Pharmaceutical Pollution in Ontario

Identifying and Assessing the Toxicity of Pharmaceutical Compounds for Environmental Release Management

Master's Research Paper

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Abstract

Pharmaceuticals have contributed to lengthening and improving the quality of life and combatting deadly diseases for humans and animals. Their continuous use and discharge has however resulted in their release into the environment, and their emergence as pseudo-persistent contaminants of concern. The current study focuses on identifying the most commonly reported pharmacologically active compounds (PHACs) in Ontario's water bodies from past literature, and evaluating whether their concentrations pose a threat to aquatic ecosystems. Through a systematic literature review, 112 PHACs were identified from 26 papers published during the period of 2003 to 2024. The top ten most frequently reported compounds (evaluated by the percent of papers that report their presence in Ontario's waters) were found to be the anticonvulsant carbamazepine, the non-steroidal anti-inflammatory drugs (NSAIDs) naproxen and ibuprofen, the antibiotics trimethoprim and sulfamethoxazole, the lipid regulators gemfibrozil and clofibric acid, the stimulants caffeine and cotinine, and the antidepressant venlafaxine. This is similar to what is observed globally, and these compounds are commonly described as "indicator compounds" in past literature. The current study finds that at least 6 of these ten PHACs are estimated to cause moderate to high ecotoxicological risk, requiring some form of action from the provincial government.

Using risk quotients (RQs), the ten PHACs were grouped into three risk categories. Caffeine, ibuprofen, and carbamazepine were categorized as high risk (RQs > 1), and should be prioritized in future investigations and management plans. The government of Ontario should not only regularly monitor these PHACs in surface waters, but also implement environmental quality standards to reduce their presence in the aquatic environment. The moderate risk category (RQs between 0.1-1) included gemfibrozil, cotinine, and sulfamethoxazole. The main recommendation for these PHACs is to implement monitoring and research programs to further assess their potential aquatic hazard. Finally, the low risk category (RQs <0.1) combined venlafaxine, naproxen, trimethoprim, and clofibric acid. This category is relatively low priority, but investment in better aquatic toxicity assessments using Canadian data would be highly relevant, especially if the data are made openly available for future researchers to use.

1 Introduction

Pharmaceuticals are used in the diagnosis, cure, or prevention of diseases in animals and humans (Schwartz et al., 2021), and have contributed to lengthening life spans, combatting deadly diseases, and improving life quality (Patel et al., 2019). The continuous use and discharge of pharmaceuticals have however resulted in their release into the environment, and their emergence as pseudo-persistent contaminants of concern (Patel et al., 2019; Schwartz et al., 2021; Wilkinson et al., 2022).

Pharmaceuticals and their active ingredients, also known as pharmacologically active compounds (PHACs) (Hua et al., 2006b), have been found in treatment plant effluent, influent, and sludge as well as in surface waters, groundwater, drinking water, soil, manure, biota, sediment, and throughout the food chain (OECD, 2019; Patel et al., 2019). For instance, traces of several pharmaceuticals were found in the drinking water of 15 southern Ontario cities (Servos et al., 2007). Chan, et al. (2014) found a variety of widely used pharmaceutical agents in streams on 17 First Nation reserves in Canada. Similarly, Schwartz and colleagues, (2021), found a total of 35 pharmaceuticals in 68% of the water bodies near or on reserve lands of 83% of First Nations. In another study, the presence of 14 steroids and hormonal compounds, as well as 88 other drugs was documented in the final effluent of two hospitals and one long-term care facility in Canada (Kleywegt et al., 2016).

Pharmaceuticals can be released into the environment via pathways such as human and animal (pets and livestock) excretion, improper dumping of unused/unwanted pharmaceuticals into sewers and landfills, as well as bathing, washing, and laundering (Zeeshan et al., 2021; Daughton & Ruhoy, 2009). Past literature indicates that municipal wastewater treatment plants are one of Canada's major sources of pharmaceutical contamination, as they are unable to completely remove many pharmaceuticals (Schwartz et al., 2021), and the final effluent released from them is typically only measured for metals, nutrients, oxygen demand and some organics (Kleywegt et al., 2019).

The presence of pharmaceuticals and their residuals in water can pose unintended negative physiological effects on non-target organisms and humans (Schwartz et al., 2021). Pharmaceuticals were created with the purpose of causing biological effects. Many can be easily hydrolyzed in the stomach's acidic pH, they are lipophilic in order to penetrate cell membranes, persistent, and have high mobility in the liquid phase. As a result, pharmaceuticals and their active ingredients can cause negative effects and bio-accumulate in aquatic and terrestrial ecosystems (Dökmeci et al., 2014).

However, it is not clear if the current concentrations of commonly reported pharmaceuticals are really of concern and require action in terms of monitoring or water quality standards and regulations. The goal of the present study was to identify the most frequently reported pharmaceutical compounds in Ontario's waters from past literature and conclude whether or not their measured concentrations are of environmental concern. The research question is: are the concentrations of the ten most commonly reported pharmaceuticals and/or pharmaceutically active compounds in Ontario's water bodies (including lakes, streams, and

rivers) considered toxic to, or result in toxic effects to aquatic life (defined as all organisms living in freshwater or reliant on it in some way (Streets & Dobbins, 2017))? This study aims to lessen the uncertainty associated with the potential effects and aquatic toxicity of pharmaceutical compounds. The results can help inform policymakers in Ontario and by identifying key compounds of concern for management and monitoring.

The following research paper is structured as follows: section 2 discusses the methodology; section 3 presents the ten most commonly reported pharmaceuticals and summarizes their behavior and toxicity in the aquatic environment; section 4 answers the research question; and sections 5 and 6 present the appendices and references respectively.

2 Methodology

The current study consisted of two stages: data collection and analysis.

In phase 1 of data collection, a systematic literature review of all the peer-reviewed literature on pharmaceutical pollution in Ontario was conducted. Figure 1 shows the search, screening, and eligibility framework that was followed. Through this stage, the ten most reported pharmaceuticals, measured using reporting frequency, were identified.

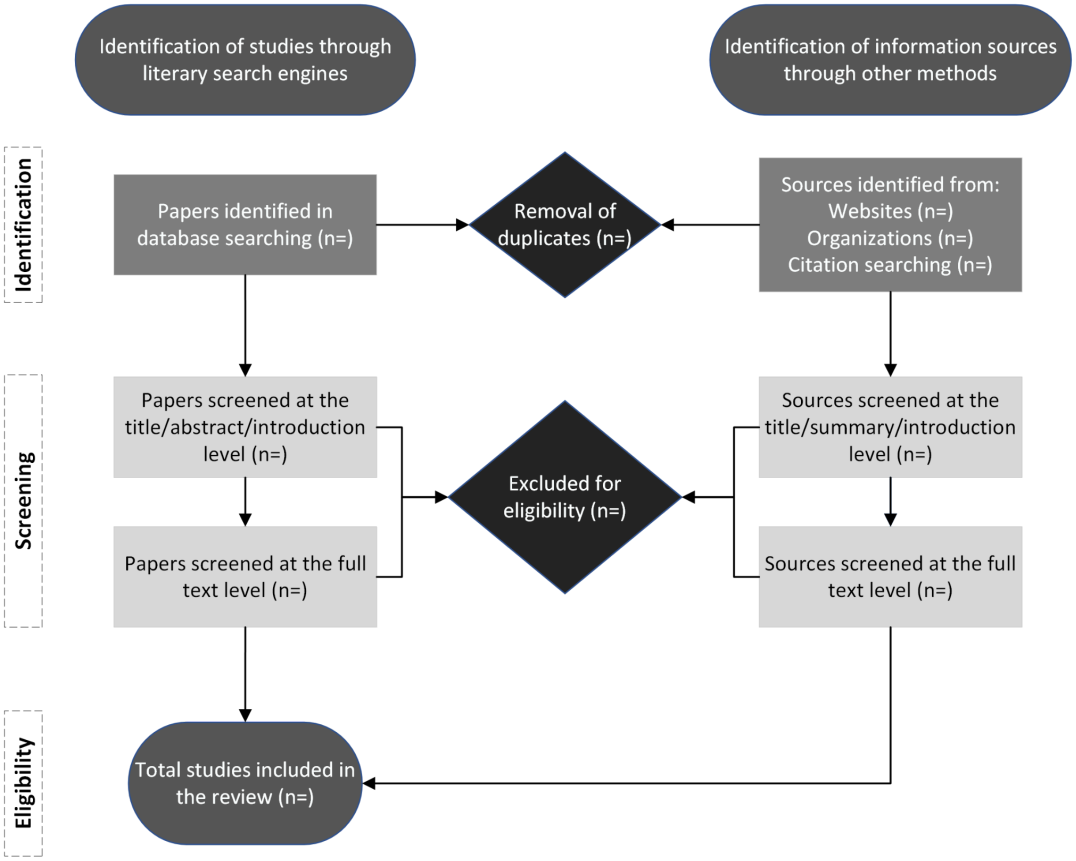


Figure 1. Showing a flow diagram of the sampling protocol used for the systematic review. Developed from Page et al., 2021.

To search for relevant studies, the following sequences were inserted into Google Scholar and Scopus through the University of Ottawa library: “Ontario AND Pharmaceutical* AND River”, “Ontario AND pharmaceutical* AND Lake”, “Ontario AND pharmaceutical* AND water”. The relevant papers were selected by looking at the title, then the abstract, then the full text. The aim of the current research paper was to identify all the relevant studies ever published, as a result there was no specific timeframe to limit the search to. Only concentrations from 1) surface water from locations not adjacent to any major wastewater effluent sources 2) raw water (influent) of drinking water stations 3) upstream of wastewater treatment plants (WWTPs) and 4) at least 100 m downstream of WWTPs. The lower limit of 100 meters was selected as it was a distance where WWTP effluent is considerably diluted and there is still available data to be extracted from past literature. The identified studies and their results were compiled into a spreadsheet (see template in Table S1, Appendix A). Concentrations of PHACs in surface waters were reported in various ways: mean, median, range, and maximum. However, several studies did not provide concentrations in numbers, instead presenting box plots, scatter plots and histograms, in that case, the concentrations were estimated from the diagrams provided. The reporting frequency, which was the criteria used to identify the ten most commonly reported compounds, of each PHAC was calculated as the ratio of papers that report its presence in Ontario’s waters out of the total number of papers identified.

A separate literature search was conducted to collect toxicity data for the identified pharmaceuticals. The following words and phrases were used in Google Scholar and Scopus: “pharmaceutical name” AND toxic* AND aquatic. Since toxicity studies using environmentally relevant concentrations are of greater significance to understanding the potential harmful effects of pharmaceutical residues on aquatic biota (Sehonova et al., 2018), only studies that examined the effects of PHACs at µg/L, ng/L and very low mg/L concentrations were reported in section 3. Additionally, studies that report acute and chronic toxicity endpoints such as LC_{50}, EC_{50}, NOEC, etc., were used and their results were summarized in Appendix B. Each PHAC in section 3 will have the following: general information, occurrence in Ontario’s water bodies, degradability and behavior in aquatic environments, and aquatic toxicity from past literature.

Finally, the information collected in stage 1 is used to answer the research question in stage 2 and conclude whether each of the investigated pharmaceuticals are of concern, and whether Ontario should conduct further research on it, monitor it, or implement regulations to stop or reduce its entrance to the environment. The risk posed by each pharmaceutical was assessed by 1) observing if the current concentrations exceed any of the reported toxicity endpoints in past literature 2) considering the significance of chronic exposure, synergistic and mixture effects and metabolites, and finally 2) calculating an ecological risk quotient (ratio of the maximum measured environmental concentrations and the lowest predicted no-effect concentrations (PNEC) (Ginebreda et al., 2010)). A risk quotient (RQ) higher than 1 indicates that the environmental concentration is higher than value below which no adverse effects are expected and thus poses high potential to aquatic biota. RQs indicate moderate ecotoxicological risk when they are between 0.1 and 1, and low or negligible risk when lower than 0.1 (Li et al., 2020). Finally, summary profiles that compile the physicochemical properties as well as the

mode of action, toxicity and recommendation for each of the ten PHACs were created and provided in Appendix C.

3 Results and Discussion

3.1 Search Results

The search resulted in 26 relevant peer reviewed papers and one PHD thesis. As seen in Figure 2, 2006 was the year with the highest number of relevant publications (5 papers), followed by the years 2010, 2016 (3 papers) and then 2011, 2013, 2014 and 2022 (2 papers).

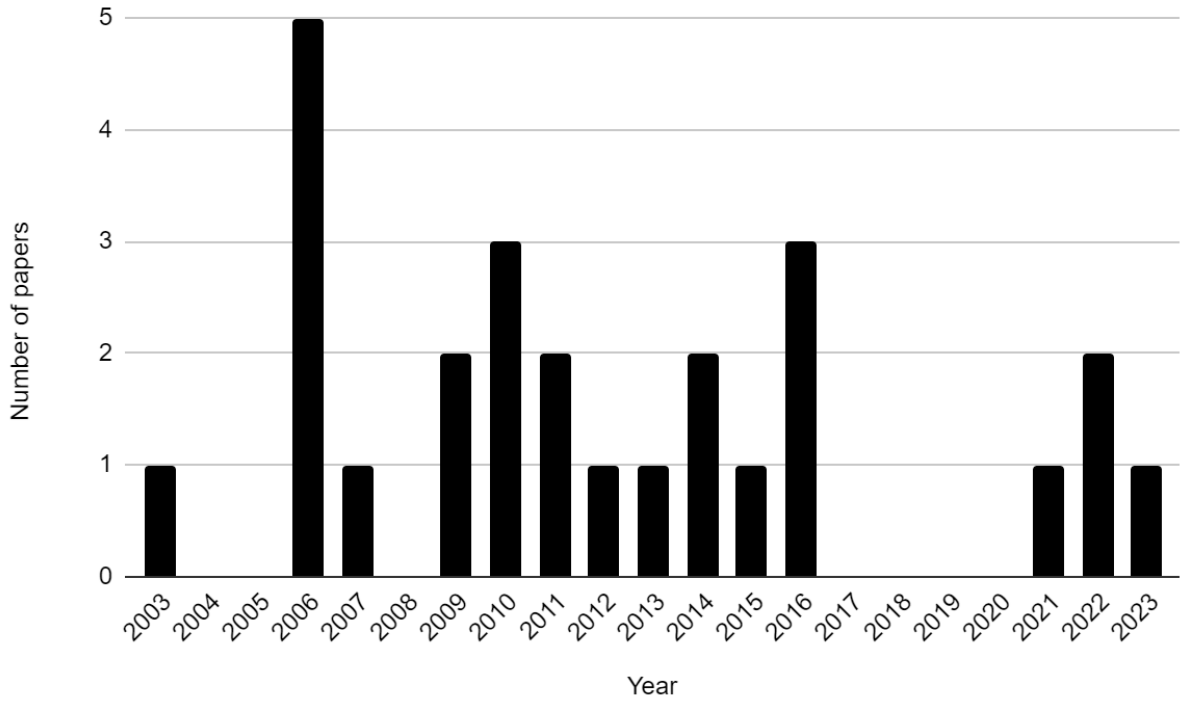


Figure 2. The number of papers published on pharmaceutical pollution in Ontario per year from 2003 to 2023.

As seen in Figure 3, the sampling locations of the identified studies all cluster in the more populated southern Ontario. It is clear that previous research was mainly interested in investigating pharmaceutical pollution in urban, and to an extent agricultural, environments. Out of the 26 studies, only two specifically sampled surface waters exposed to agricultural runoff (Lissemore et al., 2006; Hao et al., 2006). The remaining studies sampled waters receiving mainly urban input, usually near or at wastewater, sewage and drinking water treatment plants. Consequently, the most commonly investigated water bodies were the Grand River, Detroit River and Lake Ontario. The Grand River watershed is relatively large and contains around 30 municipal wastewater treatment plants (Gillis et al., 2014). Similarly, the Detroit River, an

important source of drinking water for the densely populated metropolitan area it passes through, receives substantial urban and agricultural runoff, as well as sewage treatment plant discharges (Hua et al., 2006b; Jasim et al., 2006). The A.H. Weeks Plant on the Detroit River, which is part of the Windsor Water Treatment Centre, was sampled by several studies (Boyd et al., 2003; Hua et al., 2006a; Hua et al., 2006b; Jasim et al., 2006; Tabe et al., 2009).

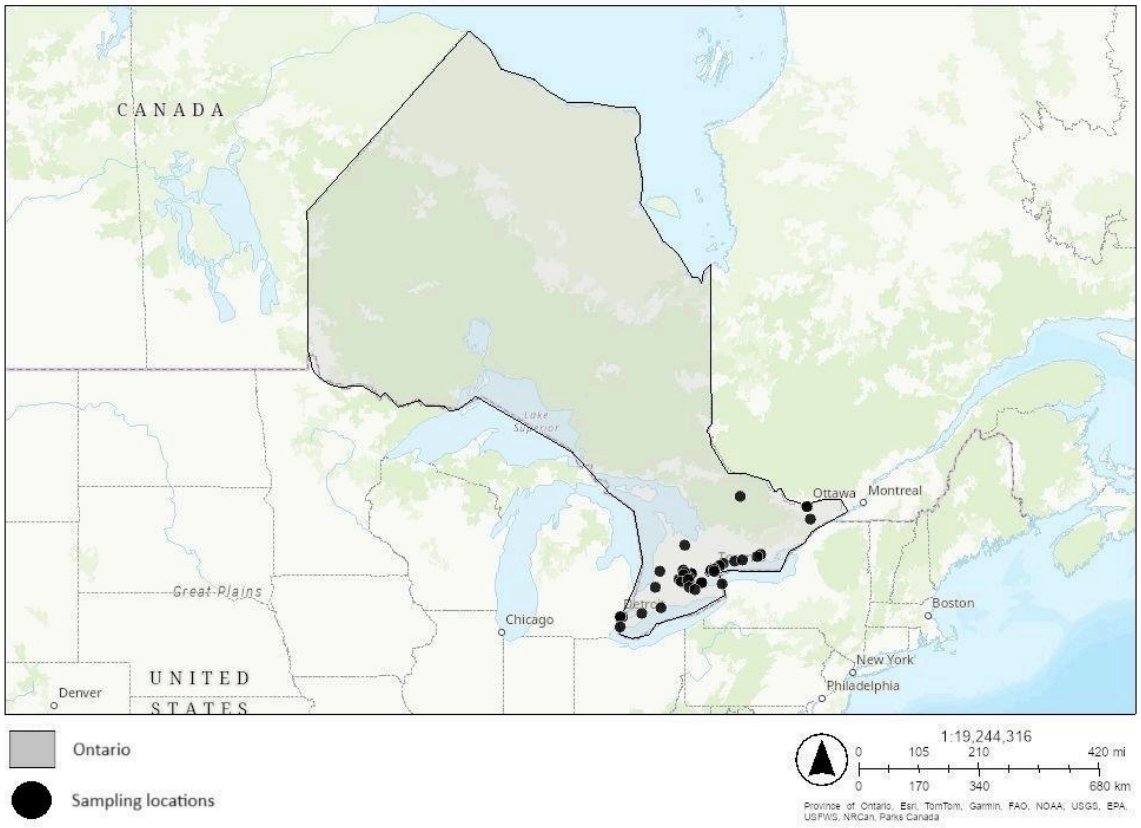


Figure 3. A map highlighting the study area, Ontario, and showing the locations sampled for the detection of PHACs by the identified published papers. Most studies did not provide exact sampling locations, thus the points on the map are estimations.

