CSCE Annual Conference

Growing with youth – Croître avec les jeunes

Laval (Greater Montreal)

June 12 - 15, 2019

OCCURRENCE OF IBUPROFEN AND 2-HYDROXY IBUPROFEN IN SAINT LAWRENCE RIVER

Pourya Zojaji[1,4], Hassan Alhachami[1], Esmaeel Kariminezhad[1], Shameem Jauffur [2], Zeinab Bakhshi[2], Marc A. Vaudreuil[3], Sebastien Sauve[3], and Maria Elektorowicz[1,4]

[1] Department of Building, Civil and Environmental Engineering, Concordia University, Montreal, Quebec, Canada

[2] Envirogenique, 780 Vezina St apt 36, Montréal, Québec, Canada

[3] Department of Chemistry, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec, Canada

[4] p_zojaji@encs.concordia.ca, mariae@encs.concordia.ca

Abstract: The widespread occurrence of pharmaceuticals at the minute concentrations in surface waters has revealed emerging anthropogenic effects on the aquatic environment. In this work, to determine the exposure of the Saint Lawrence River to the Ibuprofen (analgesic and anti-inflammatory pharmaceutical product) and its respective 2-hydroxylated metabolite, a municipal wastewater treatment plant (WWTP) and its vicinity were monitored in three locations. Composite samples were taken on an hourly basis, filtered and homogenized *in-situ*. Automated solid-phase extraction coupled to liquid chromatography tandem mass spectrometry (on-line SPE LC-MS/MS) system was utilized to measure the concentration of selected compounds. The results revealed both compounds were present at the 100% frequency in the Saint Lawrence River. Ibuprofen (IBU) and 2-hydroxy ibuprofen (2-OH IBU) were measured at the concentrations ranging 3.64- 4.40 ng/l and 330- 713 ng/l, respectively. Interestingly, the metabolite compound was present at significant higher magnitudes than the parent compound in all sampling sites alongside the river. Concentrations of 2-hydroxy ibuprofen were more remarkable in the downstream of the WWTP outfall where the metabolite level was measured 160-fold higher than the IBU. These findings indicated beside the analgesic's active compounds special attention needs to be paid to the occurrence of their stable metabolites in the Saint Lawrence River where there is a quite lack of information on their presence. Such a conclusion is important considering this riverine ecosystem serves as a food and water source for the millions of people.

1 INTRODUCTION

In Canada, analgesics are one of the most dispensed classes of pharmaceuticals (IQVIA 2017). Ibuprofen (IBU) is among the most widely used analgesics since it is sold by prescription and non-prescription.

Pharmacokinetic studies revealed IBU undergoes metabolic transformation by phase I and phase II metabolizing steps into more readily excretable compounds (Rainsford 2009). Phase I involves oxidative modification via the insertion of functional groups such as the hydroxyl, carboxyl, amines, etc. (Silva et al., 2015). In the phase II biotransformation the IBU as well as its phase I metabolites undergo conjugation

with glucuronic acid (Silva et al., 2015). Afterwards, the mixture of 25% unchanged IBU and the polar metabolites are excreted from the human body (Saunders et al., 2016). It was shown the two compounds; 2-hydroxyibuprofen (2-OH IBU) and carboxy-ibuprofen (CBX-IBU) comprise main ibuprofen metabolites in the human excretion (Larsson et al., 2014; Weigel et al., 2004).

After excretion, the unchanged parent and all the metabolite compounds end up in the sewage and wastewater treatment plant (WWTP). Two major IBU metabolites can also be formed during biodegradation of IBU by microorganisms (Boix et al., 2016; Zwiene et al., 2002). However, CBX-IBU is fairly unstable under biological operations (Ferrando-Climent et al., 2012). As a result due to their ineffective removal in conventional WWTPs, IBU and 2-OH IBU are continuously discharged with the effluent to the recipient surface waters.

Previous works showed that in the river exposed to the municipal effluent, IBU was detected in the fish's bile samples at the concentrations of a few ng/l (Brozinski et al., 2012). Exposition of Mediterranean male mussels on ibuprofen at an environmentally relevant concentration (250 ng/l) confirmed the endocrine disruption effect of this drug after two weeks (Gonzalez-Rey and Bebianno 2012). IBU at the concentration of 0.2 μg/l caused a slight cyto-genotoxicity on zebra mussel hemocytes after 96 h while the higher concentrations (2 μg/l and 8 μg/l) of IBU were able to significantly increase both genetic and cellular damage (Parolini et al., 2011). In a recent study, IBU at the concentration of 60 μg/l also induced oxidative stress in tench (*Tinca tinca*) after its 35-day exposure at the early life stage (Stancova et al., 2017).

