

**Development of robust analytical methods  
for the quantification of priority endocrine disruptors  
in water by LC-MS / MS**

**Sébastien Sauvé, Ken Goeury, Michèle Prévost  
Université de Montréal**

## Context

Large quantities of EDCs are potentially released in urban areas, linked to the human populations, and consumption of contraceptive pills, steroids, natural excretion, etc.

These EDCs are often released in the environment because of insufficient retention by wastewater treatment plants

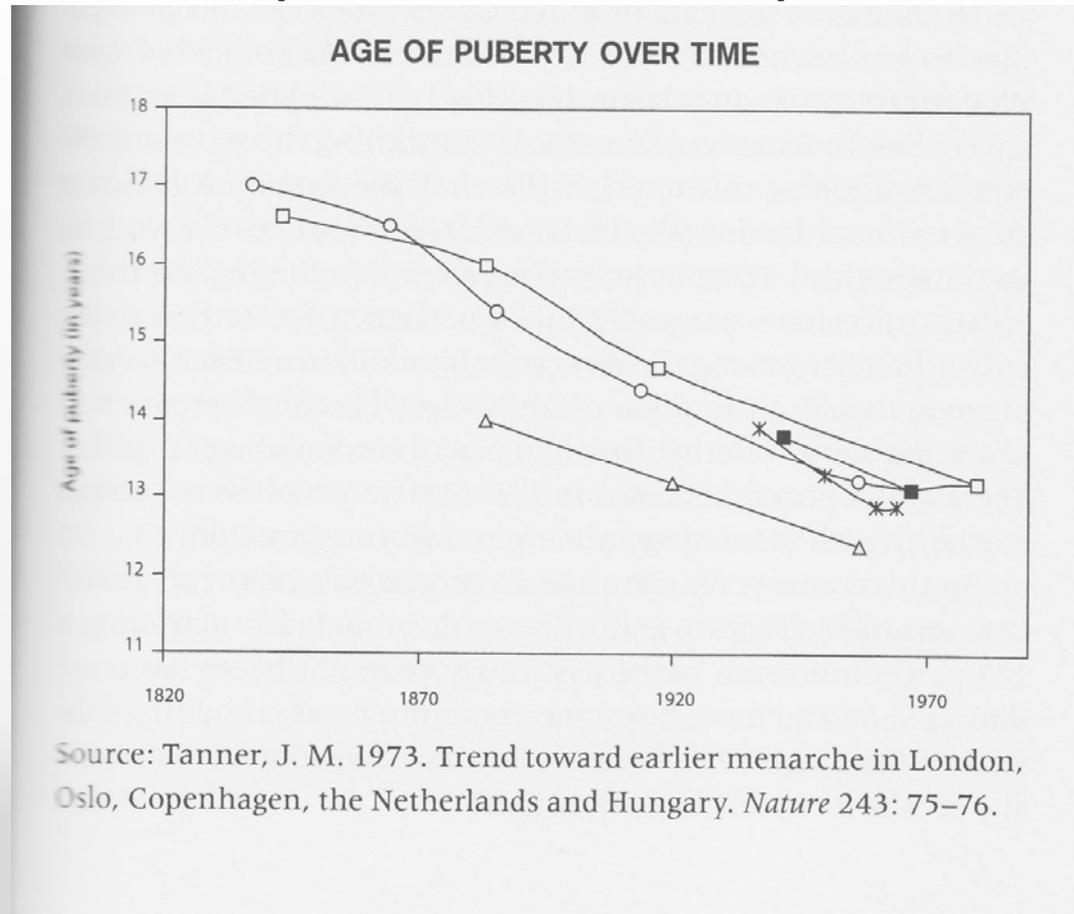
Impacted surface water concentrations are typically in the  $\text{ng L}^{-1}$  range, sufficient to elicit adverse effects on wildlife

- Behavioral impacts on fish (e.g., increased aggressiveness)
- Hormonal disorders and fertility decrease
- Effects at the population level (e.g., feminization)



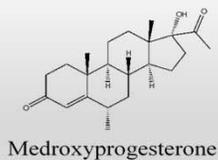
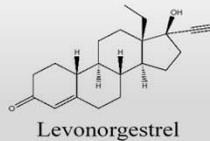
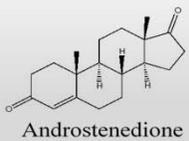
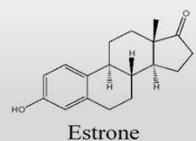
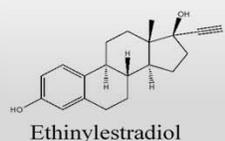
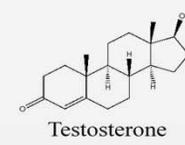
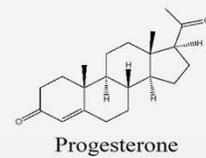
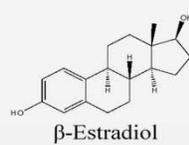
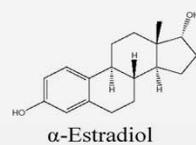
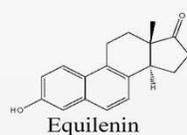
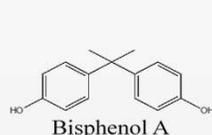
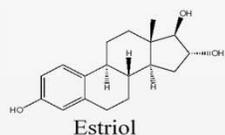
[http://www.dumez-auvergne.fr/web/genie\\_civil\\_sanitaire.html](http://www.dumez-auvergne.fr/web/genie_civil_sanitaire.html)