The laboratories used for analysis could only be identified for 20 papers, and they were a total of nine:

- Trent University (Li et al., 2010; Helm et al., 2012; Gillis et al., 2014; Metcalf et al., 2014; Metcalf et al., 2016; Sultana &Metcalf, 2022)
- Maxxam Analytics (Arlos et al., 2015; Shwartz et al., 2021; Couperus et al., 2016)
- Great Lakes Institute for Environmental Research (GLIER), University of Windsor (Hua et al., 2006b; Jasim et al., 2006)
- National Laboratory of Environmental Testing, Environment Canada (Lee et al., 2009; Staley et al., 2016)
- National Water Research Institute, Environment Canada (Servos et al., 2007; Tanna et al., 2013)

- Research and Development Centre, Agriculture and Agri-Food Canada (Littlejohn et al., 2023)
- Decon Laboratories (Boyd et al., 2003)
- Laboratory Services Branch, Ontario Ministry of the Environment (Kleywegt et al., 2011)
- Centre of Excellence in Mass Spectrometry at the University of York (Wilkinson et al., 2022)

3.2 Pharmaceuticals in Ontario's Waters

A total of 112 pharmaceutically active compounds were reported in the identified 26 papers. The ten most frequently reported PHACs are listed in Table 1. Out of these ten, there was one anticonvulsant, two NSAIDs, two antibiotics, two lipid regulators, two stimulants, and one antidepressant. Figure 4 compares the intensity and the frequency of these pharmaceuticals. The ten pharmaceuticals with the highest reported concentrations (highest intensity) in surface waters are listed in Table 2, and Figure 5 compares their frequency and intensity. Caffeine had the highest mean concentration, followed by ibuprofen and naproxen. These three compounds are ranked, respectively, the 6th, 2nd and 3rd in terms of frequency (Table 1) and the 4th, 6th, and 10th in terms of intensity (Table 2).

Table 1. The ten PHACs (eight pharmaceuticals and 2 metabolites) identified to have the highest reporting frequency in past literature on pharmaceutical pollution in Ontario's water bodies. The therapeutic class, total number of sites, frequency (calculated as the ratio of the number of reporting papers and the total number of papers) and intensity (mean concentration calculated using all mean concentrations reported in past literature) are provided.

PHAC	Therapeutic Class	# of sites	Frequency	Intensity (ng/L)		
				Minimum	Mean	Maximum
Carbamazepine	Antiepileptic[a]	68	69%	0.1	31.26	508
Ibuprofen	Anti-inflammatory[b]	47	57%	0.3	110.62	750
Naproxen	Anti-inflammatory[b]	30	57%	0.9	55.63	450
Trimethoprim	Antibiotic[c]	29	46%	0.12	22.51	274
Gemfibrozil	Lipid Regulator[a]	21	42%	0.05	17.20	364
Caffeine	CNS Stimulant[a]	40	34%	0.5	250.59	1110
Sulfamethoxazole	Antibiotic[a]	23	34%	0.05	12.9	45.7
Venlafaxine	Antidepressant[b]	31	26%	0.1	16	100
Cotinine	Metabolite of Nicotine[a]	21	23%	0.3	36.39	137

Clofibric Acid	Metabolite of lipid regulator clofibrate[d]	7	19%	0.55	34.70	103

[a]Spongberg et al 2008
[b]Arlos et al 2015
[c]Wilkinson et al., 2022
[d]Schwartz et al 2021

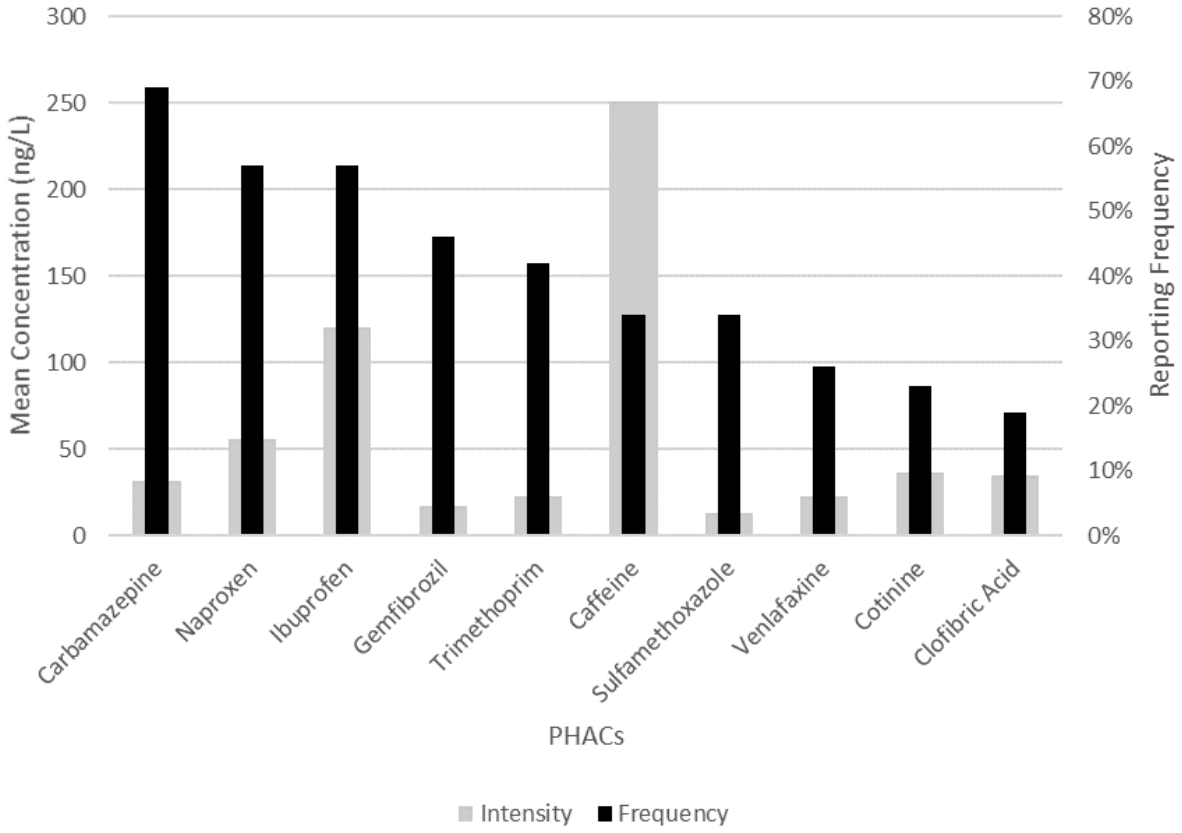


Figure 4. A histogram comparing the frequency and intensity of the top ten most frequently reported pharmaceuticals from Table 2.

Table 2. The ten PHACs identified to have the highest mean concentration in published literature on pharmaceutical pollution in Ontario's water bodies. The therapeutic class, total number of sites, frequency (calculated as the ratio of the number of reporting papers and the total number of papers) and intensity (mean concentration calculated using all mean concentrations reported in past literature) are provided.

PHAC	Therapeutic Class	# of sites	Frequency	Intensity (ng/L)
Paracetamol	Analgesic[a]	6	4%	385.71

Gabapentin	Anticonvulsant[a]	3	4%	381.33
Nicotine	Stimulant and lifestyle compound[a]	6	4%	304.033
Caffeine	CNS Stimulant[b]	40	34%	250.59
Sitagliptin	Antihyperglycemic[a]	3	4%	121.73
Ibuprofen	Anti-inflammatory[c]	47	56%	120.12
Chlorophene	Antimicrobial[c]	3	4%	116.6
Monensin	Growth promoter ionophore[d]	7	4%	104.71
Codeine	Analgesic[e]	10	8%	72.45
Naproxen	Anti-inflammatory[c]	30	60%	55.63

[a.]Wilkinson et al 2022
[b]Spongberg et al 2008
[c]Arlos et al 2015
[d]Hao et al 2006
[e]Schwartz et al 2021

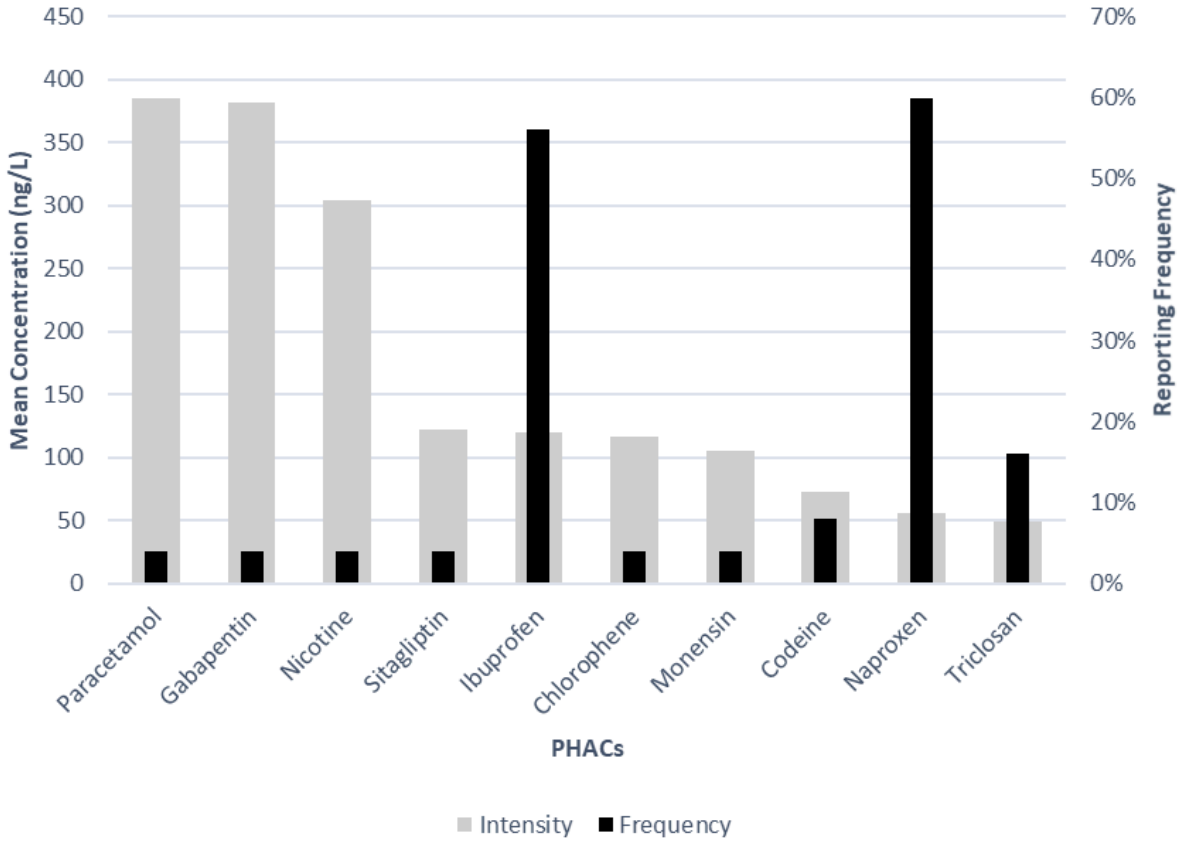


Figure 5. A histogram comparing the intensity and frequency of the ten pharmaceuticals with the highest mean concentrations listed in Table 2.

3.2.1 Anticonvulsant Carbamazepine

Carbamazepine was the most frequently reported in the identified list and was observed in the highest number of sites (see Table 1). It is a derivative of iminostilbenes (Liu et al., 2023) that was first synthesized in 1960 and is now widely prescribed for treating epilepsy, pain, and some mental disorders (Hua et al., 2006a; Li et al., 2010; Metcalfe et al., 2014; Arlos et al., 2015; Spongberg et al., 2008; Batucan et al., 2022). Its estimated worldwide consumption is around 1014 tons and, due to its frequent detection, it is commonly viewed as a marker of pharmaceutical pollution in water bodies worldwide (Baali et al., 2022). Carbamazepine works by reducing intracellular cAMP levels and blocking voltage-gated sodium channels and, to a certain extent, calcium channels, causing the inhibition of action potential and reduction of synaptic transmission (Batucan et al, 2022; Baali et al., 2022).

As seen in Table 3, the two main water bodies where studies investigated the presence of this compound were the Grand River and Detroit River. The maximum concentration, 508 ng/L, was reported by Tabe et al. (2009) in the Detroit River (Windsor), and the minimum concentration, 0.1 ng/L, was reported by Staley et al. (2016) in the Humber River. The overall average reported concentration is 31.2 ng/L (Table 1).

Table 3. Showing the mean concentrations of carbamazepine measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. RL: reporting limit, DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Lissemore et al., 2006	0.16-24[a]	RL = 0.06	2	The Grand River, primarily north and north west of Kitchener Waterloo
Hao et al., 2006	3.31	DL = 20 LOQ = 0.5	8	Grand River[b]
Hua et al., 2006a	0.5-2[a]	LOQ = 0.05	1	A.H. Weeks Water Treatment Plant (WTP) in the Detroit River
Hua et al., 2006b	9-41[a]	LOQ = 0.05	1	The Little River wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Jasim et al., 2006	1.26	DL = 10	1	A.H. Weeks WTP in the Detroit River
Tabe et al., 2009	14-508[d]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Li et al., 2010	3.4	DL = 0.1-1 LOQ = 1-10	7	Hamilton Harbour, Toronto Harbour, Humber Bay, Newcastle Harbour, Bay of

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				Quinte, MSC Buoy and the Niagara River (all in Lake Ontario)
Halle, 2010	39	DL = 5 LOQ = 16	1	Mannheim WTP in the Grand River
Kleywegt et al., 2011	3[c]	DL = 1	1	DWSs around Ontario[b]
Helm et al., 2012	0.1-7.9		2	Pickering Ajax and Port Hope in Lake Ontario
Tanna et al., 2013	13.57		7	The Waterloo, Kitchener, and Guelph municipal WWTPs in the Grand and Speed Rivers
Metcalfe et al., 2014	13.8	DL = 0.3 LOQ = 1	7	Drinking water treatment plants located on the Ottawa River, Lake Ontario, Grand River and Lake Erie
Gillis et al., 2014	2.47		1	Kitchener municipal WWTP in the Grand River
Arlos et al., 2015	18.6	DL = 3	11	Waterloo, Kitchener, Preston, Galt (Grand River) and Guelph and

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				Hespeler (Speed River) municipal WWTPs
Staley et al., 2016	0.1-1		8	Humber River and Black Creek Tributary
Schwartz et al., 2021	28.4-45.7		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[b]
Sultana & Metcalf, 2022	3.6	DL = 0.3 LOQ =1	4	Presqu'île Bay in Lake Ontario
Wilkinson et al., 2022	6.2-23.7		3	Don River and Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Exact locations were not provided
[c]Median concentration
[d]Minimum concentration

Carbamazepine is known to be ubiquitous in the aquatic environment and to display remarkable chemical stability (Trognon et al., 2023) with an estimated half-life of 100 days in double distilled water (Zhang et al., 2008). It was consistently observed at high frequencies in the 18 studies that report it in Ontario's surface waters. Halle, (2010), reported a frequency of detection of 91% for carbamazepine in the Grand River. In their global study, Wilkinson et al. (2022) found that detection frequency of carbamazepine in Lake Ontario and Don River (Toronto, ON) was 64% and noted that this pattern is similarly observed across different continents. In the Great lakes, carbamazepine had a detection frequency of 71% (Uslu et al., 2013). Carbamazepine was also associated with a poor removal efficiency in conventional biological WWTPs of <30 % (Trognon et al., 2023; Liu et al., 2023; Halle, 2010) and high persistence of 100 days in surface water (Zhang et al., 2008; Baali et al., 2022).

Carbamazepine is considered a moderate hydrophobic compound with a low ability to cross cellular membranes. It also has a neutral pH and a high pKa of >15 (Table S11 in Appendix C), which makes it have little to no adsorption affinities to sediment (Trognon et al., 2023; Yuan et

al., 2019). Carbamzepine is argued to be refractory to biodegradation in aquatic environments (Arlos et al., 2015; Liu et al., 2023). In abiotic degradation, hydrolysis seems negligible due to the compound's resistance against strong bases and acids, and photolysis is considered the main in-stream degradation pathway for the compound (Yan et al., 2019; Liu et al., 2023). However, the majority of carbamazepine's in-stream loss is argued to be due to contaminant transport processes such as advection and dispersion rather than fate mechanisms such as photolysis, biodegradation, and volatilization (Arlos et al., 2015). Helm et al. (2012) states that in a lake setting, carbamazepine can persist longer in the nearshore water column and be subjected primarily to transport via alongshore currents. Overall, this compound has a conservative behavior and high mobility in the environment (Arlos et al., 2015).

Seasonal fluctuations in concentrations were observed where higher concentrations of carbamazepine arise during the summer and early fall months (Uslu et al., 2013). Halle, (2010), observed the lowest carbamazepine concentrations in the winter and spring (January and April) and attributed this to the increased dilution effect as a result of higher flows in the Grand River at those times. This was also consistent with measurements previously performed on carbamazepine in the Grand River water and in surface water (Lissemore et al., 2006; Metcalfe et al., 2003; Halle, 2010).

Biological effects, such as mortality, oxidative stress, neurotoxicity, reproductive or growth inhibition, developmental effects and morphological changes, of the exposure to carbamazepine are observed across a wide range of concentrations.