Saint Lawrence River (SLR), with a watershed area of over 1 million km^2 is beneficial to inhabitants in its watershed since it serves as a source of food and water, power production, navigation, effluent disposal and recreational activities. Between Lake Ontario and Quebec City (550 km), effluents from variety of treatment plants serving approximately 5 million inhabitants living along the river shores are discharged into the SLR (Marcogliese et al., 2015). The impact of anthropogenic activities on the SLR aquatic ecosystem has been already revealed in the literature. For example; feminization of mussels as result of municipal effluent discharge was reported at the sites adjacent to the SLR (Gagné, et al., 2011). (Blaise et al.2002) confirmed the SLR bivalve immune response was significantly declined downstream a municipal effluent plume compared to the upstream sites.

Considering the above facts, the aim of present study was to investigate the occurrence of the well-known analgesic medicine, IBU, and its 2-hydroxylated metabolite in the SLR in the vicinity of a municipal effluent discharge. The main physicochemical characteristics of IBU and 2-OH IBU is shown in table 1.

Table 1: Target compounds name, their octanol-water partition coefficient, and disassociation constant

Compounds name	Abbreviation	Chemical formula	log K_{ow} *	pK_a ^	Solubility ($\frac{mg}{l}$)
Ibuprofen	IBU	$C_{13}H_{18}O_2$	3.97	4.9	21
2-hydroxy ibuprofen	2-OH IBU	$C_{13}H_{18}O_3$	2.41	4.63	-

* Octanol-water partition coefficient
^ Dissociation constant

2 METHODOLOGY

2.1 Description of the Study Area

The local WWTP with a conventional activated sludge (CAS) treatment, having an average hydraulic retention time (HRT) of 24 hours, serves 240,000 equivalent inhabitants. The WWTP discharges its effluent at the mean flow rate of 60,000 m^3/day to the SLR. The composite sampling of the effluent was conducted over 3-day period.

Surface water sampling stations were strategically chosen based on their ease of access and position with respect to the WWTP outfall. Two sampling sites in the downstream of the effluent outfall were located within the water basin with a surface area of approximately 53 km^2 and depth of less than 4 m. The basin exhibits semi-lacustrine ecology where the flow velocity decreases to 0.3 m/s from 3 m/s (in upstream area). Two drinking water uptake stations as well as a recreational activity centre are located in this area. Upstream rapids have the length and width of 6.8 km and 1 km, respectively. The third sampling point was selected in the upstream of the rapids. The location of each sampling station in the river was demonstrated in Figure 1. It is noteworthy; there were no other point sources of pollution nor entering tributaries between the sampling sites and the effluent outfall.

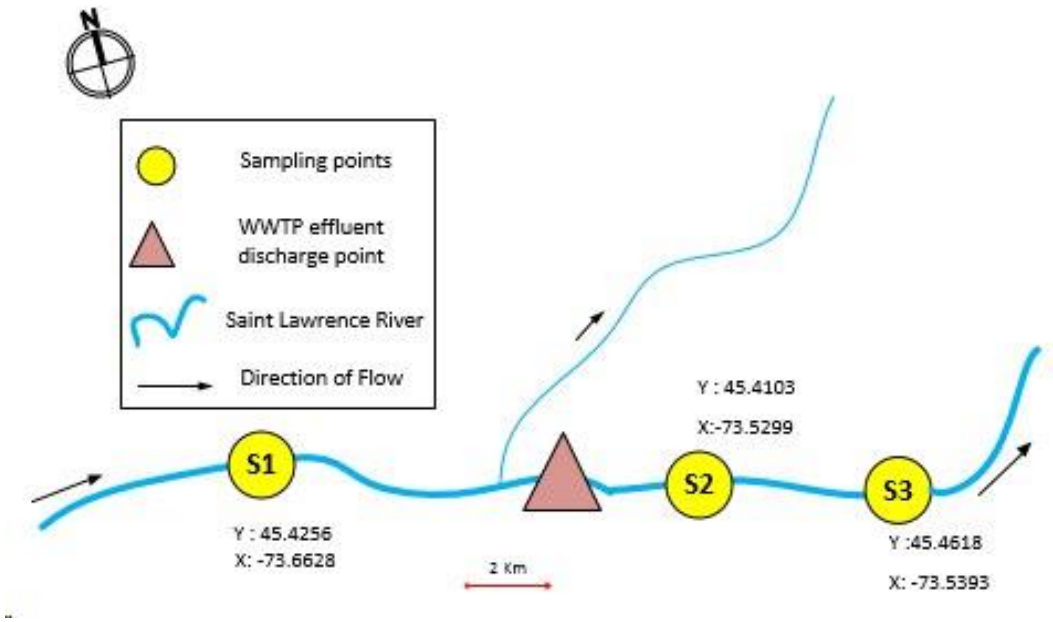

Figure 1: Locations of the sampling points in the SLR (X and Y: geographical coordinates)