# Endocrine Disruptors and Early Puberty



## Knowledge gaps

- ❑ EPA method 539 was recently expanded to include additional hormones and bisphenol A
- ❑ A fast and robust method able to capture the expanded list of priority EDCs and compliant with method 539.1 requirements would be desirable
- ❑ Performance criteria (accuracy, precision) may be easier to attain in drinking water, but a versatile method should also be functional for more complex matrixes (e.g., wastewater)



*List of EPA-priority EDCs*

## Purpose of work

- ❑ Develop fast, automatic, sensitive, and robust hormone detection methods
- ❑ Meet the EU (0.035 ng/L) and BC (0.5 ng/L) guidelines for ethinylestradiol
- ❑ Project builds upon reference work of EPA method 539.1

*Determination of Twelve Hormones and Bisphenol A in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry*

- ❑ Two approaches discussed in this presentation:
  1. On-line SPE – UHPLC-MS/MS
  2. Automated off-line SPE – UHPLC-MS/MS





Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)



Analysis of Environmental Protection Agency priority endocrine disruptor hormones and bisphenol A in tap, surface and wastewater by online concentration liquid chromatography tandem mass spectrometry



Ken Goeury<sup>a,b</sup>, Sung Vo Duy<sup>a</sup>, Gabriel Munoz<sup>a</sup>, Michèle Prévost<sup>b</sup>, Sébastien Sauvé<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, Université de Montréal, Montreal, QC, Canada

<sup>b</sup> Department of Civil, Geological and Mining Engineering, École Polytechnique de Montréal, Montreal, QC, Canada

## First approach (online SPE)

➤ Analytical column: **Hypersil Gold C18**

100 x 2.1mm (particle size: 1.9 μm)

➤ Column temperature : 50 ° C

➤ Online SPE columns (x2 in series): **Hypersil Gold C18 aQ**  
each 20 x 2.1mm (particle size: 12 μm)

➤ Source : HESI (Polarity-switching ionization)

➤ Mobile phases :

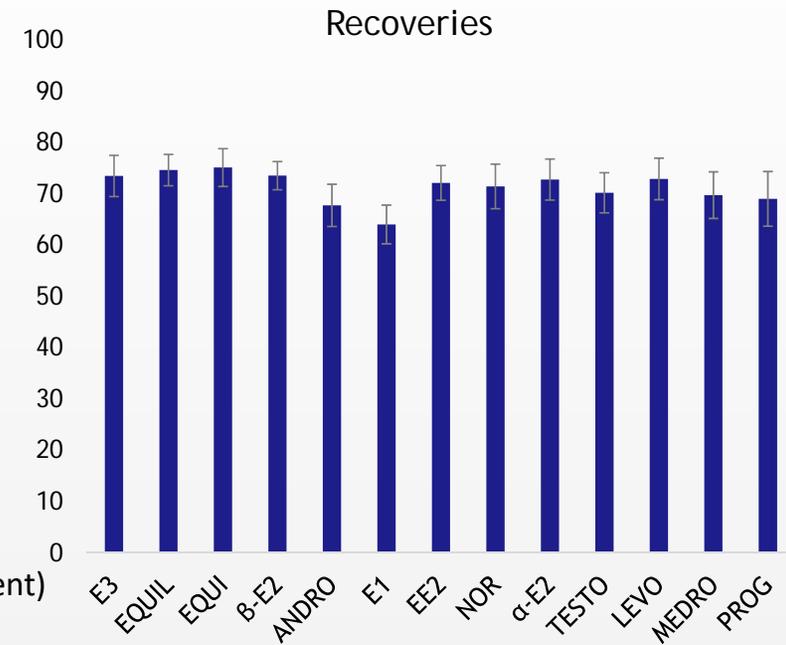
➤ analytical pump: H<sub>2</sub>O / MeOH / **NH<sub>4</sub>F (1 mM)**

➤ SPE pump : H<sub>2</sub>O; 0.1% formic acid / MeOH

➤ **Thermo TSQ Quantiva QqQ**

## Method validation (online SPE)

Parameters	HPLC water	Tap water	Surface water	Wastewater (Influent)	Wastewater (Effluent)
R <sup>2</sup>	0.9987 – 1.000	0.9982 – 0.9999	0.9930 – 0.9999	0.9990 – 0.9997	0.9906 – 0.9992
LOD (ng/L)	0.050 – 1.0	0.10 – 0.70	0.40 – 2.5	1.0 – 5.0	0.50 – 4.0
LOQ (ng/L)	0.15 – 3.0	0.30 – 2.1	1.2 – 7.5	3.0 – 15	1.5 – 12



➤ Recoveries between 64% and 75%

Detection limits ranging from 0.050 ng / L (HPLC water) and 5.0 ng / L (wastewater, influent)

Limits of quantification ranging from 0.15 ng / L (HPLC water) and 15 ng / L (wastewater, influent)

