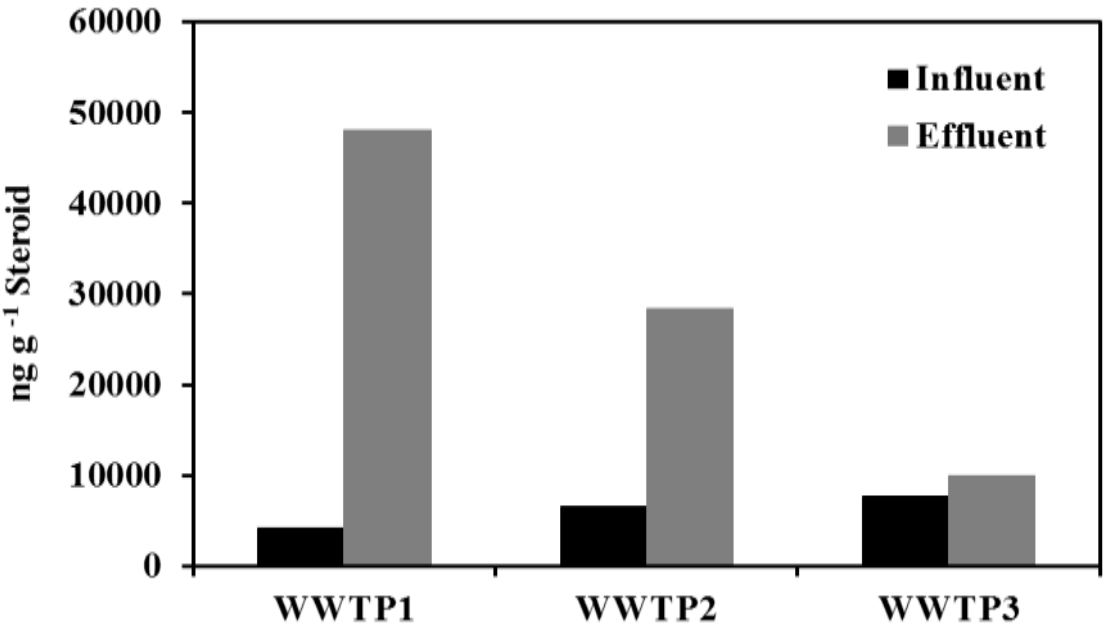


Figure 4-2. Total steroids detected in WWTPs influent and effluent.

Mass flow loadings discharged by WWTP effluents were calculated for steroids by multiplying steroids concentrations by discharge flow (Table A-1. 2). Among the 8 steroids detected in WWTP effluents, the highest mass flow discharge was related to Nore (3.3 g d^{-1}) at WWTP2 (Figure 4-3). The minimum mass flow discharged from WWTPs was related to the total mass flow from WWTP3 (2.76 g d^{-1}) with 0.36 m^3 s^{-1} discharge flow compared to 15.1 m^3 s^{-1} (WWTP1) and 37.2 m^3 s^{-1} (WWTP2).

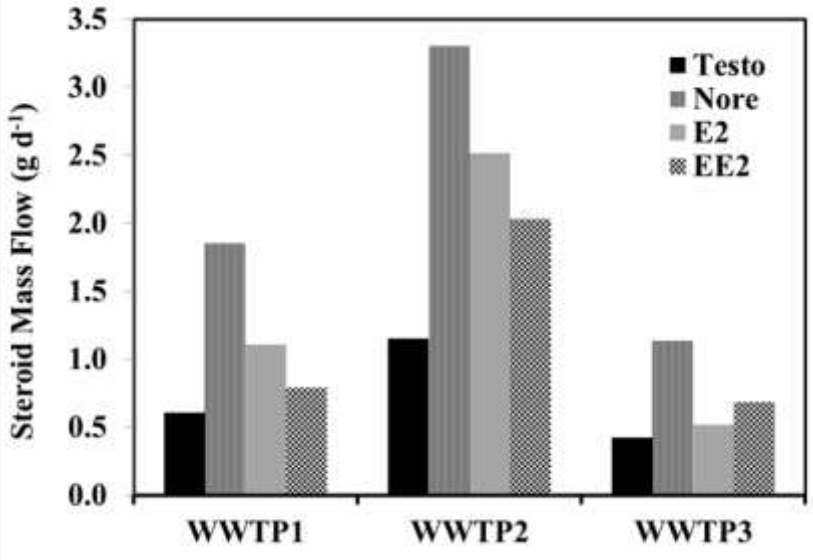


Figure 4-3. Mass flow of measured steroids in WWTP effluents.

The concentration of all the selected compounds in the dissolved phase of river water were below the detection limits in both sampling campaigns (LOD= 5-52 ng L^{-1}). In contrast, all the suspended particles contained EE2 with mean concentration of 35 and 4.7 ng L^{-1} in spring and summer samples, respectively. E2, Nore and Testo were found in suspended particles of all the spring samples whereas Prog, MDRXY-Prog, E1, and E3 were not detected at all. E2, Prog and Testo, were found in summer samples with 100% 83%, 25%, frequency of detection. Table 4-3 shows the frequency of detection and the quantified levels of steroids in particulate phase of the river water.

Table 4-3. Concentrations (ng L^{-1}) of the detected steroids in dissolved (Diss.) and particulate phase (Part.) of samples from Inf. (influent) and Eff. (effluent) of WWTPs during the spring; LOD is detection limit.

Compound	WWTP1				WWTP2				WWTP3				LOD		Literature data (Diss. Only)	
	Inf.		Eff.		Inf.		Eff.		Inf.		Eff.					
	Diss.	Part.	Diss.	Part.	Diss.	Part.	Diss.	Part.	Diss.	Part.	Diss.	Part.	Diss. (ng L^{-1})	Part. (ng g^{-1})	Inf.	Eff.
Prog	23.2	<LOD	<LOD	<LOD	26.6	<LOD	24.8	<LOD	20.8	<LOD	14.9	<LOD	9.6	24	33[a] 35-108[b]	5[a] 0.8-2.3[b]
Mdrxy-Prog	<LOD	<LOD	<LOD	7.95	1.8	11.5	0.9	10.4	5.7	30.4	2.9	5.8	1.2	18	1.08[a] 18-58[b]	0.06[a] 0.1[b]
Testo	39.3	26.35	30.1	22.18	127.4	22.69	123.6	27	146.8	67	116.9	13.5	24.17	15	62.7[a] 21-76[b] 0-95[d]	1.2[a] 0.2-1.2[b] 0-21[d]
Levo	<LOD	<LOD	<LOD	<LOD	38.4	<LOD	20.1	42	21.8	26.7	<LOD	<LOD	7	12	150-170[c] 48[d]	30[c] 93[d]
Nore	<LOD	101	<LOD	67.5	78.8	193	31.8	77	55.8	198	16.8	36.5	23	28	0-224[d] 70-205[c]	0-159[d] 30[c]
E1	<LOD	<LOD	<LOD	<LOD	81.3	<LOD	47.7	<LOD	38.9	<LOD	129.7	<LOD	23	21	56[a] 6.5-19.1[b]	12[a] 0.2-8.6[b]
E2	<LOD	46	<LOD	40	218.4	108	156.6	59	274	117.7	147	16.5	77	31	15[a] 0.9-3.8[b]	1[a] 0.2-0.8[b]
E3	<LOD	<LOD	<LOD	<LOD	295	<LOD	250	<LOD	378	<LOD	265	<LOD	71	117	26[a]	0.2[a]
EE2	<LOD	69.5	<LOD	29	28.6	116.6	15.3	47.6	19	131	13	22	6	21	<0.3[a] 0.7-1.1[b]	<0.3[a] 0.1-0.7[b]

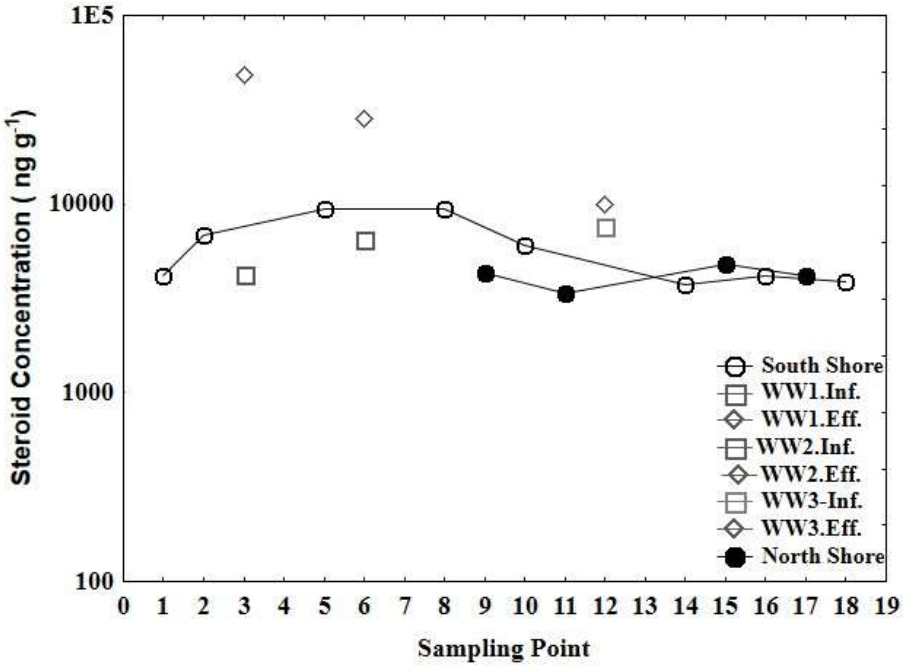
a)[166]; b) [35]; c) [68]; d) [51]

Figure 4-4 illustrates the steroid profiles in the particulate phase of spring and summer samples along the south and north shores of the river with WWTP discharges and total steroids detected in WWTP influents. The total steroid concentration in spring remained almost constant for the south shore (mean concentration of 4816 ng g^{-1}) except downstream of WWTP1 and WWTP2, which discharge 9361 and 9405 ng g^{-1} of total steroids to the river. The mean concentration of steroids for the north shore was 4153 ng g^{-1} with EE2 having the highest concentrations. In summer, the mean concentrations of total steroids for the south and north shores' particulate phase were 3918 and 5056 ng g^{-1}, respectively.

Table 4-4. Mean concentrations (ng L^{-1}) and standard deviations of steroids detected in dissolved and particulate phase of water samples taken along the river during 2 sampling campaigns. < LOD is below the detection limits.

Compound	Summer (n=12)			Spring (n=12)			LOD (µg L^{-1})	
	Diss.	Part.	% positive	Diss.	Part.	% positive	Diss.	Part.
E2	< LOD	7±5	100	< LOD	11±7	100	0.11	15
EE2	< LOD	5±2	100	< LOD	35±15	100	0.14	15
Progesterone	< LOD	3±1	83	< LOD	< LOD	0	0.13	11
Testosterone	< LOD	10±4	25	< LOD	6±2	100	0.05	15
Norethindrone	< LOD	< LOD	0	< LOD	8±4	100	0.15	14

a)



b)

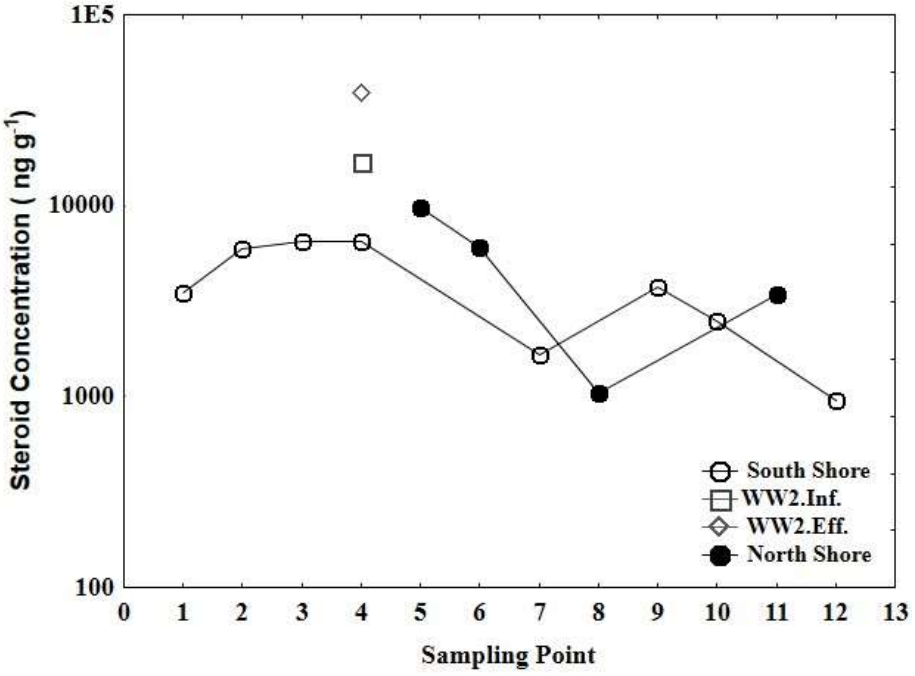
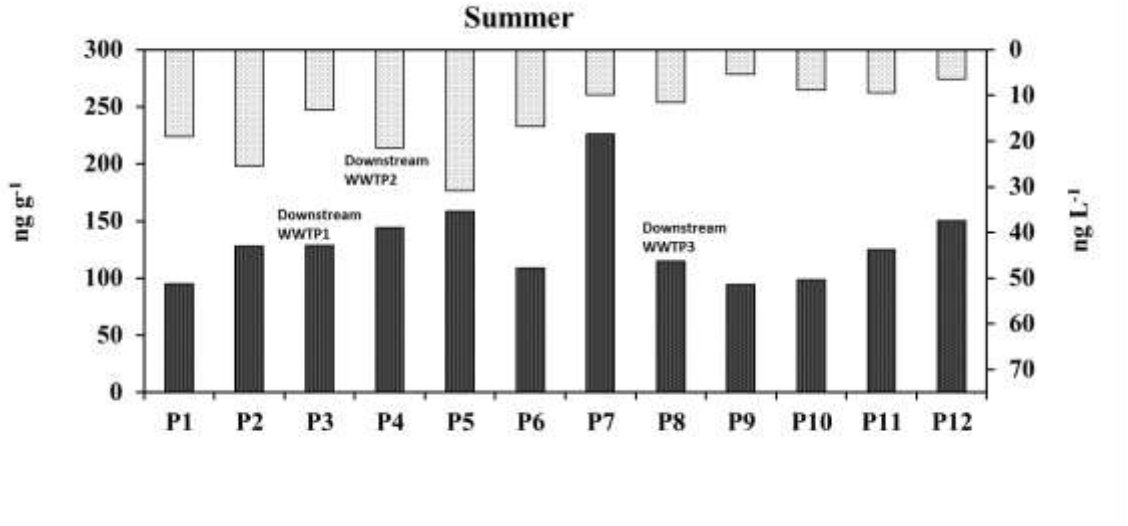


Figure 4-4.Steroid pattern in particulate phase along the both shores of the river and WWTPs influent and effluents during the a) summer and b) spring sampling.

4.3.2 Steroids in river sediment

The concentrations of detected steroids in river sediments for all sampling campaigns are shown in Table 4-5. For all seasons EE2, E2, and Prog were the only observed compounds in river sediment with a frequency of detection of 100% for EE2 (n= 36 samples from 3 campaigns), suggesting its widespread occurrence in sediments. Frequency of detection was 67% for E2 and 61% for Prog. The presence of the three detected compound is consistent with their high K_{ow} values as compared to the other selected steroids. A high seasonal variation was observed in the steroid concentrations in bed sediments. Mean steroid concentrations decreased from autumn (367 ng g^{-1}) to summer (130 ng g^{-1}) and spring (9.84 ng g^{-1}), considering that EE2 was the only steroid detected in spring sample. In autumn, steroid levels varied between 7 and 33 ng g^{-1} (E2), 13 and 51 ng g^{-1} (EE2), 22 and 1213 ng g^{-1} (Prog).

a)



a)

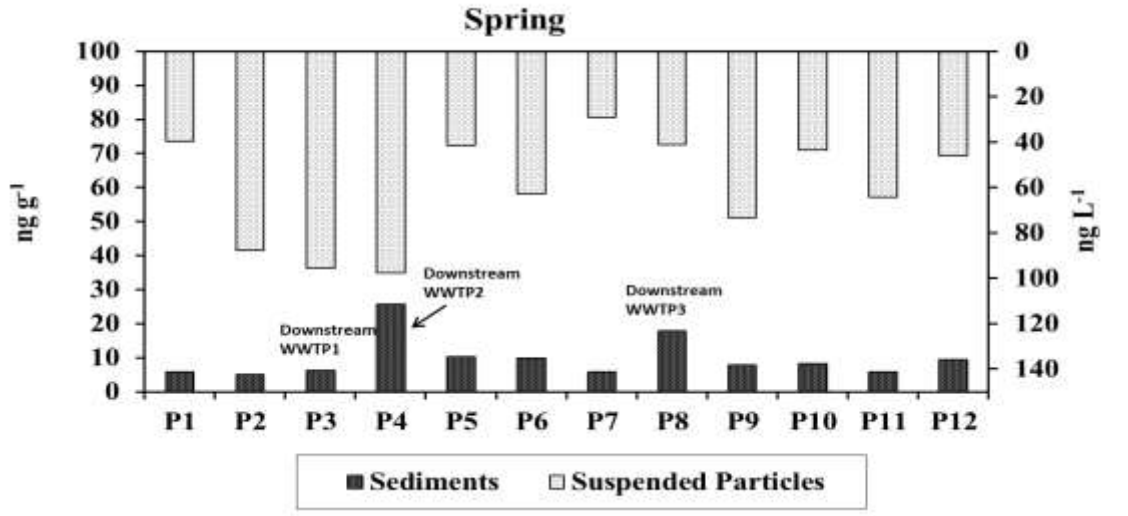


Figure 4-5. Distribution of total measured steroids between suspended particles (ng L^{-1}) and sediment bed (ng g^{-1}) along the river. a) Summer, b) Spring; Arrows indicate sampling points downstream of WWTP effluents.

Table 4-5. Mean concentration (ng g^{-1}) of steroids adsorbed on shore river sediment from the river. The mean concentration represents the mean value of 12 sampling points per sampling campaign. < LOD is below the detection limits.

Compound	Autumn (n=12)				Summer (n=12)				Spring (n=12)				LOD (ng g^{-1})
	Mean	Min	Max	% positive	Mean	Min	Max	% positive	Mean	Min	Max	% positive	
E2	18	7	33	100	56	21	110	100	<LOD	<LOD	<LOD	0	9
EE2	25	13	51	100	40	22	117	100	10	5	26	100	9
Progesterone	353	22	1213	92	68	49	91	50	<LOD	<LOD	<LOD	0	13

4.3.3 Partitioning of steroids between water and sediments

A comparison was made between the quantity of steroids adsorbed to the river water particles and the river sediments. Figure 4-5 depicts this comparison between two sampling campaigns (summer and spring). In spring, more steroids were found in suspended particles (mean concentrations ranged between 29-97 ng L^{-1}) than in the sediments (5-25 ng g^{-1} in summer) in contrast to the summer samples where mean steroid levels in suspended particles varied between 5 and 30 ng L^{-1} and between 94-226 ng g^{-1} in sediments. Differences may be explained by several factors: (i) the lower river flow rate in summer favours a higher deposition rate of particles, (ii) higher flowrates and turbidity following the snow melt period (TSS= 20.48 mg L^{-1} in spring and 9.7 mg L^{-1} in summer), (iii) the occurrence of unrestricted sewer overflow loads in spring (30 g d^{-1}, Figure 4-3), and (iv) the possibility of desorption from sediments during the period of high river flow require further investigation.

The amount of steroids sorbed to suspended particles at two temperatures (spring and summer) was compared to that sorbed on sediments. The ratio of Prog, E2, and EE2 levels ($K_p = C_{particulate}/C_{sediement}$) in the water particulate phase ($C_{particulate}$; ng L^{-1}) and in the sediments ($C_{sediement}$; ng g^{-1}) were used for this comparison (Table A-1. 3). In spring, when only EE2 was detected in sediments, the K_p for EE2 varied between 0.08 L ng^{-1} (P4) and 0.31 L ng^{-1} (P3). In summer, when higher levels of steroids were detected in sediments (Figure 4-5), the K_P for EE2 was one order of magnitude higher than that for spring with mean value of 10.5 L ng^{-1}. Among the three detected compounds in summer sediments, Prog showed the highest K_p value (31 L ng^{-1}).

4.3.4 Steroids in drinking water plants

Following steroids from the effluents of WWTPs to DWP intakes, it is expected that dilution or other processes such as biological degradation or photodegradation decrease their concentrations. However, the hormones were unexpectedly observed at the same levels in DWP intakes as in raw sewage. All the selected steroids except Prog were frequently detected in drinking water intakes. Table 4-6 shows the steroids levels detected in DWP intakes. Testo, Nore, EE2, and E2 were observed in the particulate phase of DWP intakes at concentrations ranging from 8.7 to 100.5 ng L^{-1} in spring samples and 1-72 ng L^{-1} in summer samples. Figure A-1. 2 compares the concentration of steroids detected in DWP intakes with those detected in WWTP effluents. In

spring, the maximum mean steroid concentration (46.5 ng L^{-1}) was found in DWP4 located 8 kilometers downstream of WWTP2 with the same mean concentration that was detected in effluent of the WWTP2 (43.8 ng L^{-1}).

Table 4-6. Concentration (ng L^{-1}) of the detected steroids in particulate phase of samples from DWP intakes during the spring and summer; LOD is detection limit.

Compound	Spring (n=5)					Summer (n=5)				
	DW1	DW2	DW3	DW4	DW5	DW1	DW2	DW3	DW4	DW5
E2	32.4	25.4	23.6	40.4	32.7	35.7	72	18.5	28.2	17.7
EE2	40.5	32.8	40.6	70	47.2	18	18.4	7.6	18.9	10.4
Testo	20.2	8.7	19.8	29.	32.3	4.6	2	2.9	3.8	1.9
Levo	27.1	16.2	10.3	LOD	LOD	23.3	13.8	1.9	2.4	2
Nore	100.2	81.8	94	47	55.4	7.1	4.6	2.1	2.9	1.8
MDRXY-Prog	LOD	LOD	5.3	14	8.2	LOD	LOD	LOD	1.03	LOD

4.4 Discussion

Considerable amounts of steroids were found in suspended particles of WWTP effluents and in river water. The major difference of our results with other reported values on the occurrence of steroids in WWTP effluents and surface waters is that almost all the previous studies were underestimating the particulate fraction of hormones while measuring their concentrations. Partitioning of detected steroids between dissolved and particulate phases of WWTP influents and effluents is listed in Table 4-3 and demonstrated in Figure 4-6.

Estrogens and progestogens detected in influent/ effluent of three WWTPs studied in current study are in agreement with previous reports [166]. In contrast, Testo levels are found much higher than few previous data. Testo and Prog were found in two WWTPs in China with 6.9-8.9. and 4.3-12.2 ng L^{-1} in influent and N.D.-2.5 and ND.-6.4 in effluent, respectively [4].

Steroid estrogens were detected in the dissolved phase of WWTP influents around the world over the wide range of concentrations between 0.5-845 ng L^{-1} [47, 171]. Except for Prog, E1, and E3 which were not detected in all samples, all other steroids were detected in the particulate phase of WWTP effluents with concentrations ranging between 4.5 to 77 ng L^{-1}. The absence of E3 in the particulate phase is related to its higher solubility compared to other steroids. The concentrations of progestogens detected in suspended phase of WWTP effluents are in good agreement with previously reported values for progestogens [11, 35, 51, 153] except for Levo for

which higher concentrations have been reported [5, 68]. The detected levels for testosterone and all the estrogens in the current study were much higher than those reported previously [35] but in agreement with our previously reported values for E2 and EE2 in different WWTPs [5](Table 4-3).

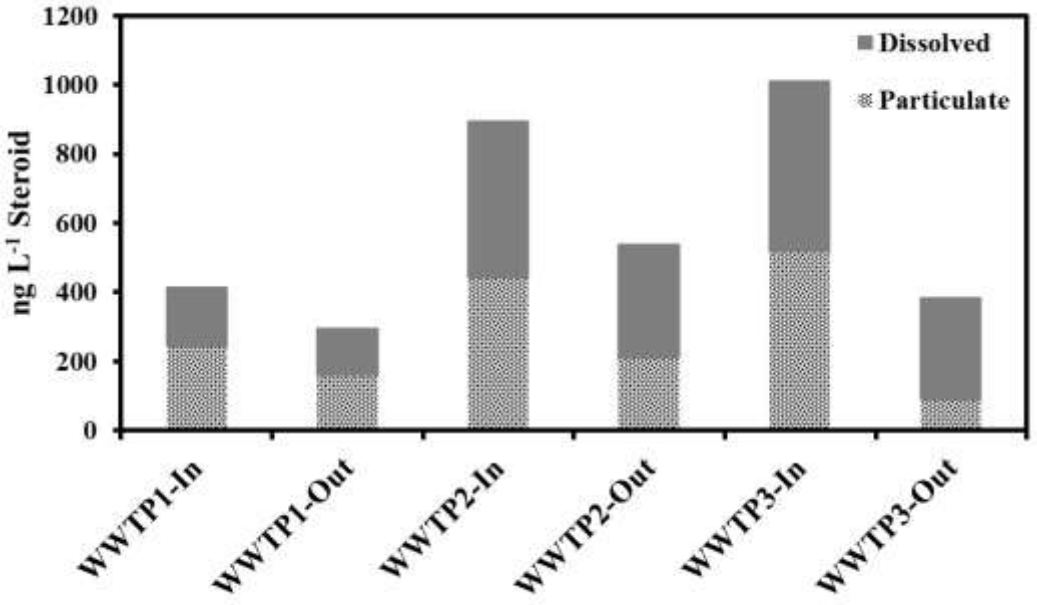


Figure 4-6. Distribution of total steroids between dissolved and particulate phases of WWTPs influent and effluent.