Neurotoxicity was one of the more sensitive endpoints. For instance, a significant decrease of 2-folds in AChE activity was observed in *Perna viridis* exposed to 0.001-0.08 µg/L carbamazepine for 7 days (Juhel et al., 2017). Similarly, female zebrafish, *Danio rerio,* exposed to 0.1-10 µg/L carbamazepine for 10 days exhibited a 1.2-fold decrease in AChE activity (Shi et al., 2019). Oxidative stress was also a sensitive endpoint. Shi et al. (2019) observed a 3-fold decrease in antioxidant enzymatic activity, specifically catalase (CAT) activity, in female zebrafish (*D. rerio*) which indicated a potential impact on cellular defense mechanisms against oxidative stress. In *P.viridis*, exposure to 0.1 µg/L carbamazepine for 7 days increased the activity of ethoxyresorufin-O-deethylase (EROD), a detoxification enzyme, by 1.6 times (Juhel et al., 2017).

In terms of energy metabolism, a 2.2-fold increase in lactate dehydrogenase (LDH) activity was observed in the zebrafish after a ten-day exposure to 0.1 µg/L carbamazepine, indicating an adaptation of the energy metabolism that is possibly linked to the metabolic demands associated with tolerance or detoxification processes (Shi et al., 2019). Potential disruption of the endocrine system was also implied by the observed 1.4-fold decrease in vitellogenin content and estradiol concentration in female *D.rerio* after exposure to carbamazepine (Shi et al., 2019). In terms of reproduction, one study found that exposure of *Hydra circumcincta* to 0.6 µg/L carbamazepine for 6 days significantly reduced budding rates by 1.4 times and reproductive success from 14% to 10% (Desbiolles et al., 2020). In another study, a six-week exposure to 10 µg/L carbamazepine resulted in male *Danio rerio* producing abnormal sperm (Galus et al., 2014).

Moving to the less sensitive endpoints development and growth, larval zebrafish (*D. rerio*) exposed to a minimum of 1 µg/L carbamazepine for up to 96 hours had greater body length, increased swim bladder appearance, and accelerated yolk sac absorption (Qiang et al., 2016). Flatheaded mayflies, *Stenonema spp,* exposed to 2 mg/L carbamazepine for 9 days experienced a 6-day delay in molting. However, lower and more environmentally relevant concentration of 0.002 µg/L did not result in any observable effects (Jarvis et al., 2014). Aguirre-Martínez et al. (2015) found that *Corbicula fluminea* bivalves chronically exposed to 0.1-1 µg/L carbamazepine had reduced lysosomal membrane stability and described them as "stressed but compensating".

Previous studies also illustrated a bioaccumulation potential for carbamazepine. Carbamazepine accumulated in wild fish (up to 33 mg CBZ per kg after exposure to polluted river water), *Mytilus galloprovincialis* (up to 400 mg CBZ per kg when exposed to 15.7 mg/L for 40 d), and *Anemonia sulcata* (up to 1100 mg CBZ per kg after 8d exposure to 80 mg/L) (Baali et al., 2022).

Internationally, standards for carbamazepine in aquatic systems include the environmental quality standard (EQS) of 2.5 mg/L suggested by the French National Institute for Industrial Environment and Risks (INERIS) and the EQS of of 0.5 mg/L established by Germany (Baali et al., 2022). Using the maximum reported concentration of 508 ng/L and the lowest PNEC of 500 ng/L, the RQ was calculated to be 1.016 (508/500). This number indicates that carbamazepine poses a significant ecotoxicological risk at concentrations commonly found in Ontario's surface waters.

3.2.2 NSAIDs Naproxen & Ibuprofen

Naproxen and Ibuprofen are the only two non-selective, nonsteroidal anti-inflammatory drugs (NSAIDs) in the list of the top ten most frequently reported PHACs compiled in this study. They both a reporting frequency of 57% and have a relatively high concentration in the aquatic environment (see Tables 1 and 2). Additionally, the studies that report them in Ontario's surface waters show similar frequency and intensity trends (Kleywegt et al., 2011; Couperus et al., 2016; Uslu et al., 2013; Arlos et al., 2015).

Naproxen is a bicyclic propionic acid derivative (Wojcieszyńska & Guzik, 2020) that works by inhibiting both cyclooxygenase isoforms that contribute to the synthesis of prostaglandins, prostacyclin and thromboxane from arachidonic acid (Wojcieszyńska & Guzik, 2020; Barcella et al., 2019). In addition to it being an over-the-counter medication, it has advantageous characteristics such as 1) rapid absorption, 2) long duration of action (due to its long biological half-life), and 3) lower vascular risk compared to other NSAIDs (Wojcieszyńska & Guzik, 2020; Barcella et al., 2019; Angiolillo & Weisman, 2017). As a result, naproxen is very commonly used to treat pain, headache, colds, and flu symptoms (Arlos et al., 2015; Wojcieszyńska & Guzik., 2020) and is one of the most frequently detected NSAIDs in the aquatic environment worldwide (Xu et al., 2019). See Table S12 Appendix C for naproxen's summary profile.

Naproxen was measured in the water bodies of Ontario by 14 papers and as seen in Table 1, its mean concentrations ranged from 0.9 ng/L in the Niagara River (Li et al., 2010) to 450 ng/L in Hamilton Harbour (Csiszar et al., 2011), whereas the overall average concentration is 55.63

ng/L. As seen in Table 4, the most commonly investigated water bodies for naproxen are the Detroit River, Grand River, and Lake Ontario.

Table 4. Showing the mean concentrations of naproxen measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. RL: reporting limit, DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Boyd et al., 2003	63	DL = 0.4	1	A.H. Weeks WTP in the Detroit River
Lissemore et al., 2006	41.7	RL = 125	1	The Grand River, primarily north and north west of Kitchener Waterloo
Hao et al., 2006	41	DL = 50 LOQ = 10	1	Grand River[a]
Hua et al., 2006b	30-50[b]	LOQ = 20	1	The Little River wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Jasim et al., 2006	63	DL = 0.03	1	A.H. Weeks WTP in the Detroit River
Servos et al., 2007	4-90[b]		2	DWSs near Burlington
Tabe et al., 2009	9-174[b]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Li et al., 2010	2.69	DL = 0.1-1 LOQ = 1-10	4	Niagara River, Hamilton

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				Harbour, Humber Bay, Toronto Harbour (all in Lake Ontario)
Halle, 2010	140[c]	DL =2 LOQ = 6	1	Mannheim WTP in the Grand River
Kleywegt et al., 2011	21[d]	DL = 2	1	DWSs in Ontario[a]
Csiszar et al., 2011	183.3	DL = 1.74-3.9	3	Hamilton Harbor
Arlos et al., 2015	32.85	DL = 3	7	Waterloo, Kitchener, Preston, Galt (Grand River) and Guelph and Hespeler (Speed River) municipal WWTPs
Schwartz et al., 2021	<5-120[b]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[a]
Sultana & Metcalf, 2022	58.6	DL = 0.8 LOQ = 2.6	4	Presqu'île Bay in Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Exact locations were not provided
[b]Minimum-maximum concentration
[C]Maximum concentration
[d]Median concentration

Ibuprofen is a non-prescription (Sultana & Metcalf, 2022) acidic drug that was first synthesized in the 1970s (Batucan et al., 2022). It is commonly used to treat pain, headache, colds, flu symptoms, arthritis, and rheumatic disorders (Arlos et al., 2015; Parolini, 2020). Ibuprofen is considered to be one of the most commonly used drugs worldwide, and is part of the World Health Organization's ''Essential Drug List'' (Das et al., 2019). Its over-the-counter sale, large prescription volume and high excretion rate (~70–80% of the therapeutic dose) has resulted in its frequent presence in aquatic ecosystems (Arlos et al., 2015; Das et al., 2019; Parolini, 2020). It works by inhibiting two cyclooxygenase enzymes involved in the synthesis of prostaglandins that lead to inflammation, pain, and fever (Batucan et al., 2022). Ibuprofen's reported mean concentrations ranged from 0.3 in Pickering-Ajax (Helm et al., 2012) to 750 ng/L in Hamilton Harbour (Csiszar et al., 2011), and its overall mean concentration in Ontario's surface waters was a high value of 110.62 ng/L (see Table 1). As seen in Table 5, it was most commonly investigated in the Grand River.

Table 5. Showing the mean concentrations of ibuprofen measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. RL: reporting limit, DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Hua et al., 2006b	13	LOQ = 4	1	The Little River wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Servos et al., 2007	3-70[a]		2	DWSs near Burlington
Tabe et al., 2009	32[b]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Li et al., 2010	8.18	DL= 0.1-1 LOQ = 1-10	6	Niagara River, hamilton Harbour, Humber Bay, Toronto Harbour, Bay of Quinte, and MSC Buoy

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				(all in Lake Ontario)
Halle, 2010	120[b]	DL= 2 LOQ = 7	1	Mannheim WTP in the Grand River
Kleywegt et al., 2011	0.98[c]	DL = 0.5	1	DWSs in Ontario[d]
Csiszar et al., 2011	316.66	DL = 1.74-3.9	3	Hamilton Harbour
Helm et al., 2012	0.3-11[a]		2	Pickering Ajax and Port Hope in Lake Ontario
Tanna et al., 2013	32.5		6	The Waterloo, Kitchener, and Guelph municipal WWTPs in the Grand and Speed Rivers
Gillis et al., 2014	2.3		1	Kitchener municipal WWTP in the Grand River
Metcalfe et al., 2014	2.9	DL= 0.6 LOQ = 2	5	Drinking water treatment plants located on the Ottawa River, Lake Ontario, Grand River and Lake Erie
Arlos et al., 2015	17.27	DL = 2	11	Waterloo, Kitchener, Preston, Galt (Grand River)

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				and Guelph and Hespeler (Speed River) municipal WWTPs
Couperus et al., 2016	57		1	Grand River main channel
Schwartz et al., 2021	<2-85[a]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[d]
Sultana & Metcalf, 2022	642	DL= 0.3 LOQ =1	4	Presqu'île Bay in Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Maximum concentration
[c]Median concentration
[d]Exact locations were not provided

NSAIDs are generally known to be easily degradable and to have relatively low environmental stability. This is especially through biotransformation in aerobic conditions and photolysis occurring in surface waters (Świacka et al., 2021). For instance, naproxen was observed by Arlos et al. (2015) to have high removals in the Grand River's wastewater treatment plants and to degrade quickly in the aquatic environment. Similarly, ibuprofen's physicochemical properties (see Table S13, Appendix C) result in its relatively high mobility but low persistence in the aquatic environment (Parolini 2020; Jan-Roblero et al., 2023). In the field, its half-life was observed to be 32 days (Das et al., 2019). A model developed by Csiszar et al. (2011) estimated a half-life of 11 to 77 days and 10 to 37 days for ibuprofen and naproxen respectively.

Ibuprofen is known for its bioaccumulative nature, water solubility, low volatility, and low tendency for adsorption to organic matter. Naproxen has a relatively low solubility of 16 mg/L at 25°C (Arlos et al., 2015) and Log K_{ow} of around 3.2 (Wojcieszyńska & Guzik, 2020). It is also an acidic pharmaceutical that is almost entirely in ionic form in the water (Table S12, Appendix C). As a result, it is less likely to undergo sorption (Świacka et al 2021), and net sediment deposition is expected to be low (Csiszar et al., 2011). In aquatic systems, the fate of the two

NSAIDs is expected to be dominated by direct/indirect photo and bio-degradation (Świacka et al 2021; Das et al., 2019; Csiszar et al., 2011; Hua et al., 2006a).

Despite their biodegradability, the constant use and consequent continuous replenishment within receiving environments makes the two NSAIDs 'pseudo persistent' PHACs that are still frequently detected in surface waters at considerable concentrations (Świacka et al., 2021; Batucan et al., 2022). As such, they are considered ideal indicators of treatment plant discharges and anthropogenic activities (Couperus et al., 2016).

In terms of seasonal patterns, Halle (2010) found similar occurrence patterns for ibuprofen and naproxen in the Grand River, with the highest concentrations occurring during the winters and the lowest concentrations during the summers. This is a result of their similar physicochemical properties; ibuprofen and naproxen are both negatively charged NSAIDs with a similar pKa value. Arlos et al. (2015) observed relatively low naproxen concentrations in the periods between August and October. Loadings for ibuprofen were highest in spring and winter and lowest during fall and summer (Csiszar et al., 2011; Uslu et al., 2013; Arlos et al., 2015). Consistently higher concentrations during the fall and winter months was explained by increased consumption of this anti-inflammatory drug during cold and flu season as well as the cold temperature that lowers its biodegradability in water (Uslu et al., 2013).

When examining specifically wastewater effluent, Hua et al. (2006a) found that concentrations of naproxen were relatively consistent among the three seasonal sampling periods. This implies that the consumption of naproxen, and thus release into wastewater, is also consistent and that seasonal variations in concentrations are due to environmental changes. Indeed, Amos Sibeko et al. (2019) found that environmental conditions such as climate can influence the fate of naproxen. For instance, stronger sunlight in the summer can increase the intensity of photodegradation of naproxen in surface waters and result in relatively lower concentrations (Wojcieszyńska & Guzik, 2020; Halle, 2010).

At a continuous exposure, even at low concentrations, NSAIDs have shown to be toxic and to contribute to the disruption of many physiological processes to vertebrates and invertebrates (Mezzelani et al., 2018; Xu et al., 2019; Świacka et al., 2021). Past studies showed that exposure to ibuprofen at environmentally relevant concentrations could result in effects such as cytotoxicity and genotoxicity, general stress, and altered growth rate, reproduction, and behavior. Fewer studies used environmentally relevant concentrations for naproxen; in general, naproxen exposure was shown to cause oxidative stress, genotoxicity, thyroid disruption and growth inhibition.

For the two NSAIDs, most endpoints were associated with considerable toxicity, with reproduction being arguably the most sensitive. The lowest endpoint concentrations of naproxen were the reproduction NOECs for *Daphnia magna* and *Moina macrocopa* (10 and 0.3 mg/L respectively) (Table S4, Appendix B; Kwak et al., 2018). Similarly, ibuprofen's reproduction NOECs were a relatively low 1.23 mg/L (Table S3, Appendix B). Japanese medaka (*Oryzias latipes*) exposed to 0.1 µg/L ibuprofen for 12 days had impaired reproduction, lower broods per pair, and higher eggs per brood and vitellogenin in males (Han et al., 2010). Additionally, in

Daphnia magna, exposure to ibuprofen concentrations of 0.5, 5, and 50 µg/L for 21 days significantly decreased the total number of eggs and broods per female and body length (Wang et al., 2016). In another study, *D.magna* exposed to ibuprofen at 1 µg/L, 2 µg/L and 4 µg/L during their whole-life-cycle showed slower growth, larger neonates, later maturation and a longer life with a higher reproduction rate and smaller broods (Adamczuk, 2022). Ibuprofen having an impact on life-history traits and population performance of the species was also suggested by Heckmann et al. (2007).

Endocrine disruption was another sensitive endpoint for naproxen. In a long-term 60-day experiment conducted by Xu et al. (2019), zebrafish exposed to 0.1-100 mg/L naproxen exhibited significant thyroid disruption and growth inhibition. Thyroid hormones (triiodothyronine and thyroxine) were dose-dependently reduced, and transthyretin (TTR), a thyroid hormone transport protein, was also significantly affected. This indicates that naproxen can have an impact on the endocrine system. The authors also argued that long-term exposure can pose high bioconcentration risks in aquatic organisms, even at low environmental concentrations (Xu et al., 2019).

Several other endpoints exhibited high sensitivity for ibuprofen. Exposure to ibuprofen for 96h induced cyto- and genotoxicity on hemocytes of the zebra mussel (*D.polymorpha*) at a concentration of 0.2 µg/L and genetic damage and lysosomal membrane instability at a concentration of 2 and 8 µg/L (Parolini et al., 2011; Parolini, 2020). Behavioral activity was also shown to be impacted in the benthic invertebrate, *Gammarus pulex,* following acute exposure to 0.01-0.1 µg/L of ibuprofen (De Lange et al., 2006). At higher concentrations, ibuprofen inhibited the regeneration of the cnidarian *Hydra vulgaris* (5 mg/L) (Quinn et al., 2008) and caused 100% lethality in *Daphnia magna* (200 mg/L for 24 hr) (Du et al., 2016).

For both naproxen and ibuprofen, oxidative stress was a moderately sensitive endpoint. Stancova et al. (2015) exposed zebrafish to 1-100 µg/L naproxen for 14 days and found that the drug had a moderate impact (in terms of weight, length and mRNA expression) on enzymes responsible for maintaining redox balance in the zebrafish intestine but not in the liver. In another study, Lucero and colleagues, (2015), investigated the impact of naproxen on *Hyalella azteca* using an artificial sediment enriched with the drug (76.60-399.28 mg/kg over 48-hour exposure period) and found that it induced both oxidative stress and genotoxicity by significantly increasing superoxide dismutase (SOD) and catalase (CAT) activity, and decreasing glutathione peroxidase (GPX) activity. On the other hand, genotoxicity was indicated by a statistically significant rise in DNA damage levels (Lucero et al., 2015).

After exposure to 0.5, 5, and 50 µg/L ibuprofen for 48h, the fish *Daphnia magna* showed increased oxidative stress through altered CAT, SOD, and GST activities (Wang et al., 2016). In the zebrafish *Danio rerio*, oxidative stress was illustrated as ibuprofen administration at concentrations of 0.1–11 µg/L for 14 days altered the antioxidant defense system and liver protein carbonylation, as well as increased lactate dehydrogenase activity (Falfushynska et al., 2022). Contrado-Jara et al. (2011) found mRNA changes of enzymes and other proteins involved in the prevention from protein damage and oxidative stress in the zebra mussel, *Dreissena polymorpha,* as a result of ibuprofen exposure (0.2-206 µg/L for 7 days). *Corbicula fluminea* also

experienced oxidative stress (increased GPX activity at 5 and 50 µg/L, GR activity at 50 µg/L, and LPO levels at 5, 10, and 50 µg/L) after exposure to carbamazepine (Aguirre-Martinez et al., 2015). In terms of general stress, chronic exposure to ibuprofen caused a reduction in lysosomal integrity in the bivalves *Unio tumidus* (0.8 µg/L for 14 days) (Martyniuk et al., 2022) and *Corbicula fluminea* (0.1-50 µg/L for 21 days) (Aguirre-Martinez et al., 2015).