Linearity ranging from LOQ to 100 ng / L (HPLC water, tap water, surface water) and up to 200 ng / L (wastewater)

Coefficients of determination (R<sup>2</sup>) superior to 0.999 for most analyte/matrix combinations

## Method validation (online SPE)

Parameters	HPLC water		Tap water		Surface water		Wastewater (Influent)		Wastewater (Effluent)	
	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>
<b>Intraday precision (%)</b>	1.9 – 12.4	0.6 – 11.6	1.0 – 4.3	0.3 – 3.0	1.3 – 11.3	0.4 – 9.3	0.1 – 4.5	0.6 – 2.2	1.0 – 28.7	1.2 – 7.5
<b>Interday precision (%)</b>	1.3 – 18.3	0.5 – 19.3	1.5 – 19.6	1.5 – 16.1	0.7 – 16.3	0.7 – 9.3	0.1 – 8.7	0.3 – 4.2	0.2 – 22.7	0.1 – 14.6
<b>Accuracy (%)</b>	83.3 – 99.9	86.8 – 99.5	80.4 – 98.9	88.6 – 99.7	75.5 – 94.4	86.2 – 98.5	83.8 – 99.7	89.7 – 99.2	74.3 – 97.2	71.7 – 95.7

Intraday precision: variability contained below 30% (QC1) and below 20% (QC2) for all matrixes

Interday precision: variability contained below 30% (QC1) and below 20% (QC2) for all matrixes

Accuracy: minimum value above 75% (QC1 and QC2) for all matrixes

**Accuracy range compliant with EPA method acceptance criteria (70-130%)**



## Matrix effects (online SPE)

	Matrix effect			
	Tap water	Surface water	Wastewater- Influent	Wastewater- Effluent
<b>E3</b>	8.0 ± 3.9	8.0 ± 7.1	-34 ± 3.3	13 ± 3.9
<b>BPA</b>	-12 ± 2.7	-1.3 ± 2.5	5.9 ± 2.0	20 ± 5.8
<b>EQUIL</b>	0.51 ± 1.3	-10 ± 3.1	-57 ± 2.4	13 ± 3.8
<b>EQUI</b>	8.1 ± 3.4	20 ± 1.6	-20 ± 4.0	39 ± 1.6
<b>ANDRO</b>	-1.3 ± 1.2	-3.4 ± 0.90	2.1 ± 0.80	-2.1 ± 0.30
<b>β-E2</b>	-3.0 ± 1.2	-3.2 ± 3.2	-3.8 ± 1.4	-2.0 ± 0.50
<b>E1</b>	2.7 ± 2.7	8.5 ± 2.0	-8.1 ± 3.0	-4.0 ± 2.4
<b>EE2</b>	-5.5 ± 1.2	-4.5 ± 5.0	1.6 ± 1.0	13 ± 7.9
<b>NOR</b>	-10 ± 2.8	-20 ± 6.2	-20 ± 2.0	-20 ± 3.8
<b>α-E2</b>	6.2 ± 5.3	-0.90 ± 1.5	2.8 ± 2.3	-5.0 ± 3.1
<b>TESTO</b>	-6.8 ± 3.3	-24 ± 1.8	-31 ± 24	-15 ± 4.5
<b>LEVO</b>	-24 ± 8.6	-23 ± 1.7	-40 ± 2.0	-24 ± 5.5
<b>MEDRO</b>	-2.0 ± 3.6	4.2 ± 4.7	24 ± 1.2	-6.0 ± 11
<b>PROG</b>	-3.5 ± 1.4	-8.2 ± 2.2	6.3 ± 1.1	3.3 ± 0.81

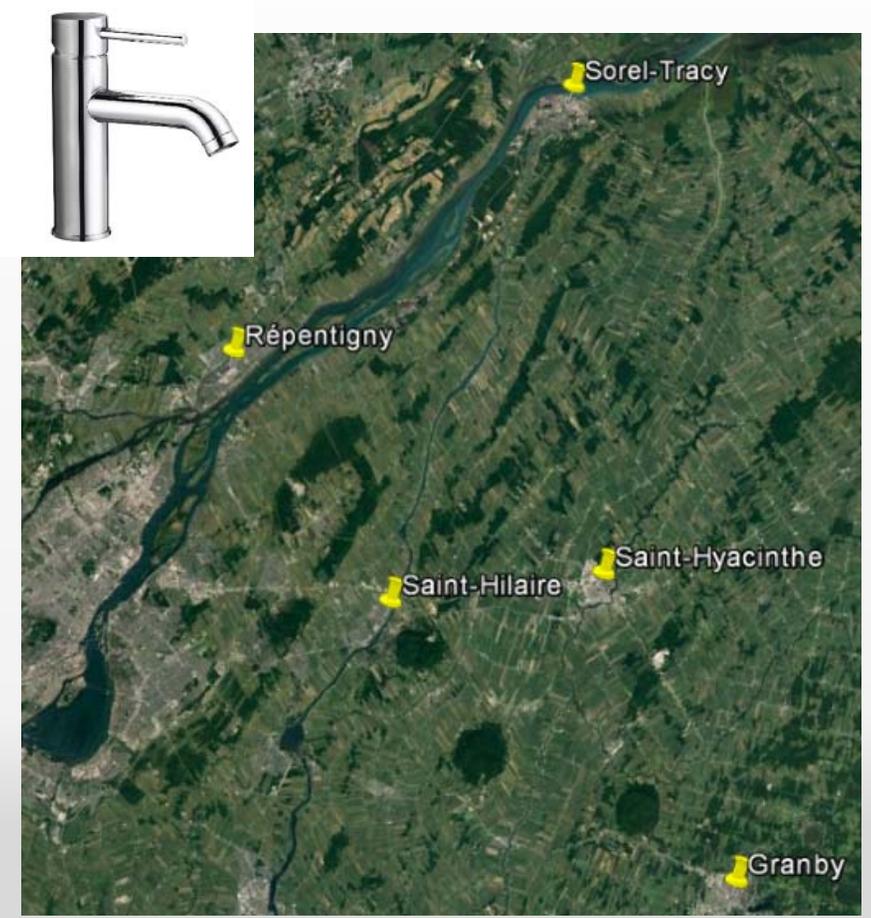
- Matrix effects below 30% for most studied compounds
- Matrix effects well compensated using matrix-matched calibration curves