In terms of removal efficiency, the highest efficiency was observed in WWTP3 with 82% mean removal rate for 6 detected compounds from the particulate phase and 45% from the dissolved phase. WWTP2 and WWTP1 removed 31% and 30% of steroids of particulate phase and 33% and 52% of dissolved phase, respectively. The highest removal efficiency was 86% for E2 from the particulate phase and 82% for Levo from the dissolved phase of WWTP3. However, the removal efficiency depends not only on the WWTP treatment processes but also on the particular compound and sampling period. Although 81% of Prog in the dissolved phase was removed in WWTP1 it was eliminated only 7% in WWTP2. Levo and Prog have been reported as nearly recalcitrant compounds in activated sludge processes, degrading within 12 days and 9 hours, respectively [4, 35]. E1 was removed by 41% in WWTP2 whereas its concentration increased from 39 to 130 ng L^{-1} from the influent to effluent of WWTP3. E1 and E3 are reported as two main degradation products of E2 [40]. A major part of the 46% removal of E2 from WWTP3 is

probably from its transformation to E1 by wastewater microorganisms. E1 production from the degradation of E2 has been reported during aerobic and anaerobic batch experiments [172]. Results suggested that E2 was readily degradable to E1 with sewage bacteria (75% transformation after 22 hr contact). Concentration of norethindrone increased after primary treatment in four WWTPs in China [173]. The mean concentration of norethindrone in four WWTPs was 25 ng L^{-1} in influent and 1.25 ng L^{-1} in effluents. Although primary treatments failed to remove norethindrone, its overall removal efficiency ranged between -70% to 100% with major removals related to biological degradation.

In terms of the effects of the types of treatment processes on the removal efficiency of steroids, the minimum mean removal rate was found for the effluent of WWTP2 (33%) which uses physical-chemical treatment processes in contrast to two other WWTPs that use biofiltration. These findings confirm that biological processes can be more effective in removing readily biodegradable steroids such as natural estrogens [172]. However, for strong hydrophobic steroids such as EE2, Prog, MDRXY-Prog and Levo with moderate to high K_{ow} values, the contribution of adsorption must be considered as another key removal mechanism. Importantly, high levels of some compounds such as estradiol, which is known as the most potent steroid estrogen and also norethindrone, were found in the effluents of two of the WWTPs. The quantity of steroids detected in suspended phase confirms the important role of the small particles on the total steroid loads from WWTP discharges, as 55% of the total hormones were detected in particulate phase of the three measured effluents. Comparing the quantity of steroids associated to suspended particles in raw sewage and effluent, the concentration of total steroids in all the effluents were increased between 1.3 and 11.5 folds between raw and treated wastewater (Figure 4-2). During wastewater treatment, large particles are majorly removed by sedimentation while recalcitrant steroids would attach to smaller particles and end up in effluents at higher concentrations. A comparison between the ratio of particles larger than 0.3 µm and TSS in raw and treated sewage shows this ratio increased in effluents confirming the higher concentrations of steroids per gram of suspended particles in effluents.

After release into the river, no specific pattern was found for steroid profiles in suspended particles. Generally, a clear gradient of micropollutants is expected when there is a single source of that compound in receiving water. In the case of the river considered in the current study, several CSOs and WWTP discharges prevent an observable gradient of steroids along the river.

However, some samples were found to have higher levels of steroids such as downstream of WWTP1 that is also located downstream of a contaminated urban creek indicating that WWTP discharges and other sewer are important point sources of steroids in the river.

Although, no clear pattern was detected in steroids levels in sediments along the river, higher concentrations were noted in sediments in the central section of the river. Such variations in steroid levels are likely due to differences in organic content of sediments and proximity of CSOs and effluent discharges. Higher levels of steroids were found downstream of WWTP 2 (P4) and WWTP3 (P8) and combined sewer discharges (P9 and P11) during the summer. The maximum concentrations were detected at P7 (226 ng g^{-1}) and P5 (150 ng g^{-1}) demonstrating steroids could travel some distance downstream from WWTPs before accumulating in bed sediments. The aerobic/ anoxic sediment conditions at the time of sampling might also influence steroids levels. Estradiol was quickly degraded in marine sediments under aerobic conditions ($t_{1/2}$= 2 days), whereas in similar conditions, EE2 degraded more slowly ($t_{t/2}$= 81 days). In anaerobic conditions, E2 and EE2 remained unchanged over a period of 70 days [174].

During low water temperatures (5°C) at the time of sampling, accumulation of steroids is expected because of lower rates of biological degradation. Clear seasonal variations were observed in total steroids in particulate phase of river water as shown in Figure 4-7. During the summer, as in the autumn campaign, three dominant steroids (E2, EE2, and Prog) were detected in sediments of all the 12 sampling points except for Prog which detected only in 6 samples (Table 4-5). Higher temperatures during summer sampling resulted in lower concentrations of Prog (48.83- 91.27 ng g^{-1}, among the points were Prog was detected) in river sediment. But E2 and EE2 were detected at higher levels; EE2 (21.9-117 ng g^{-1}), E2 (21.05-109.22 ng g^{-1}). Although higher temperature is expected to increase the possibility of biological degradation, lower river flow (135 m^3 s^{-1}) and higher contribution of WWTPs effluents in river flow (1.6%) at the time of sampling can increase accumulation of loads of E2 and EE2 which are among the ubiquitous hormones in sewage effluents and surface water. The concentrations of other hormones in sediments were below the detection limits. Higher total concentrations of steroids were found in P7 and P12 which can be attributed to two main reasons: the one is the presence of sampling point (P7) downstream of the very contaminated urban creek and the other is the proximity of several CSOs to P12 sampling point.

EE2 was the only steroid detected at concentrations higher than LOD in river sediments during the spring sampling campaign (T=12 °C). The concentration of EE2 varied between 5 and 25 ng g^{-1}. The highest concentration detected at (P4) the sample point just downstream of the WWTP2, with 21.7 g s^{-1} total steroid mass flow. Lower quantity of steroids detected during spring sampling is probably due to the rapid river flow (350 m^3 s^{-1}) following the snow melt period and high precipitation. The higher flowrates lead to scouring of the river bed that would increase steroid transport from the sediment to the water column [77]. Additionally, the contribution of WWTP effluents was lower during the spring (0.65%) sampling compared to summer.

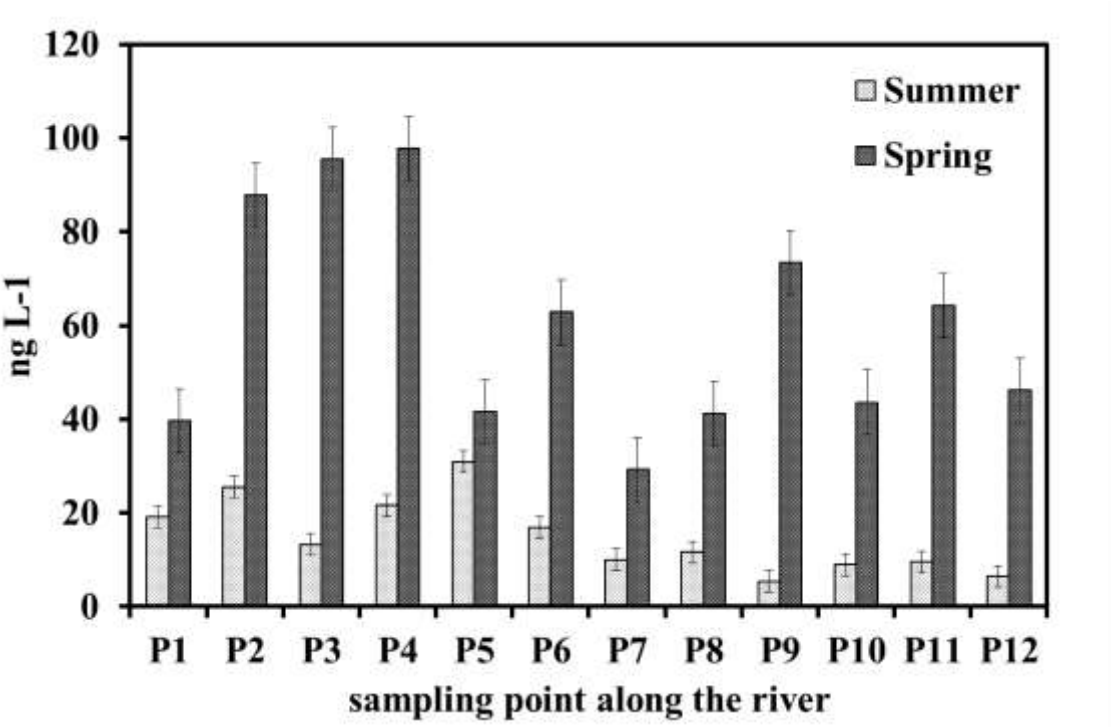


Figure 4-7. Seasonal variation in total steroids levels in suspended particles from water samples taken along the river. Error bars represent the standard errors.

Sediments particle size distribution influences the quantity of hormone adsorbed on sediments and it is previously reported that steroids sorb more to smaller particles [17]. Progesterone, testosterone, E1, and E2 were all preferentially adsorbed on the colloidal and clay fractions (particle sizes 0.87 and 1.43 µm, respectively) of silty loam sediment at concentrations of between 15-30 ng L^{-1} [17]. The fraction of particles < 80 µm ($F_{<80\mu m}$) in the current study can potentially affect the total of adsorbed hormones in each sample. This fraction varied from point to point and depended on the type of sediment and river surface. Seasonal variations were also

detected in the < 80 µm fraction of particles. Sediments sampled during the summer campaign have 1-31% $F_{<80\mu m}$ whereas spring sediments have 0-8% $F_{<80\mu m}$. The maximum $F_{<80\mu m}$ in spring was related to P5 (8%) in which 10.1 ng g^{-1} of the only detected compound (EE2) was found. The

maximum $F_{<80\mu m}$ in summer was related to P1 (31%) in which 24.5 ng g^{-1} EE2 was found. In summer samples the highest concentrations of detected steroids (226 ng g^{-1}) were related to the sediments of P7 with $F_{<80\mu m}$ =18%. These findings emphasize the importance of organic content of sediments on the amount of hormones sorbed to sediment particles. High amounts of organic content were found in sediment samples immediately downstream of WWTP discharges in all seasons.

Testo, Nore, E2 and EE2 were the most recalcitrant to WWTP processes with mean concentrations of 11.3 ng L^{-1} in summer and 37.8 ng L^{-1} in spring in the particulate phase of DWP intakes Table 4-6. These high levels can also indicate the non-point sources of hormones such as CSOs or urban creeks contaminated with mixed agricultural and urban runoff. This is evidence to the necessity of applying advanced treatment processes in order to achieve sufficient removal of potential endocrine disruptors during wastewater treatment. Occurrence of steroids in DWP intakes at such considerable levels raises the need for efficient processes to remove these compounds during water treatment and manage the sludge produced during drinking water production. Steroids were detected in sludge samples from the sedimentation tank of DWP3 with mean concentration of 653 ng g^{-1}. Norethindrone was detected at highest concentration (1016 ng g^{-1}) while MDRXY-Prog was detected with the lowest concentration 288.45 ng g^{-1}). Compounds found in DWP sludge at such high concentrations are likely to be recalcitrant and not readily biodegradable. They might be adsorbed on colloidal particles and settled on sludge bed during the coagulation process. The use or reuse of sludge for agricultural purposes brings the concerns about the endocrine disrupting effect of steroids. Consequently, application of highly effective coagulation/sedimentation process is suggested for DWPs in order to effectively remove suspended particles and prevent steroids to enter subsequent treatment steps.

This study as most studies focused on selected steroids focused on the prevalence of specific compounds. The possible interconversion occurring in sewers, wastewater treatment and in the natural environment must be considered besides investigating the occurrence and fate of steroids.

Therefore potential impacts of altered steroids on the environment, as demonstrated for E1, E2, EE2, Testo, and Prog [11, 175, 176] would account for degradation products, conjugated steroids, and parent compounds at the same time. Despite these limitations, the information provided in this study could still give valuable information on the role of small suspended particles as an important source of steroids in WWTP effluent and river water.

4.5 Conclusion

Samples were taken from WWTPs, the receiving river water, and DWP intakes to investigate the overall loadings of 9 steroid hormones by their partitioning in dissolved/particulate phases and sediments during three sampling surveys. The studied river receives effluents from several WWTPs and CSOs and also non-point sources. The new information provided by this study indicates that contamination loads of the investigated steroids can reach concentrations high enough to affect aquatic organisms. Estradiol (E2) and 17α- ethinylestradiol (EE2) showed 100% detection in all water samples in the particulate phase, indicating their extensive presence in studied river water. The concentrations of all studied compounds were below the detection limits in dissolved phase thus it was not possible to measure their distribution coefficient between dissolved and particulate phases. In bed sediments, the highest concentrations were detected for E2, EE2 and Prog during the autumn sampling campaign with mean concentrations varying between 25 and 353 ng g^{-1}. No specific trend was found in steroid profiles downstream to upstream of the river neither in the water nor in the sediments most likely due to the highly variable organic content and quantity of suspended particles in water samples and also strong effect of river surface and sediment type on the measured quantity of steroids in bed sediments. E2, EE2 were the three compounds detected in all sediments for all seasons. Progesterone was detected in 50% of sediment samples in autumn. Mean concentration of detected steroids in sediments were higher in summer than in spring contrary to the hypothesis of higher biodegradation rates at higher water temperatures presumably because of very low river flow and higher contribution of WWTPs effluents in river flow. Testo, Nore, E2, EE2, and occasionally levonorgestrel were detected in particulate phase of DWPs intake with mean concentration of 11.3 ng L^{-1} in summer and 41.5 ng L^{-1} in spring indicating the non-point sources of hormone discharges in the river. High concentrations of steroids in DWP intakes highlight the need for highly effective processes to remove recalcitrant compounds during drinking waterproduction.

4.6 Supplementary materials

Tables for DWP and WWTP water qualities, and distribution ratio for steroids between river suspended particles and sediments, and Figures for individual steroids level in influent and effluent of WWTPs, and comparison between steroids detected in DWP intakes and WWTP effluents are provided in supplementary materials.

4.7 Acknowledgements

The authors would like to acknowledge the NSERC Industrial Chair on Drinking Water Treatment and its partners (City of Montreal, City of Laval and John Meunier Inc.) for financial support and the technical staff from the Centre de Recherche, de Développement et de Validation des Technologies et Procédés en Traitement des Eaux (CREDEAU) for supporting laboratory work.

CHAPTER 5 ADSORPTION OF STEROIDS ON RIVER SEDIMENTS

5.1 Overview

Sorption to aquatic sediments is one of the important fates for steroids which directly affect their mobility in the aquatic system and their bioavailability for aquatic creatures. After sorption to sediments, steroids may biodegrade with microorganisms present in sediment, desorbed from sediments to aquatic phase or re-suspend in the system and travel from their original place. According to hydrophobic character of steroids, it is worthy to study their behavior while they come into contact with soil, sediment, or sludge particles. Extended information is available on the presence of steroids in soil, sediment and sludge. However, data are limited on their kinetic behavior and their capacity to bind with organic content of the solid phase. This Chapter studies the sorption capacity, sorption kinetics and isotherm data for the adsorption of selected steroids on different sediments. Supplementary information is provided in Appendix 2.

5.2 Introduction to adsorption of steroids on solid particles

A growing number of studies report the widespread occurrence of natural and synthetic steroid hormones in the aquatic environment in numerous countries [4, 33, 35, 37, 43, 69, 95, 177]. Focus on the occurrence and fate of progestogens and testosterone in the aquatic environment is more recent but they are considered as the most important group of environmental pharmaceuticals along with estrogenic steroids because of their potential to cause adverse effects on aquatic organisms [11, 178]. Sorption onto solids and biological degradation are two main pathways for removal of steroids from the aqueous phase. Steroid hormones are non-polar hydrophobic compounds that can be easily adsorbed onto river sediments. Sorption of steroids on aquatic sediments can directly affect their mobility, transformation, bioavailability and subsequent fate in the natural water systems including drinking water intakes. Natural and synthetic estrogens have been detected in river sediments at the ng level. Concentrations of estrogens in three river sediments in Northern China ranged from 13.4- 28.5 ng g^{-1} , with E1 showing the maximum concentrations (0.98-021.85 ng g^{-1}) [78]. More specifically, testosterone and progesterone levels were detected in river sediments from a river in middle of China at 8.1

and 2.4 ng g^{-1} [81]. Similar concentrations of natural estrogens were detected in river sediments in Spain, with values up to 3.37 ng g^{-1} and 22.8 ng g^{-1} for synthetic estrogen EE2 [77].

Solid-liquid distribution coefficient (K_d) have been determined experimentally for natural estrogens and their conjugates in sewage particles without controlling for biological degradation showing higher adsorption at neutral pH [18, 19, 32, 109]. Studies of the adsorption of steroids onto sediment and soil indicate a direct correlation between the amount of adsorbed estrogens, the organic content of sediment, and particle size distribution of sediments [109]. Particle size has been shown to be less important than the organic content of sediment, more steroids being adsorbed onto clay and colloids in the silty load sediment and fine particles of sandy sediments [17].

Results from previous experiments on the sorption estrogens suggest rapid sorption kinetics approaching an equilibrium that can be described by a pseudo second-order kinetic model [109, 113]. Yu et al. 2004, investigated the sorption of E1, E2, and EE2 onto six sediments and one soil samples from different points around the USA [179]. The required time to attain sorption equilibrium varied between 2 days and 14 days when the concentration of estrogen in aqueous phase was 50% or 20 times higher of/than their solubility limits, respectively. A pseudo second-order kinetic model was also used to describe the adsorption of estrogens onto different tropical sediments in Brazil, with adsorbed estrogens ranging between 36-153 µg g^{-1} [114]. A reverse correlation was found between the time to reach equilibrium and the particle size of the soil [180]. A steady-state equilibrium with fine particles (250 µm) was reached in a few hours, whereas for soil with larger particles (2mm), up to 48 h were required.

Several types of sorption isotherms have been applied to model the adsorption of pharmaceuticals and, to a lesser extent, of steroid hormones. The determination of sorption coefficients of natural and synthetic estrogens on a sediment were better described by a nonlinear sorption model (Freundlich) with K_f values ranging 1.33 to 2.26 ($mg^{1-1/n}$ $(m^3)^{1/n}$ g TSS^{-1}), respectively [97].

In this study, we investigated the sorption kinetic of testosterone and progestogens using batch mode testing onto river sediments, as compared to estrogenic steroids. Sediments with different organic content were collected along the river in Quebec, Canada. For kinetic studies, steroids were quantified for both liquid and solid phases. Using this approach following achievements are

expected: 1) the order of sorption amount for steroids, 2) pseudo-second order sorption constants for steroids in different sediments, and 3) production of sorption isotherms for steroids.

5.3 Experimental

5.3.1 Chemicals and standards

All steroids standards were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Mass-labeled internal standards [$^{13}C_3$] - Estradiol and [$^{13}C_6$] - Progesterone were supplied from Cambridge Isotope Laboratories, Inc. (Andover, MA). All solvents were of trace analysis grade and purchased from Fisher Scientifics (Whitby, ON, Canada). Stock solutions (1000 mg L^{-1}) of progesterone (Prog), medroxyprogesterone (MDRXY), testosterone (TESTO), levonorgestrel (LEVO), norethindrone (NOR), estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) were prepared by dissolution in HPLC grade methanol. A mixture of all individual stock solutions at 4 mg L^{-1} was prepared in methanol. All solutions were stored at -20 °C in amber glass tubes for a maximum period of 6 months. GF-75 glass fiber membrane filters (0.3 µm, 47mm diameter) were obtained from Sterlitech (Kent, WA, USA).

5.3.2 Sample collection and sample treatment

Sediment samples were collected from three different points along the Des Mille-Îles River (located in the north of Laval, Quebec, CA). Sediments were taken from surface to maximum of 20 cm at the depth of almost 1.5 meters near the shore river, transferred to autoclaved amber glass bottles, stored in an insulated chest cooler before retrieving to the laboratory. River water samples from the surface were also collected at the same time in a 1 L previously washed polypropylene containers at approximately 0.3 m below the water surface. Upon arriving to the laboratory, both sediment and water samples were sterilized using gamma radiation (30 kGy, 5.2 h) and then stored at 4°C. Sediment sterilization using gamma irradiation was confirmed by testing the total concentration of aerobic and anaerobic bacteria after irradiation, using tryptone soybean agar (TSA) at temperature of 30 °C for up to 7 days. Sediments were wet sieved (<1.25 mm) with river water from the same sampling point in order to remove debris. Wet sediments were allowed for decantation then solid fraction was used for sorption batch experiments. Sediment properties such as total solid concentration, fraction of organic carbon (f_{OC}), and

sediment pH were analysed using standard methods (Table 5-1). It can be noted that the four sediments tested differed mainly in terms of organic matter content (8-89%), organic carbon content (9900-21600 µg g^{-1}) and small particle fraction (<80 µm) (1-31%).

Table 5-1.Properties of four sediment samples.

Parameter	Sediment sample			
	S1	S2	S3	S4
pH	7.17	7.2	7.26	7.16
OM (VS/TS) %	8	37	8	89
f_{OC} (µg g^{-1})	11000 (52%)	21600 (73%)	9900 (57%)	10600 (17%)
< 80 µm particle size fraction %	14	31	14	1
Total solid (g g^{-1})	0.59	0.73	0.44	0.25

5.3.3 Sorption experiments: kinetics and isotherms

5.3.4 Sorption on sediments

The kinetics experiments were designed based on the Organisation for Economic Co-Operation and Development (OECD Guideline – test No. 106)[157]. A 1-g portion of the fresh irradiated sediment was placed in a 15-mL conical polypropylene centrifuge tubes as batch reactors and 5 mL of irradiated river water was added to each reactor. In one series of experiments a 1:1;W:v ratio of sediment to water was tested in order to verify the effect of sediment/ water (S : S) ratio on the sorption quantity. An appropriate volume of standard and steroids mix solutions was added to each reactor in order to obtain desired concentrations (5, 50, and 100 µg L^{-1}). All the experiments were carried out at ambient room temperature (25± 2 °C). The reactors were shaken on an orbital agitator for maximum of 96 h. The usage of polypropylene tubes was confirmed in previous studies to have negligible adsorption of the steroids on walls of the tube [5]. To determine the equilibrium time and kinetic parameters, samples were equilibrated for 0, 0.08, 0.25, 0.5, 1, 24, 48, and 96h. Individual reactors in duplicate were assigned to each reaction time. The liquid phase of each reactor was separated by centrifuging at 6000 rpm for 1min (for reactors at time t=0-1 h) or for 15 min (for reactors at time 24, 48, and 96 h). The solid phases were frozen at -20 °C and then freeze-dried before further analysis for mass balance calculations.

The liquid phase of each reactor was filtered through 0.3 µm pore size glass micro fiber filter to remove any residual particulate matter. The filters were also analysed for any loss of steroids

through adsorption on suspended particles on filter. The filtrate was then diluted to 50% by ultrapure water in order to prevent the HPLC column from saturation. In order to avoid any probable biological degradation, the liquid sample was acidified using formic acid (> 95% purity) as 25 µL for each 5 mL of liquid phase prior to LC-MS/MS analysis.

The freeze-dried solids from each reactor were extracted by sonication-assisted solvent extraction using a 3:1; v:v mixture of methanol and acetone. The extraction method is described in detail in previous study [5]. The extract was reconstituted to 5 mL with acidified water (0.1 % formic acid) containing 5 % methanol, sonicated at 30 °C for 10 min, and then centrifuged for 10 min at 6000 rpm. The extract was then filtered as the liquid phase and analysed by LC-MS/MS.

To determine the adsorption isotherms of steroids on sediments, 5 different concentrations of individual steroids 5, 10, 25, 50, 75, and 100 µg L^{-1} were selected for adsorption experiments.

5.3.5 Analysis and quantification of compounds

Methods used for the quantification of steroids with LC-MS/MS system were described previously by Fayad et al. 2013 [169]. Samples were extracted and purified by on-line solid phase extraction (SPE) coupled with liquid chromatography and tandem mass spectrometry (LC-MS/MS). An atmospheric pressure photoionization (APPI) source was used for steroid detection. The on-line SPE was achieved using two Hypersil Gold aQ (20 mm×2 mm, 12 µm particle size) columns in tandem and chromatographic separation was done with Hypersil Gold (100 mm ×2.1 mm, 1.9 µm particle size). Ionization of steroids was achieved by the Ion Max API source mounted on a Quantum Ultra AM triple quadrupole mass spectrometry Thermo Fisher Scientific (Waltham, MA) operated in selected reaction monitoring (SRM) mode for quantification and detection. A sample loading volume varied between 1-mL and 5-mL for wastewater and river water, respectively. Ionization of hormones was achieved with an APCI source in positive (PI) mode. The MS/MS peaks were integrated using the Xcalibur 2.2 SP1.48 software from Thermo Fisher Scientific and concentrations were measured from the ratio of the analyte area to that of the internal standard. The limits of detection (LOD) were determined using a six point calibration curve, analysed in duplicate, in analyte-free water matrices. The LOD were calculated by multiplying by 3.3 the error on the y-intercept and divided by the slope of the regression line equations.

The suspended particles on filters were analysed for any loss of steroids via filtration. Steroids were analysed and quantified with the LDTD-APCI ionization source developed and manufactured by Phytronix Technologies (Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were spotted (5 µL) into the LazWell 96-well plate and dried at 40 °C for 10 min. the plate is then placed in the apparatus for analysis of samples and then introduced directly into the mass spectrometer. The detailed analysis procedure is available in previous studies [5, 168]. Results of the MS/MS peaks were interpreted using the Interactive Chemical Information System algorithm of Xcalibur 2.2 SP1.48 software from Thermo Fisher Scientific and concentrations were measured from the ratio of the analyte area to that of the internal standard.