Table S12 and S13 in Appendix C, show many examples of acute and chronic toxicological endpoints investigated in past literature for naproxen and ibuprofen respectively. Huang et al. (2018) reported PNECs for ibuprofen of 1400, 1000, and 26 ng/L based on mortality, growth and reproduction respectively, noting that the reproduction endpoint is much more sensitive compared to the others. Naproxen's PNEC on the other hand, was higher at 21000 ng/L (Uslu et al., 2013; Straub & Stewart, 2007). Using these values, the RQ was calculated to be 0.02 (450/21,000) and 28 (720/26) for naproxen and ibuprofen respectively in Ontario's surface waters respectively. These results indicate that while naproxen poses a low ecotoxicological risk at the current concentrations, ibuprofen poses a very high one.

3.2.4 Antibiotics Trimethoprim & Sulfamethoxazole

The 4th most commonly reported pharmaceutical is the antibiotic trimethoprim, a diaminopyrimidine that is used for human and agricultural applications (Gillis et al., 2014, Kleywegt et al., 2011, Metcalfe et al., 2014, Hua et al., 2006b, Wilkinson et al., 2022). This antibiotic is known to be widely detected in wastewater, and is considered part of the suite of "indicator compounds" (Metcalf et al 2014; Helm et al 2012). One global consumption survey completed between 2000 and 2010 found that trimethoprim is among the five most used antibiotics worldwide (Krauper et al., 2020).

In agricultural applications, trimethoprim is used primarily in swine and cattle production (Lissemore et al., 2006). Whereas in human applications, it is prescribed for the treatment of infections of the respiratory and urinary tract in humans (Couperus et al 2016). Trimethoprim acts on dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolic acid (Kolar et al., 2014). Approximately 46% of the ingested dose of trimethoprim is excreted through urine and feces, 22% of which is unchanged (Kolar et al., 2014). In the present study, trimethoprim had a detection frequency of 46%; its concentrations ranged from 0.12 ng/L in the Niagara River (Li et al., 2010) to 274 ng/L in the Detroit River (Tabe et al., 2009), with the overall mean concentration being 22.51 ng/ (Table 1). The most commonly investigated surface waters were Lake Ontario and the Grand River (Table 6).

Table 6. Showing the mean concentrations of trimethoprim measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Lissemore et al., 2006	0.2-15[a]	RL = 0.2	1	The Grand River, primarily north

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				and north west of Kitchener Waterloo
Hua et al., 2006b	14-17[a]	LOQ = 0.3	1	The Little River wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Tabe et al., 2009	16-274[a]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Li et al., 2010	1.23	DL = 0.1-1 LOQ = 1-10	6	Niagara River, Hamilton Harbour, Humber Bay, Toronto Harbour, Newcastle Harbour, and MSC Buoy (all in Lake Ontario)
Kleywegt et al., 2011	0.4[b]	DL = 1	1	DWSs in Ontario[c]
Helm et al., 2012	0.2-5.4[a]		2	Pickering Ajax and Port Hope in Lake Ontario
Gillis et al., 2014	1.89		1	Kitchener municipal WWTP in the Grand River
Metcalfe et al., 2014	4.42	DL = 0.6 LOQ = 2	5	Drinking water treatment plants located on the

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				Ottawa River, Lake Ontario, Grand River and Lake Erie
Couperus et al., 2016	6.3		2	Grand River main channel and tributary (Canagagigue Creek, Conestogo River, Nith River, Speed tributary, Whiteman's Creek)
Schwartz et al., 2021	6-10.2[a]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[c]
Sultana & Metcalf, 2022	0.7	DL = 0.7 LOQ =2.4	4	Presqu'île Bay in Lake Ontario
Wilkinson et al., 2022	9.9		3	Don River and Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Median concentration
[c]Exact locations were not provided

Trimethoprim is commonly prescribed and used in combination with a sulphonamide; for example, sulfamethoxazole and trimethoprim are commonly prescribed for treating infections (Couperus et al., 2016). It is unsurprising then, that sulfamethoxazole was the 7th most commonly reported pharmaceutical in the current study (freq. 34%). Its overall mean concentration was 12.9 ng/L and the measured concentrations ranged from 0.05 ng/L in MSC Buoy (Li et al., 2010) to 45.7 ng/L in surface water of a First Nation located in southern Ontario

(Schwartz et al., 2021) (Table 1 and 7). Like trimethoprim, sulfamethoxazole was most commonly investigated and reported in the Grand River and Lake Ontario (Table 7).

Sulfamethoxazole is an acidic antibiotic/antimicrobial that is used for both humans and animals (Gillis et al., 2014; Metcalfe et al., 2014;, Couperus et al., 2016). It is considered a potent pharmaceutical and it works by reducing the uptake of p-aminobenzoic acid needed for dihydrofolic acid synthesis, which results in completely inhibiting the enzyme dihydropteroate synthetase in microorganisms. It is one of the top five most prescribed antimicrobials in Canada, commonly used to treat urinary tract infections, bronchitis, and sinusitis.

Table 7. Showing the mean concentrations of sulfamethoxazole measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. RL: reporting limit, DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Lissemore et al., 2006	0.3-9[a]	RL = 0.3	1	The Grand River, primarily north and north west of Kitchener Waterloo
Li et al., 2010	0.36	DL = 0.1-1 LOQ = 1-10	6	Niagara River, Hamilton Harbour, Humber Bay, Toronto Harbour, Newcastle Harbour, and MSC Buoy (all in Lake Ontario)
Kleywegt et al., 2011	0.17[b]	DL = 2	1	Raw water of 17 DWSs around Ontario
Gillis et al., 2014	0.23		1	Grand River
Metcalfe et al., 2014	3.06	DL = 0.3 LOQ = 1	5	Drinking water treatment plants located on the Ottawa River, Lake Ontario, Grand River and

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
				Lake Erie
Couperus et al., 2016	20.35	DL = 1	2	Grand River main channel and tributary (Canagagigue Creek, Conestogo River, Nith River, Speed tributary, Whiteman's Creek)
Schwartz et al., 2021	44.4-45.7		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[c]
Wilkinson et al., 2022	17.7		2	Ottawa (Ottawa River), and Toronto (Don River and Lake Ontario)
Sultana et al., 2022	1.46	DL = 1 LOQ = 3.3	3	Presqu'île Bay in Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Median concentration
[c]Exact locations were not provided

Metcalf et al. (2014) detected trimethoprim and sulfamethoxazole at similar frequencies, and found that they exhibited the highest rates of loss compared to other compounds investigated (gemfibrozil, ibuprofen and carbamazepine). The authors also stated that the two antibiotics belong to the list of “indicator compounds” that are most commonly observed in surface waters (Metcalf et al., 2014). Similarly, Couperus et al. (2016) found that sulfamethoxazole and trimethoprim were statistically well-correlated (R = 0.9, p b 0.01) in river samples from the Grand River main channel. The frequencies of detection of trimethoprim in past literature

ranged from 10 to 75%, whereas for sulfamethoxazole they ranged from 18% to 84%. Interestingly, Couperus et al. (2016) found sulfamethoxazole at much lower concentrations in agricultural tributaries compared to the urban areas of the channel despite its agricultural use.

Trimethoprim is considered to be relatively more persistent and resistant to wastewater treatment processes compared to other antibiotics (Nguyen et al., 2015; Yang et al., 2020). In their experiment, Nguyen et al. (2015) observed that degradation rate for trimethoprim was slow in the water phase, with a half-life of around 30 days, which indicated that photodegradation was unlikely to cause dissipation for the antibiotic. On the other hand, they observed rapid dissipation in the water and sediment phase, which illustrated that biodegradation is the main mechanism of removal for trimethoprim. This is supported by its relatively low Log K_{ow} value of 0.91 (Table S14, Appendix C).

Sulfamethoxazole is water-soluble, hydrophilic, amphoteric and polar (Chen et al., 2024), expected to exhibit moderate mobility (Duan et al., 2022), as well as high solubility and chemical stability in water (Luo et al., 2011). Unlike trimethoprim, it is resistant to natural biodegradation and hydrolysis (Trovó et al., 2009; Chen et al., 2024). Instead, photolytic processes, mainly direct photolysis, dominate sulfamethoxazole's fate in the aquatic environment. The rate of these processes is influenced by the pH, salinity and the presence of other substances (such as dissolved organic matter) (Duan et al., 2022). For instance, in one laboratory experiment, photodegradation of sulfamethoxazole was observed to be faster under lower pH values (between 3 and 10) and at lower initial concentrations of the antibiotic, fulvic acids and suspended sediments. At pH values between 7.9 and 8.7 sulfamethoxazole is mainly at its anionic form and photodegradation rates will be slower (Oliveira et al., 2019). Sulfamethoxazole undergoing photodegradation in surface water has a half-life ranging from 10h to over 100h (Kovalakova et al., 2020; Duan et al., 2022). Sulfamethoxazole may also undergo adsorption and bioaccumulation processes (Chen et al., 2024).

Antibiotics were usually found at higher concentrations in the dry seasons compared to the wet seasons due to dilution (Yang et al., 2020). Similarly, Hua et al. (2006b) found that trimethoprim concentrations were considerably higher in the summer sampling periods relative to the fall ones.

In general, antibiotics were shown to have the potential to induce chronic effects, such as changes in growth, behavior, and reproduction (Kovalakova et al., 2020). Standard ecotoxicity bioassays indicated that primary producers (e.g. algae) were particularly sensitive to antibiotics (Duan et al., 2022). Accordingly, sulfamethoxazole and trimethoprim were argued to have high toxicity to microorganisms in previous prioritization lists (Lacaze et al., 2015). This is concerning, since long-term alteration of the microbial community composition can lead to the alteration of biogeochemical cycling and the disruption of the aquatic environment (i.e. algal blooms) (Kovalakova et al., 2020).

There was not much data on the non-therapeutic (low-concentration) effects of antibiotics on aquatic biota. The majority of studies were based on acute toxicity assessments at high concentrations (Kovalakova et al., 2020). This is especially the case with trimethoprim.

Kovalakova et al. (2020) found that, compared to other studied antibiotics, trimethoprim imposed relatively low toxicity toward all the tested organisms and was the only antibiotic that was not associated with a high risk to the aquatic environment (Kovalakova et al., 2020). Similarly, Yang et al. (2020) concluded that trimethoprim was not toxic to zebrafish; it was however toxic to green algae at concentrations well above average environmental exposure (Yang et al., 2020). Toxic effects of trimethoprim, including genotoxicity at 200 µg/L and immunotoxicity at 20 mg/L, were observed in the bivalve *Mytilus edulis* (Lacaze et al., 2015). In another study, a 20−40% higher inhibitory effect of trimethoprim and sulfamethoxazole was observed in an acute toxicity test employing the luminescent bacterium *Vibrio qinghaiensis* (Zhang et al., 2016).

Unlike trimethoprim, sulfamethoxazole was associated with more sensitive endpoints. At a concentration of 0.26 µg/L, sulfamethoxazole increased mortality and induced an inflammatory response in zebrafish (Zhou et al., 2016; Yang et al., 2020). It also altered nutrient metabolism, suppressed the innate immune system, inhibited antioxidant capacity, stimulated inflammatory and detoxification responses, and caused lipid peroxidation in the liver and intestine of the fish *Oreochromis niloticus* (Limbu et al., 2018). Exposure to 0.3 µg/L of sulfamethoxazole for 30 days significantly inhibited SOD and CAT activities, causing oxidative stress, in the fish *Ctenopharyngodon idellus* (Zhao et al., 2020). Similarly, GST activity decreased significantly in the liver tissue of *D. rerio* after exposure to 1 and 10 µg/L of sulfamethoxazole for 7 days (Lin et al., 2014).

Evidence of the neurotoxicity of sulfamethoxazole was also observed in fish and rotifera. The antibiotic was found to be genotoxic to goldfish (*Carassius auratus*) (Liu et al., 2014). In the amphipod *Hyalella azteca*, exposure to sulfamethoxazole induced the activity of AChE, indicating genotoxicity (Duan et al., 2022). At a relatively high concentration of 1000 µg/L, exposure to sulfamethoxazole for 24 hours significantly downregulated AChE activity in the monogonont rotifer, *Brachionus koreanus* (Rhee et al., 2013). Yang et al. (2019) investigated the effect of sulfamethoxazole on AChE, SOD and EROD activities in the freshwater crucian carp *Carassius auratus* and found that AChE activity was only inhibited at concentrations higher than 20 µg/L. These endpoints were considered less sensitive and were dependent on high concentrations of the antibiotic.

In terms of metabolites and transformation products, one study argued that the toxicity of the intermediate products of sulfonamides, including sulfamethoxazole, after degradation was lower than the initial compounds (Baran et al., 2006). However, trimethoprim seemed to be an exception, as Arvaniti et al. (2020) performed an in silico toxicity evaluation and found that the toxicity of its transformation products was higher than the parent compound.

As seen in the toxicity endpoint table (Table S5, Appendix B), EC_{50} values for trimethoprim ranged between 0.691 mg/L to the algae *Pseudokirchneriella subcapitata* (Arvaniti et al., 2020) and >200 mg/L to the cyanobacterium *Synechococcus sp.* (Ando et al., 2007). The LC_{50} ranged from 58.8 to 65.7 mg/L for the crustaceans *D.magna* and *Pimephales promleas* respectively (Arvaniti et al., 2020). These concentrations are well beyond the average concentrations reported in Ontario's surface waters in the present study (Table 6). Kovalakova et al. (2020) used

environmental concentrations of trimethoprim in Canada to calculate risk quotients for algae, daphnids and fish. The quotients ranged from 0.0002 for fish to 0.09 for algae, and the authors concluded that the antibiotic is expected to pose low risk at the current concentrations. Uslu et al. (2013), reported a PNEC of 16,000 ng/L for trimethoprim. Using this value with the maximum reported concentration, 274 ng/L, the current study calculates the RQ of 0.02 for trimethoprim, which indicates low risk.

For sulfamethoxazole, past literature reports risk quotients of 17, 0.02, and 0.0009 for algae, daphnids and fish respectively, which illustrated a high risk for algae (Kovalakova et al., 2020). Additionally, Zhou et al. (2016), calculated a risk quotient that is larger than 1. Unlike trimethoprim, sulfamethoxazole is argued to be one of the antibiotics that can pose a great risk to the aquatic system (Duan et al., 2022). Aquatic PNECs for sulfamethoxazole ranged from 520 ng/L (Zhou et al., 2016) to 890 ng/L (Huang et al., 2018). Using the lowest PNEC, the RQ of sulfamethoxazole in Ontario's surface waters was calculated to be 0.09 (45.7/520). This quotient is higher than that of trimethoprim but is still low, suggesting that the current concentrations of sulfamethoxazole in Ontario's aquatic environment pose low ecotoxicological risk.

3.2.5 Lipid Regulators Gemfibrozil & Clofibric Acid

Gemfibrozil (GEM) is a lipid regulator (Lissemore et al., 2006), or a cholesterol reducing agent (Li et al., 2010) that has been clinically prescribed since the early 1980s to patients with high risk of coronary heart disease (Zurita et al., 2007; Hao et al., 2006; Metcalfe et al., 2014). It belongs to the fibric acid derivative class, which work by increasing the activity of lipoprotein lipase and activating peroxisome proliferators and receptors (PPARγ), resulting in reductions in the level of serum triglycerides and very low-density lipoproteins (Blonç et al., 2023; Hu et al., 2022; Zurita et al., 2007). Gemfibrozil is metabolized in the liver after ingestion, and up to 70% of it is excreted with the urine, mostly as gemfibrozil acyl glucuronide (Zurita et al., 2007).

Gemfibrozil was found to be the 4th most reported PHAC in Ontario's surface waters, with a reporting frequency of 44%. The concentration at which it was reported ranged from 0.05 ng/L in the Grand River to 364 ng/L in the Detroit River and averaged 17.2 ng/L (Table 1 and 8). Like several other PHACs in the current study, gemfibrozil is considered an "indicator compound" due to its widespread detection in past literature and can be used to assess contributions of wastewater effluent in receiving waters (Helm et al., 2012; Uslu et al., 2013). Lake Ontario and the Grand River were the most commonly investigated surface waters for gemfibrozil (Table 8).

Table 8. Showing the mean concentrations of gemfibrozil measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. RL: reporting limit, DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Lissemore et al., 2006	13.7	RL =2	1	The Grand River, primarily north and north west

				of Kitchener Waterloo
Hao et al., 2006	13	DL = 12 LOQ = 5	1	Grand River[a]
Servos et al., 2007	6.5		2	DWSs near Burlington
Table et al., 2009	4-364[c]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Li et al., 2010	9.76	DL = 0.1-1 LOQ = 1-10	3	Hamilton Harbour, Humber Bay, and Toronto Barbour (all in Lake Ontario)
Csiszar et al., 2011	33.3	DL = 1.74-3.9	3	Hamilton Harbour
Kleywegt et al., 2011	0.7[b]	DL = 1	1	DWS in Ontario[a]
Helm et al., 2012	0.1-25[c]		2	Pickering Ajax and Port Hope in Lake Ontario
Gillis et al., 2014	0.05		1	Kitchener municipal WWTP in the Grand River
Schwartz et al., 2021	<1-2.9[c]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[a]
Sultana &	22.27	DL = 0.4	4	Presqu'île Bay in

Metcalf, 2022	LOQ = 1.3	Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Exact locations were not provided
[b]Median concentration
[c]Minimum-maximum concentration

Clofibric acid (CA) was the second most reported lipid regulating agent in the current study (reporting frequency of 19%). The reported concentrations ranged from 0.55 ng/L (Servos et al., 2007) to 103 ng/L (Boyd et al., 2003; Jasim et al., 2006) and averaged 34.7 ng/L (Table 1 and 9). Interestingly, more recent studies report relatively lower concentrations of clofibric acid. Clofibric acid is an active metabolite of lipid regulators such as clofibrate, etofyllinoclofibrate, and etofibrate (Ighalo et al., 2020; Boyd et al., 2003; Uslu et al., 2013). It works by binding to the nuclear receptor peroxisome proliferator-activated receptor (PPAR) which increases the number, size, enzyme load and activity of the peroxisomes (Rebelo et al., 2020).

The fact that gemfibrozil and clofibric acid are in the list of the top ten most commonly reported PHACs is not surprising, because following NSAIDs, lipid lowering drugs (e.g. clofibrate, and gemfibrozil) are the most commonly prescribed pharmaceuticals globally (Falfushynska et al., 2022)

Table 9. Showing the mean concentrations of clofibric acid measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. DL: detection limit.