## Comparison of first approach (online SPE) with other methods from literature

Literature	Studied compounds	Matrixes	Detection	Analytique column	SPE cartridge	Sample volume	Running time	Recoveries (%)	LOD (ng/L)
Vega – Morales et al. (2010)	BPA, E1, E2, E3, EE2	Sewage sample	ESI (+-)/MS	C18	/	250 mL	29	60 -108	0.5 - 6
Fayad et al. (2013)	E1, E2, E3, EE2, LEVO, MEDRO, PROG, NORE	Affluent, Effluent	APCI (+)/MS	C18	C18	10 mL	15	64 - 101	5 - 90
Ciofi et al. (2013)	E1, $\alpha$ -E2, $\beta$ -E2, E3, EE2	Milli-Q water (validation), surface water, wastewater	ESI (-)/MS	C18	Strata C 18E	2.5 mL	10	71 - 95	0.15 - 0.95
Guedes-Alonso et al. (2015)	E1, E2, E3, LEVO, PROG, TESTO	Effluent	ESI (+-)/MS	C18	Oasis HLB	2.0 mL	15	43 - 95	0.5 - 13.2
Xue Lin Goh et al. (2016)	E1, E2, E3, EE2, EQUI	Affluent, Effluent	ESI (-)/MS	C18	HyperSep	5.0 mL	17	67 - 103	0.16 - 2.14
Čelić et al. (2017)	E1, E2, E3, EE2	Milli-Q water (validation), surface water, wastewater	ESI (-)/MS	C18	Hypersil Gold aQ	5.0 mL	10	76 - 106	0.030 - 0.26
<b>This study</b>	E1, E2, E3, EE2, EQUI, EQUIL, ANDRO, LEVO, NOR, TESTO, MEDRO, PROG, NORE, ALTRE	Milli-Q water (validation), tap water, surface water, wastewater affluent and effluent	ESI (+-)/MS	C18	C18	10 mL	15.5	64 - 75	0.05 - 5.00

# Online SPE method sensitive enough to detect EDCs in tap water

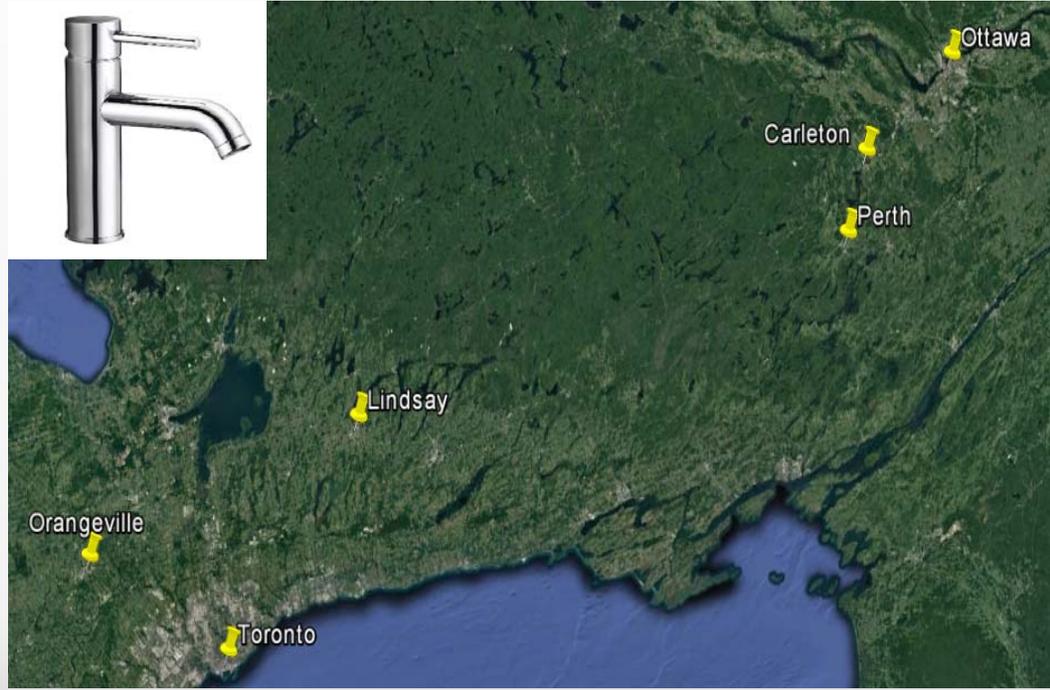
Québec					
Compounds	Repentigny	Saint-Hilaire	Sainte-Hyacinthe	Sorel	Granby
<b>E3</b>	0.78* ± 0.42	1.2 ± 0.37	0.54* ± 0.29	ND	ND
<b>EQUIL</b>	ND	ND	ND	ND	ND
<b>EQUI</b>	ND	ND	ND	ND	ND
<b>ANDRO</b>	ND	ND	ND	ND	ND
<b>β-E2</b>	ND	ND	ND	ND	1.1* ± 0.12
<b>E1</b>	ND	ND	ND	ND	ND
<b>EE2</b>	ND	ND	ND	ND	ND
<b>NOR</b>	0.59* ± 0.05	0.35* ± 0.01	ND	0.43* ± 0.04	0.87* ± 0.11
<b>α-E2</b>	ND	ND	ND	ND	ND
<b>TESTO</b>	ND	ND	ND	ND	ND
<b>LEVO</b>	ND	ND	ND	ND	ND
<b>MEDRO</b>	ND	ND	ND	ND	ND
<b>PROG</b>	ND	ND	0.76*	ND	ND