2.2 Sample Collection and Preservation

The sampling campaign was undertaken in the September 2018, on the days without precipitation. Composite river water samples were collected based on hourly intervals. Each composite sample was comprised of 12 grab samples filtered and homogenized in-situ. To this end, the DWK life science grab sampler was deployed inside the water in desired distance localized by GPS from the shoreline. At the specific depth of water, the rubber stopper has raised from the bottle permitting on water sample collection. Subsequently, a 50 ml aliquot of each grab sample was filtered *in situ* by Chromafil syringe filter (glass fibre/polyester, 1/0.45 µm, 25 mm). The composite filtrate was stored in an amber glass bottle at the temperature 4℃. Blank samples (Milli-Q water) were also used to monitor the likelihood of artifacts introduction during sample collection. Blank samples mimicked the sampling and pretreatment procedure.

Moreover, 24-h time proportional composite effluent sample was collected over 3 consecutive days at the WWTP. ISCO refrigerated autosampler was used to generate these samples, while the same sampling pretreatment techniques were applied to all samples.

2.3 On-line SPE LC-MS/MS

The selected compounds were analyzed by automated solid-phase extraction coupled to liquid chromatography tandem mass spectrometry (on-line SPE LC-MS/MS). In this method the extraction, purification, and detection steps are integrated into one instrument. The preconcentration was performed using the EQuan (Thermo Fisher Scientific, Waltham, Massachusetts) system. This system is based on column switching between the SPE and chromatographic columns. The ionization of the selected

compounds was achieved by electrospray ionization (ESI) interface. The triple quadrupole mass spectrometer was operated in selected reaction monitoring (SRM) mode for the detection of transitions at their respective m/z ratios. Quantification for both compounds was performed using a standard addition calibration with linear regression and isotopically-labelled internal standards for river water and effluent samples. The analytical method (under publication) demonstrated high trueness (recovery), sensitivity, and precession for IBU and 2-OH IBU in both matrices.

2.4 Mass Loading of Target Compounds in the Municipal Wastewater

To obtain the mass loads of IBU and 2-OH IBU within the treated wastewater, the concentration of target compounds on each day of sampling was multiplied to the mean flow rate of the WWTP effluent. Then the mass loads were normalized by the population equivalent served by the WWTP using equation 1 (Paíga et al., 2016)

[Equation 1] Normalized mass loads (mg/day/1000 inhabitants)=($\frac{\text{mass loads (mg per day)}}{\text{population served by WWTP}}$ ×1000)

3 RESULT and DISCUSSION

3.1 IBU and 2-OH IBU in the Effluent Discharge

The target compounds were present at the 100% frequency in composite samples collected in the effluent discharge. Generally speaking, the IBU exhibits relatively high removal efficiency in the conventional WWTP. The removal efficiency of more than 90% was reported previously in the literature (Clara et al., 2005; Ferrando-Climent et al., 2012). Nonetheless, the presence of IBU in the municipal effluent discharge at the minute concentrations was reported worldwide (Archer et al., 2017; Guerra et al., 2014; Larsson et al., 2014). In this study, IBU was measured in the WWTP effluent at the concentrations ranging from 15.4 ng/l to 22.7 ng/l.

Simultaneously, significant higher concentrations of 2-OH IBU than IBU were detected in the effluent outfall since its magnitude was ranging between 512 ng/l and 1362 ng/l. On the last day of sampling, the concentration of 2-OH IBU was quantified up to 60-fold higher than the parent compound. These results are in agreement with the previous studies which reported higher concentrations of 2-OH IBU in the municipal outfall (Guerra et al., 2014; Larsson et al., 2014). This might be attributed to the higher levels of 2-OH IBU in the WWTP inflow (Larsson et al., 2014) and its lower removal efficiency (Gagnon et al., 2012). More importantly, in the case of IBU, transformation products similar to human metabolite compounds were observed in the biological operation which could enhance the probability of 2-OH IBU presence in the effluent discharge (Ebele, et al., 2017).