Citation	Concentration (ng/L)	Detection limit	# of sites	Location/water body
Boyd et al., 2003	103[a]	DL = 0.6	1	A.H. Weeks WTP in the Detroit River
Jasim et al., 2006	103	DL = 0.06	1	A.H. Weeks WTP in the Detroit River
Servos et al., 2007	0.55		2	DWS sites in Ontario[b]
Tabe et al., 2009	5-12[c]		1	Little River WWTP and A.H. Weeks WTP in the Detroit River
Schwartz et al., 2021	<1-1.4[c]		2	Two First Nations (FN)

			located in the Mixedwood Plains in the Ontario region[b]

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum concentration
[b]Exact locations were not provided
[c]Minimum-maximum concentration

Jasim et al. (2006) argue that clofibric acid is one of the drugs that have very low removal efficiencies from STPs, which results in their concentrations in surface waters being mainly governed by dilution rather than degradation. Compared to other indicator compounds, clofibric acid has a relatively long half-life ranging from 20.2 and 433.2 days (Shwartz et al., 2021; Araujo et al., 2014), and low degradation rates (Ighalo et al., 2020). Gemfibrozil's half-life was similarly high, ranging between 119 and 288 days in surface water (Araujo et al., 2009; Araujo et al., 2011; Blonc et al., 2023).

Zhang et al. (2018) found that the main mechanism of removal of clofibric acid in the aquatic environment is photodegradation. Factors that affected the photodegradation of clofibric acid include the initial concentration, pH and dissolved oxygen. Photolysis rates decreased with higher initial concentrations of clofibric acid, and higher concentrations of dissolved oxygen (Zhang et al., 2018). For gemfibrozil, Csiszar et al. (2011) estimated 44 to 70% of its loss in surface water to be via degradation and 27 to 51% via export. Like clofibric acid, photodegradation was the main form of degradation governing the persistence of gemfibrozil with volatilization also playing a role (Araujo et al., 2009). Gemfibrozil also exhibited a low potential for adsorption to organic matter, despite what its Log K_{ow} value of 4.77 suggests. This is mainly because gemfibrozil exists mainly in ionic form in water and will thus have negligible net sediment deposition (Araujo et al., 2009; Csiszar et al., 2011).

Gemfibrozil can act as a stress inducing agent and may alter reproduction, development, and overall fitness. The lipid regulator showed a moderate toxic effect on zebrafish fish embryo development, affecting their survival and hatching success and impairing locomotor activity (Henriques et al., 2016). Embryonic malabsorption syndrome in zebrafish larvae was induced after a 5-28 day exposure to 0.00038-0.015 µg gemfibrozil/L (Raldúa et al., 2008). A direct impact on oocyte development was also observed as atretic oocytes and altered ovarian histology occurred in the female zebrafish after a 6-week exposure to 0.5 and 10 µg gemfibrozil/L (Galus et al., 2013).

Reproduction was an endpoint of low to moderate sensitivity for gemfibrozil, depending on the species. When exposed to 1.5 µg gemfibrozil/L, fathead minnows showed no significant physiological or reproductive impacts. After exposure to 1,500 mg/L for 21 days, a considerable but non-significant reduction in fecundity was observed (Skolness et al., 2012). On the other hand, zebrafish exposed to 0.5 and 10 µg/L of gemfibrozil for 6 weeks showed 35% and 41%

decline in fecundity respectively (Galus et al., 2013). In another study, a 67-day exposure of zebrafish to 17.5 µg/L of gemfibrozil caused a similar 44% decline in fecundity (Fraz et al., 2018). In *D. magna*, short-term exposure to gemfibrozil (5-5000 µg/L for 24-48h) was associated with oxidative stress and DNA damage and long-term exposure (5-5000 µg/L for 21 days) delayed first brooding and hatching times, decreased egg production number, and altered the heart rate and the swimming behavior of *D.magna* (Hu et al., 2022).

Lipid regulators have been reported to have a similar mode of action in aquatic vertebrates to the one in humans. As such, exposure to gemfibrozil in adult zebrafish (0.14-1.14 µg/g body weight for 30 days) caused a reduction in cholesterol, triglycerides, cortisol, testosterone, and estradiol. This reduction in cortisol can result in impaired ability to mount a suitable stress response in fish, whereas the reduction of sex steroids may negatively affect reproduction (Al-Habsi et al., 2016). Similarly, fathead minnows exposed to the high concentration of 600 mg gemfibrozil/L for 8 days showed reduced plasma cholesterol levels in males and altered expression of several hepatic genes important to lipid metabolism (Skolness et al., 2012). Adult goldfish (*Carassius auratus*) were exposed to 1.5 µg/L and 15 mg/L of gemfibrozil for 28 days and exhibited mild antioxidant responses only at the high dose and reduced plasma cortisol levels. The lipid regulator was also observed to accumulate in the liver and muscle of *Carassius auratus* (Blonç et al., 2023a). Conversely, Mimeault et al. (2005) illustrated gemfibrozil's potential to be taken up from water (mainly through the gills) and concentrated in goldfish blood and reported a plasma bioconcentration factor (BCF) as high as 113 (Zurita et al., 2007; Araujo et al., 2009). In another study, goldfish exposed to 1.5 and 1,500 µg/L of gemfibrozil for 14 days showed reduced testosterone levels by 49 and 72% respectively, which indicated endocrine disruption and reproductive effects (Mimealt et al., 2005; Araujo et al., 2009).

Like the previous PHACs, the environmental concentrations of gemfibrozil reported in Ontario's surface waters are much lower than the identified concentrations for the toxicity endpoints (see Table S6, Appendix B). However, the calculated risk quotient, using the lowest reported PNEC of 900 ng/L, is 0.4 (17.2/900), which indicates that the current concentrations of gemfibrozil in Ontario surface waters poses a moderate ecotoxicological risk.

Clofibric acid is argued to be less hazardous to the aquatic environment compared to its main parent compound clofibrate (Ighalo et al., 2020). Runnalls et al. (2007) suggest that the toxicological impact of clofibric acid to be mainly reproductive. Long-term exposure to clofibric acid (1 and 10 mg/g via food over the entire lifetime) resulted in a decrease in fecundity, altered male gonad development and increased frequency of embryo abnormalities. Additionally, it showed evidence for multigenerational effects (Coimbra et al., 2015). In fathead minnows (*Pimephales promelas*), exposure to clofibric acid impaired spermatogenesis, reduced sperm count, and altered various measures of sperm mobility including curvilinear velocity and straight-line velocity (Runnalls et al., 2007). Another study also observed declined fecundity in fathead minnows at high concentrations of the metabolite after 21 days of exposure (108.9 mg/L) (Weston et al., 2009).

Other sensitive endpoints for clofibric acid include growth, behavior and oxidative stress. Chronic exposure of zebrafish (*D. rerio*) to clofibric acid resulted in considerable reductions in

growth and triglyceride muscle content (Coimbra et al., 2015). Another study investigated acute (120h) and chronic (60 days) toxicity of clofibric acid (10-170 µg/L) on zebrafish (*D. rerio*) (Rebelo et al., 2020). A suite of effects were observed, including significant behavioral alterations (such as swimming time and total distance traveled), oxidative stress (increased SOD and GPx), and the increase and decrease of the biotransformation enzyme GST at low and high concentrations respectively. The authors did not observe any alterations in terms of sex ratio and maturation stages from chronic exposure to clofibric acid, which suggests that clofibric acid is not an endocrine disruptor (Rebelo et al., 2020).

Overall, both acute and chronic toxicities for clofibric acid were estimated to not be below 14 mg/L (Ighalo et al., 2020). As seen in Table S10, Appendix B, acute toxicity endpoints for clofibric acid ranged from EC_{50} (luminescence) of 91.8 mg/L to EC_{50} (mobility) of >200 mg/L. On the other hand, chronic endpoints ranged from NOEC (reproduction) of 0.246 mg/L to NOEC (embryo and larvae mortality) of 700 mg/L (Ferrari et al., 2003; Ighalo et al., 2020). The PNEC value for clofibric acid is approximately 4920 ng/L (Ferrari et al., 2004; Uslu et al., 2013). Using that value, the RQ is calculated to be 0.02 (103/4920) indicating low ecotoxicological risk at the current concentrations.

3.2.6 Stimulants Caffeine & Cotinine

Caffeine is an alkaloid that is synthesized by more than 60 different plant species. However, its concentration in aquatic ecosystems is argued to be mainly a result of anthropogenic activity (Bruton et al., 2010; Li et al., 2020). In addition to being a lifestyle consumable, caffeine is widely used as a component of several pharmaceuticals (Wilkinson et al., 2022). For example, it is a component of the analgesic acetaminophen (Schwartz et al., 2021). Due to its psychoactive nature, it has also been used for treatment of neurasthenia and coma recovery (Li et al., 2012). It is considered a human activity marker and it is one of the most ubiquitous PHACs in the environment, consistently being detected at high frequencies and concentrations (Wilkinson et al., 2022, Schwartz et al., 2021). Caffeine stimulates the central nervous system as well as the cardiovascular, respiratory, and renal systems (Committee on Military Nutrition Research, 2001; Bruton et al., 2010; Li et al., 2010). It works by 1) inhibiting adenosine receptors 2) inhibiting phosphodiesterase enzymes in skeletal muscle and adipose tissues (increasing intracellular concentrations of cAMP 3) mobilizing intracellular calcium and 4) antagonizing benzodiazepine receptors (Committee on Military Nutrition Research, 2001).

The only other CNS stimulant in the current study's list is cotinine, the main metabolite of the neuroactive compound nicotine (Hua et al., 2006b; Wilkinson et al., 2022; Bellot et al., 2024). Nicotine is also a naturally occurring alkaloid, mainly in tobacco and other Solanaceae plants (Barreto et al., 2014). Around 80% of it is metabolized in the human body to cotinine and excreted through urine. Photodegradation of nicotine in surface waters also produces cotinine (Lian et al., 2017). Like its parent compound, cotinine is a psychoactive compound; it causes neuropharmacological and behavioral effects by crossing the blood brain barrier and interacting with both nAChRs and non-nAChRs in the nervous system (Tan et al., 2021). It is found together with nicotine in many aquatic ecosystems (Bellot et al., 2024), however, in the current study, nicotine was only reported by 1 paper (Wilkinson et al., 2022), whereas cotinine was reported

by 6 out of 26 papers, and was the 9th most reported PHAC (see Table 1 and 11). The reason for this may be that cotinine can be measured concomitantly with a suite of pollutants via solid phase extraction and liquid chromatography/mass spectrometry, whereas nicotine has a higher pK value that requires different methodology and is less likely to be measured by researchers (Beutel et al., 2021).

Cotinine's reported concentration in Ontario's surface waters ranged from 0.3 ng/L in the Detroit River (Hua et al., 2006a) to 137 ng/L in Don River (Wilkinson et al., 2022), with an average of 36.39 ng/L (Table 1). Caffeine had a higher reporting frequency (34%) than cotinine (23%) (Table 1), and its minimum and maximum concentrations (0.5-1110 ng/L) were reported by the same papers (Hua et al., 2006a; Wilkinson et al., 2022). The overall mean concentration of caffeine was 250.59 ng/L. The two stimulants were most commonly reported in the Detroit River and Lake Ontario (Tables 10 and 11).

Table 10. Showing the mean concentrations of caffeine measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Hua et al., 2006a	0.5-10[a]	LOQ = 1	1	The Little River wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Hua et al., 2006b	10-42[a]	LOQ = 1	1	A.H. Weeks Water Treatment Plant (WTP) in the Detroit River
Jasim et al., 2006	4.7		3	A.H. Weeks WTP in the Detroit River
Li et al., 2010	12.36	DL = 0.1-1 LOQ = 1-10	7	Niagara River, Hamilton Harbour, Humber Bay, Toronto Harbour, Newcastle Harbour, Bay of

				Quinte and MSC Buoy (all in Lake Ontario)
Helm et al., 2012	2.9-28[a]		2	Pickering-Ajax and Port Hope
Staley et al., 2016	1.5-2.7[a]		8	Humber River and Black Creek Tributary
Schwartz et al., 2021	21.4-502[a]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[b]
Wilkinson et al., 2022	525.27		12	Ottawa (Ottawa River) and Toronto (Don River and Lake Ontario)
Sultana & Metcalf, 2022	158.52	DL = 1 LOQ = 3.3	4	Presqu'île Bay in Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Exact locations were not provided

Table 11. Showing the mean concentrations of cotinine measured in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. LOQ: limit of quantification.

Citation	**Concentration (ng/L)**	**Detection limit**	**# of sites**	**Location**
Hua et al., 2006a	0.3-0.6[a]	LOQ = 0.1	1	A.H. Weeks Water Treatment Plant (WTP) in the Detroit River
Hua et al., 2006b	1-14[a]	LOQ = 0.1	1	The Little River

				wastewater treatment plant (WWTP) and the A.H. Weeks WTP in the Detroit River
Jasim et al., 2006	0.43		3	A.H. Weeks WTP in the Detroit River
Staley et al., 2016	0.9-1.7[a]		8	Humber River, Black Creek Tributary
Schwartz et al., 2021	10.4-50.7[a]		2	Two First Nations (FN) located in the Mixedwood Plains in the Ontario region[b]
Wilkinson et al., 2022	60.15		6	Toronto (Don River and Lake Ontario

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Minimum-maximum concentration
[b]Exact locations not provided

Cotinine is known to have a higher stability and half-life than its parent compound nicotine (Valcárcel et al., 2011). However, its half-life, an average of 19 hours, is still relatively low compared to other PHACs in the current study (Gray & Hall, 2014). Cotinine displays high mobility in the aquatic environment. For instance, it was detectable in all extracted surface water samples, including those at the shoreline sites and across a wide range of several kilometers across the Detroit River (Hua et al., 2006b). Buerge et al. (2008) explained that the aerial transport, photodegradation, and deposition of nicotine results in this widespread occurrence of cotinine. In a similar fashion, Helm et al. (2012) found caffeine's distribution to be widespread and with little variation over the study area, suggesting that background concentrations were stronger than the point source (WWTP effluent release) they were investigating.

Caffeine generally shows excellent removal efficiencies during wastewater treatment of above 94% (Bruton et al., 2010; Li et al., 2020). It also exhibits rapid removal in the aquatic

environment, with a relatively low average half-life of 1.5 days. Once it enters the environment, it is quickly degraded mainly via photodegradation caused by sunlight (Bruton et al., 2010). As laboratory experiments previously indicated, caffeine is less likely to be removed by hydrolysis or biodegradation (Lam et al., 2004; Bruton et al., 2010; Korekar et al., 2020). It also exhibits a moderate adsorption affinity in sediment (Li et al., 2020). Contrary to what its low lipophilicity implies, caffeine was observed to bioaccumulate in organic tissues after long-term exposure; it was detected in tissues of clams, fishes, macroalga and other aquatic plants (Li et al., 2020). Li et al. (2020) theorized that this bioaccumulation potential can be influenced by other lipid-independent mechanisms. Despite its rapid removal, concentrations of caffeine are still expected to be relatively high and stable as it is continuously released into the aquatic environment at considerable concentrations (Bruton et al., 2010). In terms of seasonal variation, caffeine was observed to be elevated in early/late spring by Hua et al. (2006b); and consistent between the months of June and September (Helm et al., 2012).

Due to its stimulant nature, caffeine can impact behavioral endpoints in fish. For instance, both fathead minnow and zebrafish exhibited lower photomotor responses in dark conditions and higher responses in light conditions after a 4-day exposure to caffeine (Steele et al., 2018). Various other biological effects were also observed with exposure to environmentally relevant concentrations of caffeine. Freshwater teleost (*Prochilodus lineatus*) exposed to 0.3-30 µg/L of caffeine for 7 days had a significant reduction in the activity of the biotransformation enzyme EROD in the brain but a significant increase in the liver (Santos-Silva et al., 2018). When exposed to caffeine at variable concentrations for 21 days, the bivalve *Corbicula fluminea* showed decreased lysosome stability (15 and 50 µg/L) as well as increased EROD and BDF activities in the digestive gland (15 and 50 µg/L) and increased DNA damage (50 µg/L) (Aguirre-Martinez et al., 2015). In a following study, caffeine exposure to the same species caused inflammation (5-50 µg/L), increased dopamine levels in the gonads (50 µg/L) and energy expenditure (15-50 µg/L). Additionally, it significantly inhibited AChE activity at 5 µg/L and increased it at 50 µg/L (Aguirre-Martinez et al., 2018). In the goldfish (*Carassius auratus*), exposure to 0.08 mg/L caffeine for 7 days caused genotoxicity and general stress by decreasing AChE activity, and increasing EROD, GST and SOD activities in the liver. At higher concentrations of 2 mg/L, serum levels of vitellogenin, a biomarker for detecting estrogenic contamination, increased in male goldfish (Li et al., 2012). Finally, the stimulant reduced lysosomal membrane stability and induced embryogenesis in *Hydra attenuata* (Quinn et al., 2008) and *Paracentrotus lividus* respectively.

Exposure to cotinine has been shown to cause neuroactive effects and mild developmental toxicity (Tan et al., 2021). Like nicotine, cotinine is able to quickly the blood-brain-barrier and accumulate in the central nervous system; unlike nicotine, it is considered a weak agonist of nAChRs indicating less potency (Tan et al., 2021; Bellot et al., 2024). One non-peer reviewed study exposed zebrafish *Danio rerio* to environmentally relevant concentrations of cotinine and found that it resulted in neurobehavioral disorder, significantly altering 11 neurotransmitters (Yang et al., 2023). When exposing zebrafish embryos to nicotine (0.002-2.5 µg/L) and cotinine (50 pg/L-10 µg/L) for 24 hours, nicotine resulted in hyperactivity, increased light-off visual motor response, decreased vibrational startle response and increased non-associative learning.

Whereas cotinine only influenced the increase of light-off visual motor (VMR) response in zebrafish embryos. Interestingly though, the impact cotinine had on VMR was 40-fold that of nicotine (Bellot et al., 2024).

All the acute and chronic toxicity endpoint concentrations for caffeine (Table S7, Appendix B), the lowest of which is a NOEC of 5000 ng/L, were much higher than measured environmental concentrations. Using the lowest reported PNEC, 4 ng/L estimated by Rodriguez-gil et al. (2018), the RQ for caffeine is calculated to be 277 (1110/4). As a result, high environmental risk is expected from chronic exposure to caffeine from surface water. Like caffeine, the acute and chronic toxicity endpoints for cotinine (Table S9, Appendix B) were much higher than the concentrations reported in Ontario's surface waters. However, using the lowest reported PNEC, 589 for algae (Valcárcel et al., 2011), the RQ for cotinine was calculated to be 0.2 (137/589), which suggests that cotinine poses moderate risk to exposed aquatic communities.