- Tap water samples from the province of Quebec
- Relatively low occurrence for the studied sites
- Contamination levels
  - Individual maximum level : 1.2 ng/L (E3)
  - Cumulative maximum level : 2.0 ng/L (Granby)

# Online SPE method sensitive enough to detect EDCs in tap water

Ontario						
Compounds	Carleton	Lindsay	Orangeville	Ottawa	Perth	Toronto
<b>E3</b>	ND	ND	ND	ND	ND	ND
<b>EQUIL</b>	ND	ND	ND	ND	ND	ND
<b>EQUI</b>	ND	ND	ND	ND	ND	ND
<b>β-E2</b>	ND	ND	ND	2.8	ND	ND
<b>ANDRO</b>	1.0 ± 0.02	0.81 ± 0.07	ND	ND	ND	ND
<b>E1</b>	ND	ND	ND	ND	ND	ND
<b>EE2</b>	ND	ND	ND	ND	ND	ND
<b>NOR</b>	ND	0.87* ± 0.20	ND	ND	ND	ND
<b>α-E2</b>	ND	ND	ND	ND	ND	ND
<b>TESTO</b>	0.37* ± 0.02	ND	ND	0.37* ± 0.25	ND	2.1
<b>LEVO</b>	ND	ND	ND	ND	ND	ND
<b>MEDRO</b>	ND	ND	ND	ND	ND	ND
<b>PROG</b>	ND	ND	ND	ND	ND	ND

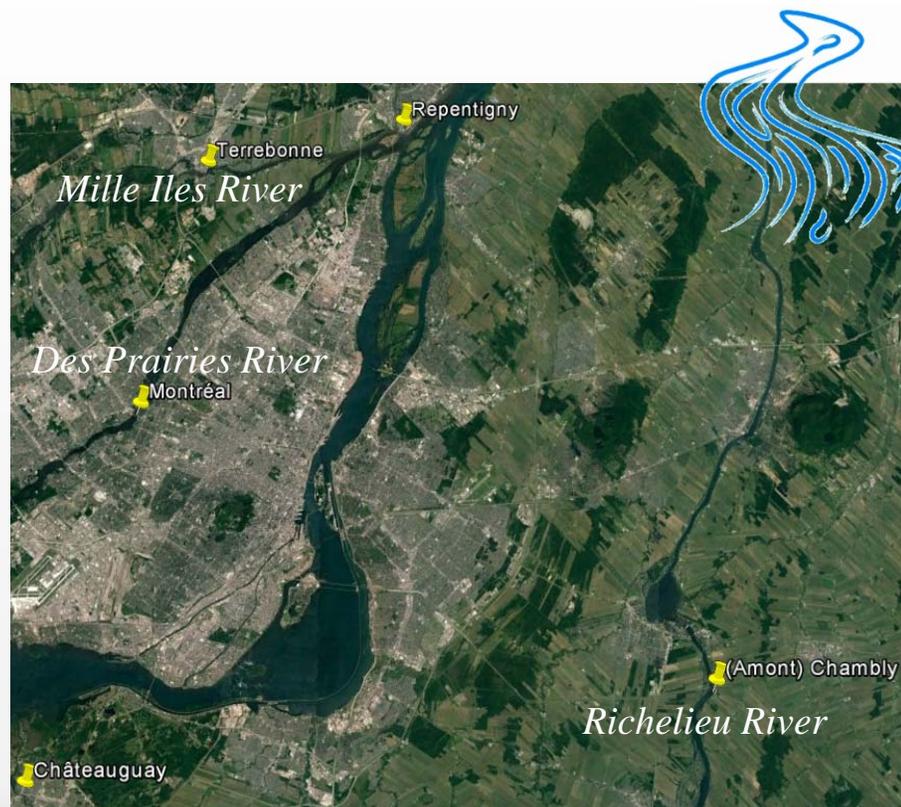


- Tap water sampling in the province of Ontario
- Low occurrence of EDCs regardless of sampling site location
- Contamination levels
  - Individual and cumulative maximal concentration : 2.8 ng/L (β-E2)