5.3.6 Data Analysis of sorption isotherms

The equilibrium between the concentration of steroids in aqueous and solid phases can be described by sorption isotherms [24]. The sorption isotherms are constructed with batch experiments over a wide range of steroids concentrations. The adsorption of steroids onto sediment was verified by the most frequently used isotherm models. The Langmuir isotherm that is based on assumption of homogeneity of the adsorption sites on the monolayer of the adsorbent surface has the form:

$$q_s = \frac{k\, q_{max} C_e}{1 + kC_e} \qquad 6$$

Where q_{max} (µg L^{-1}) is the maximum adsorbent loading, C_e (µg L^{-1}) concentration of steroid at supernatant and k (L μg^{-1}) is the Langmuir adsorption coefficient related to the adsorption affinity. At low concentrations ($C_e << 1$) the Langmuir isotherm reduces to the linear isotherm:

$$q_s = k\, q_{max} C_e \qquad 7$$

The Freundlich isotherm is more representative at medium concentrations (neither at very low nor at the saturation levels of sorbent) and is most often the most appropriate model for adsorption processes in water treatment.

$$q_s = k_f C_e^n \qquad 8$$

Where, q_s is the amount of steroid adsorbed (µg kg^{-1}); K_f (L kg^{-1}) is the adsorption coefficient which describes the strength of adsorption. The higher adsorbent loadings are achieved at higher K_f values. The exponent *n* is Freundlich constant parameter describing the degree of nonlinearity and is related to the heterogeneity of the adsorbent surface. The value of *n* is usually lower than 1 which is favorable isotherm with *n* <1 show high adsorbent loadings at low concentrations. At *n* =1 the Freundlich isotherms becomes linear and the loading is equal to K_f. Freundlich, Linear, and Langmuir sorption isotherms were generated and fit to determine which isotherms best fits the sorption of steroids on sediments. The Statistica. Ink 13 (Dell Inc., OK, USA) was used for data evaluation, using 95% confidence interval for the best-fit sorption isotherms. Furthermore, in order to qualify the best fit the R^2-value for the curve should be > 0.8, otherwise no fit was made.

5.3.7 Solid-liquid distribution coefficient calculation

The solid- liquid distribution coefficient (K_d, L kg^{-1}) is an important parameter to determine the adsorption capacity of the sorbate. The K_d is defined as the ratio of the concentration of a dissolved substance in aqueous phase and solid phase at the equilibrium condition. The K_d value is identical for each solid phase type. A normalized form of K_d, the organic carbon partitioning coefficient (K_{OC}) is defined for natural systems. The K_{OC} is deducted from total organic carbon content of the adsorbent and K_d. The K_d and K_{OC} can be calculated with Equation 9 and Equation 10.

$$K_d = \frac{q_s}{q_e}$$ Equation 9

$$K_{OC} = \frac{K_d}{f_{OC}} \times 100$$ Equation 10

5.4 Results and discussion

5.4.1 Sorption Experiments

The results of sorption kinetic experiments for steroids presented in Figure 5-1 show the significant differences in steroid sorption as a function of time especially in terms of sorption

amount at equilibrium (q_e). Although the adsorption of steroids on sediments starts instantaneously, the adsorbed amount at equilibrium time is different between samples. The time to reach equilibrium varied between hormones and sediments with different organic carbon content. As illustrated in Figure 5-1, in sediment S2 adsorption profile was similar for Prog and estrogens increasing to 2 min then decrease to 5 min then increase to 15 min and then remained constant. While the adsorption profile for synthetic progestogens and testosterone was reduced sorption until 5min then increased sorption to 15 min then sorption amount remained constant. It would be concluded that for S2 all the hormones reached equilibrium with solid phase within 15 min. for S1 and S3 with similar and lower organic carbon content, adsorption of Prog, Testo, Levo, and estrogens increased up to 5 min then remained constant. While adsorption of MDRXY-Prog and Nore decreased until 2min, increase to 5 min then remained constant until 30 min. except for Prog with highest instant sorption on all sediments; synthetic hormones were more readily adsorbed as compared to natural hormones.

Sorption of steroids estrogens to soil and sediments has been evaluated in several studies but varying results are reported in terms of sorption amount and time to reach equilibrium [97, 181, 182]. Time for estrogens to reach equilibrium in sediments range widely from within one hour [97] to 170 hours [182].

The competition for sorption sites increase with decreasing OC content as shown by the minimal values sorption at the t = 0 is 14 % for E2 and the maximum is 56 % for progesterone measured for S4 with (f_{OC} = 17 %). Prior studies have investigated the extent of competitive sorption of estrogens at different initial concentrations, organic content of sediment, and particle size distribution of sediment [97, 183, 184]. In one study by Yu et al 2004, a mixture of estrogens exhibited lower sorption capacities on soil and sediments than in their single solute systems. The isotherm nonlinearity, sorption capacity and desorption of each compound was affected in the presence of other EDCs. Competition was suggested to be a function of the physicochemical properties of the compounds and compounds with similar molecular structure exhibited greater competitive sorption than those with very different structures and properties [179]. Li et al. investigated the competitive adsorption of BPA and EE2 in presence of E1, E2, and E3 onto river sediments. The effect of coexisting of compounds in binary or multiple compound systems on the amount of sorption was evaluated according to the linear isotherm data for compounds in different coexisting system [184]. Addition of E1, E2, and E3 to the system prompted the

adsorption of BPA indicating that these compounds do not compete with BPA for sorption. While the adsorption of EE2 was reduced in presence of E1, E2, and BPA suggesting that these compounds compete with EE2 for adsorption.

The order of partitioning of hormones was similar in three sediments. The amount of steroids sorbed to S4 with the lowest organic carbon content was in the order: E2 < EE2< E1< Levo< Testo< MDRXY< Prog. This behavior was slightly different for the S2 with higher organic content with the order: E2 < Nore < EE2<E1< Testo< Levo < MDRXY < Prog. In experiments with equal sediment/water ratio (1:1), Prog and EE2 were still the mostly adsorbed compounds. The sorption amounts for two sediment/water ratios were compared in Figure A-2. 2. The average sorbed amount for eight steroids for the first hour of contact with three sediment samples was 68 % ± 0.009 with 1:5 ratios while the average sorption increased up to 95% in the first hour of sorption process in sediment/water ratio of 1:1; w:v. Owing to high Kd values of steroids, the 1:5; w:v is the appropriate sediment: water ratio in order to keep the aqueous concentrations of compounds at detectable levels along the 95 h sorption experiments.

For all the sediment samples tested, Prog showed a higher sorption affinity than the other steroids. The high affinity of Prog to soil and sediment has previously been reported [17, 185]. Yamamoto et al. 2003 compared the sorption of E2, EE2, and some alkylphenols into the various types of dissolved organic matter (DOM) [186]. The sorption coefficients of estrogens by DOM were larger than those of alkylphenols. The authors suggested the hydrogen bonding between phenolic group of estrogens and DOM was a dominant sorption mechanism. In contrast, those alkylphenols with ester groups showed lower affinity with DOM. Since ester groups have less hydrogen donor contribution to sorption compare to phenolic groups. Therefore, given the chemical structure of steroids in this study, it is reasonable that Prog and E1 which have a ketone group in their structure show higher interaction with organic matter in sediments. The hydrogen bonding ability of substituent groups on selected steroids which is suggested to play an important role on their sorption affinity can better explain their sorption order. Although E1, E2, and EE2 all have phenolic group substituent, E2 has hydroxyl group on its C-17 position while E1 has ketone group which is more hydrogen donator. Progesterone and testosterone have very similar structures; however progesterone has acetyl substituent on its C-17 and also high electron donator ketone substituent on its C-20, while testosterone only has hydroxyl.

The difference between orders of sorption in different samples may be due to the different composition of organic matter in the sediments from the three sites. In sediments S2 for which highest sorption amounts were measured, the inorganic carbon content of sample is 27%. While in three other sediments the inorganic carbon content is lower (S1: 48%, S3: 43%, and S4: 83%). In one study by Neala et al. 2009, the interaction of E1, E2, Prog, and Testo with humic acid, alginic acid and tannic acid was evaluated at different pH ranging between 4 and 12 [185]. The sorption of Prog and E1 to humic acid and tannic acid at natural pH= 7 (as in current study) was found 2.3 to 7.9 times greater than E2 and Testo respectively. The strength of partitioning of hormones at natural pH was influenced by functional groups, and the strongest sorption observed for progesterone and estrone to tannic acid (TA). These authors attribute these phenomena to the dissociated form of humic acid and alginic acid at this pH (7) while tannic acid was in a non-dissociated form. Consequently, non-dissociating steroids interacted better with tannic acid.

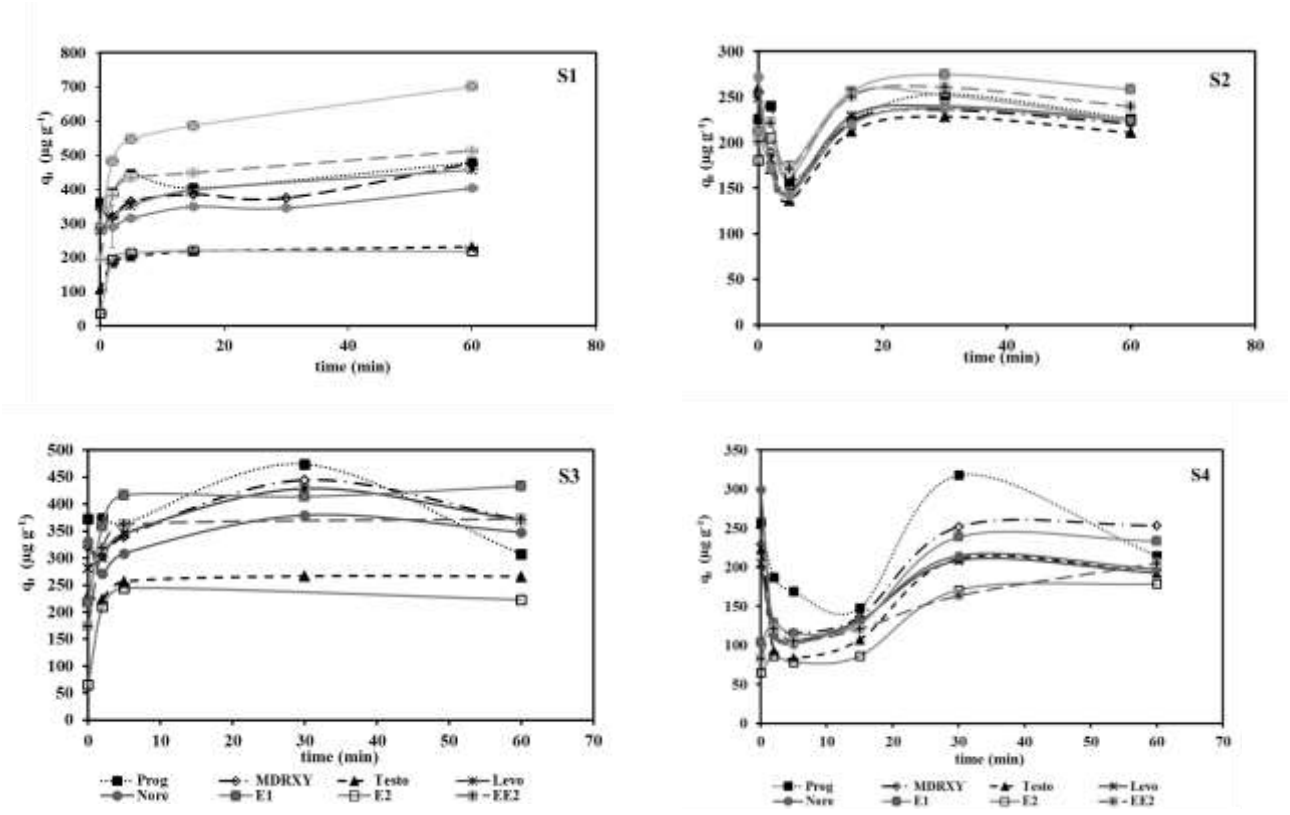


Figure 5-1. Sorption of steroids onto sediment samples at different organic carbon contents S1 f_{OC}= 52%, S2 f_{OC} = 73%, S3 f_{OC} =57%, S4 f_{OC} =17%. Conditions: mass of sediment=1 g; volume of solution= 5 mL; temperature= 25 °C; q_t values from duplicate analysis of duplicate measurements.

5.4.2 Solid- liquid distribution coefficients (K_d)

The results of adsorption experiments showed that sorption of steroids on sediments started instantaneously and sorption equilibrium has been reached after about 15 min or 30 min depending on the sample and hormone (Figure 5-1). Therefore, the solid-water distribution coefficients (K_d, L kg $^{-1}$ solid) values were calculated based on data after the equilibrium at both 1:1 and 1:5 sediment/ water ratio. The normalized organic carbon partition coefficients (K_{OC}, L kg^{-1} OC) were also estimated from K_d values using Equation 9. The Log K_{OC} and K_d values and associated standard deviations are summarized in **Error! Reference source not found.** and lotted in

Figure 5-2 for three types of sediments and eight steroids. Data related to sediment/ water ratio 1:1 are plotted in Figure A-2. 2. The sediment sample with the lowest organic content (S4) has the lowest values of K_d as low as 5 L kg^{-1} following by S1 and S2 with higher OC contents. However, the Log K_{OC} values remained constant between steroids and different samples, ranging between 2.4- 3.2. As shown in Figure A-2. 2, the same trends were observed for equal ratio of sediment/ water.

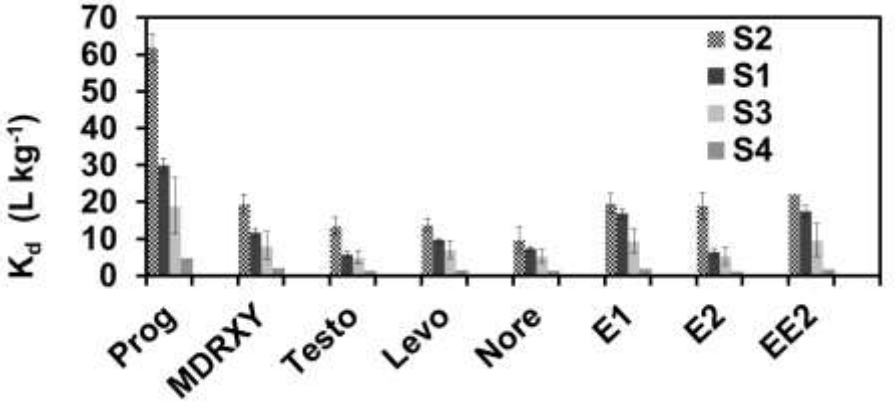
Table 5-2. Solid-liquid sorption coefficient (K_d ± SD) and organic carbon partitioning coefficient (K_{OC}) values measured at equilibrium time for eight steroids. C_0 hormone= 100 µg/L, mass of sediment= 1.0 g, volume of liquid= 5 mL.

Compound	S1		S2		S3		S4		Log K_{OW}
	K_d (L kg^{-1})	Log K_{OC}	K_d (L kg^{-1})	Log K_{OC}	K_d (L kg^{-1})	Log K_{OC}	K_d (L kg^{-1})	Log K_{OC}	
Prog	30±1.9	1.76	61±15	1.93	19±7.5	1.52	5.1±3	1.47	3.9
MDRXY	11.8±1	1.35	19.5±3.8	1.43	8.1±3.7	1.15	2.3±1	1.13	2.69
Testo	5.7±0.9	1.04	13.5±2.5	1.28	5.05±1.8	0.95	1.5±1.2	0.94	3.3
Levo	9.7±0.6	1.27	13.9±2.2	1.27	6.9±2.5	1.09	1.8±0.7	1.02	3.08
Nore	7.3±0.6	1.14	9.8±1.5	1.13	5.3±1.7	0.97	1.5±0.6	0.95	2.97
E1	16.9±1.3	1.51	19.5±3.4	1.43	9.46±3.3	1.22	2.1±1.9	1.08	3.4
E2	6.3±1	1.09	19.03±2.7	1.48	5.39±2.30	0.98	1.3±1	0.91	3.9
EE2	17.4±1.6	1.52	22.2±3.5	1.42	9.7±4.4	1.23	1.9±1	1.06	4

When comparing the obtained K_d values with the previously published data, other parameters than organic content should also be considered such as initial concentration of adsorbates, particle size distribution, surface area, and competition with other existing components. Qi et al. 2016 investigated the sorption of Testo to different soil particles [110]. At low Testo concentrations, the major part of Testo is sorbed to small particles. While at higher Testo

concentrations, small particles are saturated and larger particles may have contribute more to sorption. In three sediments analysed in current study, S2 with highest sorption capacities has the total solids content of 25% with 31% of particles smaller than 80 µm. While S1 and S3 with lower sorption capacities, contained 40 % and 55% total suspended solid, respectively; but only 14 % of particles were smaller than 80 µm. A compensating effect of the total solid content and fraction < 80 µm could explain the similar sorption amounts with steroid initial concentrations varying from 5 µg L^{-1} to 100 µg L^{-1}.

a)



b)

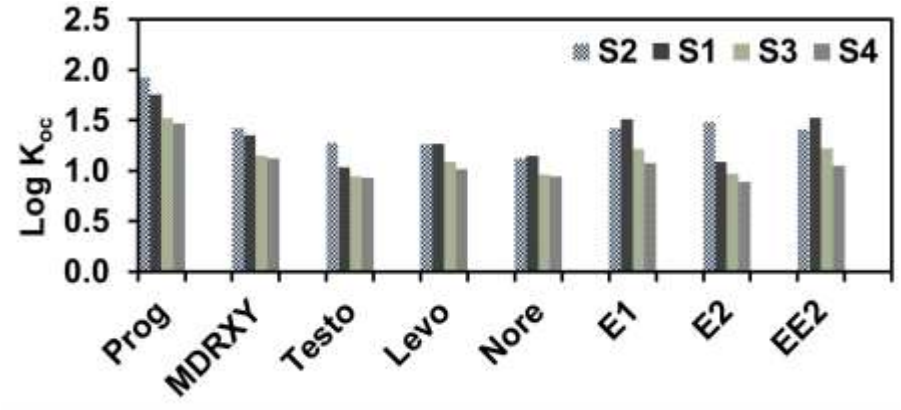


Figure 5-2. Comparison of a) K_d and b) K_{OC} values for 8 steroids in four sediment types. Conditions: mass of sediment=1 g; volume of solution= 5 mL; temperature= 25 °C.

No significant relationship was found between Log K_{OC} and Log K_{OW} confirming the previous statement from Yamamoto et al (2003) that the sorption process is dominantly controlled by hydrogen bonding rather than hydrophobic interactions between steroids and organic matter

[186], [185], [97], [187]. The independence of Log K_{OC} from Log K_{OW} in three sediment samples is shown in Figure 5-3. The obtained K_d values for steroids in this study are within the range of previously published data, with the exception of synthetic progestogens which no other data is available for comparison. Studies were performed to investigate the sorption of E2 and EE2 onto suspended and bed sediments from 5 rivers in UK [188]. Under anaerobic conditions, up to 90% of estrogens were sorbed to bed sediments in less than one day. Higher distribution coefficient (k_d, L kg^{-1}) values were obtained in bed sediments compared to suspended sediments due to the smaller particle size distribution and higher organic carbon content. Also the k_d values were up to three factors higher for EE2 than E2.

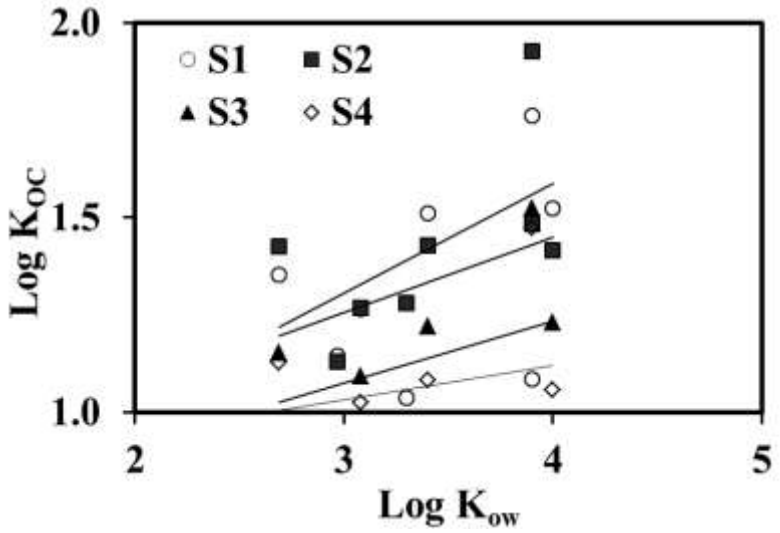


Figure 5-3. Relationship between sorption coefficients and octanol-water partitioning coefficients of selected steroids in sediment samples.

Sangster et al were determined the K_d and Log K_{OC} values for E1, E2, Prog, and Testo in silty loam and sandy sediments. The reported K_d values in sandy sediment with K_d values of 1.10, 2.54, 33.3, and 3.45 L kg^{-1} for E1, E2, Prog, and Testo respectively, are in same order with the values reported in **Error! Reference source not found.**. Better compatibility is notable between eported Log K_{OC} values with 2.60, 3.04, 4.16, and 3.18 for E1, E2, Prog, and Testo respectively. Neala et al. (2009) also reported higher Log K_{OC} values compared to this study [185]. Given the differences in the experimental setup and differences between DOM compositions of sediments, the K_{OC} values are not directly comparable. Further investigations on the DOM composition such as ratio of oxygen and nitrogen to carbon, ratio of hydrogen to oxygen, molecular weight of

components, and UV absorption provide more data to better compare the K_{OC} values between different samples from different studies.

5.4.3 Pseudo-second order kinetics

The sorption process can also be described by evaluating the kinetic of adsorption of steroids onto the sediments. Various sorption models have already been used to model sorption processes in the environment, the most comprehensive as the diffusion models [189]. Although application of chemical reaction kinetics leads to several simplifications in the overall sorption system, these models are widely applied to environmental systems. A pseudo-second order kinetic is reported to better fit for natural samples with high organic content [114, 190]. Therefore, the regression of the adsorption process was conducted in current study with two kinetic models, including first-order kinetic model and the pseudo-second-order kinetic model in order to evaluate sorption of the steroids by sediments. The derived rate constants together with the correlation coefficient from two models for sediment (S1) were showed in Table 5-3 and plotted in Figure 5-4. Kinetic data for other sediment samples are presented in Table A-2 1. Results of kinetic experiments demonstrate that the pseudo-second-order kinetic model could best fit the derived kinetic data with correlation coefficients over 0.99. In multi-sorbent systems, the interactions and competition of sorbents for the existing sorption sites are suggested to affect the sorption of individual compounds [189]. In case of steroids considered in current study, progestogens with relatively higher sorption affinities toward sediment's organic content demonstrate higher sorption constants in S2.

Table 5-3. Kinetic parameters for adsorption of the steroids onto the sediment sample (S1), mass of sediment = 1 g; volume of solution = 5 mL, hormones initial concentration = 100 µg L^{-1}.

Kinetic model	Model equation	Compound	Equation	Rate constant	r^2
First-order kinetic models	$\frac{dq_t}{dt} = kq_t$	E1	y=0.0092x+6.0606	9.20E-03	0.46
		E2	y=0.0124x+4.7976	1.24E-02	0.16
		EE2	y=0.0089x+5.7805	8.90E-03	0.34
		Prog	y=0.0034x+5.9681	3.40E-03	0.61
		MDRXY-Prog	y=0.0048x+5.8508	4.80E-03	0.88
		Testo	y=0.0071x+5.0874	7.10E-03	0.31
		Levo	y=0.0048x+5.7157	4.80E-03	0.87
		Nore	y=0.0065x+5.7671	6.50E-03	0.72
Pseudo-second-order kinetic model	$\frac{dq_t}{dt} = k(q_e - q_{t)}{}^2$	E1	y=0.0014x+0.0018	1.09E-03	0.99
		E2	y=0.0046x+0.0003	7.05E-02	1
		EE2	y=0.0019x+0.0018	2.01E-03	0.99
		Prog	y=0.0021x+0.0017	2.59E-03	0.99
		MDRXY-Prog	y=0.0021x+0.0031	1.42E-03	0.99
		Testo	y=0.0043x+0.0023	8.04E-03	0.99
		Levo	y=0.0025x+0.0037	1.69E-03	0.99
		Nore	y=0.0022x+0.0025	1.94E-03	0.99

The calculated values of sorption capacity at equilibrium (q_e) were similar to those obtained from experiments. Figure 5-4 compares the modeled sorption capacity of the studied steroids with predictions made using experimentally obtained q_e within the 95th prediction intervals. These results are interesting since there are limited previous studies in the literature on sorption kinetics of progestogens and testosterone for sediments. Cunha et al. 2012, studied the sorption kinetics of E1, E2, and EE2 in tropical sediment samples under different pH and sediment amount [114]. The reported rate constants in sediment (S1, f_{OC}= 52%) were 9.27 E-03, 7.08 E-03, 2.56 E-02 for E1, E2, and EE2 respectively. While in sediment (S2) with higher organic content (f_{OC} = 73%)

the reported rate constants were about an order of magnitude lower with 2.06, 3.32, 2.35 E-04 for E1, E2, and EE2 respectively.

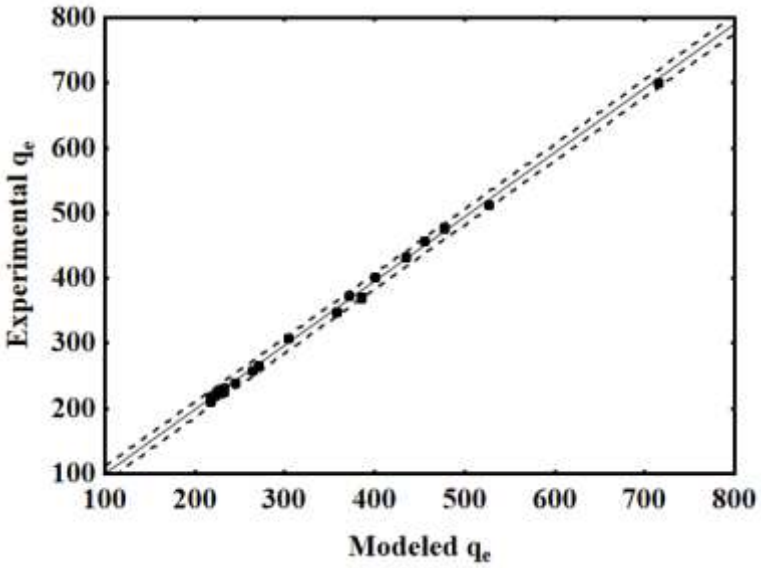


Figure 5-4. Modeled vs experimentally obtained q_e values from pseudo second-order kinetics for the steroids; dotted lines indicate the 95th prediction intervals.