3.2.7 Antidepressant Venlafaxine

Venlafaxine is a neuro-active drug that is widely prescribed as a first-line antidepressant (Helm et al., 2012; Arlos et al., 2015; Couperus et al., 2016; Ribeiro et al., 2022) and to manage anxiety (Sehonova et al., 2018). It is derived from bicyclic phenylethylamine and is part of the serotonin and noradrenaline (norepinephrine) reuptake inhibitors class (Qu et al., 2019; Sehonova et al., 2018). Venlafaxine mainly acts at the synaptic junction to prevent the reuptake of serotonin and, to a lesser extent, noradrenaline. It also has a weak effect on dopamine reuptake (Sehonova et al., 2018). In the current study venlafaxine had a reporting frequency of 26%, and it was ranked number 8 on the list of the most commonly reported PHACs. The measured concentrations of this compound ranged from 0.1 ng/L in Pickering-Ajax (Helm et al., 2012) to 100 ng/L in the Speed River (Arlos et al., 2015) and the overall mean concentration was 16 ng/L (see Table 1 and Table 21). Venlafaxine was most commonly reported in the Grand River and Lake Ontario (Table 21).

Table 21. Showing the measured mean concentrations of venlafaxine in surface waters upstream or at least 100 m downstream of WWTPs and in raw water of drinking water stations (DWSs) across the Ontario Region. DL: detection limit, LOQ: limit of quantification.

Citation	Concentration (ng/L)	Detection limit (ng/L)	# of sites	Location
Li et al., 2010	2.69	DL = 0.1-1 LOQ = 1-10	7	Niagara River, Hamilton Harbour, Humber Bay, Toronto Harbour, Newcastle Harbour, Bay of Quinte and MSC Buoy (all in Lake Ontario

Metcalf et al., 2010	0.13		3	Two WWTPs and one DWS in the Grand River[a]
Helm et al., 2012	0.1-6.7[b]		2	Pickering-Ajax and Port Hope
Tanna et al., 2013	32.5		6	The Waterloo, Kitchener, and Guelph municipal WWTPs in the Grand and Speed Rivers
Arlos et al., 2015	38.3	DL = 1	9	Waterloo, Kitchener, Preston, Galt (Grand River) and Guelph and Hespeler (Speed River) municipal WWTPs
Couperus et al., 2016	16.5		2	Grand River main channel and tributary (Canagagigue Creek, Conestogo River, Nith River, Speed tributary, Whiteman's Creek)
Wilkinson et al., 2022	31.6		2	Toronto (Don River and Lake Ontario)

*Blank cells in the detection limit column indicate that none were provided by the study
[a]Exact locations not provided
[b]Minimum-maximum concentration

Like several other PHACs in the present study, venlafaxine is difficult to remove completely in the wastewater treatment process (Qu et al., 2019), and it is known for its recalcitrant

(Couperus et al., 2016) and persistent nature (Arlos et al., 2015). It is also considered a little more persistent compared to other antidepressants in the aquatic environment (Lacaze et al., 2015). In the surface waters of Ontario, venlafaxine usually spiked near WWTPs and persisted several kilometers downstream (Couperus et al., 2016; Arlos et al., 2015). Studies indicated that direct photodegradation and biodegradation of venlafaxine occurs at very slow rates in surface water and are not a significant removal pathway (Rúa-Gómez & Püttmann, 2013; Singh et al., 2022). Additionally, hydrolysis was unlikely to occur (Rúa-Gómez & Püttmann, 2013). On the other hand, biosorption seems to be more likely (Singh et al., 2022), and indirect photodegradation is suggested to be the key mechanism for the ongoing removal and degradation of the antidepressant in surface waters (Rúa-Gómez & Püttmann, 2013).

Since it is a neuroactive drug that affects the central nervous system and causes decreased psychomotor activity and a sedative effect, venlafaxine is expected to have various behavioral impacts on aquatic biota (Ribeiro et al., 2022). Its metabolite, O-desmethylvenlafaxine, has a similar chemical construction and is thus predicted to have a similar toxicological effect (Qu et al., 2019). Painter et al. (2009) found that escape responses were slowed in larval fathead minnows when exposed to 5 µg/L of venlafaxine over a period of 12 days. Similarly, on the sixth day of exposure to venlafaxine concentrations of 50, 250, and 500 µg/L, hybrid striped bass (*Morone saxatilis x Morone chrysops*) experienced a significant increase in the time to capture prey (Bisesi et al., 2014). Chabenat et al. (2021) found alterations in the locomotor activities (cryptic behavior) of the crustacean *Carcinus Maenas* after exposure to 5 ng/L for 25 days. Adult mosquitofish (*Gambusia holbrooki*) exposed to 100 µg venlafaxine/L for 7 days exhibited a disturbed circadian rhythm with decreased locomotion during the day (Melvin, 2017). When exposed to 31-313 µg/L of venlafaxine for one hour, *Ilyanassa obsoleta* snails took significantly more time to right themselves (Fong et al., 2017). Thompson et al. (2017) wanted to investigate the impact of potential maternal transfer of venlafaxine by injecting the antidepressant in the eggs of zebrafish (*Danio rerio*). They found that venlafaxine altered brain development, which resulted in changed behavior and disruption of neurogenesis in the preoptic area and the hypothalamus.

In terms of oxidative stress, Qu et al. (2019) exposed loach (*M. anguillicaudatus*) to venlafaxine (0-500 µg/L) for 4 days and observed elevated levels of oxidative stress indicators SOD and MDA in the liver. Reproduction was another sensitive endpoint for venlafaxine. Adult zebrafish exposed to the antidepressant at a concentration of 10 µg/L for 6 weeks had significantly reduced embryo production. Additionally, increased malformations in the larvae of the fish *D. rerio* were observed after their exposure to 0.3-3000 µg/L for 96 hours (Ribeiro et al., 2022). The chemical structure of antidepressants generally includes the aromatic ring, the fluorobenzene group and the nitro group, which are considered potentially mutagenic and carcinogenic components able to induce DNA damage. Accordingly, DNA damage was observed in mussel hemocytes exposed to venlafaxine (Lacaze et al., 2015).

Heart development was disrupted and cardiovascular performance was compromised when newly fertilized *D.rerio* fish were exposed to venlafaxine (0-10 ng/L) for 48 hours (Thompson et al., 2022). The zygotic venlafaxine exposure of 1–10 µg/L for 6–8 months in *D.rerio* attenuated the cortisol levels (critical for restoring animal homeostasis) in females and indicated long-term

and generational repercussions in terms of cortisol stress activity disruption (Thompson & Vijayan, 2021). However, in one study by Parrott and Metcalfe (2017), fathead minnows (*P. promelas*) exposed to 0.88 and 8.8 µg/L venlafaxine over their full life cycle exhibited no significant adverse effects in terms of survival, development, and reproduction. Sehonova et al. (2018), argue that this might be due to the fact that when exposed to venlafaxine over their whole life cycle, the fish were able to adapt. This indicates that development can be a relatively less sensitive chronic endpoint, and highlights the importance of long-term tests.

Venlafaxine and its metabolite, O-desmethylvenlafaxine were recently added to the Water Framework Directive in the 3rd European Union Watch List (Gomez Cortes et al., 2020; Ribeiro et al., 2022) and are viewed as potential priority substances that need to be monitored and assessed for environmental risk in European aquatic waters (Ribeiro et al., 2022). Due to the small amount of endpoint toxicity data for venlafaxine in the past literature, it doesn't have a toxicity endpoint table in Appendix B. Briefly, Minguez et al. (2014) exposed the crustacean *Daphnia magna* to venlafaxine and found the EC_{50} for immobilization and the LC_{50} for lethality to be close at ~141.3 mg/L. A following study by Minguez et al. (2018) calculated the IC_{50} for population growth inhibition for the green algae *Raphidocelis subcapitatai* to be 47.5 mg/L. Singh et al. (2022) provided PNEC values of 653, 1060, and 7680 ng/L for algae, daphnids and fish respectively. They also stated that venlafaxine's RQ is lower than 0.6 and it therefore poses a medium risk to the environment. The RQ of venlafaxine calculated by the current study was 0.06, based on the maximum environmental concentration of 38.3 ng/L and the lowest reported PNEC of 653 ng/L. This quotient indicates a low ecological risk.

4 Conclusion

The current study was interested in identifying the ten most commonly reported PHACs in Ontario's water bodies and concluding whether or not their measured concentrations are of concern to aquatic environments and require regulatory action. Twenty-six papers published between 2003 and 2024 were selected through a systematic literature review and a total of 112 pharmaceutical compounds were identified to exist in surface waters mainly in southern Ontario. The ten most frequently reported PHACs were carbamazepine, naproxen, ibuprofen, trimethoprim, gemfibrozil, caffeine, sulfamethoxazole, venlafaxine, cotinine, and clofibric acid (listed in terms of highest to lowest reporting frequency). The compound with the highest average environmental concentration was caffeine followed by ibuprofen, naproxen, cotinine, clofibric acid and carbamazepine, with the rest of the compounds having average concentrations <25 ng/L. Unsurprisingly, most of these identified PHACs are also commonly detected at high frequencies around the world and have been previously coined "indicator compounds" that can be used as indicators for anthropogenic activities and wastewater effluent release (Helm et al., 2012; Metcalf et al., 2014).

There is a general consensus that despite the varying influencing factors and toxicity mechanisms, an agent that was observed to have a toxic effect on one organism is expected to have a similar effect on others (Dökmeci et al., 2014). However, assessing the aquatic toxicity of PHACs can be a very complex subject as it is highly influenced by various factors such as the

physicochemical properties of the compound, the species and their developmental stages, as well as the environmental conditions. For instance, salinity, temperature, and pH were found to influence the toxicity of antibiotics in the aquatic environment, however, no consistent trends have been identified (Huang et al., 2020; Duan et al., 2022). Seasonal considerations are another example of the influence of environmental conditions; carbamazepine, the most commonly reported PHAC, was found to exhibit its highest concentrations in the summer and early fall as a result of lower flow rates. A lot can be inferred from the physicochemical properties of PHACs: some studies find the Log K_{ow} to be positively correlated with toxicity (Cleuvers, 2004; Minguez et al., 2014), due to the fact that most PHACs act by nonpolar narcosis (Cleuvers, 2004). However, as seen in Table 22, the current study did not observe this trend.

Table 22. A comparison between the calculated risk quotients (RQs), listed in descending order, and the octanol-water partition coefficients (Log K_{ow}) for the top ten most reported pharmaceuticals in Ontario's surface water.

Pharmaceutical	RQ	Log K_{ow}
Caffeine	277	-0.13
Ibuprofen	28	3.5
Carbamazepine	1.016	2.45
Gemfibrozil	0.4	4.77
Cotinine	0.2	0.34
Sulfamethoxazole	0.1	0.89
Venlafaxine	0.06	3.2
Naproxen	0.02	3.18
Trimethoprim	0.02	0.91
Clofibric Acid	0.02	2.6

All of the ten PHACs identified in the current study were found to impact several acute and chronic toxicity endpoints and biomarkers on at least two taxonomic classes (see Appendix D for toxicity endpoints). Most of these toxic effects were however observed at concentrations of µg-mg/L, which is at least x1000 times higher than the reported environmental concentrations of ng/L. Studies rarely investigate the environmentally more relevant concentrations lower than 100 ng/L, as they are less likely to result in observable effects (Jarvis et al., 2014; Bali et al., 2020). Despite that, it is important to note that the observed effects at higher concentrations indicate that it is likely that prolonged exposure to typical environmental concentrations will still result in the same adverse effects, potentially influencing future generations and altering biodiversity (Baali et al., 2022; Trognon et al 2023). It is also important to consider the impact of

1) exposure to the various and potentially interacting PHACs and other pollutants, 2) metabolites and transformation products, 3) synergism and additivity and 4) bioaccumulation.

Pharmaceuticals in the environment can get diluted, transported, bio-accumulated in organic matter, and degraded (bio- and/or photodegradation). The degradation process can produce a variety of active metabolites and transformation products that may have vastly different fates, biological activities, and toxicities compared to the parent compound. Conversely, more than 30 carbamazepine metabolites, including pharmacologically active or genotoxic compounds, have been identified in humans (Liu et al., 2023). In one study investigating the chronic toxicity of some of these transformation products, 95% were found to be toxic, and an average of 38% were found to be very toxic (Trognon et al., 2023). These compounds will be present in complex mixtures that can have unpredictable effects on sensitive life stages or populations and the impact of their long-term exposure will be very difficult to assess (Servos et al 2007).

These active metabolites, along with other PHACs and pollutants can also exhibit synergistic or additive toxicity to the aquatic biota (Desbiolles et al., 2020; Baali et al., 2022). Indeed, mixtures can exhibit significant toxicities, even at concentrations at which the individual compounds showed no or very slight effects. For instance, one study found that the toxicity ($EC_{50\text{-}80}$ values) of the mixture of diclofenac, ibuprofen, naproxen, and acetylsalicylic acid was equal to or higher than what was predicted by concentration addition (Cleuvers, 2004). Yang et al. (2019) also suggested that the effects of ofloxacin, sulfamethoxazole and ibuprofen can be additive on several physiological indices (AChE, EROD, and SOD activities) in *Carassius auratus*. Conversely, fish exhibited altered diurnal activity patterns when exposed to a combination of antidepressants (fluoxetine, sertraline, and venlafaxine) at doses of 1 µg/L and 100 µg/L (Melvin et al., 2017). Finally, when considering the effect of other pollutants, Qu et al. (2019) found that co-exposure of PHACs, specifically venlafaxine and its metabolite, with microplastics increases their toxicity, bioavailability and bioaccumulation factor (10-fold increase). The authors concluded that microplastics acted as a "vehicle" for the antidepressant (Qu et al., 2019).

Understanding the bioavailability of contaminants is fundamental in the assessment of their potential aquatic toxicity. Bioavailability is defined as "the fraction of the chemical present in sediment and water that can potentially be accumulating in the tissues of organisms during their lifetime" (Chen et al., 2024). In general, compounds that have a Log K_{ow} > 3 have a relatively high capacity for bioaccumulation in the organism's tissues (Dökmeci et al., 2014). In the current study, naproxen, ibuprofen, gemfibrozil, and venlafaxine all have Log K_{ow} > 3 and therefore have a potential to bioaccumulate. Additionally, other compounds, such as carbamazepine, sulfamethoxazole, and caffeine were found to exhibit considerable bioaccumulation factors despite their relatively low Log K_{ow} values (see sections 3.2.1, 3.2.4, and 3.2.6 respectively). The fact that the majority of the commonly reported PHACs in this study have the ability to bioaccumulate in organic tissue is concerning and increases their potential toxicological risk.

Finally, another interesting point is the range of sensitivities different species have to different PHACs. Most of the PHACs in the current list were associated with a large range for L(E)C_{50} values reported in the past literature, indicating that their toxicity differs across species (Duan et

al., 2022). For instance, lipid regulators (gemfibrozil and clofibric acid) seem to be more toxic to fish, whereas antibiotics (trimethoprim and sulfamethoxazole) pose a higher risk to primary producers. Toxicity to primary producers, such as microbial community composition, can have cascading effects on the rest of the food chain, leading to the alteration of biogeochemical cycling and the disruption of the aquatic environment (Kovalakova et al., 2020).

To quantitatively assess the toxicity of the identified PHACs, the current study used risk quotients, which are a function of the environmental concentration of the compounds and their potential toxicity. In general, RQs higher than 1 pose high ecotoxicological risk, whereas RQs between 0.1-1 and lower than 0.1 indicate moderate and low ecotoxicological risk respectively. As seen in Table 22 six out of the ten PHACs had RQs indicating moderate to high ecotoxicological risk. Caffeine, ibuprofen, and carbamazepine were the only PHACs in the list with RQs > 1. Gemfibrozil, cotinine, and sulfamethoxazole had RQs ranging from 0.1 to 1, and venlafaxine, naproxen, trimethoprim, and clofibric acid all had RQs lower than 1.

Caffeine was the PHAC with the highest measured concentrations across Ontario's surface waters. It is highly degradable but the level of its consumption and release continues to generate high concentrations in the environment. At environmentally realistic concentrations, it had the potential to cause lethality, oxidative stress, neurotoxicity, and lipid peroxidation, as well as affect energy reserves and metabolic activity, reproduction and development (see section 3.2.6). Ibuprofen and carbamazepine were also two of the most concerning PHACs from the list compiled in the current study, as they both had high 1) reporting frequencies 2) intensities and 3) RQs. Past studies have shown that exposure to ibuprofen at environmentally relevant concentrations could result in effects such as cytotoxicity and genotoxicity, general stress, and altered growth rate, reproduction, and behavior (section 3.2.2). Whereas carbamazepine was shown to cause mortality, oxidative stress, neurotoxicity, reproductive or growth inhibition, developmental effects and morphological changes (section 3.2.1). It was previously described by the European legislation on the classification and labeling of chemicals as "harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment" (Zhang et al., 2008). However, it is not included in the list of priority substances in surface water under Annex X of the European Water Framework Directive (Moermond & Smit, 2016; Water Framework Directive (2000/60/EC)). Carbamazepine was also associated with highly toxic metabolites. Ibuprofen was the only PHAC that had a toxicity endpoint (NOEC for *O. latipes* of 100 ng/L) that was lower than the maximum concentrations reported in Ontario's surface waters (see Table 1, section 3.2 and Table S4, Appendix B).

Regarding these three high risk category PHACs, the current study recommends that the government of Ontario not only implement monitoring programs to evaluate their ambient concentrations across Ontario, but also develop measures to reduce their concentration in the environment.

The moderate risk category included the lipid regulator gemfibrozil, the nicotine metabolite cotinine, and the antibiotic sulfamethoxazole. The main recommendation for this category is to implement monitoring programs and to conduct research that allows for a better understanding of their potential toxicity. Gemfibrozil, as concluded in section 3.2.5, 1) has a relatively high

persistence in the aquatic environment, 2) is more toxic to fish (vertebrates) and 3) mainly influences lipid metabolism, reproduction, genotoxicity, development, and immunity. Sulfamethoxazole, on the other hand, was shown to be more toxic to microorganisms (i.e. algae). It is a compound that exhibits moderate mobility and high chemical stability in the aquatic environment, and its transformation products were argued to have lower aquatic toxicity (section 3.2.4). The current study did not investigate the toxicity of cotinine as much as the other PHACs since most of its concentration in the environment is a result of the contamination of the urine of tobacco smokers and not pharmaceutical pollution. There was also little data on its aquatic toxicity as an individual rather than in a mixture with other compounds that regularly leach from cigarettes. However it exists at concentrations high enough to produce a considerable RQ, and its parent compound nicotine was considered more toxic (section 3.2.6). Nicotine in the current study was only reported by Wilkinson et al. (2022), but it had the highest average concentration out of the 112 PHACs reported in past literature (see Table 2, section 3.2). As a result, the current study argues that, out of the two, nicotine should be prioritized over its metabolite in the monitoring and research initiatives.