## Method application to rivers

Compounds	Surface water				
	Chateauguay	Upstream Chambly	Repentigny	Terrebonne	Montréal
E3	ND	ND	ND	ND	ND
EQUIL	ND	ND	ND	ND	ND
EQUI	ND	ND	ND	ND	ND
ANDRO	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
β-E2	ND	ND	ND	ND	1.7 ± 0.01
E1	< LOQ	0.50 ± 0.37	< LOQ	< LOQ	< LOQ
EE2	ND	ND	ND	ND	< LOQ
NOR	1.7 ± 0.05	2.7 ± 0.17	2.1 ± 0.2	2.1 ± 0.03	2.3 ± 0.05
α-E2	ND	< LOQ	ND	< LOQ	ND
TESTO	ND	ND	< LOQ	ND	ND
LEVO	ND	ND	ND	ND	ND
MEDRO	ND	ND	0.40	ND	ND
PROG	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

- Surface water sampling in the Greater Montreal area
- Low occurrence except for norethindrone systematically detected across sites



- Contamination levels
  - Individual maximum level : 2.3 ng/L (β-E2)
  - Cumulative maximum level : 4.0 ng/L

## Method application to wastewater effluents and influents



Compounds	Wastewater (Effluent)					Wastewater (Influent)					
	Site A	Site B	Site C	Site D	Site E	Site A	Site B	Site C	Site D	Site E	Site F
<b>E3</b>	110 ± 1.6	95 ± 8.7	75 ± 11.3	ND	240 ± 21.9	ND	ND	ND	92 ± 9.0	ND	ND
<b>EQUIL</b>	4.7 ± 0.5	8.6 ± 0.6	6.4 ± 0.2	ND	7.0 ± 0.7	ND	ND	ND	ND	ND	ND
<b>EQUI</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.6 ± 0.1
<b>ANDRO</b>	ND	ND	ND	ND	ND	74 ± 5.2	ND	21 ± 2.4	ND	10 ± 3.4	8.3 ± 1.8
<b>β-E2</b>	120 ± 4.5	45 ± 0.3	43 ± 0.2	31 ± 0.7	94 ± 4.3	ND	ND	ND	15 ± 1.3	ND	6.3 ± 1.7
<b>E1</b>	14 ± 0.1	15 ± 0.1	14 ± 0.3	44 ± 0.5	33 ± 1.5	ND	ND	ND	22 ± 2.8	ND	ND
<b>EE2</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>NOR</b>	ND	ND	25 ± 0.2	ND	132 ± 2.2	ND	ND	ND	ND	ND	ND
<b>α-E2</b>	< LOQ	ND	ND	ND	ND	ND	ND	ND	< LOQ	ND	ND
<b>TESTO</b>	42 ± 4.6	41 ± 3.6	23 ± 0.8	ND	59 ± 2.5	13 ± 0.3	< LOQ	ND	ND	ND	14 ± 0.5
<b>LEVO</b>	ND	ND	ND	ND	ND	150 ± 42.7	73 ± 3.7	300 ± 17.0	ND	27 ± 1.8	26 ± 1.7
<b>MEDRO</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>PROG</b>	7.3 ± 0.3	8.6 ± 0.2	14 ± 0.7	< LOQ	13 ± 1.4	ND	< LOQ	< LOQ	ND	ND	ND

- Wastewater sampling in the Greater Montreal area
- 11/13 EDCs detected at least once
- Contamination levels
  - Individual maximum level : 300 ng/L (LEVO; influent) and 240 ng/L (E3; effluent)
  - Cumulative maximum level : 578 ng/L (site E; effluent) and 320 ng/L (site C; influent)

## Method settings using second approach (automated off-line SPE)

➤ During field collection

➤ NaCl (116 mg/L)

➤ Omadine Salt (70 mg/L)

➤ Sample clean-up

Off-line SPE using Si-1 cartridges (Phenomenex)



Dionex AutoTrace 280 (Automated SPE)

- Dry concentration (N<sub>2</sub> ; 40°C)
- 250 µL H<sub>2</sub>O; 40% MeOH
- IS:
  - Androstenedione-<sup>13</sup>C<sub>3</sub>
  - Estradiol-<sup>13</sup>C<sub>6</sub>
  - Ethinylestradiol-<sup>13</sup>C<sub>2</sub>
  - Progesterone-D<sub>9</sub>
  - Mestranol-D<sub>4</sub>



Matrixes	HPLC water	Tap water	Surface water	Wastewater (Influent)	Wastewater (Effluent)
Sample volume (mL)	1000	1000	500	250	250
Steps	Solvents		Solvents		
Cartridge washing	MeOH		MTBE		
Conditioning	H <sub>2</sub> O		MeOH		
Sample cleaning	H <sub>2</sub> O		H <sub>2</sub> O		
Cartridge rinsing	H <sub>2</sub> O; 2% AA		H <sub>2</sub> O/MeOH (9:1), 2% NH <sub>4</sub> OH		
Elution	H <sub>2</sub> O		H <sub>2</sub> O		
	MeOH				