5.4.4 Sorption isotherms Data

Sorption isotherms for the steroid compounds produced from compound equilibration were constructed form directly measured sorbed (C_s, µg g^{-1}) and solution (C_e µg L^{-1}) concentrations. Table 5-4 lists the isotherm parameters obtained for the mixed solute systems. The data listed in Table 5-4 confirm that all the sorption isotherms of mixed solute systems can be effectively fitted with the linear model with the r^2 values greater than 0.89. Sangster et al 2015 also obtained linear isotherms for E1, E2, Testo, and Prog for adsorption on the sediment with steroids initial concentrations in the same range (5-500 µg L^{-1} and 0.22-2.5 % OC) as in current study.

Sediment S3 with the most linear isotherms has the lowest organic content among the four sediments, suggesting that the isotherm nonlinearity may be greatly influenced at higher OC [114]. These results are consistent with previous reports on linear isotherms for estrogens and testosterone sorption in soil and sediment [109]. As mentioned in section 5.2.6, the Freundlich model becomes as linear model when the exponent *n* is near one and K_f will represents the distribution coefficient (k_d). From Table 5-4, the K_f and *n* of Freundlich isotherm ranged in 3-67 L kg^{-1} and 0.57-1.6, respectively.

Due to experimental conditions and analytical limitations the sorption isotherms were obtained only for some of compounds in each sample. The reason could not be due to sorption on tube surface since it was previously confirmed by Darwano et al. [5] that steroids has less sorption on polypropylene tubes than any other substance. In contrast low sorption affinity on the sediment can explain the reason to make it impossible to determine sorption isotherms. When isotherms created, the K_f values were higher in S2 with higher OC% for all the compounds confirming the higher interaction between OC and steroids. The K_f values varied in the range 5.5 (Nore)- 67.6 (Prog) for S1, 11.01 (Testo)- 34.76 (Levo) for S2, and 3.03 (EE2)- 31.6 (Pro) for S3, and 1.57 (E2)-17.81 (Prog) for S4 indicating the large difference between sorption affinity of steroids for sediments.

Table 5-4. Sorption isotherm parameters for the steroids and sediment samples.

Compound	Sample	K_f (L kg $^{-1}$)	n	r^2 (Freundlich)	r^2 (linear)
Prog	S1	67.63	1.05	0.97	0.91
	S3	31.67	1.09	0.93	0.94
	S4	17.81	0.88	0.95	0.96
MDRXY	S1	14.67	1.06	0.94	0.98
	S3	13.58	1.04	0.98	0.99
	S4	8.98	0.74	0.92	0.97
Testo	S1	6.31	0.91	0.81	0.89
	S2	11.01	0.57	0.90	0.91
	S3	4.02	0.89	0.62	0.94
	S4	4.52	0.89	0.85	0.93
Levo	S1	16.76	0.98	0.93	0.99
	S2	34.76	0.76	0.94	0.93
	S3	8.48	1.17	0.94	0.99
Nore	S1	5.51	1.09	0.72	0.98
	S3	5.21	1.29	0.81	0.96
E1	S1	6.91	1.27	0.96	0.94
	S2	13.35	1.24	0.98	0.97
	S3	7.65	0.8	0.69	0.73
	S4	5.57	0.94	0.95	0.96
E2	S2	14.83	0.98	0.90	0.95
	S4	1.57	1.18	0.97	0.94
EE2	S1	18.45	1.18	0.96	0.97
	S2	28.49	1.07	0.94	0.92
	S3	3.03	1.64	0.96	0.97
	S4	7.53	0.91	0.85	0.90

The difference in sorption affinities are confirmed by the order of sorption as discussed in previous sections. The experimental k_d values where compared with k_d- s from linear isotherms. Figure 5-5 compares the sorption coefficients (k_d) obtained from linear isotherms (when isotherms were producible) with experimentally obtained k_d s within the 95th confidence intervals.

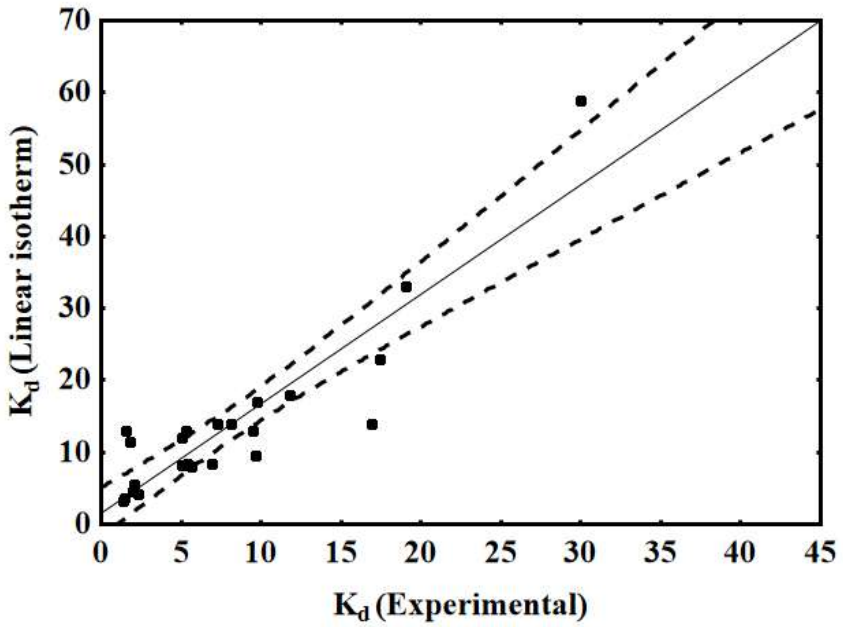


Figure 5-5. Experimentally obtained K_d values vs K_d s from linear isotherm within 95% confidence intervals.

5.4.5 Environmental implications

The interactions of steroid hormones with sediments are of high importance in determining their fate in aquatic environments. The fast adsorption of all the selected steroids onto river sediments containing different content of organic matter demonstrate the high affinity of steroids specially progestogens to sorb onto river sediment. Additionally, high K_{OC} values in samples with higher organic content reveal the mobility of these compounds in sediment which might determine the presence of steroids in sediments of receiving water downstream of wastewater treatment plant discharges. Investigation of single compound sorption process for each steroid on sediment is highly recommended in order to better compare the effect of sorption competition between compounds with high sorption affinities. Additionally, the biodegradation kinetic of

progestogens and androgens in sediments beside their sorption kinetic would provide better understanding of the combined fate of steroids through sorption and biodegradation.

CHAPTER 6 ARTICLE 2: IMPACT OF TEMPERATURE ON OXIDATION KINETICS OF TESTOSTERONE AND PROGESTOGENS BY OZONE

Ozone has been approved as a strong oxidant in water treatment processes to breakdown large molecules to small, easily degradable ones or to mineralize biodegradation refractory compounds. Despite its strong selective characteristics, some micropollutants including some steroid hormones are found as ozone refractory compounds. This chapter presents the degradation kinetics of the oxidation of progestogens and, for the first time, of testosterone with ozone. This study is the first to investigate the effect of temperature on oxidation of ozone-refractory steroids and also present rate constants for testosterone as one of the ubiquitous steroids in surface waters and also as an ozone refractory compound under the conditions occurring within water treatment plants. The results of this study are presented as a research paper submitted to *Water Research*. Supplementary data is provided in APPENDIX 3.

IMPACT OF TEMPERATURE ON OXIDATION KINETICS OF TESTOSTERONE AND PROGESTOGENS BY OZONE

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ABSTRACT

Increasing presence of endocrine disrupting compounds (EDCs) in water sources and their adverse health effects on aquatic life are major concerns for water utilities and authorities worldwide. The oxidation kinetics for degradation of ozone resistant steroid hormones were investigated to quantify their removal in natural water under varying water temperatures and pH. Studying impact of temperature on oxidation of these compounds is one of the novel aspects of this research. The fate of four progestogens (progesterone, Medroxyprogesterone, levonorgestrel, and norethindrone) and, for the first time, of the androgenic steroid testosterone, in the presence of ozone was measured at bench scale in ultrapure, natural surface water and wastewater. The estimated second order constant rate for testosterone of 590± 0.13 $M^{-1}s^{-1}$ was comparable to our estimates and previous reports for similar structure progesterone (444- 601$M^{-1}s^{-1}$) and medroxyprogesterone (532± 0.04) and significantly lower than for levonorgestrel (2233 $M^{-1}s^{-1}$) and norethindrone (2292 $M^{-1}s^{-1}$). The removal of selected compounds was changed from 1% for norethindrone to 8% for medroxyprogesterone from pH 6 in presence of radical scavenger to pH 8. For all compounds the second-order rate constants increased from 3 folds for norethindrone to 5.5 folds for progesterone with temperatures ranging from 5- 35 °C. The required activation energy was estimated for the five selected steroids and ranged from 30 kJ (norethindrone) to 39 kJ (progesterone). The removal rates of the five selected compounds were accurately predicted in natural water and wastewater. Finally, we showed that ozonation processes using typical water treatment dosages required for disinfection (Ct_{O3}= 2 mg min L^{-1}) were capable of removing 77% (progesterone) to 99% (levonorgestrel) at 21 °C and even less (47% medroxyprogesterone to 96% norethindrone) at 5 °C of the selected compounds.

KEYWORDS

Water and wastewater treatment, ozone, oxidation kinetic, steroid hormones, endocrine disruptors, testosterone

6.1 Introduction

The adverse health effects of endocrine disrupting compounds (EDCs) on aquatic life have resulted in increased concern regarding their occurrence in the aquatic environment [10, 44, 191]. Various hormones and their metabolites, considered as EDCs, have been detected in wastewater treatment plant (WWTP) effluents and surface waters around the world [52, 95, 192]. Progestogens were detected in surface waters at similar levels as in treated wastewater with concentrations ranging between 1- 100 ng L^{-1} [11, 66]. Testosterone and progesterone were measured in French surface waters with concentrations in the range of 0.1- 15.6 ng L^{-1} [43] with effluents from treatment plants being recognized as the main source of these compounds [3]. The occurrence of low concentrations of steroidal hormones in treated drinking water is poorly documented partly because of the costly and complex analytical methods required for their detection [193]. Oxidation has been identified as a treatment barrier to steroids treatment and ozonation has been identified as a most promising oxidant to consider for their removal in water and wastewater [141]. The kinetics of hormone oxidation by common oxidants has been described for the chlorination of estrogens [91, 123] and the oxidation of four progestogens with potassium permanganate [93]. The oxidation of steroid estrogens (estrone, estradiol, estriol, and 17α-ethinylestradiol) by ozone over a wide range of pH 2.5-10.5 revealed very high reactivity of estrogens with ozone (1.05E+05 for estriol to 2.21E+05 for estradiol) [119]. Under the water treatment conditions considered (pH 7, 21 °C), estrogens were easily removed (>95%) with a very low ozone exposure of 2.0E-03 mg min L^{-1}.

As for more recalcitrant hormones which are the focus of this investigation, limited information is available about the actual second order kinetic ozonation constants, the effect of pH and water quality (see Table 6-2 for available data). Furthermore, no information is available concerning the effect of water temperature on oxidation of these compounds. Barron and al. (2006) first estimated the second-order rate constant for progesterone at pH ranging from 2-8 showing that this compound was much less reactive than estrogenic hormones. Some oxidation by-products for the reaction of progesterone with ozone were also identified [138]. Second order oxidation constants of four progestogens were estimated by [130] who showed that a Ct of 2 mg min L^{-1} O_3 removes more than 90% of natural and synthetic progestagens in filtered surface water.

Observational studies reporting overall removals of hormones by ozone have focused mostly on natural estrogens [86, 87, 92, 137, 145] and at removal of some progestogens [130, 138]. The oxidation of pharmaceuticals and EE2 in WWTP effluents with variable suspended particle concentrations (0-20 mg L^{-1}) was tested at ozone dosages of 2 mg L^{-1} [145]. Ozonation of seven steroids, including progesterone and testosterone, was studied during surface water and wastewater ozonation in Nevada, USA [124]. Up to 99%, 84% and 87% of estrogenic steroids, progesterone and testosterone, respectively, were removed with an ozone dose of 2.5 mg L^{-1} in surface water ozonation (TOC= 3.23 mg L^{-1}, T= 21°C). For WW, a much higher dose of 7.1 mg L^{-1} was required to remove 99% of steroids, including testosterone. All these studies confirm the interest of ozonation to remove recalcitrant steroids including testosterone. Although ozonation appears to be a most promising treatment process, it must be acknowledged that here is limited information on the fate and risks in terms of biological activity associated with the transformation products of ozone oxidation of steroid hormones [194]. For example, two ozonation by-products of progesterone were found resistant to further reaction with ozone but are likely to be more biodegradable than the parent compound based on their structure [195].

The objectives of this study were to fill several data gaps on the kinetics of five natural (progesterone and testosterone) and synthetic (medroxyprogesterone, norethindrone, and levonorgestrel) steroid hormones which are considered to be recalcitrant to ozonation by : (1) providing the first estimate of a second order constant rate for the ozonation of testosterone; (2) quantifying the impact of pH on the oxidation of these compounds; (3) estimating the activation energy for the range of temperature typical in water treatment; (4) applying the confirmed rate constants for the four progestogens and the newly estimated rate constant for testosterone in natural water and wastewater and finally (5) quantifying expected removal of the five target compounds under typical ozonation operational conditions set by disinfection.

6.2 Materials and methods

6.2.1 Standards and Reagents

Target compounds, including progesterone, medroxyprogesterone, levonorgestrel, norethindrone and testosterone, were purchased from Sigma-Aldrich, Canada (Oakville, ON, Canada). ($^{13}C3$)-Estradiol, which is used as an internal standard, was supplied by ACP Chemical Inc. (Montreal,

QC, Canada). Stock solutions of hormones were prepared by dissolving 25 mg of high purity hormone in 25 mL of LC-grade acetone, and the stock solutions were stored in the dark at 4°C. Diluted solutions (1 mg L^{-1}) were prepared using ultrapure water to achieve an initial hormone concentration of 10 µg L^{-1} in the ozonation reactor. The final concentration of acetone in reactor is 0.6 mM L^{-1} which is negligible. Also, acetone has a low reactivity toward ozone (k_{O3}, acetone= 0.032 $M^{-1}s^{-1}$ [196]) and pre-tests showed that such a low quantity of acetone did not interfere with hormone oxidation. Para-chlorobenzoic acid (ρCBA) and tert-butanol (tertBuOH) solutions were prepared using commercial compounds dissolved in ultrapure water to yield final concentrations of 200 µg L^{-1} (1.28 µM) and 50 mM, respectively.

6.2.2 Surface water and WWTP effluent samples

Water samples were collected (i) after conventional treatment (alum coagulation, settling and sand/anthracite filtration) at a surface water drinking water plant (DWP) (eastern Canada) and (ii) from the effluent of aerated lagoons with (90,000 m^3 d^{-1} capacity). Samples were collected in 5 L polypropylene containers that had been washed and rinsed with distilled water and then ultrapure water. The characteristics of the water samples are provided in Table 6-1. These samples were spiked with concentrations of 10 µg L^{-1} of each hormone. Based on a previous study [153], the background concentrations of hormones are much lower than the spiked concentrations.

Table 6-1.Characteristics of DWTP filtered water and WWTP effluent.

Parameters	pH	Alkalinity ($mgCaCO_3$ L^{-1})	DOC (mg L^{-1})	UV absorbance (254 nm) (cm^{-1})	Turbidity (NTU)	COD (mgO_2 L^{-1})
Natural Filtered Water	6.8	34	2.82	0.049	0.11	2.7
Treated WW	7.2	90	11.5	0.248	0.57	42

6.2.3 Dissolved Ozone Analysis

The dissolved ozone concentrations in the stock solution and the residual ozone concentration following oxidation were both determined using the standard colorimetric method 4500-O_3 [160] using indigo trisulfonate (ε_{600nm}=20,000 M^{-1} cm^{-1}). The absorbance was measured at 600 nm in a 1-cm or 2-cm quartz cell using a Varian (Cary 100, Victoria, Australia) spectrophotometer.

6.2.4 Quantification of Hormones and ρCBA

Hormones were analyzed using on-line solid phase extraction (SPE) coupled with liquid chromatography/tandem mass spectrometry. Under the experimental condition the detection limit of steroids was 0.13±0.03 (1500 µL injection volume). The atmospheric pressure photoionization (APPI) was used as an ionization source for the detection of hormones. The complete detection and quantification methods used for hormones are described previously [153]. The hydroxyl radical exposure was measured indirectly by oxidizing an ozone-resistant molecule, ρCBA. To analyze the degradation of ρCBA, 1 mL sub-samples were also collected from reactor in parallel and filtered on 0.45 µm (Millex-HV) for analysis of ρCBA concentration using reverse-phase HPLC (60% acidified water at pH 2 with H_2SO_4, and 40% methanol). The detection of pCBA was made using diode array detection at 236 nm with detection limit of 0.2 µg L^{-1} (Elite LaChrom, Hitachi). The detailed description of the column characteristics and mobile phases are described elsewhere [159].

6.2.5 Ozonation Experiments

Ozonation experiments were successively conducted on three types of test waters: buffered ultrapure water, un-buffered filtered water samples collected from a DWTP and diluted WWTP effluent. The ozonation of WW effluents is usually tested in continuous systems due to the high initial ozone demand and to maintain a sufficient residual ozone concentration during the experiment. The WW must be diluted during batch experiments in order to reduce the initial demands for ozone to detectable levels and follow the ozone decomposition during the reaction (1 L filtered WW was diluted with 1 L ultrapure water and the alkalinity was adjusted using 0.5 M sodium bicarbonate). During experiments with ultrapure water, the pH was held constant at 6 or 8 using a phosphate buffer (final concentrations of 0.817 and 0.35 M, respectively). Ozone stock solutions (50-60 mg L^{-1}) were prepared by sparging gaseous ozone produced with an oxygen-fed ozone generator (Ozone Service, BC, CA) through 1 L ultrapure water flasks places in ice bath. All tests were conducted in a true batch reactor consisting of a 2 L beaker covered with a floating *Teflon* lid to prevent ozone from degassing. An appropriate volume of ozone stock solution was added in each reactor to yield the target ozone doses (0-10 mg L^{-1}). Hormone and ozone residuals were measured at defined time intervals over a 10 min period by collecting 5

mL samples that were dispensed in 20 mL of indigo solution (0.02, 1 or 3%). The reaction of hormones with ozone was stopped by using ascorbic acid (final concentration 2 mg L^{-1}).

6.2.6 Determining the rate constants for the reaction of ozone with steroids

The reaction kinetic of ozone with hormones is first order with respect to both ozone and hormone concentration [141] and is given by the following equation:

$$Ln\left(\frac{[P]}{[P_0]}\right) = -k_{O_3,P}[O_3]t = -k_{O_3,P}Ct_{O3} \quad \text{Equation (11)}$$

Where, *P* is the target compound and $k_{O_3,P}$ is the second-order rate constant with ozone ($M^{-1}s^{-1}$) obtained from experiments in ultrapure water with radical scavenger tert-BuOH at constant pH 6. Ozone exposure (Ct_{O_3}) values (expressed in M s) were calculated using the effective Ct concept [197], which represents the area under the decay curve at time (t).

$$Ct_{O3} = Ct_{effective} = \int C(t)dt = \frac{C_0}{k'}[1 - \exp(-k' \times t)] \quad \text{Equation (12)}$$

Where *C* and C_0 are the residual ozone at time (t) and the initial residual ozone (mg L^{-1}), respectively, and k´ is the pseudo-first-order ozone decay rate (min^{-1}).

The observed second order rate constants for reaction of hormones with both ozone and hydroxyl radicals can be estimated without radical scavenger and using ρCBA as reference compound. The observed rate constants can be obtained using the following equation:

$$ln\left(\frac{[P]}{[P]_0}\right) = -K_{obs}Ct_{O3} = -\left(k_{O_{3,P}} + k_{OH,P}R_{Ct}\right)Ct_{O3} \quad (13)$$

Where $k_{OH,P}$ is the rate constant for the reaction of hormone (P) with hydroxyl radicals, K_{obs} is the observed rate constant for reaction of hormone with both oxidants (O_3 and OH), and R_{Ct} is the ratio of ozone exposure and radical exposure $R_{Ct} = \frac{Ct_{OH}}{Ct_{O_3}}$.

6.2.7 Activation energy of the reaction of the ozone-hormone

The activation energy, E_{act}, for ozone-hormone reactions are calculated in natural and ultrapure water using the Arrhenius's law varying the temperature from 5 to 35 °C.

$$k = \mathrm{A}\exp\left(-\frac{E_{act}}{RT}\right) \quad (14)$$

Where E_{act} is the activation energy (J.mol^{-1}), R is the universal gas constant (8.314 J.mol^{-1} K^{-1}), A is the frequency factor (M^{-1} s^{-1}), and T is the absolute temperature (K).

6.3 Results and discussion

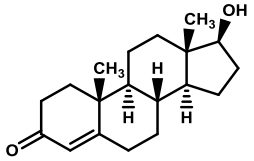
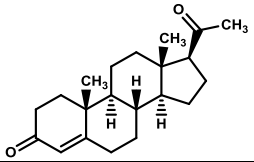
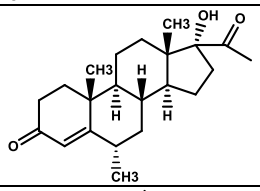
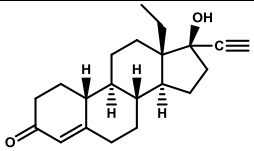
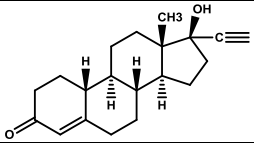
6.3.1 Rate Constants for the Reactions of the Hormones with Ozone

The second-order rate constants for the reactions of four progestogens and, for the first time, for testosterone with ozone in ultrapure water at pH 6 in the presence of a radical scavenger are determined using Equation (11) and results are presented in Table 6-2. Testosterone exhibited similar reactivity towards ozone as progesterone and medroxyprogesterone, c.a. k_{O3}= 532~594 M^{-1}s^{-1}, potentially due to their very similar chemical structures and more importantly because they have the same substituents at their C-C double bound, which probably results in similar reactive intermediates (Table 6-2). No significant difference was found between the reactivity of levonorgestrel and norethindrone towards ozone ($P< 0.05$) with reaction rate constants of $k_{O3,Levo}$= 2233 M^{-1} s^{-1} and $k_{O3,Nore}$= 2292 M^{-1} s^{-1}. On the other hand, the acetyl group has electron withdrawing (additional-) substituent in the progesterone structure and reduces its reactivity towards ozone compared to the ethynyl group in the levonorgestrel and norethindrone structure. This effect was observed before in galaxolide and tonalide, as two similar musk fragrances. Tonalide (with an acetyl group) shows a smaller reaction rate constant ($k_{O3,\ tonalide}$= 8 M^{-1}s^{-1}) compared to galaxolide ($k_{O3,\ galaxolide}$ = 140 M^{-1}s^{-1}) [198].

As of two compounds with moderate reactivity toward ozone, levonorgestrel has a methyl as the additional reactive site, while in norethindrone; ethyl is the additional reactive group (Table 6-2). Although methyl and ethyl group are both nonreactive towards ozone, this difference in the additional reactive groups causes slightly different reaction constants in these two compounds. Both norethindrone and levonorgestrel have a hydrogen atom on their C10 carbon atom (one of the substituents at C-C double bound) whereas progesterone, medroxyprogesterone, and testosterone have methyl on the same carbon. Although the methyl group has no direct effect on the reactivity of C-C double bond towards ozone, the steric hindrance of the methyl group can

decrease the reactivity of the steroid hormones towards ozone or prevent further oxidation of intermediate oxidation products. Hence, the higher reaction rate constants observed for norethindrone and levonorgestrel compared to the other three steroid hormones could be explained by: i) higher reactivity of the ethynyl group toward ozonation or, ii) lower steric hindrance on C=C bond due to the presence of –H substituent instead of $–CH_3$. However, further research is required to speculate which of the two reasons is the prevalent.

Table 6-2. Kinetic rate constants for reaction of ozone with steroid hormones at T= 21°C and pH= 6 in ultrapure water. Errors show the standard errors from duplicate experiments and duplicate analyses. **a)** [130], **b)** [138].

Compound	k_{O3} ($M^{-1}s^{-1}$)	MDL ($\mu g.\ L^{-1}$)	References	Chemical Structure
Testosterone	590±0.13	0.133	This study	
Progesterone	594±0.11 601 444	0.177	This study a b	
Medroxyprogesterone	532±0.04 558	0.177	This study a	
Levonorgestrel	2233±0.5 1427	0.107	This study a	
Norethindrone	2292±0.57 2215	0.105	This study a	

Comparing the reactivity of testosterone and progestogens with estrogens, the reaction rate constants are about 2-orders of magnitude lower than those of estrogenic steroids ($k_{O3,\ estrogens}$~10^5 $M^{-1}s^{-1}$). Although all the steroid hormones have three hexagonal rings (A,B, and C) and one pentagonal ring (D), there is significance differences between their functional groups. Estrogens have a phenolic group with high reactivity towards ozone ($k_{O3,\ phenol}$= 1.3×10^4 M^{-1} s^{-1} [196] in the A-ring position; while the progestogens and testosterone are olefinic compounds

with carbonyl group in the same ring. The carbonyl group is considered to reduce the reactivity of C-C double bond in these compounds [199]. Carbamazepine, an antiepileptic drug commonly detected in WWTP effluents and surface waters that has an olefin group as its electron rich moiety is highly reactive with ozone ($k_{O3} = 3 \times 10^5$ M^{-1} s^{-1} [145]). Another olefinic compound that in contrast has a low reactivity with ozone is the artificial sweetener acesulfame, with k_{O3}= 88 M^{-1} s^{-1} [200]. Therefore, being an olefinic compound is not the determining factor in reactivity towards ozone. The electron withdrawing or donating properties of substituents at the C-C double bound and their corresponding reactivity with ozone can strongly affect their second-order rate constants. Even, the different conformations of olefins, isomeric olefins for instance, can strongly affect the produced ozonide and consequently the related reaction rate constant. As an example, the reaction rate constant of 1, 1-dicholoroethylene (k_{O3}= 22 M^{-1} s^{-1}) is much smaller than that of 1, 2-dichloroethylene (k_{O3}= 591 M^{-1} s^{-1}) [201].