Finally, for the low risk category that includes venlafaxine, naproxen, trimethoprim, and clofibric acid, the current study recommends that the government of Ontario invests in better assessments of their aquatic toxicity using Canadian data and allow for the results to be openly available for future researchers to use. This recommendation is arguably applicable to all the PHACs discussed in the present study. In answer to the research question posed at section 1, the current study finds that 1) pharmaceuticals and their active ingredients are present at considerable frequencies and intensities in Ontario's surface waters and 2) at least 6 of them are estimated to cause moderate to high ecotoxicological risk, requiring some form of action from the provincial government.

As a result of the large level of uncertainty associated with pharmaceutical pollution and its socioeconomic impacts (OECD, 2019), there is usually little global interest in establishing drug detection programs in aquatic ecosystems (Jan-Roblero et al., 2023) and few legally binding regulations or guidelines on how to dispose of pharmaceutical waste properly (Zeeshan et al., 2021). In Canada, there is no formal centralized surveillance system to track the presence of pharmaceutical contaminants in water bodies. However, some provincial, municipal, and community initiatives exist for the collection and disposal of pharmaceuticals. Examples are Alberta's environmentally friendly medication disposal program that is funded by the Alberta Pharmacists' Association, and British Columbia's Medications Return Program, which is managed by the Health Products Stewardship Association (Gagnon 2009; de Oliveira Souza et al., 2021). In Ontario, O. Regulation 298/12 mandates that the producers are the ones responsible for the collection, recycling and/or disposal of the pharmaceuticals, and their packaging, purchased by their customers. Overall, pharmaceutical disposal legislation is considered decentralized (de Oliveira Souza et al., 2021) and these initiatives are estimated to only collect a small fraction of unused and expired pharmaceuticals (Zeeshan et al., 2021; Kelvin & Art, 2014). There are also no mandatory monitoring procedures or emission limits on pharmaceutical disposal into lakes, and no provincial or federal regulations on pharmaceuticals and their residues in hospital sewage waters (Zeeshan et al., 2021). The results of the current

paper provides information that will hopefully facilitate the prioritization of pharmaceuticals and their active compounds in the process of making appropriate and much needed protection policies in the future.

Internationally, there is a similar lack of regulations and guidelines for these indicator compounds in aquatic systems. One of the most comprehensive water quality legislations is the European Water Framework Directive (2000/60/EC), which aims to ensure the health of inland, transitional, coastal and ground waters. This directive applies to all European Union member states and covers a wide range of water-related issues, including pollution control, water quality monitoring, ecological assessment, and river basin management (European Commission, 2023). Annex X of the WFD provides a list of priority substances in surface waters (Moermond & Smit, 2016; Water Framework Directive (2000/60/EC)), and environmental quality standards (EQSs) are established for these substances, serving as concentration limits in water bodies that member states must abide by. In some cases, EQSs can lead to compounds being phased out of production (Johnson et al., 2013). Surprisingly, none of the ten PHACs discussed in the current study are in this list. Currently, only three pharmaceuticals, diclofenac, E2 (17β-estradiol) and EE2 (17α-ethinylestradiol), are part of the watch list and require regular monitoring in all European Union member states (Johnson et al., 2013; Moermond & Smit, 2016).

To conclude, the design of the present study presented a few limitations. First, the sampling locations used in the eligible studies were all clustered in southern Ontario near densely populated urban areas and regions with high agricultural runoff potential (Figure 3, section 3.1). As a result, the data used was really only representative of a small portion of the identified study area. Second, due to the diverse methodologies used for sampling and analysis in previous studies, the data collected for the current study was highly unstandardized. For instance, as discussed in section 3.1, samples were analyzed in at least nine different laboratories, introducing lab-specific biases. Finally, past research really only observed and reported PHACs that were specifically measured for. Due to budget and time constraints, studies rarely measure for a diverse and large suite of PHACs, opting instead for a select few. Consequently, the ten most commonly reported PHACs were typically those previously recognized as “indicator compounds” for anthropogenic activities due to their high detection frequencies and concentrations on a global scale.

5 Appendixes

Appendix A. Methods

Table S1. Showing the synthesis matrix used for phase 1 of the data collection stage.

Pharmaceutical	**Citation**	**No. of observations**	**Site ID/#**	**Concentration**	**Detection limit**	**Region/ Location**	**Comments**
	Study authors and publication date of the study that reports the pharmaceutical	The count of studies that report the pharmaceuticals	The site or sample names, numbers or IDs as reported in the study	Minimum, mean, median, maximum concentration	The study's detection limit, limit of quantification, limit of detection, or reporting limit as provided by the study	Where the observation was made in Ontario Type of waterbody	Any mentions of toxicity Other considerations such as strength of evidence

Appendix B. Toxicity Endpoints

The following tables list a number of acute and toxicity endpoints for carbamazepine, naproxen, ibuprofen, trimethoprim, gemfibrozil, caffeine, sulfamethoxazole, cotinine and clofibric acid. EC_{50} denotes the half maximal effective concentration, LC_{50} is the lethal concentration 50, NOEC and LOEC stand for no observed effect concentration and lowest observed effect concentration, and finally, IC_{50} is half maximal inhibitory concentration.

Table S2. Acute and chronic toxicity endpoints of carbamazepine to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Bacteria	*V.fischeri*	30min	EC_{50} (luminescence)	>81	Ferrari et al., 2003
	Crustaceans	*D.magna*	48h	EC_{50} (mobility)	>13.8	
		C.dubia	48h	EC_{50} (mobility)	77.7	
		Ceriodaphnia dubia	48h	LC_{50} (mortality)	7.07	Batucan et al., 2020
	Algae		96h	$L(E)C_{50}$	0.26	Lin et al., 2020
		Chlorella pyrenoidosa	96	EC_{50}(growth)	49.4	Batucan et al., 2020
	Daphnid		48h	$L(E)C_{50}$	14.07	Lin et al., 2020
	Fish		96h	$L(E)C_{50}$	40.92	Lin et al., 2020
		Danio rerio	72h	LC_{50}	>245	Batucan et al., 2020
Chronic	Algae	*P. subcapitata*	96h	NOEC (growth)	100	Ferrari et al., 2003
		Chlorella pyrenoidosa	30d	EC_{50} (growth)	7	Batucan et al., 2020

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
		Scenedesmus obliquus	30d	EC_{50} (growth)	0.8	
	Rotifers	*B. calyciflorus*	48h	NOEC (reproduction)	0.377	Ferrari et al., 2003
	Crustaceans	*C. dubia*	7d	NOEC (reproduction)	0.025	Ferrari et al., 2003
	Fish	*Pimephales promelas*	28d	NOEC (mortality)	0.862	Batucan et al., 2020
				NOEC (growth)	0.68	
		D. rerio	10d	NOEC (embryos and larvae mortality	25	Ferrari et al., 2003

Table S3. Acute and chronic toxicity of ibuprofen to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Algae	*D. subspicatus*		EC_{50}	342.2	Cleuvers, 2004
	Cnidarian	*Hydra vulgaris*	96h	LC_{50} (mortality)	22.36	Huang et al., 2018
				LC_{50} (inhibits 50% of the embryos to develop)	3.84	Quinn et al., 2008
	Platyhelminthes	*Dugesia japonica*	96h	LC_{50} (mortality)	11.1	Huang et al., 2018
	Crustaceans	*T. battagliai*	48h	LC_{50}	49.7	Trombini et al., 2016
		A. desmarestii	96h	LC_{50}	13.3	Nieto et al., 2013
		T. platyurus	24h	LC_{50}	19.59	Kim et al., 2009
		D. magna	48h	EC_{50} (immobilization)	51.4	Han et al., 2010
					101.2	Cleuvers, 2004
			24h	EC_{50}	3.97	Du et al., 2016
		M. macrocopa	48h	EC_{50} (immobilization)	72.6	Han et al., 2010
	Molluscs	*P. carinatus*	48, 72h	LC_{50}	17.1	Pounds et al., 2008
	Fish	*Cirrhinus mrigala*	24h	LC_{50} (mortality)	142	Huang et al., 2018

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
		O. latipes	96h	LC_{50}	>100	Kim et al., 2009
	Bacteria	*A. fischeri*	30 min	EC_{50} (reduction in luminescence)	39.93	Dökmeci et al., 2014
Chronic	Crustaceans	*D. magna*	21d	NOEC (reproduction)	<1.23 mg/L	Han et al., 2010
			14d	EC_{50}	13.4	Heckmann et al., 2007
		M. macrocopa	7d	Reproduction NOEC	25	Han et al., 2010
	Molluscs	*P. carinatus*	21d	NOEC (survival)	5.36	Pounds et al., 2008
				NOEC (reproduction)	2.43	
				NOEC (growth)	1.02	
	Fish	*O. latipes*	120d	NOEC (survival)	0.0001 mg/L	Han et al., 2010

Table S4. Acute and chronic toxicity of naproxen to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Hydrozoa	*Hydra magnipapillata*	24h	LC_{50}	51.99	Yamindago et al., 2019
			48h		44.935	
			72h		42.50	
	Algae	*Cymbella sp.*	72h	EC_{50} (growth)	102.76	Ding et al., 2017
		Scenedesmus quadricauda	72h		101.45	
		Scenedesmus subspicatus	72h		625.5	Cleuvers, 2004*
	Crustacean	*Ceriodaphnia dubia*	48h	EC_{50} (immobilization)	66.4	Isidori et al., 2005*
		Daphnia magna	48h		46.72	Gheorghe et al., 2016
		D. magna	48h		166.3	Cleuvers, 2004*
			48h		85.34	Kwak et al., 2018
		Moina macrocopa	48h		74.13	
	Fish	*Cyprinus carpio*	96h	LC_{50}	269.15	Gheorghe et al., 2016
		Danio rerio (embryo)	96h		115.2	Li et al., 2016
		D. rerio (larvae)	96h		147.6	
		D. rerio (embryo)	96h	EC_{50} (malformation)	98.3	
		D. rerio	96h		149	

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
		(larvae)				
Chronic	Algae	*S. subspicatus*	72h	EC_{50} (growth)	321.5	Cleuvers, 2004*
	Crustacean	*D. magna*	21d	NOEC (survival)	30	Kwak et al., 2018
			21d	NOEC (reproduction)	10	
			21d	NOEC (growth)	10	
		M. macrocopa	7d	NOEC (survival)	30	
			7d	NOEC (reproduction)	0.3	
	Fish	*C. carpio*	32d	NOEC (juvenile survival)	>0.2	Sehonova et al., 2017*
		O. latipes	40d		0.5	Kwak et al., 2018
			40d	NOEC (juvenile growth)	50	

*Naproxen Sodium Salt (CAS number: 26159-34-2) was used

Table S5. Acute toxicity of trimethoprim to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Bacteria	*A.flos-aque*	72h	ErC_{50}	253	Kolar et al., 2014
			72h	ErC_{10}	26	
		V. fisheri	15 min	EC_{50}	176.7	Santos et al., 2010
	Algae	*P.subcapitata*	72h	ErC_{50}	129	Kolar et al., 2014
			72h	EC_{50}	0.691	Arvaniti et al., 2020
		C.vulgaris	72h	EC_{50}	90.89-123.22	Kovalakova et al., 2020
	Crustacean	*D.magna*	48h	ErC_{50}	100	Kolar et al., 2014
			48h	ErC_{10}	66	
			48h	LC_{50}	58.8	Arvaniti et al., 2020
		P.promelas	96h	LC_{50}	65.7	
	Cnidaria	*A.salina*	48h	EC_{50}	>100	Kovalakova et al., 2020
	Amphibian	*X.Laevis*	96h	EC_{50}	>100	Brausch et al., 2012
	Fish	*O.latipes*	48h, 96h	EC_{50}	>100	
Chronic	Algae	*M.aeruginosa*	6d	EC_{50}	150	Kovalakova et al., 2020
			7d	EC_{50}	112	Valitalo et al., 2017
		Synechococcus sp.	6d	EC_{50}	>200	Ando et al., 2007

Table S6. Acute and chronic toxicity of gemfibrozil to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Crustacean	*D.magna*	72h	EC_{50}	120 uM	Zurita et al., 2007
			96h	LC_{50} (mortality)	11.3	Raldua et al., 2008
			72h	LC_{50} (survival)	14.61	Henriques et al., 2016
				LC_{50} (hatching success)	11.26	
		Ceriodaphnia dubia	48h	EC_{50} (population growth rate)	0.53	Isidori et al., 2007
	Fish	*Oncorrynchus mykiss*	96h	LC_{50}	22	Henriques et al., 2016
		Danio rerio		EC_{50} (mortality)	0.85	Kalasekar et al., 2015
	Algae	*Anabaena sp.*	24h	EC_{50} (physiology)	4.42	Rosal et al., 2010
Chronic	Crustacean	*Ceriodaphnia dubia*	7d	NOEC (population growth rate)	0.078	Isidori et al., 2007
	Fish	*Pimephales promelas*	2d		0.014	Skolness et al., 2012
	Algae	*Pseudokirchneriella subcapitata*	3d	NOEC (genetics)	3.125	Isidori et al., 2007

Table S7. Acute and chronic toxicity endpoints of caffeine to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Invertebrate	*Dugesia japonica*	96h	LC_{50} (mortality)	377.6	Li, 2013
		Plationus patulus	48h		419	Martinez Gomez, 2012
		Brachionus calyciflorus	24h		1018	Zarrelli et al., 2014
	Amphibian	*Xenopus laevis*	96h		220	Bantle et al., 1996
	Crustacean	*Daphnia magna*	24h		159.624	Calleja et al., 1994
		Streptocephalus proboscideus			409.74	
	Fish	*Danio rerio*	144h		306.8	Selderslaghs et al., 2012
Chronic	Algae	River biofilms	56d	NOEC (population)	0.005	Lawrence et al., 2012
					0.01	Lawrence et al., 2005
	Plant	*Lemna gibba*	35d	NOEC (injury)	1	Brain et al., 2004
	Amphibian	*Lithobates pipiens*	21	NOEC (growth)	0.06	Lu et al., 2013
		Bufo americanus	14d		0.6	Smith and Burgett, 2005

	Invertebrate	*Hydra vulgaris*	LOEC (regeneration)	100	Quinn et al., 2008

Table S8. Acute and chronic toxicity of sulfamethoxazole to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Bacteria	*V. fisheri*	15 min	EC_{50}	43.56	Ortiz de García et al., 2014
	Algae	*P.subcapitata*	72h	EC_{50}	1.12	Minguez et al., 2016
			72h	EC_{50}	1.53	Eguchi et al., 2004
			72h	EC_{50}	1.9	Yang et al., 2008
			96h	EC_{50}	0.15	Ginebreda et al., 2014
		C.vulgaris	72h	EC_{50}	0.98-1.51	Kovalakova et al., 2020
			48h	EC_{50}	1.57	Baran et al., 2006
			48h	EC_{50}	0.98	Xiong et al., 2019
		Scenedesmus sp.	72h	EC_{50}	1.54	Kovalakova et al., 2020
	Crustacean	*D.magna*	48h	EC_{50}	123.1-189.2	Santos et al., 2010
			48h	EC_{10}	25.2	Waiser et al., 2011
			48h	EC_{50}	98.01	Minguez et al., 2016
		P.promelas	96h	LC_{50}	65.7	Arvaniti et al., 2020

	Cnidaria	*H.attenuata*	96h	EC_{50}	>100	Santos et al., 2010
				NOEC	5	
	Amphibian	*X.Laevis*	96h	EC_{50}	>100	Brausch et al., 2012
	Fish	*O.latipes*	48h, 96h	EC_{50}	>100	
Chronic	Fish	*D.rerio*	10d	NOEC (embryo mortality)	>8	Ferrari et al., 2004

Table S9. Acute and chronic toxicity endpoints of cotinine to aquatic organisms from past literature. EC_{50}: , LC_{50}: , TEC: threshold effect concentration.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Bacteria	*Vibrio Fisheri*	15min	IC_{50}	369.3	Blaise et al., 2006
	Micro-invertebrates	*Hydra attenuata*	96h	EC_{50}	>375	
		Thamnocephalus platyurus	24h	LC_{50}	>700	
	Fish/fish cells	*Oncorhynchus mykiss*	48h	TEC*	88.1	
Chronic	Algae	*Pseudokirchneriella subcapitata*	72h	IC_{50}	589.5	

***TEC: threshold effect concentration.

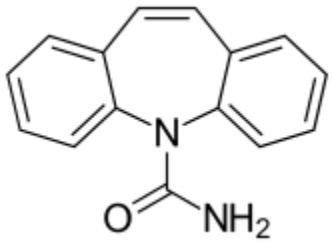
Table S10. Acute and chronic toxicity endpoints of clofibric acid to aquatic organisms from past literature.

Test Type	Taxonomic class	Species	Duration	Endpoint	Toxicity value (mg/L)	Reference
Acute	Bacteria	*V.fischeri*	30min	EC_{50} (luminescence)	91.827	Ferrari et al., 2003
	Crustaceans	*D.magna*	48h	EC_{50} (mobility)	>200	
		C.dubia	48h	EC_{50} (mobility)	>200	
Chronic	Algae	*P. subcapitata*	96h	NOEC (growth)	75	
	Rotifers	*B. calyciflorus*	48h	NOEC (reproduction)	0.246	
	Crustaceans	*C. dubia*	7d	NOEC (reproduction)	0.640	
	Fish	*D. rerio*	10d	NOEC (embryos and larvae mortality)	700	

Appendix C. Summary Profiles

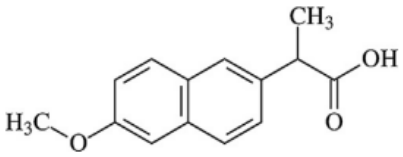
The following tables are summary profiles for carbamazepine, naproxen, ibuprofen, trimethoprim, gemfibrozil, caffeine, sulfamethoxazole, cotinine and clofibric acid. Each table provides some of the compounds' physicochemical properties, usages, and action mechanisms, as well as their recommendations. The Log K_{ow} stands for the octanol-water partitioning coefficient and pKa is the acid dissociation constant.