Tandem with HRPHS SolEx cartridges (Dionex)

Elution : DCM/Acetone (7:3)

## Method validation in 5 water matrices (automated off-line SPE)

Parameters	HPLC water		Tap water		Surface water		Wastewater (Affluent)		Wastewater (Effluent)	
LOD	0.10 – 1.0		0.030 – 0.5		0.10 – 0.5		0.50 – 1.0		0.16 – 1.0	
LOQ	0.30 – 3.0		0.090 – 1.5		0.30 – 1.5		0.50 – 3.0		0.48 – 3.0	
Range	LOQ – 100 ng/L		LOQ – 100 ng/L		LOQ – 200 ng/L		LOQ – 200 ng/L		LOQ – 200 ng/L	
Level	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>	QC <sub>1</sub>	QC <sub>2</sub>
Intraday precision (%)	1.0 – 9.3	1.5 – 6.2	0.3 – 3.8	0.4 – 2.5	0.6 – 5.8	0.5 – 7.9	1.2 – 14.9	0.2 – 5.9	0.6 – 8.5	0.1 – 10.3
Interday precision (%)	2.0 – 24.2	1.2 – 14.6	0.5 – 13.3	1.1 – 11.8	2.9 – 21.0	0.4 – 10.4	0.9 – 29.7	1.6 – 29.9	0.6 – 16.1	0.8 – 15.3
Accuracy (%)	84.2 – 98.2	92.7 – 100	78.8 – 99.5	90.7 – 99.8	75.3 – 99.1	87.5 – 99.9	61.4 – 97.2	76.7 – 99.0	73.1 – 99.0	86.3 – 98.6

- **Intraday precision** : values below 15 % for studied compounds (QC<sub>1</sub> et QC<sub>2</sub>)
- **Interday precision** : values below 30 % for studied compounds (QC<sub>1</sub> et QC<sub>2</sub>)
- **Accuracy** : Values superior to 70 % for the majority of analytes and matrixes



Contents lists available at ScienceDirect

## Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



### Seasonal variations of steroid hormones released by wastewater treatment plants to river water and sediments: Distribution between particulate and dissolved phases



Hadis Yarahmadi <sup>a,\*</sup>, Sung Vo Duy <sup>b</sup>, Mounia Hachad <sup>a</sup>, Sarah Dorner <sup>a,d</sup>, Sébastien Sauvé <sup>b</sup>, Michèle Prévost <sup>a,c</sup>

<sup>a</sup> Civil, Geological and Mining Engineering Department, Polytechnique de Montréal, CP 6079, succ. Centre-Ville, Montréal, QC H3C 3A7, Canada

<sup>b</sup> Department of Chemistry, Université de Montréal, P.O. Box 6128, succ. Centre-Ville, Montréal, QC H3C 3J7, Canada

<sup>c</sup> NSERC Industrial Chair on Drinking Water, Civil, Geological and Mining Engineering Department, Polytechnique de Montréal, QC, Canada

<sup>d</sup> Canada Research Chair in Microbial Contaminant Dynamics in Source Waters, Civil, Geological and Mining Engineering Department, Polytechnique de Montréal, QC, Canada

# Dissolved vs. Particulate

**Table 3**

Mean concentrations ( $\text{ng g}^{-1}$ ) 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 limit.

Compound	Spring (n = 12)			Summer (n = 12)			LOD	
	Diss.	Part.	% positive	Diss.	Part.	% positive	Diss. ( $\text{ng L}^{-1}$ )	Part. ( $\text{ng g}^{-1}$ )
E2	<LOD	1000 ± 741	100	<LOD	646 ± 457	100	51	44
EE2	<LOD	3258 ± 1338	100	<LOD	460 ± 181	100	5	23
Prog	<LOD	<LOD	0	<LOD	316 ± 211	83	5	33
Testo	<LOD	503 ± 212	100	<LOD	310 ± 112	25	3	27
Nore	<LOD	736 ± 439	100	<LOD	<LOD	0	23	34