The oxidation of olefins with ozone usually follows the Criegee mechanism [201]. The referred mechanism suggests an ozone-olefin adduct, named ozonide, which is very unstable and usually cleaves to a carbonyl compound and a hydroxyhydroproxide.

The produced hydroxyhydroproxide itself could stabilize through several reactions and could produce hydrogen peroxide and carbonyl compounds depending on the structure of the main olefinic compound and the oxidation environment. The transformation products of oxidation reactions could further react with ozone or resist toward ozone reaction. While oxidation products of progesterone are reported to resist further reaction with ozone, transformation products of levonorgestrel and norethindrone could probably be further oxidized by ozone since cleavage of the C-H bond on C10 atom in levonorgestrel and norethindrone is more likely than the methyl-C at same carbon in progesterone.

The biological activity of oxidation products could greatly change through the oxidation with ozone compare to parent compounds. Ozone transformation products from oxidation of olefinic compounds, such as progesterone, which usually results in production of aldehydes, ketones or carboxylic acids are more reported easily biodegradable than the parent compounds [195]. Recently, the application of computer–aided programs using model compounds in combination with available reaction rate constants is developing rapidly. Hence, product formation mechanisms as well as their reaction rate constants can be estimated via predictive *in-silico* tools

[195, 202]. Recent advances in molecular modeling has significantly improved the ability to predict the reactivity if chemical compounds with different oxidants. Assessing the electronic structures of selected compounds could provide a better understanding of their oxidation reactivity towards ozone. Reactivity of two compounds depends on the energies of the frontier molecular orbitals, that is, ε_{HOMO} (the highest occupied molecular orbital) and ε_{LUMO} (the lowest unoccupied molecular orbital) which reflect the potential reactivity between two compounds. These energies can be calculated for any chemical compound using computational quantum chemistry techniques. The smaller the energy difference ($\Delta\varepsilon$) between the HOMO of the nucleophile reactant and the LUMO of the electrophile oxidant, the higher is the reactivity between two compounds. Ozone is a strong electrophile compound and has ε_{HOMO} of -9.07 eV and ε_{LUMO} of -5.5 eV [199]. The $\Delta\varepsilon$ between ozone and progesterone is 1.2 eV, while it is 1.07 eV between ozone and levonorgestrel. The experimentally observed greater reactivity of levonorgestrel compared to progesterone is in agreement with this theoretical explanation. Also, $\Delta\varepsilon$ between ozone and estrogens is 0.52 eV which confirms their higher reactivity with ozone compared to progestogens [199].

6.3.2 Impact of pH on oxidative transformation of steroids

The oxidation rate of steroids (testosterone and progestogens) using 2 mg L^{-1} of ozone at 21 °C was evaluated at pH 6 in presence of a radical scavenger (50 mM tertBuOH) and at pH 8 without radical scavenger (Figure A-3. 1). Higher removal rates are expected at pH 8 since more hydroxyl radicals are produced from ozone decomposition at pH 8 [203]. However, in this study which ozone decomposition was the only source of hydroxyl radical and no hydrogen peroxide was added, the concentration of radicals was very low to impact the oxidation rate of ozone resistant compounds. Therefore, the difference between removal rates at pH 6 and 8 was limited between 1% for norethindrone and 8% for medroxyprogesterone.

This result is consistent with the fact that steroid hormones are not pH sensitive because their chemical structure does not contain acidic or basic moieties. The independence of the k_{O3} relative to pH was also noted in another study of progesterone oxidation with ozone [138]. The rate constant for the direct reaction of progesterone with ozone (594 $M^{-1}s^{-1}$ at pH 6) was compared with a previously reported value (444±11 $M^{-1}s^{-1}$ at pH 6.49) [138]. The higher reaction rate obtained in this study potentially resulted from differences in the experimental conditions such as

temperature which has an important effect on ozone decomposition (18 °C vs. 21 °C), radical scavenger concentration which controls the radical production (10 mM vs. 50 mM) and analytical accuracy. Other reaction rate constants agree with previously reported values, except for testosterone, for which no previous comparisons were found and also for levonorgestrel for which a higher rate constant was obtained in this study compared to previously reported value (Table 6-2). This difference is explained by different experimental conditions (reported value was obtained at pH 8 in presence of radical scavenger and data at pH 6 are not published) and different method of data analysis. Although oxidation of progestogens is found independent of the pH of reaction environment, hydroxyl radicals produced from faster ozone decomposition at pH 8 can result in slightly higher reaction kinetics compared to pH6.

6.3.3 Impact of Temperature on Kinetic Rate Constants

Figure 6-1 and Figure A-3. 2 demonstrate the effects of temperature on the kinetics of hormone oxidation by ozone and the oxidant decay rates of ozone, respectively, in ultrapure water at four temperatures from 5 to 35 °C. The ozone decay rates decreased with decreasing temperature. Pseudo first-order reaction rates for ozone decay show that ozone depletion rates increased up to one order of magnitude with temperature increase from 5 °C (k_{O3}= 0.58 s^{-1}) to 35 °C (k_{O3}= 1.76 s^{-1}). At higher temperatures the solubility of ozone in water decreases and its decomposition rate increases by the factor of 1.2-1.8 for every 10 °C increase in temperature (3.02-folds from 5 to 35 °C). In absence of radical scavengers, the ozone decomposition rate is accelerated by hydroxyl radicals from 1.44 min^{-1} to 4.46 min^{-1} with and without radical scavenger, respectively.

To investigate the effect of temperature, the second-order rate constants ($K_{O3,hormone}$) for testosterone and progestogens must be quantified at different temperatures (Figure 6-1). The temperature revealed significant effect on removal rate of testosterone and progestogens oxidation. The $K_{O3,hormone}$ values increased from 3-folds for norethindrone (Figure 6-1-d) to 5.5-folds for progesterone (Figure 6-1-a) following the temperature increase from 5 to 35°C. The reaction of medroxyprogesterone and testosterone showed similar behavior as progesterone (Figure 6-1-b, 6-1-c). At temperature of 35 °C with Ct_{O3} of 2 mg $min\ L^{-1}$, progesterone, medroxyprogesterone, and testosterone were removed almost 1log; whereas at 5 °C the removal rate reduced significantly below 0.5 log. These results indicate that very high Ct values, up to 10 mg $min\ L^{-1}$, would need to be applied at lower temperatures in order to obtain at least 1 log

removal of such recalcitrant compounds. Levonorgestrel removal rate increased 4-folds at 30 °C. In case of norethindrone and levonorgestrel, complete removal of these compounds could be achieved at 35 °C with Ct_{O3} of 8 mg min L^{-1}. However, like three other compounds more than 10 mg min L^{-1} should be applied for complete removals of norethindrone and levonorgestrel.

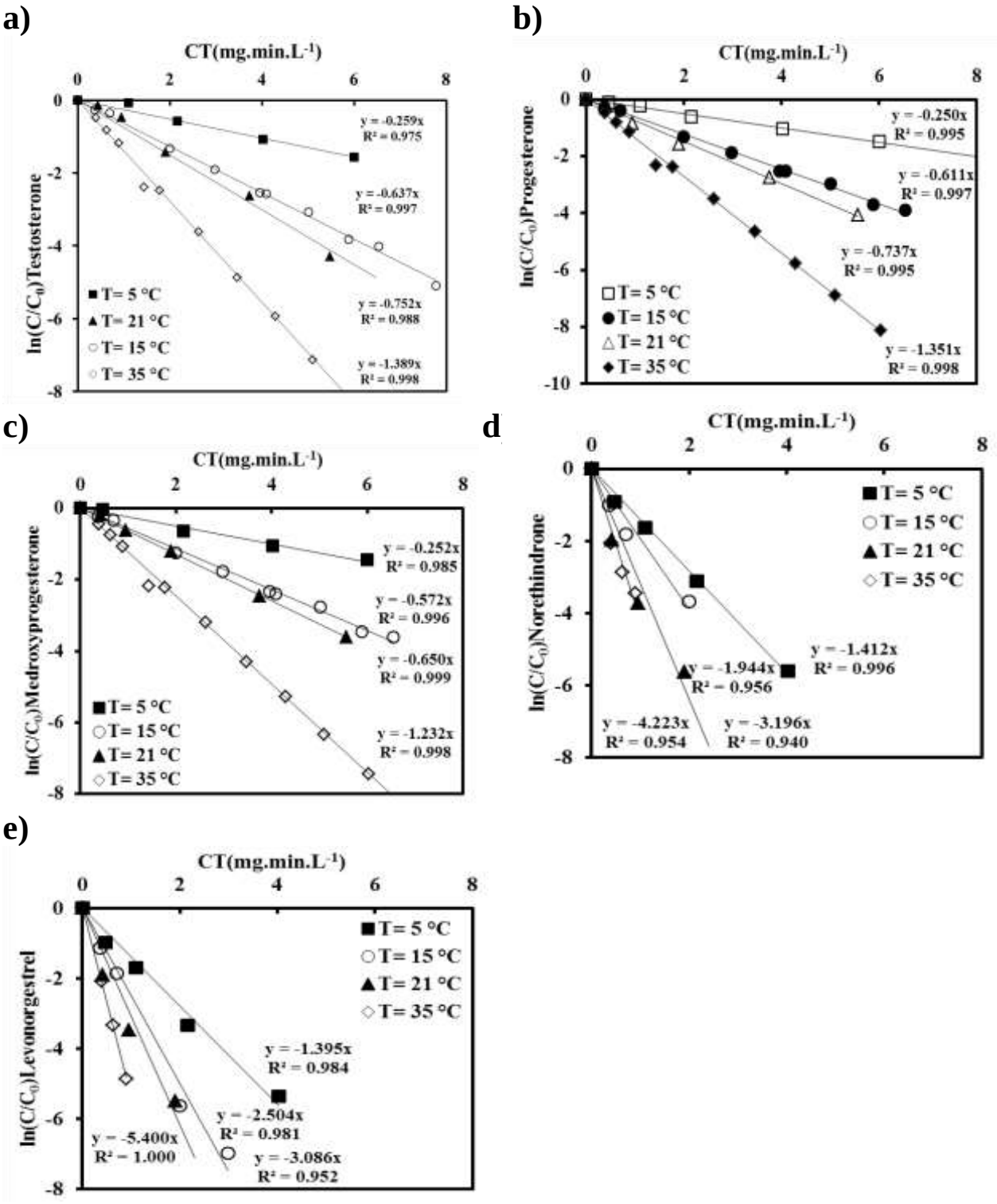


Figure 6-1.Effects of temperature on the second-order decay of (a) testosterone, (b) progesterone, (c) medroxyprogesterone, (d) norethindrone, and (e) levonorgestrel, in ultrapure water at pH 6 and in presence of radical scavenger with 2 mg O_3 L^{-1}. Solid lines represent the linear regression.

The activation energies *(E_{act})* in ultrapure water at pH 6 were obtained from linear regression between temperature (1/T, K^{-1}) and second order rate constants of ozone-hormone reaction (ln k_{O3}) (Figure 6-2). The obtained results varied between 30 kJ mol^{-1} (norethindrone) and 39 kJ mol^{-1} (progesterone). As shown in Figure 6-2, the selected compounds have same trend of increasing decomposition rate from 5 °C to 35 °C. However, for defined Ct_{O3}, the removal rate for testosterone, progesterone, and medroxyprogesterone were more variable with temperature increase than two other compounds. For instance, at Ct_{O3}= 2 mg min L^{-1}, testosterone removal increased from 56.4% to 98.4% when temperature increase from 5 °C to 35 °C; while for norethindrone the removal rate changed only from 98.3% to 99.9%. Figure 6-2 also compares the variation of removal rates with temperature for selected steroids. The activation energy can provide better understanding of the removal rates of recalcitrant compounds by giving information on temperature sensitivity of such compounds. As the higher is the activation energy means the more sensitive is removal rate to the temperature [204]. The obtained E_{act} are in agreement with Hoigne *et al*. 1983 [196], which states the activation energy required for the reaction of organic compounds such as phenolic compounds, carboxylic acids and nitrogenous compounds with ozone is between 35 and 50 kJ.mol^{-1}. To the best of our knowledge, no reports of the activation energies for the temperature dependent rate constants of ozone-steroid reactions exist. These results are valuable specially, during the critical periods of cold weather which biodegradation becomes very limited and the role of oxidation becomes more important for removal of such a recalcitrant compounds. Accordingly, the impacts of temperature must be considered when evaluating the performance of ozone in water treatment plants subjected to large seasonal temperature variations.

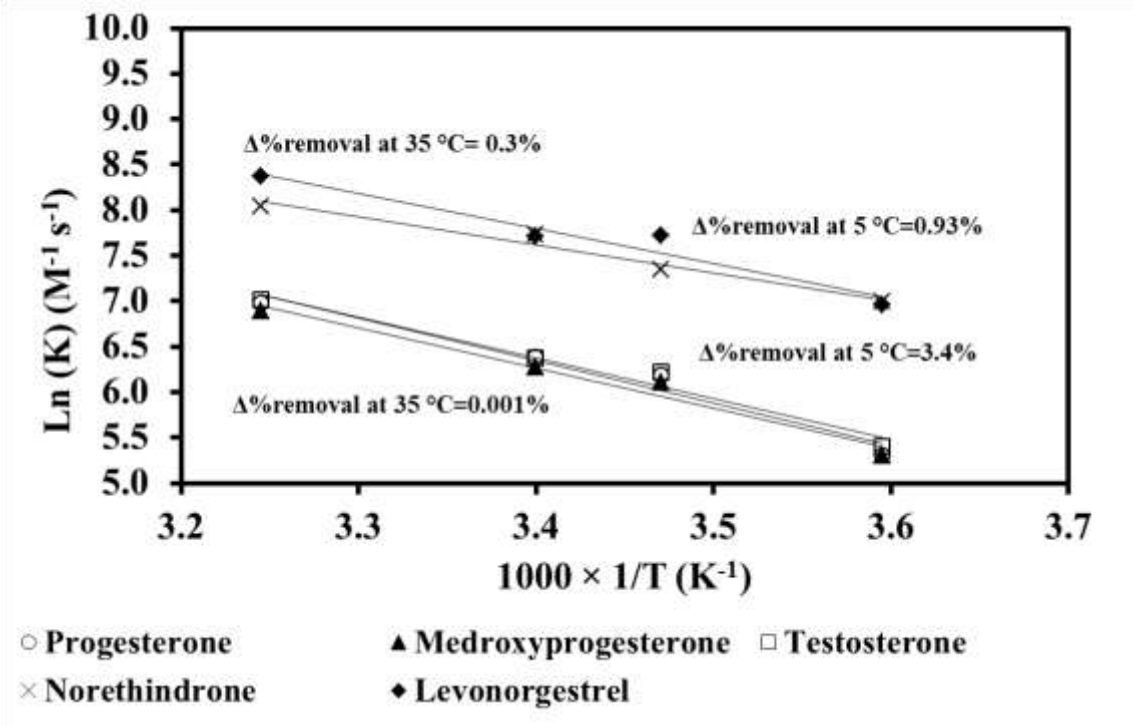


Figure 6-2. Impact of temperature on measured rate constants of progestogens and testosterone in ultrapure water at pH 6 and in presence of radical scavenger. Solid lines represent the linear regression of the measured data. Δ% removals represent the removal rate differences for steroids at 35 °C and 5 °C.

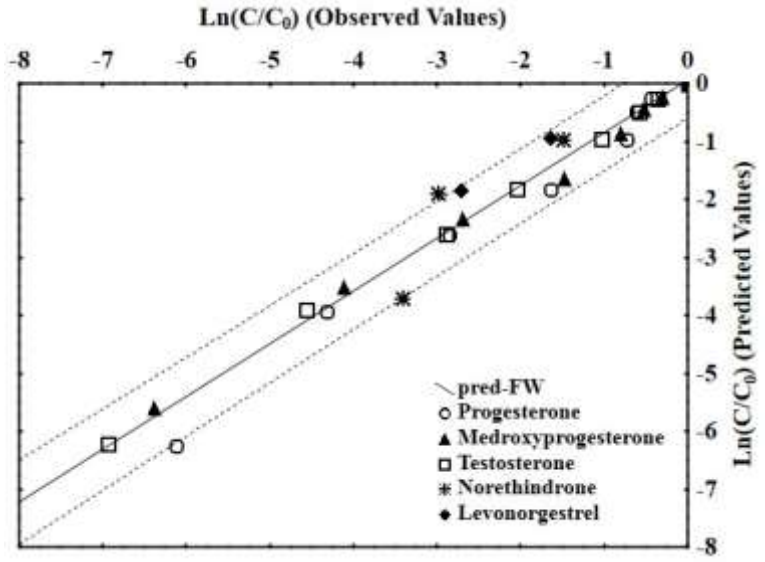
6.3.4 Oxidation of Hormones by Ozone in Real Water Matrices

The effects of the water matrix on the kinetics of oxidation of hormones by ozone were studied during ozonation of natural filtered water and diluted filtered WW effluent spiked with 10±3 µg L^{-1} of hormones in the presence of ρCBA and/or a radical scavenger (tertBuOH) and different ozone doses (2 mg L^{-1} for filtered water and 10 mg L^{-1} for diluted WW).

From oxidation experiments in real water matrices it was observed that ozone decay starts with a rapid decomposition of ozone due to the 30% and 49% initial demand of ozone in the natural water and WW effluent, respectively (Figure A-3. 3, Figure A-3. 4). The immediate ozone demand was calculated as the difference between the applied ozone dose (2 and 10 mg L^{-1}) and the initial concentration of ozone by assuming a pseudo-first-order reaction rate for ozone decomposition [159]. Initial ozone decay was followed by slower decay rates which fit with first-order kinetics for both natural water and WW effluent (inset of Figure A-3. 3, Figure A-3. 4).

Kinetic rate constants obtained from oxidation of studied hormones in ultrapure water in presence of radical scavenger were used to predict removal rates during ozonation of natural filtered water and diluted wastewater effluent in presence of radical scavenger. Figure 6-3.a and b present the results of comparisons in natural water and WW effluent, respectively. For the five measured compounds in natural water, all removal rates were within the 95^{th} prediction intervals (Figure 6-3.a). The data related to norethindrone and levonorgestrel were placed on the edge of regression bands of the 95^{th} prediction intervals for which rapid removal during the first minutes of reaction reduced the accuracy of the observed data. These findings indicate that the kinetic behavior of these compounds is highly predictable in different natural water conditions based on kinetic data from oxidation tests conducted in ultrapure water.

a)



b)

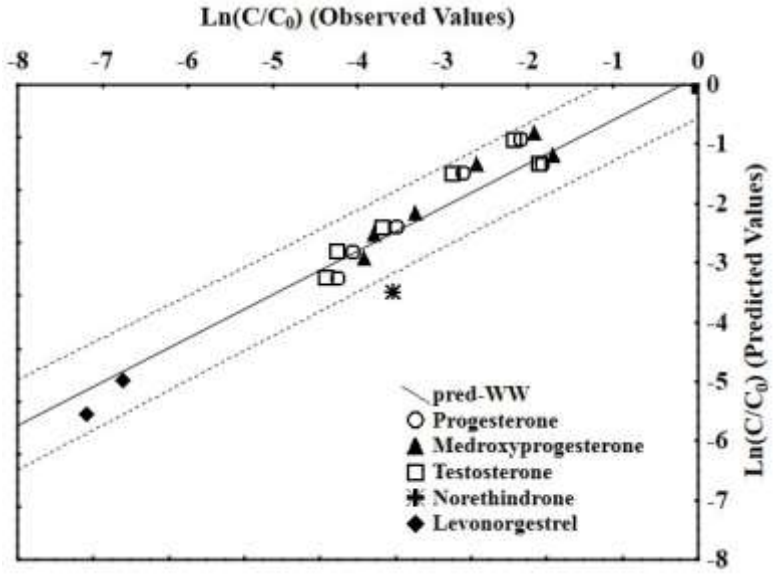


Figure 6-3. Predicted vs. observed removal rates for the five steroids in the filtered water (a) and WW effluent (b); Dotted lines indicate the 95th prediction intervals.

During the oxidation of natural waters, hydroxyl radicals are formed as secondary oxidants from the oxidation of natural organic matter [141]. The R_{Ct} value can elucidate the relative importance of hydroxyl radicals on the oxidation of steroid hormones. R_{Ct} is also a key parameter when modeling the oxidation of micropollutants in natural waters when assuming that its value usually remains constant during the second phase of ozone decomposition [158]. The values of R_{Ct} was measured from the oxidation of hormones spiked in natural filtered water and diluted wastewater

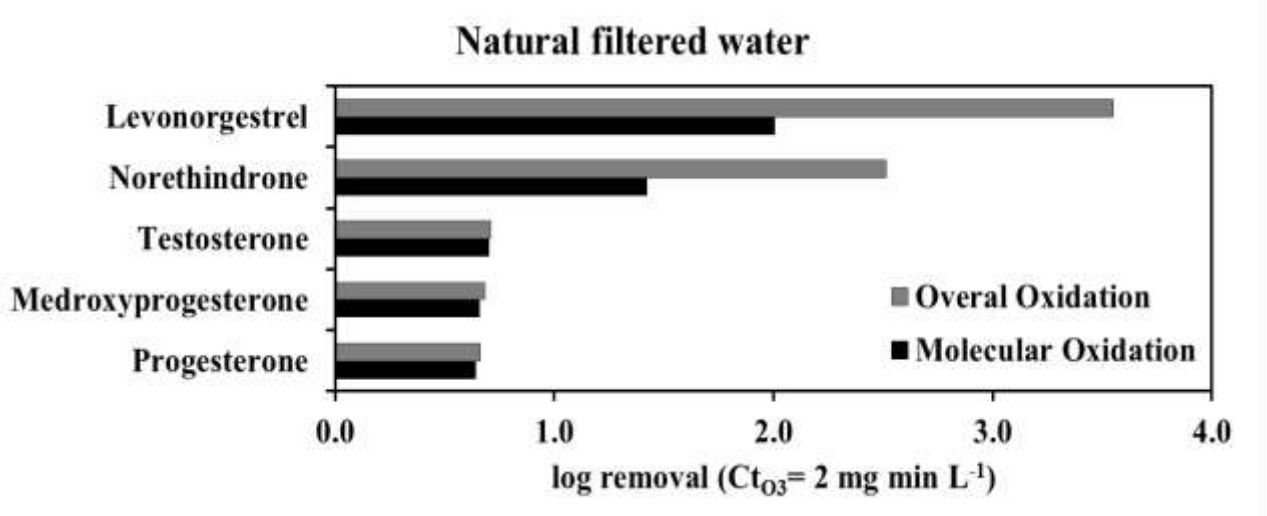
effluent using the slope of $\ln(\rho CBA/\rho CBA_0)$ vs ozone exposure (Ct_{O3}) as demonstrated in Figure A-3.5. The R_{Ct} value in the first phase of ozone decomposition (4.6E-08 and 4.3E-08 for natural water and wastewater) is higher than the R_{Ct} of the second phase (3.1E-09 and 1.02E-08 for natural water and wastewater). The hydroxyl radical exposure (Ct_{OH}) was measured indirectly using R_{Ct} and Ct_{O3}. Since there was no extra source of hydroxyl radicals, such as hydrogen peroxide or using UV lamps to enhance ozone decomposition, the Ct_{OH} values were very low (Ct_{OH}~10^{-12}) and contribution of radicals in hormone oxidation was negligible. Consequently, oxidation of steroids is governed by direct reaction with ozone. Precise estimation of rate constants for reaction of hormones with radicals becomes problematic at such low concentrations of radicals. Additionally, no data was available on rate constants of progestogens and testosterone with radicals. Therefore, the contribution of radicals in hormone oxidation can be estimated by comparing the observed rate constants which contains both K_{O3} and K_{OH} with rate constants from direct reaction with ozone K_{O3}.