The permanent presence of IBU and its metabolite in the municipal wastewater causes a continuous discharge of xenobiotic mass to the SLR. The normalized mass loads of IBU and 2-hydroxy IBU in the effluent discharge each day of sampling were shown in table 1.

Table 2: Normalized Mass loads of IBU and 2-OH IBU in the municipal effluent discharge

Compound	Day 1st (mg/day/1000 inhabitants)	Day 2nd (mg/day/1000 inhabitants)	Day 3rd (mg/day/1000 inhabitants)
IBU	4.2	5	6.1
2-OH IBU	139	262	369

3.2 IBU and 2-OH IBU in the SLR

Both IBU and 2-OH IBU were detected in the three sampling stations alongside the SLR; their concentrations are shown in the table 3.

Table 3: Concentrations of IBU and 2-OH IBU in the St Lawrence River

Compound	Upstream (Site 1) Concentration ($\frac{ng}{l}$)	Downstream (Site 2) Concentration ($\frac{ng}{l}$)	Downstream (Site 3) Concentration ($\frac{ng}{l}$)
IBU	3.64	4.40	4.27
2-OH IBU	330	710	713

IBU and its respective metabolite were measured at higher levels (over 4 ng/l) in the downstream of the municipal discharge where the concentration of 2-OH IBU (over 700 ng/l) showed more than twice increase in relation to the upstream site. This may confirm the contribution of municipal discharge on the release of analgesics to the aquatic environment.

So far data about the occurrence of IBU metabolites in the Canadian aquatic environment is rather scarce. To the best of author's knowledge, this is the first time the presence of 2OH-IBU was reported in the SLR. Interestingly, in all sampling sites 2-OH IBU was present at remarkable higher levels than the parent compound. In the vicinity of the municipal effluent discharge 2OH-IBU was detected at the concentrations of *circa* 160 times more than IBU. The significant higher concentration of 2OH-IBU could be justified when the ratio of this compound to its respective parent in the WWTP outfall is remarkable. Furthermore, 2OH-IBU was reported as a transformation product from the IBU biodegradation in recipient surface waters (Boix et al., 2016). Significant concentrations of metabolite compounds in the surface water were previously reported in the Europe. (López-Serna et al. 2012) reported the overall significant contribution of metabolites of target compounds representing as an average 30–50% of the total pharmaceutical load (parent compounds+ metabolites) into the surface water. Also, the concentrations of IBU metabolites were measured in the Ter River in Spain, where 2OH-IBU was present at the level of 4 times higher than IBU.

4 CONCLUSION

In this study to investigate the presence of IBU and its 2-hydroxylated metabolite in the Saint Lawrence River (SLR), composite water samples were collected from three sites in highly urbanized area. The sampling locations were chosen in the vicinity of a wastewater treatment plant outflow. The results revealed the IBU and 2-OH IBU were measured at the 100% frequency in the river. Significant higher concentrations of the metabolite (over 700 ng/l) than the parent compound (4 ng/l) were present in the SLR. 2-OH IBU magnitude was 160-fold higher than IBU in the farthest downstream sampling point. Both compounds were present at the similar frequency in the samples collected from municipal effluent discharge itself. However, the IBU and 2-OH IBU were measured at the concentrations approximately 4 and 2 times higher than SLR, respectively. The IBU and 2-OH IBU levels were increased in the downstream of the WWTP outfall suggesting the effluent contribution in releasing the analgesics to the aquatic systems. The results revealed future monitoring studies should also include pharmaceuticals metabolite and transformation products in order to gain better overlook of these xenobiotics presence in the SLR aquatic ecosystem. Such conclusion is important considering the fact that this riverine ecosystem serves as a source of food and water for the millions of people.

5 ACKNOWLEDGMENT

The authors gratefully thank Environment and Climate Change Canada (ECCC) for the funding of this research (EDF-PQ-2017G005).

6 REFERENCE

Archer, E., Petrie, B., Kasprzyk-Hordern, B., and Wolfaardt, G. M. 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere, **174***, 437-446.

Blaise, C., Trottier, S., Gagné, F., Lallement, C., and Hansen, P. 2002. Immunocompetence of bivalve hemocytes as evaluated by a miniaturized phagocytosis assay. *Environmental Toxicology: An International Journal,* ***17***(3), 160-169.

Boix, C., Ibáñez, M., Sancho, J. V., Parsons, J. R., de Voogt, P., and Hernández, F. 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: Identification and occurrence of transformation products. *Journal of Hazardous Materials,* ***302***, 175-187.