# Data on Particulate Phase

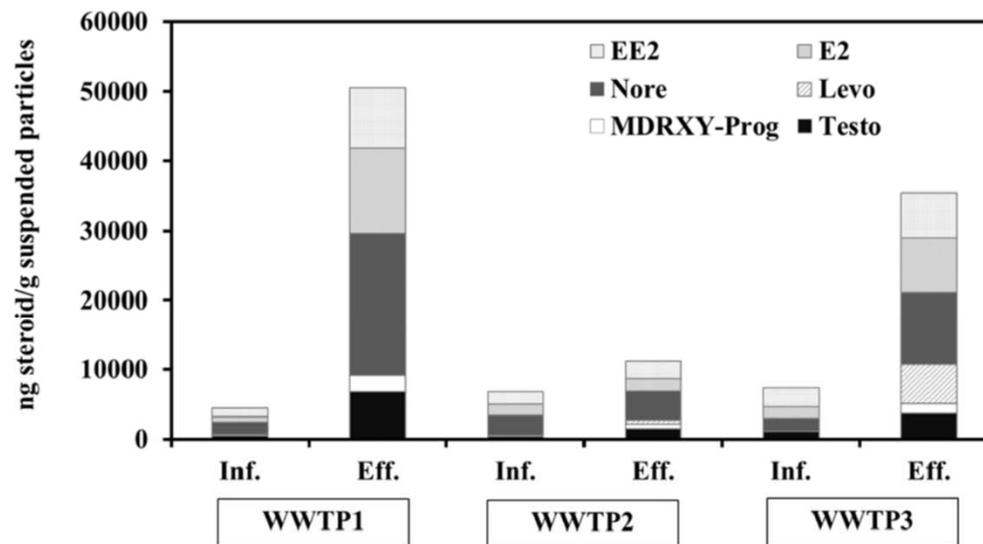


Fig. 2. Steroids detected in particulate phase per unit gram of suspended particles (>0.3  $\mu\text{m}$ ) of raw sewage (Inf.) and WWTP effluent (Eff.).

# Seasonal Variations

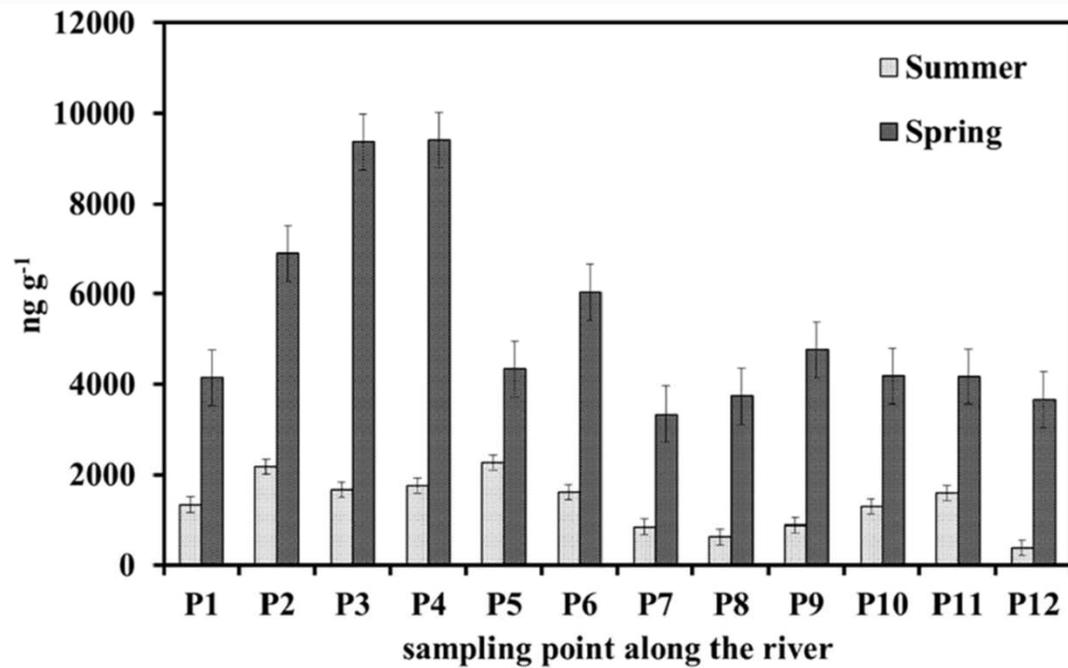
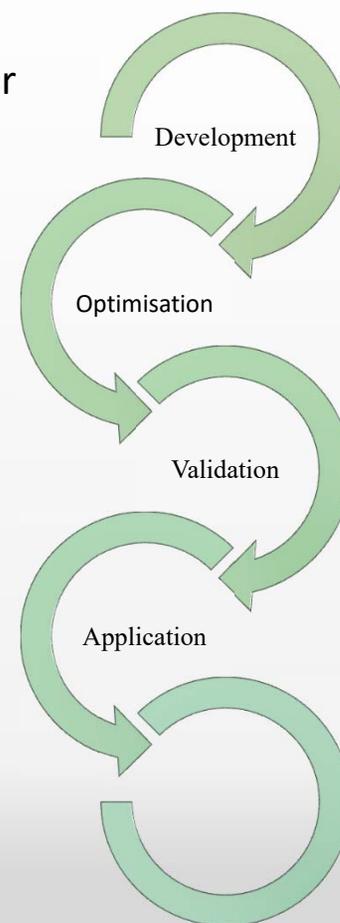


Fig. 4. Seasonal variation in total steroids levels ( $\text{ng g}^{-1}$ ) in suspended particles from water samples taken along the river. Error bars represent the standard errors from duplicate samples and duplicate analysis.

Yaramadhi et al 2018, Environmental Pollution

## Conclusions

- Optimisation, development and validation of efficient analytic method for detection and quantification of hormones at trace levels
- Method applicability in several matrixes :
  - HPLC water
  - Tap water
  - Surface water
  - Wastewater
  - Suspended particulates must be considered
- SPE automation, using either online or off-line (Autotrace) methods
- High sensitivity methods : Sub-ng/L and ng/L
- Fast analysis time



## Acknowledgments



[sebastien.sauve@umontreal.ca](mailto:sebastien.sauve@umontreal.ca)