Addition of radical scavenger provides the conditions to evaluate the impact of hydroxyl radical production through oxidation of natural organic matter. Radical scavenger did not greatly modified the ozone decomposition rate in filtered water due to the low concentration of DOC (2.8 mg C L^{-1}) but reduced the decomposition of ozone in diluted wastewater effluent for about 23 percent. The overall removal rates of steroids were compared to the fraction of compounds reacted with ozone (

Figure 6-4). The results of such comparison illustrated in

Figure 6-4.a indicates the role of direct reaction of steroids with ozone, specially testosterone, progesterone, and medroxyprogesterone with lower reaction rates. Testosterone was removed by 80% during the ozonation of natural filtered water in presence of radical scavenger and Ct_{O3}= 2 mg min L^{-1}. The removal rate increased only 1% when no radical scavenger was added in the oxidation reactor. The 2 mg min L^{-1} ozone exposure with no radical scavenger was enough to remove 2.5 and 3.5 log of norethindrone and levonorgestrel, respectively. After adding the radical scavenger, the removal logs reduced to 1.4 and 2log for norethindrone and levonorgestrel, respectively. Direct reaction with ozone can consequently be considered as the main removal mechanism of testosterone, progesterone, and medroxyprogesterone as the most recalcitrant compounds among the studied hormones during natural water treatment.

a)



b)

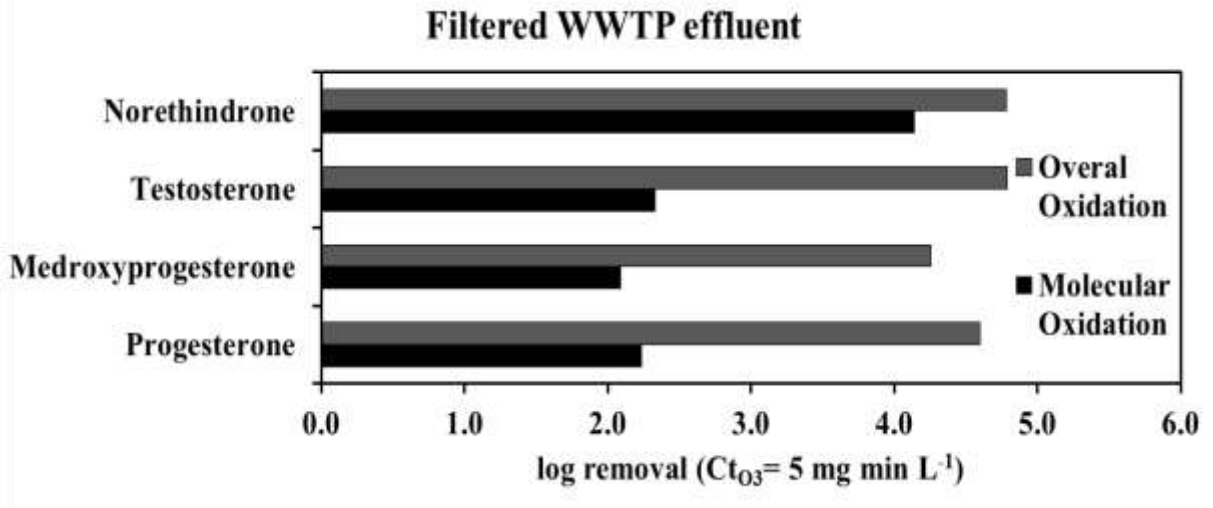


Figure 6-4. Predicted removal of steroids with direct reaction with ozone and combination of ozone and radicals from natural filtered water and WWTP effluent with the typical Ct_{O3} values used in water treatment (Ct_{O3}= 2 mg min L^{-1} for natural filtered water and Ct_{O3}= 5 mg min L^{-1} for WWTP effluent at T=21 °C).

The role of radicals becomes important during the oxidation of wastewater with higher loads of organic matter (11.5 mg C L^{-1}). As

Figure 6-4.b shows, steroids where removed more than 4 log with Ct_{O3}= 5 mg min L^{-1} during ozonation of WWTP effluent at T= 21 °C. When 50 mM of radical scavenger was added to the oxidation reactor, the removal log reduced to 2log for testosterone, progesterone, and medroxyprogesterone and 4log for norethindrone. The direct reaction of levonorgestrel and

ozone could not be measured during oxidation of WWTP effluent. Because of rapid decomposition of this compound at the first minutes of reaction, monitoring the residual concentration of levonorgestrel (< LOD) became difficult.

According to new information provided in this study, if one wishes to use the ozonation potential to remove such micropollutants, high Ct values or the implementation of advanced oxidation (using H_2O_2 and/or UV) are necessary to obtain sufficient removal of recalcitrant hormones or production of less harmful and more biodegradable by-products. Application of AOPs can enhance ozone decomposition and produce more hydroxyl radicals which can unselectively oxidize ozone resistant compounds like testosterone.Incomplete removal of recalcitrant steroids in this study (< 1log in natural water,

Figure 6-4.a) and necessity of modified treatment processes for complete removal of such compounds are in accordance with the previous observation on partial removal of other recalcitrant compounds such as anti-inflammatory drug ibuprofen (9.6 $M^{-1}s^{-1}$) and anti-anxiety agent diazepam (0.75 $M^{-1}s^{-1}$) [145].

Since complete mineralization of selected compounds could not be reached with conventional doses of ozone used in water treatment processes, further studies on the identification and quantification of potential oxidation byproducts will provide better perspective on the efficiency of removal of refractory steroids to ozone oxidation. To date, no standard regulations exist regarding the quantity of steroid hormones released in surface waters and their maximum allowable concentrations in drinking water. The results obtained in this study confirm the persistence of recalcitrant steroids after ozonation at typical dosages applied by industry. These relatively recalcitrant compounds could be used as indicator compounds for assessing the efficacy of WWTPs and DWTPs to reduce environmental risks and the possible health risks of human exposure to trace concentrations of steroid hormones.

6.3.5 Conclusion

The main aim of this study was to evaluate the efficiency of ozone as powerful oxidant in water treatment processes for oxidation of steroid hormones. The effect of temperature, pH, and natural organic matter were investigated. The following conclusions can be drawn:

- Testosterone is moderately reactive towards ozone with rate constant of 590±0.13 $M^{-1}s^{-1}$ in ultra-pure water in the same range of rate constant for progestogens (532- 2593 $M^{-1}s^{-1}$).
- The effect of temperature on the performance of ozone in water treatment plants is significant in the range of water temperatures encountered by utilities. The removal rate of selected steroids increased 3 to 5.5-fold when the temperature increased from 5 to 35°C.
- Testosterone, progesterone, and medroxyprogesterone oxidation rates were more sensitive to temperature change as compared to levonorgestrel and norethindrone. This fact confirms the importance of temperature on the potential of ozonation to oxidize recalcitrant steroids, particularly during the cold weather.
- The activation energies were calculated for the first time for ozone-hormone reactions in ultrapure water at pH 6 using the corresponding second-order rate constants at different temperatures (E_{act} = 30- 39 kJ mol^{-1}).
- The oxidation constants were successfully predicted in natural filtered water and treated WW. Using established kinetic constants, it is possible to adjust water treatment processes to ensure desired hormone removal from source waters.
- The results of this study suggest that progestogens and testosterone will be removed by less than 1 log if typical Ct values (2 and 5 mg min L^{-1} at 20 °C) are applied during water treatment. Consequently, high Ct values or the implementation of advanced oxidation (using H_2O_2 and/or UV) are necessary for obtaining sufficient removal of such ozone resisting compounds.
- Comparing the overall reaction of steroids with ozone and hydroxyl radicals, direct reaction with ozone was found as governing removal mechanism of selected steroids with > 97% removal with ozone for testosterone, medroxyprogesterone, and progesterone and 56% for norethindrone and levonorgestrel.
- Partial oxidation of progestogens and testosterone could lead to the formation of oxidation byproducts. The identification and quantification of byproducts could help better understand the oxidation mechanisms involved and identify possible biological effects of such compounds especially when ozone is used for WW disinfection before discharge into surface waters.

6.4 Acknowledgments

The authors would like to acknowledge the NSERC Industrial Chair on Drinking Water Treatment and its partners (City of Montreal, City of Laval and John Meunier Inc.) for financial support and the technical staff from the Centre de Recherche, de Développement et de Validation des Technologies et Procédés en Traitement des Eaux (CREDEAU) for supporting laboratory work.

CHAPTER 7 GENERAL DISCUSSION

This chapter discusses the main findings presenting the different aspects of the research included in this thesis. Strength and limitations for each part of this work and any further work that could address the limitations are identified in the conclusion and recommendations.

The main objective of the work described in this thesis was to evaluate the environmental occurrence and potential treatment processes for the removal of selected steroids present in drinking water sources. Figure 7-1 summarizes the different aspects of the research work conducted as a function of the specific objectives listed in Chapter 2. The first step was to generate field data on the overall presence and sources of a group of steroids in an urban river subjected to multiple wastewater and sewage discharges. Multiple drinking water intakes are present along this urban river. We generated data to better understand the distribution of steroids between their dissolved and particulate phases and their seasonal variations along the river, in sewage and in treated wastewater. Once the concentrations and forms of steroids in surface water and sediments were established, we conducted adsorption tests to better quantify the potential of surface shore river sediments to act as a sink for steroids. The last part of the work focused on the most promising and commonly available water treatment process, namely ozonation, to remove the more recalcitrant steroids, confirming and extending early work from our group by including additional steroids. Investigations were carried out through bench scale and field study. Sorption and oxidation kinetics were determined through laboratory scale experiments to establish the capacity of ozonation to remove any dissolved steroids remaining after filtration.

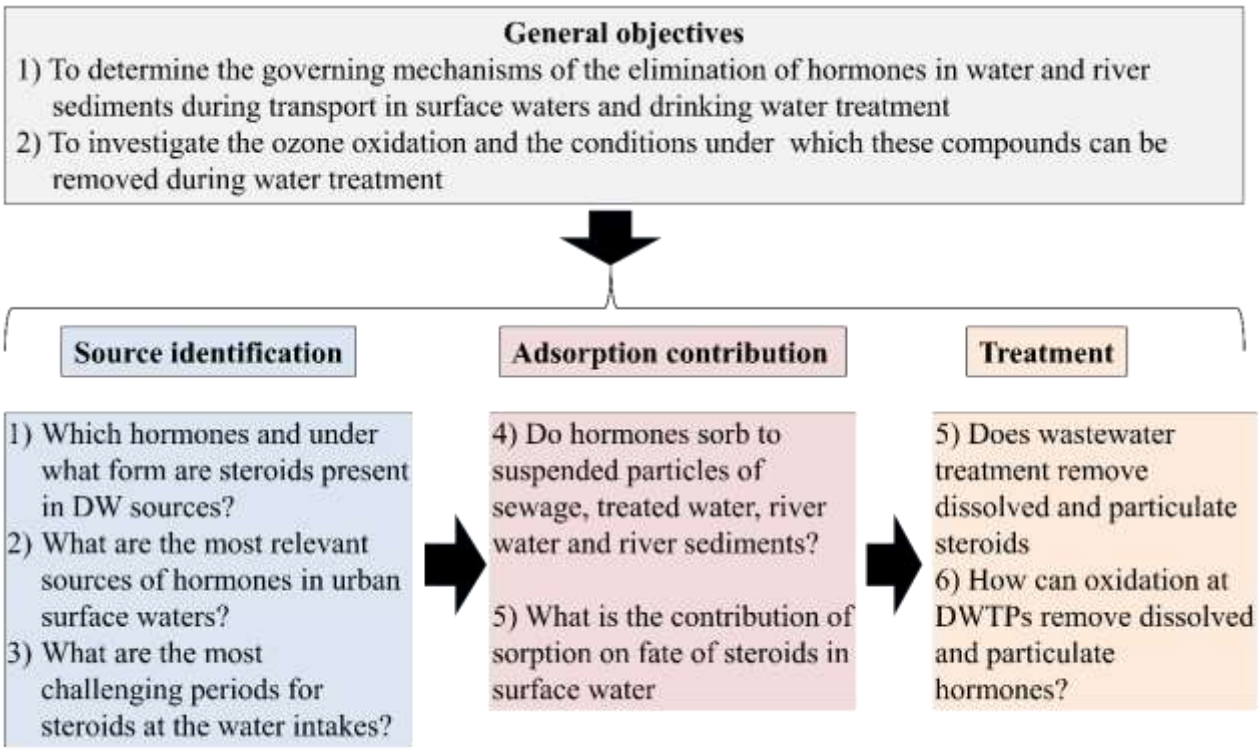


Figure 7-1. Summary of the research conducted.

Key findings of this research are discussed hereafter considering:

1) The estimation of the total concentration of steroid hormones in surface water.

2) The improved demonstration of the key role of the particulate fraction of steroid hormones in surface water.

3) The investigation the adsorption behavior of steroid hormones onto shore river sediments.

4) The kinetic assessment of ozone oxidation of steroid hormones during water treatment.

7.1 Estimation of the total concentration of steroid hormones in surface water

The presence of steroid hormones in different matrices of aquatic environments and their adverse effects on aquatic creatures is widely recognized. Most of steroid hormones are partially soluble in water, thus the fraction of hormones attached to suspended particles and sediments must be considered when monitoring these compounds in environmental waters. Quantification of particulate associate steroids is environmentally relevant since steroids adsorbed to suspended particles and sediments can bioaccumulate [163]. Furthermore, the association with particles determines the potential for removal of steroids by processes that target particle removal in water treatment plants, such as chemically enhanced filtration. There are few studies on the distribution of steroids between the dissolved and particulate phases in natural waters, raw sewage and WRRFs treated effluents. Most studies have focused on estrogens and there is a significant gap in studies on natural and synthetic progestogens and testosterone [53, 164, 205]. Furthermore, there are no reports that identify the sources of dissolved and particulate phases of steroids and relative contribution at drinking water intakes. This is an important data gap for drinking water systems with sources in rivers impacted by significant wastewater and sewage discharges. Another important consideration to understand the limitations of existing reports of the fate of steroid hormones is the significant challenges of effective extraction and reproducible analytical method at the low ng L^{-1} levels. During the work described in this research, the solid samples (river particle, sewage particles, treated WW particles, and sediments) were analysed using a new extraction method developed in our group by our co-researchers [5]. The method includes two cycle extraction of lyophilised solids or suspended particles from water samples combined with a clean-up step and ultrafast mass spectrometry quantification through an LDTD interface

allowing the processing of the large number of particulate samples which was the major focus of my work.

7.1.1 Occurrence of dissolved and particulate steroids in raw sewage and treated wastewater

To understand the role of WRRFs as the source of steroids at drinking water intakes, the occurrence of steroid hormones in influent/effluent of three different WRRFs discharging their effluent in river was assessed. Although not the focus of this work, the investigations on raw and treated wastewater at three WRRFs provided interesting insights in the efficacy of the WWT to remove dissolved and particle associated steroids. When comparing experimental results presented in this work with the literature, it became obvious that most prior reports focused on the detection of the dissolved steroids, while some investigated the particulate phase or the presence in sludge [33, 47, 48, 163, 171, 206, 207]. As shown in Table 4-4, dissolved testosterone and progesterone were found in all raw sewage samples with mean concentrations of 105 and 24 ng L^{-1} and lowered to 90 and 15 ng L^{-1}, respectively after treatment. These levels are coherent with data reported by prior studies. Fan et al. detected 62 and 33 ng L^{-1} of testosterone and progesterone in a large treatment plant in Beijing, China [166]. The concentrations of testosterone in the influent and effluent of 6 WRRFs studied by Huang et al. ranged between 7-53 ng L^{-1} and n.d.-2.5 ng L^{-1}, respectively [74]. While, progesterone concentrations varied between 18-69 ng L^{-1} and n.d.-6.4 ng L^{-1} in influent and effluent, respectively. Dissolved estrogens and synthetic progestogens have also been detected in raw sewage. In this work, the mean concentration of estrogens in the dissolved phase of sewage and WRRFs effluents were 181 and 151 ng L^{-1}, respectively. The apparent poor removal of E1 is probably caused by the conversion of E2 to E1 and E3. It is also the result of the cleavage of steroid conjugates by glucuronidase enzymes which increased average E1 levels from 118 to 145 ng L^{-1} in the WRRFs influent and effluent, respectively as shown in Table 4-3. The mean dissolved concentration of levonorgestrel and norethindrone detected in influents (30 and 67 ng L^{-1}) and effluents (12-16 ng L^{-1}) were higher than those reported in previous study by Chang et al. [4]. Medroxyprogesterone was the compound presenting the lowest average concentration (4 ng L^{-1} influent and 2 ng L^{-1} in effluents) among the steroids measured in our work.

The focus of this work and by far the most important form of steroids in surface water is the particulate phase. E2, EE2, norethindrone, and testosterone were found in the particulate phase of all raw sewage and WRRFs effluent/effluent samples. Levonorgestrel was only detected in WRRF3 at a concentration of 27 ng L in the influent and reduced to 4.5 ng L^{-1} after treatment. The mean particulate concentration of medroxyprogesterone in influents and effluents were 17.5 and 8 ng L^{-1}, respectively. WRRF2, which uses physical treatment, was the least efficient to remove medroxyprogesterone (10%), EE2 (59%), norethindrone (60%) and testosterone (-19%) compared to two other treatment plants with biofiltration process. Biological treatment processes can remove estrogens from wastewater to varying levels depending on the type of plant, initial concentration of compounds for removal, location of plant and also temperature [118]. Degradation of estrogens and testosterone in wastewater biosolids can lead to mineralization to $^{14}CO_2$ with 55-65% and >90% efficiency, respectively [118]. E2 was readily degraded to E1 after 22 hr contact with activated sludge from WRRF in Burlington, Ontario [172]. Degradation of norgestrel and progesterone were compared in activated sludge from WRRFs in southern China [107]. For norgestrel, its aerobic biodegradation followed first order reaction kinetics with $t_{1/2}$= 12.5 d while progesterone followed zero order reaction kinetics with $t_{1/2}$= 4.3 h. Previous studies also confirmed degradation of progesterone under aerobic conditions [35]. However, norgestrel was found to behave as a recalcitrant compound to biodegradation such as EE2. Degradation of EE2 and E2 under different activated sludge systems was studied by Li et al. 2011[208]. E2 was significantly degraded under anaerobic condition (71%removal) and its concentration dropped below the detection limit while it was still detected in solid phase of aerobic reactor t a concentration of 0.057 µg L^{-1} suggesting that E2 may adsorb to the sludge particles and remain in the solid phase of the reactor. EE2 was more persistent to biodegradation as its concentration increase after anaerobic reactor and its final removal efficiency was less than 80%.

Among the most important findings of our investigations is the steep increase in the amount of steroids adsorbed per gram of particles in the treated WW as discussed in chapter 4 and illustrated in Figure 4-2. The highest variation corresponded to norethindrone with its mean concentration increasing from 2.5 µg g^{-1} (raw sewage) to 11.6 µg g^{-1} (treated WW). These findings illustrate the important contribution of treated WW particles as a source of particulate associated hormones released to the river.

7.1.2 Contribution of combined sewage overflows (CSOs)

With the knowledge of the concentration of dissolved and particulate steroids in raw sewage, it is also possible to discuss the contribution of CSOs to the river contamination by these compounds. CSOs have been considered to be an important source of micropollutants in urban river in the area of this study [209, 210]. This contribution becomes more significant during rainfall when sewage combines with storm water and is discharged into surface waters. High loads of micropollutants can then enter surface water during the CSO discharges reaching drinking water intakes. The river selected for this research receives discharges from 14 WRRFs, 194 CSOs, and several urban creeks. All these discharges are introduced to the river along the 42 kilometer and definitively increase the loads of micropollutants and consequently impact the quality at the drinking water intakes. Concentrations of EE2 in samples taken downstream of all the WRRFs were higher than found in the WRRRFs effluents. EE2 discharge loadings from WRRFs were 0.8, 2, and 0.7 g d^{-1} from WRRF1, WRRF2, and WRRF3, respectively; while its mass flow in samples taken downstream of WRRFs discharge point were 1693, 1756, and 670 g d^{-1} at , P3, P4, and P5, respectively. The mass loading s of EE2 discharged from WRRF2 were similar in summer (1.8 g d^{-1}) and spring (2.1 g d^{-1}), indicating that despite the higher river flow in spring (350 m^3 s^{-1}) and possibility of dilution downstream of WRRFs discharge point, EE2 would still be likely to be detected in the river at high levels. Untreated sewage discharged to the river through CSOs could also have contributed in the persistent loadings of EE2.

7.1.3 Profile of steroid hormones concentration along the river

The results presented in Chapter 4 show the trends of 9 steroid hormones concentrations measured during two seasons along the studied urban river. Extensive sampling was conducted from the entry point of the river to various points downstream of WRRFs discharges and of major CSO discharge areas. Our sampling and analytical efforts were directed toward the quantification of particulate associated steroids concentrations. Significant amount of steroids were found in particles suspended in river water with mean concentrations ranging between 3 to 35 ng L^{-1} (Table 4-4), confirming the importance of the particulate fraction of natural and synthetic estrogens, progestogens and testosterone in the total amount of each compound in water samples.

Most studies reporting the occurrence of steroids in environmental waters processed samples with filters (0.7-1.2 µm pore size) and only considered compounds present in the filtrate [3, 56, 60-62, 64-70][64]. However, colloidal particles (<1 µm) have been found to be strong sorbents for estrogens [182] and progesterone [17]. Gong et al. [164] studied the partitioning of some endocrine disrupting compounds including steroid estrogens in river water and suspended particles > 0.7 µm. E1 was the only estrogen detected in the dissolved phase at a mean concentration of 3.2 ng L^{-1}. Particulate estrone (E1) was detected in one sample receiving two very contaminated streams at 1.1 ng L^{-1} (14.4 ng g^{-1}).

Our analyses of particles suspended in river (>0.3 µm) emphasize the important role of small particles on the total amount of steroids detected in surface waters. Considerable amounts of hormones were detected in the suspended phase of river water. EE2, E2, norethindrone and testosterone were found in suspended particles in all spring samples. In summer, E2, progesterone and testosterone were the most frequently detected compounds (100%, 83%, [179]and 25%, respectively). Considerable amounts of dissolved E2 (9 ng L^{-1}) and trace concentrations of dissolved progesterone (3 ng L^{-1}) and medroxyprogesterone have been reported in the same river by our collaborators [68, 211]. These levels of E2 were reported to be sufficient to induce estrogenic activity affecting aquatic life [161]. Progesterone, norethindrone, and levonorgestrel were found in suspended particles of another river in Quebec with concentration of progesterone as high as 97 ng g^{-1} [5]. The mean concentrations of norethindrone and levonorgestrel were reported as 29 and 28 ng g^{-1}, respectively.

Owing to the properties of steroid hormones, it appeared likely that sediments act as an important sink for these compounds [17]. Analyses of sediments also provide useful information on the long-term occurrence of the steroids as opposed to grab water samples. Estrone is most frequently reported steroid in sediment [52, 205, 212]. In this study, E2, EE2, and Prog were the only steroids detected in river sediments with 100%, 67%, and 61% frequency of detection for the total of 36 samples collected in 3 seasons. In spring samples, only EE2 was detected in sediments with concentrations ranging between 10-26 ng g^{-1}. Progesterone levels measured during this study are coherent with results reported by Viglino et al. (12 ng g^{-1}) [76] and Mulabagal et al., but higher than those reported by López de Alda et al. (0.08- 6.82 ng g^{-1}) [77]. Progesterone and testosterone were detected in sediments of Gulf of Mexico at a depth of 0-46 cm at concentrations ranging 6.47-22.3 and 4.8-12 pg g^{-1}, respectively [213]. In the case of

estrogens, wide ranges of concentration have been reported in aquatic sediment [17, 37, 72, 77, 78, 81, 108, 179, 205, 212, 214-216]. Differences between the reported levels may reflect different locations, varying organic content, and the particle size distribution of sediments. A direct relation was found between the TOC of the sediment sample and the amount of estrogens adsorbed onto the sediment particles [205]. Our findings of the estrogen levels in sediment are in accordance with results of the study by Viglino et al. (70 and 30 ng g^{-1} for E2 and EE2, respectively) [76] and higher than levels reported in Spain (n.d for E2 and 22.8 ng g^{-1} EE2, respectively) [77] and in China (1.58 and 2.1 ng g^{-1} for E2 and EE2, respectively) [52]. However, contrary to other reports, we did not detected estrone (the most frequent steroid in sediments) in any of our sediment samples. Synthetic progestogens, levonorgestrel, norethindrone, and medroxyprogesterone have been detected in river sediments at concentrations up to 19, 90, and 29 ng g^{-1}, respectively [76, 77, 217].

Although multiple discharges were present along the river, no clear gradient of steroids content in river was observed in sediments up flow to down flow of the river (Figure 4-6,Table 4-5). As an example, sediment associated concentration of E2 increased in the first part of the river (P1-P7) and then decreased down flow (P7-P12). A different pattern was observed for EE2 with pick concentrations measured in the middle of river. The patterns for sediment associated progesterone concentration was even more variable with high levels detected downstream of two WTPs. Additionally, the maximum concentrations of particulate E2 and Prog were found downstream of WRRF3 (P7) confirming the presence of additional discharge source near this point while the minimum was at P11 almost at the end of the river. The lack of clear patterns up flow to down flow of the river suggest that local conditions, the discharge of a mixture of treated wastewater, and naturally attenuated untreated wastewater during the study period may govern the variable levels of steroids.

7.1.4 Seasonal variations of concentration/loading of steroid hormones in the river water solids and sediments

The frequency of detection and the levels of steroids in water samples taken along the river during summer and spring campaigns are presented in Table 4-4. The two sampling campaigns were timed to examine the effect of both snowmelt/high precipitation season and also dry season on steroid profiles. Higher loads of steroids were found in particulate phase of water samples

during the spring campaign (722 ng L^{-1}), compared to overall steroids measured in summer campaign (179 ng L^{-1}). In Quebec, April/March is the beginning of snowmelt melting season which leads to increased river flow. Also higher loads of raw sewage will discharge through CSOs into the river because of infiltration and heavy rainfall during spring. Additionally, lower temperatures decrease the performance of WRRFs, especially those using biological processes. Consequently, more steroids are expected to release suspended phase in water samples.

Shore river sediments were sampled during spring, summer, and fall. The average concentration of steroid hormones was highly variable between sampling campaigns with 367 ng g^{-1} (fall), 130 ng g^{-1} (summer), and 9.84 ng g^{-1} (spring). Petrovic et al.[217], observed a seasonal fluctuation of the concentration of steroids in river sediments, where the total concentration of steroids in winter was one order of magnitude higher than in spring and summer. E1, norethindrone, and progesterone were detected in samples with 79, 79, and 54% frequency of detection.

EE2 was the only compound detected in both the particulate phase of water and sediments during the spring and summer sampling with 100% detection frequency. Contrary to Nie et al. which found E3 as the most abundant estrogen in suspended particles and sediment during both cold and warm weather, we were found distinctive difference between the partitioning of EE2 (the most frequently detected compound in our samples) between suspended particles and sediment at different sampling periods. The maximum concentrations of EE2 in suspended phase and sediments reached 8 ng L^{-1} and 117 ng g^{-1} in summer and 58 ng L^{-1} and 25 ng g^{-1} in spring, respectively, resulting in higher particle loads during spring (3 mg L^{-1} in summer vs 11 mg L^{-1} in spring).