Brozinski, J., Lahti, M., Meierjohann, A., Oikari, A., and Kronberg, L. 2012. The anti-inflammatory drugs diclofenac, naproxen and ibuprofen are found in the bile of wild fish caught downstream of a wastewater treatment plant. *Environmental Science and Technology,* ***47***(1), 342-348.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., and Kroiss, H. 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research,* ***39***(19), 4797-4807.

Ebele,J. Mohamed, A., and Stuart, H. 2017. Pharmaceuticals and Personal Care Products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants* **3** (1): 1-16.

Ferrando-Climent, L., Collado, N., Buttiglieri, G., Gros, M., Rodriguez-Roda, I., Rodriguez-Mozaz, S., and Barceló, D. 2012. Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. *Science of the Total Environment,* ***438***, 404-413.

Gagné, F., Bouchard, B., André, C., Farcy, E., and Fournier, M. 2011. Evidence of feminization in wild elliptio complanata mussels in the receiving waters downstream of a municipal effluent outfall. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* ***153***(1), 99-106.

Gagnon, C., and Lajeunesse, A. (2012). Low removal of acidic and hydrophilic pharmaceutical products by various types of municipal wastewater treatment plants. *Journal of Xenobiotics,* ***2***(1), 3.

Gonzalez-Rey, Maria and Maria João Bebianno. 2012. "Does Non-Steroidal Anti-Inflammatory (NSAID) Ibuprofen Induce Antioxidant Stress and Endocrine Disruption in Mussel Mytilus Galloprovincialis?" Environmental Toxicology and Pharmacology **33** (2): 361-371.

Guerra, P., Kim, M., Shah, A., Alaee, M., and Smyth, S. 2014. Occurrence and fate of antibiotics, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. *Science of the Total Environment,* ***473***, 235-243.

Larsson, E., al-Hamimi, S., and Jönsson, J. Å. 2014. Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry. *Science of the Total Environment,* ***485***, 300-308.

López-Serna, R., Petrović, M., and Barceló, D. 2012. Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the ebro river basin (NE spain). *Science of the Total Environment,* ***440***, 280-289.

Marcogliese, D. J., Blaise, C., Cyr, D., De Lafontaine, Y., Fournier, M., Gagné, F., and Hudon, C. 2015. Effects of a major municipal effluent on the St. Lawrence River: A case study. *Ambio,* ***44***(4), 257-274.

Paíga, P., Santos, L. H., Ramos, S., Jorge, S., Silva, J. G., and Delerue-Matos, C. (2016). Presence of pharmaceuticals in the lis river (Portugal): sources, fate and seasonal variation. *Science of the Total Environment,* ***573***, 164-177.

Parolini, Marco, Andrea Binelli, and Alfredo Provini. 2011. "Chronic Effects Induced by Ibuprofen on the Freshwater Bivalve Dreissena Polymorpha." Ecotoxicology and Environmental Safety **74** (6): 1586-1594

Rainsford, KD. 2009. "Ibuprofen: Pharmacology, Efficacy and Safety." Inflammopharmacology **17** (6): 275-342.

Saunders, L. J., Mazumder, A., and Lowe, C. J. 2016. Pharmaceutical concentrations in screened municipal wastewaters in Victoria, British Columbia: A comparison with prescription rates and predicted concentrations. *Environmental Toxicology and Chemistry,* ***35***(4), 919-929.

Silva, B., Costa, F., Neves, I. C., and Tavares, T. 2015. *Psychiatric Pharmaceuticals as Emerging Contaminants in Wastewater.* Springer International Publishing.

Stancova, V., Plhalova, L., Blahova, J., Zivna, D., Bartoskova, M., Siroka, Z., and Svobodova, Z. 2017. Effects of the pharmaceutical contaminants ibuprofen, diclofenac, and carbamazepine alone, and in combination, on oxidative stress parameters in early life stages of tench (tinca tinca). *Veterinarni Medicina,* ***62***(2), 90-97

Weigel, S., Berger, U., Jensen, E., Kallenborn, R., Thoresen, H., and Hühnerfuss, H. 2004. Determination of selected pharmaceuticals and caffeine in sewage and seawater from tromsø/Norway with emphasis on ibuprofen and its metabolites. *Chemosphere,* ***56***(6), 583-592.

Zwiener, C., Seeger, S., Glauner, T., and Frimmel, F. 2002. Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments. *Analytical and Bioanalytical Chemistry,* ***372***(4), 569-575.