When comparing the amount of steroids attached to the particulate phase ($C_{particulate}$; ng L^{-1}) and that in the sediments ($C_{sediement}$; ng g^{-1}), the ratio of the three compounds detected in both suspended particle phase and in the sediments of summer samples, namely progesterone, E2, and EE2 ($K_p = C_{particulate} / C_{sediement}$) varied between 10.5 L ng $^{-1}$ for EE2 to 31.3 for progesterone (Table A-1. 3). The K_p of EE2 was compared between spring and summer with K_p in summer one order of magnitude hier than that in spring (0.31 L ng^{-1}).

7.1.5 Steroids present in drinking water plants

Treated wastewater treatment plant discharges represented 0.65 and 1.6% of the total river flow during spring and summer sampling periods, respectively. Although dilution is expected to reduce steroid levels along the river, they could still be detected in DWP intakes at concentrations as high as raw sewage (Figure A-1. 2). All selected steroids except progesterone were frequently detected at drinking water intakes. Testosterone was the only steroid detected in the dissolved phase of DWP intakes with concentrations between 17-35 ng L^{-1}. Testo, Prog, and estrogens were previously detected in the dissolved phase of DWP intakes of the American DWP intake at concentrations much lower than our findings. The average concentration of testosterone in 19 DWPs intakes and treated waters were low as 1.2 ng L^{-1} and below the detection limits [193]. In the same study, progesterone and E2 were detected at 3.1 and 17 ng L^{-1} while progesterone was also found in treated water at 0.57 ng L^{-1}. In another study, Prog was found in one DWP intake at 0.15 ng L^{-1} and one treated drinking water at 0.2 ng L^{-1} among the 29 DWP analyzed [63].

In suspended particles from DWP intakes, testosterone, norethindrone, EE2, and E2 were found at concentrations ranging from 8.73 to 100.52 ng L^{-1}. The high concentrations of the steroids detected during this work are coherent with the volumes and concentrations of treated WW and sewage discharged into the river (Table 4-3). Of key findings from Chapter 4 are demonstrations of adsorbed steroids concentration in treated WW particles are 3.5 folds higher than that in DWP intakes.

The presence of particle associated steroids at DWP intakes will lead to their accumulation in sludge of coagulation/sedimentation processes. We conducted a series of tests to quantify steroids in settled sludge of DWP3. All steroids detected in particulate phase of water intake were found in the sludge particles with concentrations ranging between 1016 ng g^{-1}(Norethindrone) and 288.45 ng g^{-1} (MDRXY-Prog). Consequently, optimising coagulation/sedimentation processes appears to be a major barrier to remove steroids present in raw water and prevent steroid passage to treated water. However, the use or reuse of sludge for agricultural purposes raises concerns about the endocrine disrupting effect of steroids.

7.2 Adsorption of steroid hormones onto shore river sediments

Once WRRFs effluent and other non-point sources of hormones are discharged into surface waters, they undergo several fate and transport processes such as degradation, sorption, and mobility [8, 32, 97]. Steroid hormones have limited water solubility. Hence, adsorption to organic content of aquatic systems will largely determine the fate of these compounds.

The second objective of this thesis was to investigation the adsorption potential of selected steroid hormones onto the shore river sediments. The sorption experiments were performed on different sediments with different organic carbon contents (S1= 11000 µg g^{-1}, S2= 21600 µg g^{-1}, S3= 9900 µg g^{-1}, and S4=10600 µg g^{-1}). Our findings evidence the high potential of natural sediments in adsorption of different steroids and the importance of the sediments as a potential sink which affect the removal of steroids form water. Results presented in Figure 5-1, also demonstrate the effect of organic carbon content of sediments on the amount of sorbed steroids. The sorption amount to sediment sample with highest organic carbon content (S2) was almost similar for all compounds (61- 78%) whereas in S4 with lower organic content the minimum sorption was 14% for E2 and the maximum was 56% for Prog.

The solid-water distribution coefficients (K_d, L kg^{-1} solid) values and normalized organic carbon partition coefficients (K_{OC}, L kg^{-1} OC) were estimated based on the data from equilibrium. All the steroids showed rapid adsorption onto sediments within 15 min which indicate that major part of sorption occurred right after the contact of steroid and sediment. The sorption equilibrium varied between steroids and sediments with different organic carbon content.**Error! Reference ource not found.** summarized the Log K_{OC} and K_d values and associated standard deviations. Highest sorption coefficients were related to the sediment sample with higher OC. The obtained K_d s were in rang of relatively high Kd s previously reported considering that several parameters such as sediment particle size distribution, available surface area, pH, and initial concentration of steroids can obviously change the partitioning coefficient of compounds [32, 109, 179].

No distinctive relationship was found between Log K_{OW} and Log K_{OC} of compounds (Figure 5-3). This sentence confirms the fact that hydrophobic interactions are not the main mechanism for sorption of steroids [186], i.e., the phenolic group of estrogens is suggested to make hydrogen bonding with humic acids and mineral surfaces [179].

Adsorption isotherms were applied to predict adsorption behavior of steroids on sediments. The sorption isotherms were produced in batch mode over a 3 order of magnitude of steroid concentrations (5, 10, 25, 50, 75, 100 µg L^{-1}). The adsorption data for all steroids better fitted with linear isotherms. Table 5-4 lists the isotherm parameters obtained for the mixture of steroids. Higher linearity observed in the sediment with lowest organic carbon content (S3), suggesting that amount of organic content has direct effect on the sorption linearity Previous study on sorption of steroids onto natural sediments also used linear isotherms for E1, E2, and testosterone [17].

Kinetic experiments were conducted in batch mode with 1g of irradiated sediment and 1-5 ml of irradiated river water at three initial concentrations of steroids (5, 50, and 100 µg L^{-1}). Results of sorption kinetics studies revealed that adsorption of all the selected steroids on sediments follow a pseudo-second order kinetic with correlation coefficient over 99%. The quantities of steroids sorbed at equilibrium (q_e) from model were similar to those obtained from experiments. The rate constants varied between 1.09E-03 and 8.04E-03 g ng^{-1} min. comparing between sediment samples with most different characteristics, S2 and S4, higher rate constants were obtained for sorption of steroids on S4 with lower organic carbon content.

7.3 Kinetic assessment of ozone oxidation of steroid hormones during water treatment

Oxidation and advanced oxidation are considered as promising processes for the removal of micropollutants [86, 130, 145, 195, 218-220]. Organic micropollutants may oxidize through either the direct reaction with ozone or indirectly through the reaction with hydroxyl radicals [141]. However, complete removal of micropollutants is typically not achieved and many compounds are only transformed. Therefore, the toxicity of the transformation products must also be verified. In case of wastewater treatment, a subsequent biological filtration can remove most of oxidation by-products which are usually more biodegradable compared to parent compounds [142, 221].

One of the main objectives of this research work was to evaluate the potential for ozone oxidation of recalcitrant steroid hormones under different conditions of pH and temperature. Kinetic constants were measured for 4 progestogens (progesterone, medroxyprogesterone,

norethindrone, and levonorgestrel) and for the first time for testosterone in ultra-pure water. The activation energy for the reaction of selected steroids was also estimated for the first time.

7.3.1 Ozone oxidation kinetic constants of steroid hormones in ultra-pure water (K_{O3})

The second-order rate constants for the reaction of ozone with progesterone, testosterone, medroxyprogesterone, levonorgestrel, and norethindrone were determined in batch mode using ultra-pure water at pH 6 and 8 and at temperatures in range of 5 to 35 °C. As demonstrated in Table 6-2, all the five compounds have moderate reactivity toward ozone. The rate constant were 594, 590, 532, 2233, 2292 $M^{-1}s^{-1}$ for progesterone, testosterone, medroxyprogesterone, levonorgestrel, and norethindrone, respectively. The obtained rate constants for recalcitrant steroids were compared in Table 6-2 with previously reported values except for testosterone which no previous data were found [130, 138]. The obtained kinetic data in current study were in good agreement with sparse data available from previous studies. Barron et al. [138] investigated the oxidation of progesterone at different pH values. The average oxidation rate for this compound was 469±21 $M^{-1}s^{-1}$ compared to 594 $M^{-1}s^{-1}$ in our study and 601 $M^{-1}s^{-1}$ reported by Broséus et al.[130].

The estimated rate constants for progestogens and testosterone were about 2 orders of magnitude lower than that for estrogens. Higher reactivity of estrogens is related to the phenolic group in their structure ($k_{O3,}$ phenol= 1.3×10^{4} M^{-1} s^{-1} [196]). Whereas, testosterone and progesterone both have C-C double bond attached to the carbonyl group which reduces the reactivity toward ozone. Progesterone has also an acetyl group which has low reactivity with ozone. The rate constants for progesterone, testosterone, and medroxyprogesterone are similar since they have very similar structures. However, levonorgestrel and norethindrone showed higher reactivity toward ozone. The two latter compounds have hydrogen atoms on their C10 carbon while three compounds with lower reactivity have methyl on the same carbon which may induce steric hindrance on C-C double bond. Overall, the electron withdrawing or donating properties of substituents at the C-C double bound and their corresponding reactivity with ozone can strongly affect their reactivity toward ozone.

7.3.2 Effect of pH, temperature, and organic matter on the oxidation rates of steroid hormones

Experimental conditions have direct effect on the oxidation of micropollutants with ozone. The rate of ozone decomposition varies with pH. At higher pH values, ozone decomposes to hydroxyl radicals and oxidation will be governed by non-selective OH° radicals. While, at lower pH values, direct reaction with ozone is the main mechanism of micropollutant removal. In our experiments at pH 8, no significant difference observed in steroids oxidation rate compared to pH 6. The reason is suggested the low concentration of hydroxyl radicals since no external source of hydroxyl radicals (such as hydrogen peroxide or UV lamps) was added during our experiments. Our aim was to evaluate the oxidation of steroids under real water treatment process without advanced oxidation process application. The independence of ozone oxidation of progesterone to pH was reported previously as the rate constant varied from 485-469 M^{-1} s^{-1} when pH increased from 2 to 7.96 [138].

The effect of temperature on the efficiency of ozone in water treatment is especially important in Northern climates where water temperature show large seasonal variations. Figure A-3. 1 illustrates the direct effect of temperature variations on decomposition rate of ozone. The pseudo first-order ozone decay rates increased up to one order of magnitude when temperature increased from 5 to 35 °C. For every 10 degree increase in water temperature, the ozone decomposition increased by the factor of 1.2-1.8. Consequently, the removal rate of steroids varied with temperature variations. Progesterone removal increased 5.5-folds with temperature variation from 5 to 35 °C. The oxidation constants at different temperatures (Figure 6-1) were used to calculate the activation energies for reaction of steroids with ozone in ultra-pure water at pH 6. The E_{act} were obtained for the first time from linear regression with minimum of 30 kJ mol^{-1} for norethindrone and maximum of 39 kJ mol^{-1} for progesterone. Similar activation energies are acceptable for five measured steroids because of their comparable structures. The obtained E_{act} are in agreement with the previously reported values for the reaction of most organic compounds with ozone which are expected to vary between 35 and 50 kJ.mol^{-1} [196]. As shown in Figure 6-2, steroids showed increasing decomposition rate from 5 °C to 35 °C. However, for defined Ct_{O3}, the removal rate for testosterone, progesterone, and medroxyprogesterone were found more variable with temperature variation than two other compounds. For instance, at

Ct_{O3}= 2 mg min L^{-1}, testosterone removal increased from 56.4% to 98.4% when temperature increased from 5 °C to 35 °C; while for norethindrone the removal rate changed only from 98.3% to 99.9%.

7.3.3 Predicted rate constants for ozone oxidation of steroid hormones in natural water and wastewater

Oxidation rate of steroids with ozone were evaluated in drinking water filtered water samples and diluted WRRF effluent. Removal rates were predicted in both water samples using kinetic data from ultra-pure water. The obtained results were compared with observed removal rates from oxidation of natural water and diluted WW effluent. The results of this comparison were placed within the 95^{th} prediction intervals (Figure 6-3.a and b).

The role of hydroxyl radicals becomes more important during the oxidation of natural waters since more radicals are produced from oxidation of natural organic matter with ozone. The R_{Ct} value (the ratio of hydroxyl radial exposure to ozone exposure) can explain the relative importance of hydroxyl radicals on the oxidation of steroid hormones. The R_{Ct} value is also a key parameter when modeling the oxidation of micropollutants in natural waters when assuming that its value usually remains constant during the second phase of ozone decomposition [203]. The R_{CT} value is also useful to indirectly measure the hydroxyl radical exposure. As no external source of radicals was added to the reaction, direct reaction with ozone is considered as the main mechanism of steroids oxidation.

Tert-butanol was used as radical scavenger to evaluate the impact of hydroxyl radical production through oxidation of natural organic matter. The addition of a radical scavenger did not affect the ozone decomposition in the filtered drinking water due to the low concentration of DOC (2.8 mg C L^{-1}). The fraction of steroids removed by ozone, obtained from experiments in the presence of a radical scavenger, was compared with overall removals without a radical scavenger. The results are illustrated in

Figure 6-4.a indicating the role of direct reaction of steroids with ozone, specially testosterone, progesterone, and medroxyprogesterone with lower reaction rates. Less than one log (80%) of testosterone was removed from natural filtered water with Ct_{O3}= 2 mg min L^{-1} and in presence of tert-butanol. A Ct_{O3} of 2 mg min L^{-1} with no radical scavenger was enough to remove 2.5 and 3.5

log of norethindrone and levonorgestrel, respectively. Addition of the radical scavenger reduced the removal logs to 1.4 and 2log for norethindrone and levonorgestrel, respectively. Consequently, direct reaction with ozone can be considered as the main removal mechanism of testosterone and progestogens during water treatment. The role of radicals becomes important during the oxidation of wastewater with higher loads of organic matter (11.5 mg C L^{-1}). As

Figure 6-4.b shows, steroids where removed more than 4 log with Ct_{O3}= 5 mg min L^{-1} during ozonation of WRRF effluent at T= 21 °C. When 50 mM of radical scavenger was added to the oxidation reactor, the removal log reduced to 2log for testosterone, progesterone, and medroxyprogesterone and 4log for norethindrone. According to the results of this study, high Ct values or the application of advanced oxidation (using H_2O_2 and/or UV) are necessary to obtain sufficient removal of recalcitrant hormones or production of less harmful and more biodegradable by-products. Incomplete removal of recalcitrant steroids in this study are in accordance with the previous observation on partial removal of other recalcitrant compounds such as anti-inflammatory drug ibuprofen (9.6 $M^{-1}s^{-1}$) and anti-anxiety agent diazepam (0.75 $M^{-1}s^{-1}$) [145].

CHAPTER 8 CONCLUSION AND RECOMMENDATIONS

The results of this doctoral research bring us to the following conclusions:

- Our literature review on the occurrence of steroid hormones in different water matrices revealed that few studies performed the analysis of particulate fraction of these compounds. Since steroids are present in water matrices at low concentrations (maximum of hundreds ng L-1), specific analytical techniques are required for detection and quantification of these compounds in aqueous and solid matrices at low concentrations. Online SPE coupled with LC/MS-MS and Laser Diode Thermal desorption tandem MS-MS (LDTD/MS-MS) were used to quantify steroid hormones. Using these advanced methods allowed us to evaluate the concentrations of steroids in dissolved, particulate, and sediment part of each sample from different points along the river and from water treatment plants.

- Our results show the overwhelming importance of particulate associated fraction of steroids in water treatment plants and river.

- The particulate contents of treated wastewater were clearly higher compared to raw sewage and sediment particles. Therefore, advanced WWT processed capable of removing dissolved and particulate steroids are required for effective removal of particles and consequently particle associated steroids released to surface water.

- The presence of the steroids studied varied depending on the type of particle considered. While testosterone, progesterone, and norethindrone were frequently detected in river suspended particles, Estradiol (E2) and 17α- ethinylestradiol (EE2) were systematically detected in all water samples, indicating their extensive presence in studied river water.

- In bed sediments, E2, EE2 and progesterone were the only detected steroids with the highest levels observed during autumn.

- No specific trend was found in steroid profiles downstream to upstream of the river neither in water nor in sediments most likely due to the highly variable organic content and quantity of suspended particles in water samples and also strong effect of river surface and sediment type on the measured quantity of steroids in bed sediments.

- Steroids were detected in suspended particles of DWP intakes; they were removed by particle removing processes and found in drinking water sludge. The presence of these compounds in sludge raises potential risks in the case of sludge bed reactors and should be considered when considering best environmental practices for their disposal.

- The extent of sorption varied between sediment samples with different organic content. In sample with higher organic carbon content steroids have higher molecular interactions with organic matter and greater sorption. The order of interactions with sediments was nearly similar for steroids in all sediments with the highest sorption for progesterone while testosterone and E2 showed the lowest interactions with sediments. Sorption coefficients (K_d, K_{oc}) for selected compounds were similar in three sediments and the Log K_{OC} was almost constant between steroids and different samples, reflecting their similar structure and physicochemical properties.

- Ozone has been used to remove micropollutants during water treatment. The effectiveness of ozone to oxidize recalcitrant steroid hormones was investigated under drinking water production and WW treatment conditions. Ozone oxidation of testosterone was studied for the first time and confirmed the low reactivity of testosterone. The oxidation of steroids was found to be governed by the direct reaction with ozone and the contribution of radicals was insignificant under the testing conditions used. Activation energies of selected progestogens and testosterone were estimated. The oxidation constants were successfully predicted in natural filtered water and treated WW. Using estimated kinetic constants, it is possible to adjust water treatment processes to ensure sufficient hormone removal from source waters.

Some limitations of the work and further perspectives of the work have been identified, in the form of recommendations to further advance our understanding of the fate of steroid hormones during their long trip from the raw sewage to drinking water intakes. As mentioned in results of the Chapter 4, no hormone were detected in dissolved phase of samples taken from the river water. Compared to other studies on the occurrence of dissolved steroids in surface waters, the quick analytical method used had relatively high detection limits of steroid (3-52 ng L^{-1}). This precluded us from detecting these compounds in dissolved phase of river water but facilitated the treatment of large number of particulate samples. Whereas, according to their considerable total levels in water suspended particles (180-720 ng L^{-1}), relatively high amounts of steroids are

expected to be detected in dissolved phase of river. Some challenges remain and further investigation on the quantity of steroids in dissolved phase of river water should be considered:

- An improved new analytical method with detection limits of below 1 ng L^{-1} for steroids in dissolved phases is under development with our colleagues in the analytical chemistry team. The application of this new method is recommended to analyse samples from the river studied in this research project along with new samples from bed sediment to re-evaluate the presence of steroids in dissolved phase and if any was found, to complete the steroids profile along the river.

- Due to the highly variable organic content and quantity of suspended particles in water samples, no specific trend was found in steroid profiles downstream to upstream of the river neither in water nor in sediments. Our preliminary works revealed the higher presence of steroids in fraction of sediments less than 80 µm compare to the fraction bigger than 120 µm. It is recommended to investigate the partitioning of steroids between different particle size fractions and different types (silt, loam, clay) of sediment.

- Considering that hormones are usually transformed during wastewater treatment and there is always chance of surviving the conventional treatment processes, sorption studies are highly recommended to be carried out on transformation product, in order to evaluate the fate and transport of these compounds.

- Attached hormones to the suspended particles in river water can settle as river bed sediment or can enter water treatment plants. Desorption may occur after high precipitation and end up in higher levels of hormones downstream of the river or in the treated wastewater. We recommend studying the desorption kinetics of steroid hormones from river sediment and sludge particles under different conditions.

- Our studies on ozonation of steroids with conventional doses of ozone (2 mg O_3 L^{-1}) confirmed that complete mineralization of selected compounds could not be reached. Therefore, further studies are recommended on the identification and quantification of potential oxidation by-products to better understand the efficiency of removal of refractory steroids to ozone oxidation.

- Considering the Ct value (0.32 mg.min.L^{-1} at 20°C for the filtered water used in this study) required to achieve typical disinfection goals (4 log virus removal and 2 log *Giardia*

removal), the results of this study suggest that steroids would be removed by less than 1 log under such scenarios. Therefore, high Ct values or the implementation of advanced oxidation (using H_2O_2 and/or UV) are necessary for obtaining sufficient recalcitrant hormone removal.

- To date, no regulations or guidance have been proposed for steroid hormones released in surface waters and their maximum allowable concentrations in drinking water. The results obtained in this study confirm the persistence of recalcitrant steroids after ozonation at typical dosages applied by industry. These compounds could be used as indicator compounds for assessing the efficacy of WRRFs and DWPs to reduce environmental risks and the possible health risks of human exposure to trace concentrations of steroid hormones.

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APENDICES

APPENDIX A. SUPPLEMENTARY INFORMATION, ARTICLE1: SEASONAL VARIATIONS OF STEROID HORMONES RELEASED BY WASTEWATER TREATMENT PLANTS TO RIVER WATER AND SEDIMENTS: DISTRIBUTION BETWEEN PARTICULATE AND DISSOLVED PHASES

Journal: (accepted with major revision received by 12/01/2018) Science of the Total Environment

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Number of pages: 6

Table A-1. 1. Applied treatment processes and water quality of the five DWPs involved in this study

DWP	Treatment process	TOC (mg C L^{-1})	TSS (mg L^{-1})	Turbidity (NTU)	Particles >0.3 µm (mg L^{-1})
DWP1	ozonation/activated carbon and UV	7.6	22	28	24
DWP2	ozonation and activated carbon filtration	8.2	10	15	11
DWP3	chlorination/filtration and activated carbon	8	24	33	25
DWP4	ozonation and activated carbon filtration	7.9	16	36	25
DWP5	chlorination/filtration and ozonation	8.25	29	36	32

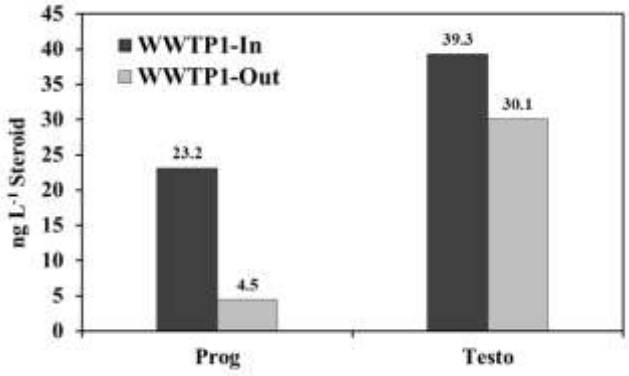
Table A-1. 2. Applied treatment processes, discharge flow and water quality of Inf. (influent) and Eff. (effluent) of the five WWTPs involved in this study

Treatment plant	Applied treatment	Eff. Flow (m^3 d^{-1})	DO (mg O_2 L^{-1})		DOC (mg C L^{-1})		TSS (mg L^{-1})		Turbidity (NTU)		Particles >0.3 µm (mg L^{-1})	
			Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.
WWTP1	Biofiltration UV	27447	6.6	5.4	19	9.7	104	9	51	8	97	9.8
WWTP2	Physico-Chemical UV	42692	3.6	6.5	16	8.2	192	12	56	6	79	11
WWTP3	Biofiltration UV	31153	6.7	3.9	11	12	148	12	44	14	110	15

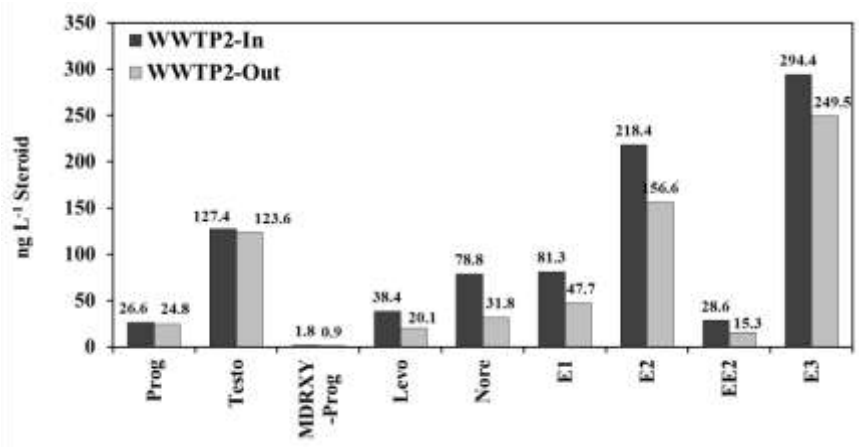
Table A-1. 3. Distribution of steroids between suspended particles and sediments of the river for the three steroids detected in sediments

Compound	C_p/C_s (L ng^{-1})	
	Spring	Summer
Prog	-	31.3
E2	-	16.3
EE2	0.31	10.5

a)



b)



c)

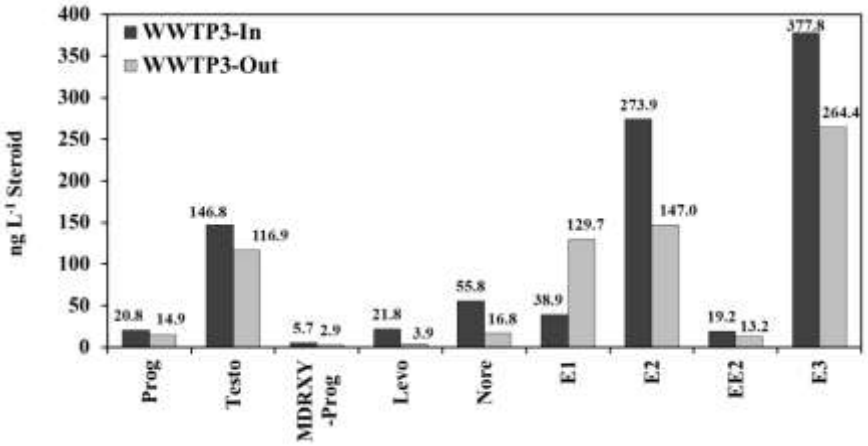


Figure A-1. 1.Steroid levels in dissolved phase of samples from influent/effluent of a) WWTP1, b) WWTP2, and c) WWTP3 in spring. Data labels show concentration of each steroid in influent and effluent.

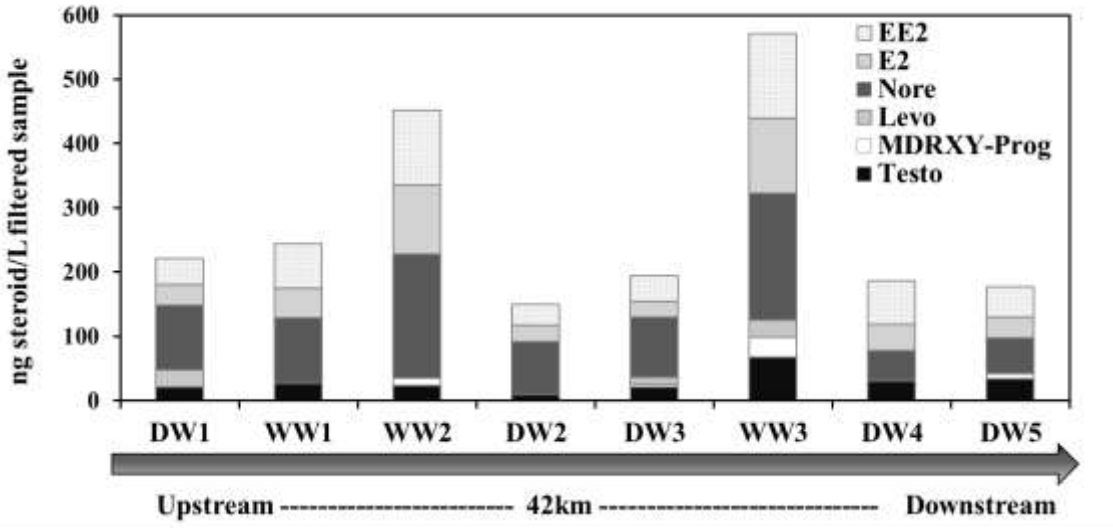


Figure A-1. 2. Steroid levels in particulate phase of effluent of WWTPs and DWP intakes in spring. River flow = 350 m^3 s^{-1}, T= 12 °C Points are in order of WWTP effluent or DW intake from upstream to downstream.

APPENDIX B. SUPPLEMENTARY INFORMATION, CHAPTER 5: ADSORPTION OF STEOIRDS ON RIVER SEDIMENTS

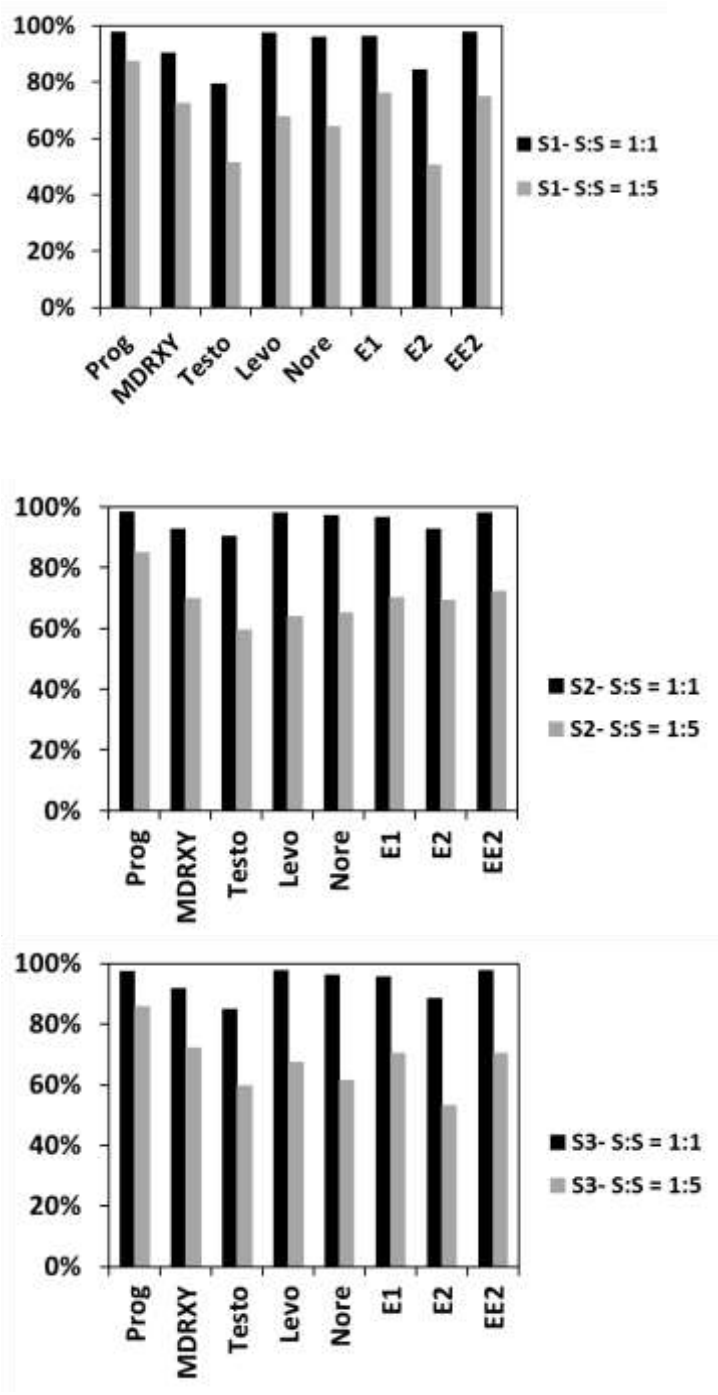


Figure A-2. 1. Comparison of amount of steroids sorbed on sediments at two different sediment/water (S:S) ratios.

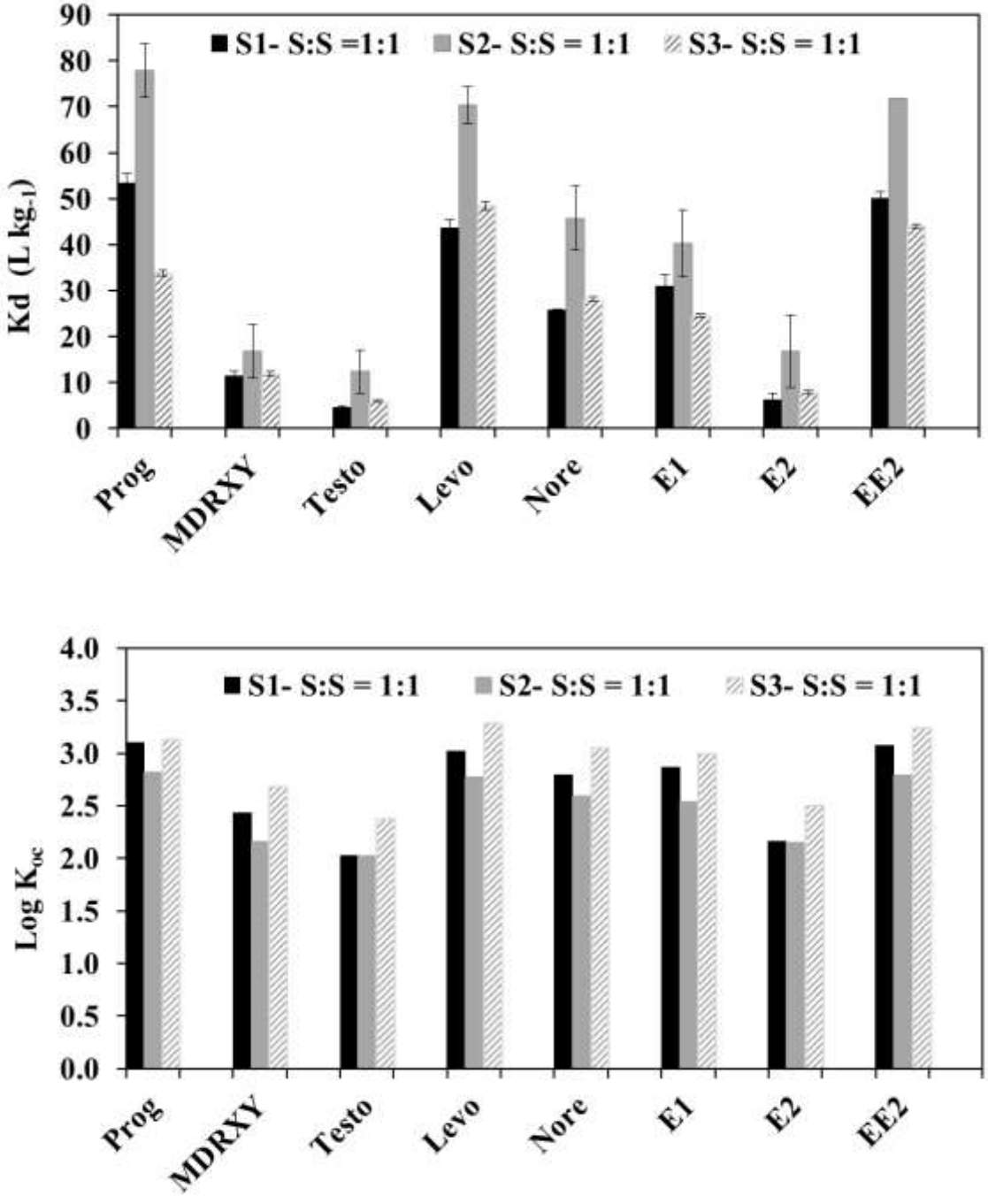


Figure A-2. 2.K_d and Log K_{OC} values for sediment/ water (S:S) ratio 1:1.

Table A-2 1. Kinetic parameters for adsorption of the steroids onto the sediment sample (S2), mass of sediment = 1 g; volume of solution = 5 mL, hormones initial concentration = 100 µg L^{-1}.

Kinetic model	Model equation	Compound	Equation	Rate constant	r^2
First-order kinetic models	$\frac{dq_t}{dt} = kq_t$	E1	y=0.0031x+5.4273	3.10E-03	0.51
		E2	y=0.0027x+5.3392	2.70E-03	0.22
		EE2	y=0.0023x+5.4031	2.30E-03	0.31
		Prog	y=0.0024x+5.4332	2.40E-03	0.34
		MDRXY	y=0.0017x+5.3356	1.70E-03	0.24
		Testo	y=5e-5x+5.3674	5.00E-05	7.0E-05
		Levo	y=0.0007x+5.3918	7.00E-04	0.009
		Nore	y=0.001x+5.389	1.00E-03	0.04
Pseudo-second-order kinetic model (a)	$\frac{dq_t}{dt} = k(q_e - q_t)^2$	E1	y=0.0038x+0.0023	6.28E-03	0.99
		E2	y=0.0044x+0.001	1.94E-02	0.99
		EE2	y=0.0041x+0.0009	1.87E-02	0.99
		Prog	y=0.0044x+0.0027	7.17E-03	0.99
		MDRXY	y=0.0045x+0.0036	5.63E-03	0.99
		Testo	y=0.0046x+0.003	7.05E-03	0.99
		Levo	y=0.0044x+0.0036	5.38E-03	0.99
		Nore	y=0.0043x+0.0029	6.38E-03	0.99

Table A-2 2.Kinetic parameters for adsorption of the steroids onto the sediment sample (S3), mass of sediment = 1 g; volume of solution = 5 mL, hormones initial concentration = 100 µg L^{-1}.

Kinetic model	Model equation	Compound	Equation	Rate constant	r^2
First-order kinetic models	$\frac{dq_t}{dt} = kq_t$	E1	y=0.0063x+5.7565	6.30E-03	0.32
		E2	y=0.0069x+4.9568	6.90E-03	0.11
		EE2	y=0.0062x+5.5802	6.20E-03	0.26
		Prog	y=0.0086x+5.8964	8.60E-03	0.94
		MDRXY	y=0.0036x+5.7957	3.60E-03	0.38
		Testo	y=0.0027x+5.4186	2.70E-03	0.23
		Levo	y=0.003x+5.7282	3.00E-03	0.37
		Nore	y=0.0043x+5.7514	4.30E-03	0.44
Pseudo-second-order kinetic model (a)	$\frac{dq_t}{dt} = k(q_e - q_t)^2$	E1	y=0.0023x+0.0009	5.88E-03	0.99
		E2	y=0.0045x+0.004	5.06E-03	0.99
		EE2	y=0.0027x+0.002	3.65E-03	0.99
		Prog	y=0.0033x+0.0011	9.90E-03	0.99
		MDRXY	y=0.0026x+0.0009	7.51E-03	0.99
		Testo	y=0.0037x+0.0049	2.79E-03	0.99
		Levo	y=0.0028x+0.0002	3.92E-02	0.99
		Nore	y=0.0026x+0.0007	9.66E-03	0.99

APPENDIX C. SUPPLEMENTARY INFORMATION, ARTICLE2: IMPACT OF TEMPERATURE ON OXIDATION KINETICS OF TESTOSTERONE AND PROGESTOGENS BY OZONE

Journal: Water Research

Title: IMPACT OF TEMPERATURE ON OXIDATION KINETICS OF TESTOSTERONE AND PROGESTOGENS BY OZONE

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Number of pages: 3

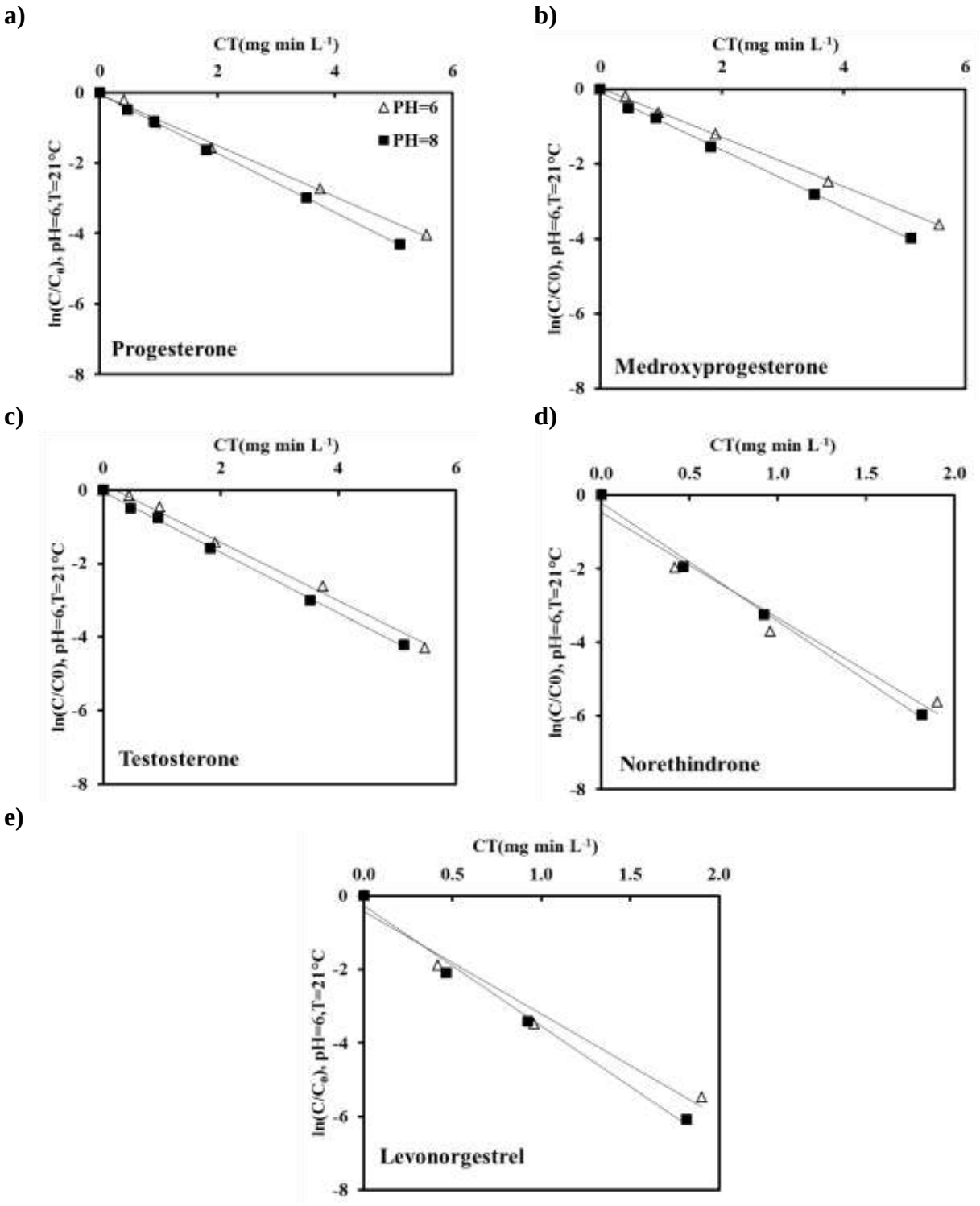

Figure A-3. 1. Oxidation of testosterone and progestogens at pH 6 and 8 at 21 °C with 2 mg L^{-1} ozone in ultrapure water.

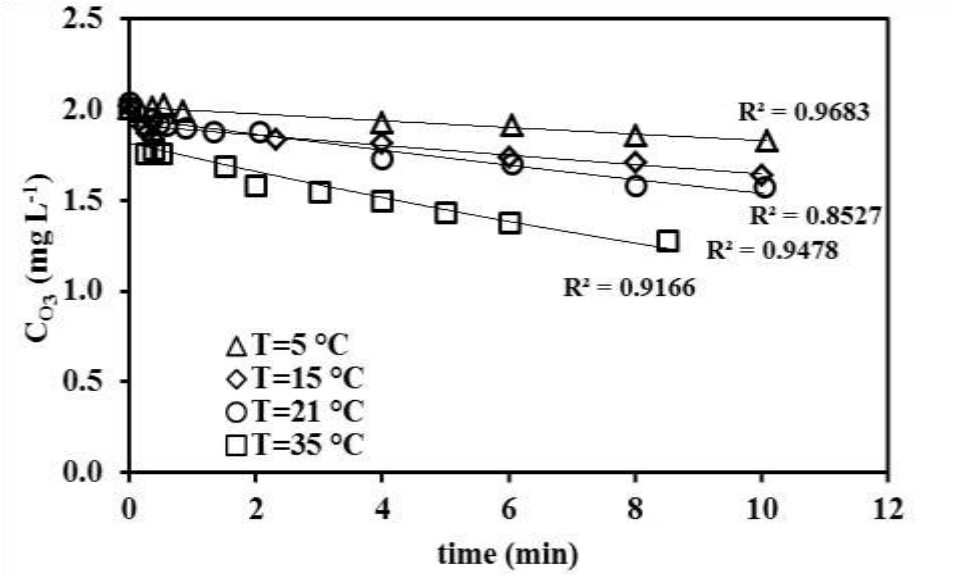

Figure A-3. 2. Ozone decay in ultrapure water at pH 6 and different temperatures, applied O_3 dose = 2 mg L^{-1}.

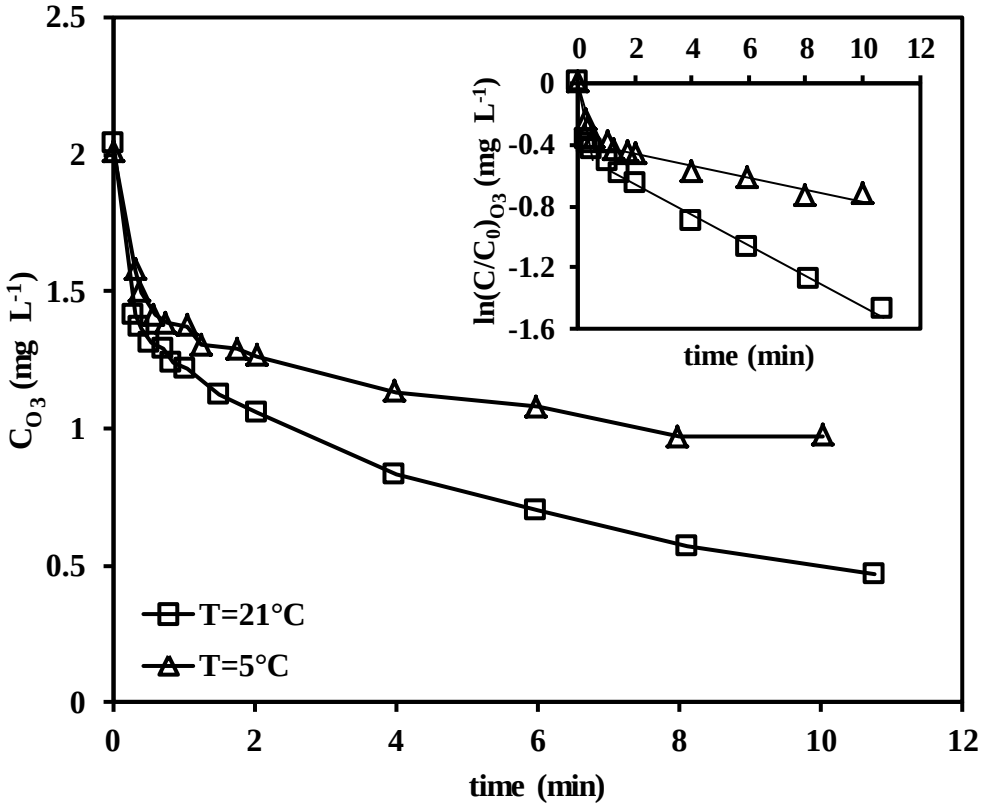

Figure A-3. 3.Ozone decay in natural filtered water as a function of time at 21°C (□) and 5°C (Δ). Inset: first-order kinetic plots for the ozone decomposition indicating two phase depletion reaction. Applied O_3 dose= 2 mg L^{-1}.

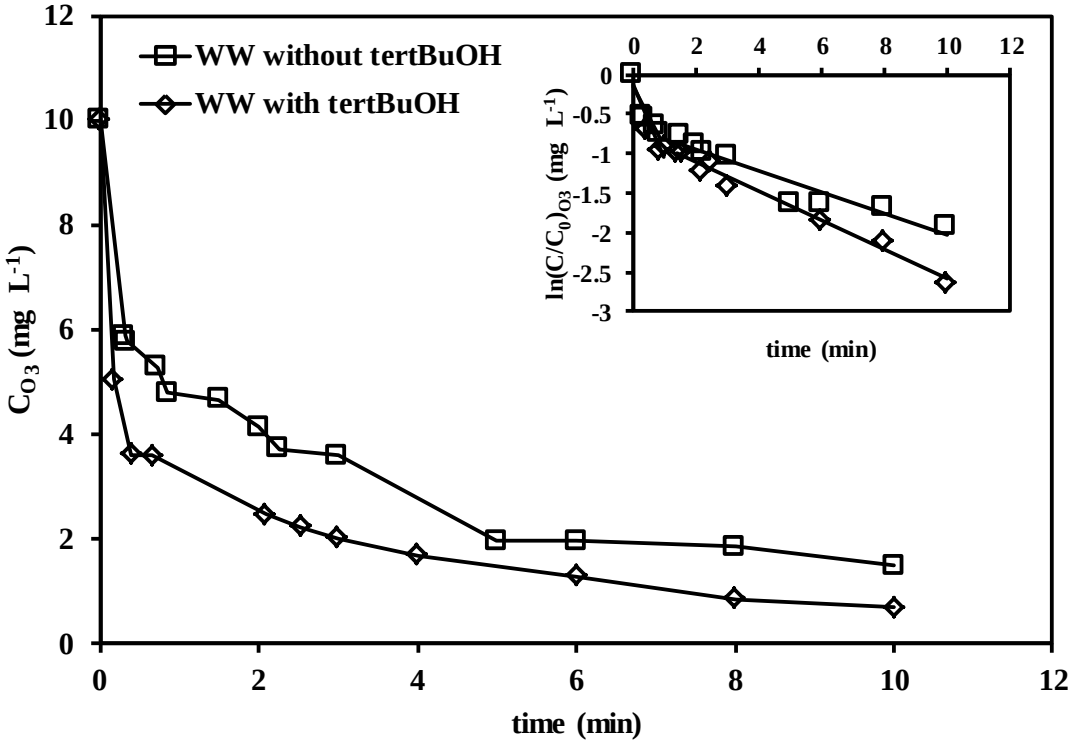

Figure A-3. 4. Ozone decay in the diluted WW effluent at 21 °C with/without a radical scavenger; Inset: first-order kinetic plots for the ozone decomposition indicating two phase depletion reaction. Applied O_3 dose= 10 mg L^{-1}.

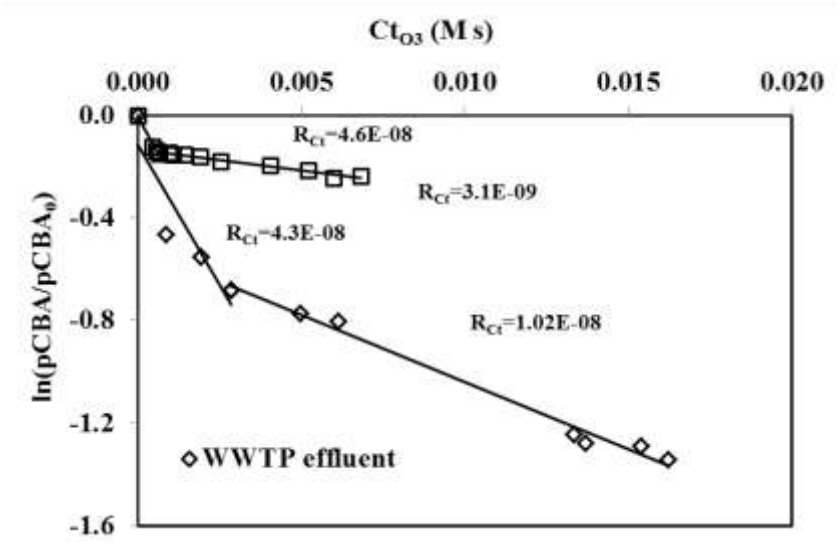

Figure A-3.5. R_{Ct} plots for the two phases of oxidation reaction in natural filtered water and diluted WWTP effluent at T= 21 °C over the 10 min reaction time. Ozone dose= 2 mg L^{-1} for natural filtered water and 10 mg L^{-1} for diluted WWTP effluent. Concentration of ρCBA= 200 µg L^{-1}.