Table S11. Summary profile of carbamazepine.

Pharmaceutical Compound	**Carbamazepine**
Structure	 (Aguirre-Martínez et al., 2015)
Contaminant Overview	**Uses**: anticonvulsant, antimanic, antiepileptic, psychiatric agent
	pKa: 13.9 (Arlos et al 2015) 15.96 (Liu et al., 2023)
	Solubility: 18 mg/L at 25°C (Arlos et al 2015)
	Alkalinity: neutral
	Log K_{ow} : 2.45 (Arlos et al 2015)
	PNEC: 500 ng/L (Uslu et al, 2013)
	Toxicity species/organism endpoint: Crustacean - NOEC (Uslu et al, 2013, Ferrari et al, 2004)
	Sources: WWTPs and STPs (Batucan et al., 2022)
	Important Degradates: Oxcarbazepine (OxCBZ) and acridine 9-carboxylic acid (9-CAA) (Desbiolles et al., 2020)
Mode of Action	Binds to voltage-gated sodium channels and, to a certain extent, calcium channels, inhibiting action potential and decreasing synaptic transmission (Batucan et al., 2022).
Relevant Media for Monitoring	Water, sediment, and biota

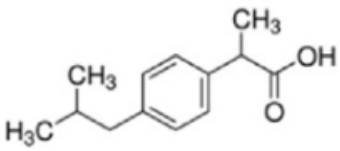
Seasonal considerations	Concentrations increase in the summer and early fall due to lower flow rates
Recommendations	- RQ was >1, which indicate high ecotoxicological risk - It has the highest reporting frequency of 69% - Toxicological endpoints included oxidative stress, neurotoxicity, immunity, cellular stress and detoxification - Its metabolites were found to be more toxic - Shown to have synergistic and additive impacts with other pollutants in the environment - Monitoring as well as environmental quality standards should be implemented by the government of Ontario

Table S12. Summary profile for naproxen.

Pharmaceutical Compound	Naproxen
Structure	 (Madikizela et al., 2016)
Contaminant Overview	**Uses**: non-steroidal anti-inflammatory and an analgesic (Hua et al., 2006a)
	pKa: 4.2 (Arlos et al., 2015)
	Alkalinity: Acidic (Li et al., 2010)
	Solubility: 16 mg/L at 25^{o}C (Arlos et al., 2015)
	Log K_{ow}: 3.18 (Li et al., 2010)
	PNEC: 21,000 ng/L (Uslu et al., 2013; Straub & Stewart, 2007)
	Sources: WWTPs and STPs
	Important Degradates: O-desmethylnaproxen and naproxen glucuronide (Addison et al. 2020)
Mode of Action	Inhibits both cyclooxygenase isoforms that are involved in the synthesis of prostaglandins, prostacyclin and thromboxane from arachidonic acid (Wojcieszyńska et al., 2020).
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	Concentrations are consistently lower in the summer compared to other seasons
Recommendations	- RQ is 0.02, indicating low ecotoxicological risk - It has a relatively high frequency and intensity - The government of Ontario should invest in better assessments of their aquatic toxicity using

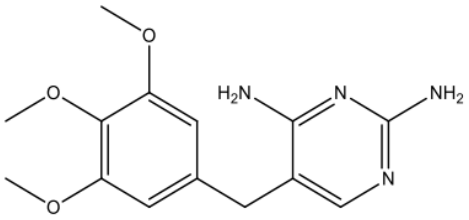
	Canadian data and allow for the results to be openly available for future researchers to use

Table S13. Summary profile for ibuprofen.

Pharmaceutical Compound	**Ibuprofen**
Structure	 (Aguirre-Martínez et al., 2015)
Contaminant Overview	**Uses**: it is used as an antipyretic, analgesic, and anti-inflammatory to reduce fever, headache, muscle pain, menstruation discomfort, neurological pain, and post-surgical pain (Jan-Roblero et al., 2023)
	pKa: 5.2 (Couperus et al 2016)
	Alkalinity: Acidic (Li et al 2010)
	Solubility: 21 mg/L at 25 ^{o}C (Arlos et al 2015)
	Log K_{ow}: 3.5 (Couperus et al 2016)
	PNEC: 26 (reproduction), 1400 (mortality) (Huang et al., 2018)
	Sources: WWTPs and STPs
	Important Degradates: Carboxy-ibuprofen (CX-IBU), Hydroxy-ibuprofen (OH-IBU) (Das et al., 2019)
Mode of Action	It inhibits cyclooxygenase and subsequent prostaglandin synthesis, which reduces the release of inflammatory substances and mediators and prevents the activation of pain receptors (nociceptors) (Jan-Roblero et al., 2023)
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	The highest concentrations occurred during the winters and the lowest concentrations have been measured during the summers (Halle 2010)
Recommendations	- High biodegradability compensated by high use and release

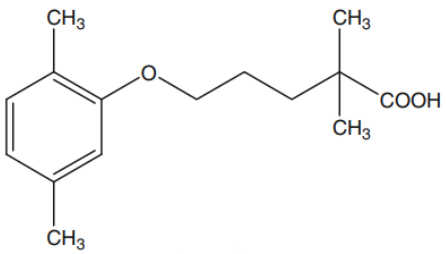
	- RQ was >1, indicates that it poses high ecotoxicological risk at the current concentrations - It has a relatively high frequency and intensity - Lowest toxicity endpoint was survival NOEC for *O.Latipes* of 100 ng/L. This value is lower than the observed maximum concentration in the current study - Monitoring as well as environmental quality standards should be implemented by the government of Ontario

Table S14. Summary profile for trimethoprim.

Pharmaceutical Compound	**Trimethoprim**
Structure	 (Arvaniti et al., 2020)
Contaminant Overview	**Uses**: antibiotic (Kolar et al., 2014)
	pKa: 7.1 (Couperus et al., 2016)
	Alkalinity: basic to neutral (Couperus et al., 2016)
	Solubility: 400 mg/L at 25 ^{o}C (Couperus et al., 2016; Kolar et al., 2014)
	Log K_{ow}: 0.91 (Couperus et al., 2016)
	PNEC: 16,000 ng/L (Uslu et al., 2013)
	Sources: WWTPs and STPs
	Important Degradates: α-hydroxytrimethoprim and α-ketotrimethoprim (Ji et al., 2016)
Mode of Action	Acts on dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolic acid (Kolar et al., 2014)
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	Trimethoprim concentrations were considerably higher in the early spring and summer 2003 sampling periods relative to the fall 2002 sampling period (Hua et al., 2006b)
Recommendations	- A relatively persistent compound with a half-life of 30 and a main removal mechanism of biodegradation - Frequency and intensity on the higher end - RQ based on aquatic PNEC is a low 0.02, suggesting a low ecotoxicological risk.

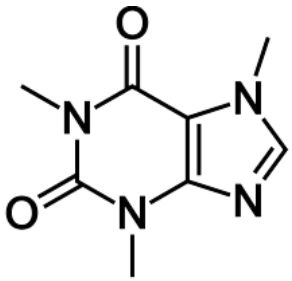
	- Transformation products are argued to be more toxic than the parent compound - The government of Ontario should invest in better assessments of their aquatic toxicity using Canadian data and allow for the results to be openly available for future researchers to use

Table S15. Summary profile for Gemfibrozil.

Pharmaceutical Compound	**Gemfibrozil**
Structure	 (Araujo et al., 2009)
Contaminant Overview	**Uses**: lipid regulator and cholesterol reducing agent (Lissemore et al., 2006; Li et al., 2010)
	pKa: 4.5 (Santos et al., 2020)
	Alkalinity: acidic (Li et al., 2010)
	Solubility: 500 mg/L
	Log K_{ow}: 4.77 (Li et al., 2010)
	PNEC: 900 ng/L (Uslu et al., 2013)
	Sources: WWTPs and STPs
	Important Degradates: gemfibrozil acyl glucuronide (Zurita et al., 2007)
Mode of Action	It works by increasing the activity of lipoprotein lipase and activating peroxisome proliferators and receptors (PPARγ), resulting in reductions in the level of serum triglycerides and very low-density lipoproteins
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	
Recommendations	- Relatively high persistence - 4th highest reporting frequency and relatively moderate concentrations in the aquatic environment - RQ is 0.4, indicating moderate ecotoxicological risk at the current concentrations

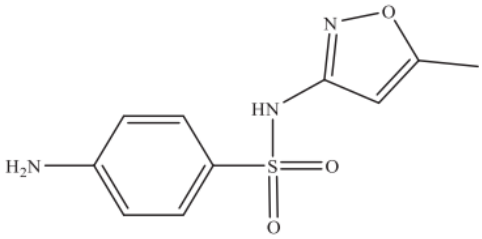
	- It is recommended that the government of Ontario implements monitoring programs and further assess its potential aquatic hazard

Table S16. Summary profile for caffeine.

Pharmaceutical Compound	**Caffeine**
Structure	 (Rodriguez-gil et al., 2018)
Contaminant Overview	**Uses**: psychoactive stimulant, which has been used for treatment of neurasthenia and coma recovery (Li et al., 2012)
	pKa: 10.4 (Rodriguez-gil et al., 2018)
	Alkalinity: neutral (Hua et al 2006b)
	Solubility: 21.6 g/L at 25^{o}C (Rodriguez-gil et al., 2018)
	Log K_{ow}: -0.13 (Rodriguez-gil et al., 2018)
	PNEC: 4 (chronic) and 15 (acute) ng/L (Rodriguez-gil et al., 2018)
	Sources: WWTPs and STPs
	Important Degradates: paraxanthine (Rodríguez-Gil et al., 2018)
Mode of Action	It works by 1) inhibiting adenosine receptors 2) inhibiting phosphodiesterase enzymes in skeletal muscle and adipose tissues (increasing intracellular concentrations of cAMP 3) mobilizing intracellular calcium and 4) antagonizing benzodiazepine receptors (Committee on Military Nutrition Research. 2001)
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	Was observed to be elevated in early/late spring (Hua et al., 2006b)

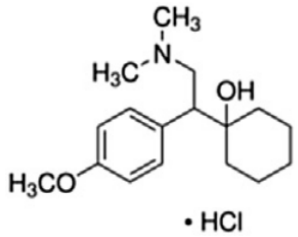
Recommendations	- Calculated RQ was >1, indicating high ecotoxicological risk - Recommendation to government of Ontario is to implement monitoring programs as well as take measures to reduce the concentration of caffeine in the environment

Table S17. Summary profile for sulfamethoxazole.

Pharmaceutical Compound	**Sulfamethoxazole**
Structure	 (Duan et al., 2022)
Contaminant Overview	**Uses**: antibiotic/antimicrobial (Couperus et al., 2016; Duan et al., 2022)
	pKa: 5.8 (Li et al., 2010, Couperus et al., 2016)
	Alkalinity: acidic (Metcalfe et al., 2014)
	Solubility: 610 mg/L (37 °C) (Kovalakova et al., 2020)
	Log K_{ow}: 0.89 (Li et al., 2010, Couperus et al., 2016)
	PNEC: 890 ng/L (Huang et al., 2018) 520 ng/L (Zhou et al., 2016)
	Sources: WWTPs and STPs, agricultural runoff
	Important Degradates:
Mode of Action	It works by reducing the uptake of p-aminobenzoic acid needed for dihydrofolic acid synthesis, which results in completely inhibiting the enzyme dihydropteroate synthetase in microorganisms (Couperus et al 2016)
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	Higher concentrations are expected in the drier seasons
Recommendations	- A compound that exhibits moderate mobility and high chemical stability in the aquatic environment - Its intensity and frequency are on the lower end of the current study's list. - Its RQ is ~0.1 indicating moderate ecotoxicological risk. However, past studies calculated an RQ for

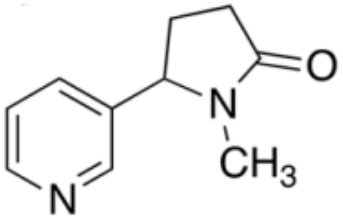
	Canadian data and found that it poses high ecotoxicological risk to algae (RQ = 17) - Its transformation products have been shown to be less toxic than the parent compound - The recommendation is that the government of Ontario should implement monitoring programs as well as conduct further studies on the potential toxicity this compound has on the aquatic biota

Table S18. Summary profile for venlafaxine.

Pharmaceutical Compound	**Venlafaxine**
Structure	 (Lacaze et al., 2015)
Contaminant Overview	**Uses**: Antidepressant (Couperus et al 2016)
	pKa: 10.1 (Arlos et al., 2015)
	Alkalinity: Basic (Couperus et al., 2016)
	Solubility: 267 mg/L at 25 °C (Arlos et al 2015)
	Log K_{ow}: 3.2 (Arlos et al., 2015)
	PNEC: 653, 1060, and 7680 ng/L for algae, daphnids and fish respectively (Singh et al., 2022)
	Sources: WWTPs and STPs
	Important Degradates: desmethyl venlafaxine (O-desmethyl-venlafaxine (Helm et al 2012) and N-demethyl venlafaxine (Metcalfe et al 2010)) **Enantiomers:** (R)-venlafaxine (VEN) and (S)-VEN inhibits (Ribeiro et al., 2022)
Mode of Action	Acts at the synaptic junction to prevent the reuptake of serotonin and noradrenaline, albeit to a lesser extent. It also has a weak effect on dopamine reuptake (Sehonova et al., 2018)
Relevant Media for Monitoring	**Media**: surface water, sediment, biota
Seasonal considerations	
Recommendations	- Frequency and intensity are relatively low - RQ (0.06) is lower than 0.1 which indicates low ecotoxicological risk

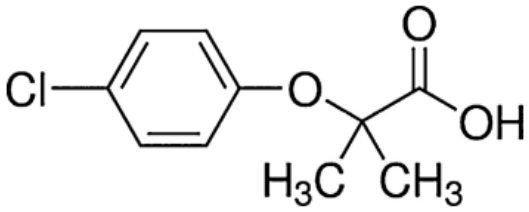
	- Recommendation is to monitor the compound regularly in Ontario's surface waters

Table S19. Summary profile for cotinine.

Pharmaceutical Compound	**Cotinine**
Structure	 (Chang et al., 2015)
Contaminant Overview	**Uses**: no uses, it is metabolite of neuroactive compound nicotine
	pKa: 4.72 (Chang et al., 2015)
	Alkalinity: neutral (Hua et al., 2006b; Hua et al., 2006a)
	Solubility: 48910 mg/L (ChemSpider. 2024)
	Log K_{ow}: 0.34 (ChemSpider. 2024)
	PNEC: 970 ng/L (Bellot et al 2024), 589 (Valcárcel et al., 2011)
	Sources: WWTPs and STPs
Mode of Action	It causes neuropharmacological and behavioral effects by crossing the blood brain barrier and interacting with both nAChRs and non-nAChRs in the nervous system (Tan et al., 2021).
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	
Recommendations	- Relatively low half-life and high solubility - Low reporting frequency and moderate average concentration in Ontario's surface waters - There is not enough evidence on the toxicity of cotinine and it seems that it is less toxic than its parent compound - RQ is 0.2 which indicates that the current concentrations may pose moderate risk, however, little aquatic toxicity data exists for cotinine

	- It is recommended that the government of Ontario implements monitoring programs and further assess potential aquatic hazard of cotinine and its parent compound nicotine

Table S20. Summary profile for clofibric acid.

Pharmaceutical Compound	**Clofibric acid**
Structure	 (Ighalo et al., 2020)
Contaminant Overview	**Uses**: metabolite and used in the production of anti-cholesteremic, antilipemic and an antineoplastic agent (Ighalo et al., 2020)
	pKa: 3.18 (Ighalo et al., 2020)
	Alkalinity: acidic
	Solubility: 583 mg/L at 20^{o}C (Ighalo et al., 2020)
	Log K_{ow}: 2.6 (Ighalo et al., 2020)
	PNEC: 4920 ng/L (Ferrari et al., 2004; Uslu et al., 2013)
	Sources: WWTPs and STPs
Mode of Action	Clofibric acid works by inducing the enzymatic activity of hepatic peroxisomes (Rebelo et al., 2020)
Relevant Media for Monitoring	Surface water, sediment, biota
Seasonal considerations	
Recommendations	- Relatively high persistence and slow degradability - Lowest reporting frequency and average concentration on the lower range - Concentrations show a decreasing trend in more recent studies - RQ is 0.02 indicating low ecotoxicological risk at the current concentrations - The government of Ontario should invest in better assessments of their aquatic toxicity using Canadian data and allow for the results to be openly available for future researchers to use

Appendix D. Second Reader comments

This section is intended to list how the substantive comments (ones that potentially influence the research question or hypotheses) made by the second reader at the proposal stage were incorporated in the current research paper. The substantive comments provided by Dr. Jules Blais resulted in two main alterations:

1. The removal of the hypothesis and predictions
2. The use of risk quotients (RQs) as a measure of ecotoxicological risk rather than just frequency and intensity

Appendix E. Glossary

PHACs: pharmacologically active compounds

NSAIDs: non-steroidal anti-inflammatory drugs

WWTP: wastewater treatment plant

WTP: sewage treatment plant

DWS: drinking water station

DWTP: drinking water treatment plant

pKa: acid-dissociation constant

Log K_{ow} : the octanol-water partitioning coefficient

EC_{50} : the half maximal effective concentration

LC_{50} : the lethal concentration 50

NOEC: no observed effect concentration

LOEC: lowest observed effect concentration

IC_{50} : half maximal inhibitory concentration.

WFD: European Water Framework Directive (2000/60/EC)

6 References

Addison, R. S., Parker-Scott, S. L., Hooper, W. D., Eadie, M. J., & Dickinson, R. G. (2000). Effect of naproxen co-administration on valproate disposition. Biopharmaceutics & drug disposition, 21(6), 235-242.

Ando, T., Nagase, H., Eguchi, K., Hirooka, T., Nakamura, T., Miyamoto, K., & Hirata, K. (2007). A novel method using cyanobacteria for ecotoxicity test of veterinary antimicrobial agents. Environmental Toxicology and Chemistry: An International Journal, 26(4), 601-606.

Araujo, L., Villa, N., Camargo, N., Bustos, M., García, T., & Prieto, A. D. J. (2011). Persistence of gemfibrozil, naproxen and mefenamic acid in natural waters. Environmental Chemistry Letters, 9, 13-18.

Araujo, L., Troconis, M. E., Espina, M. B., & Prieto, A. (2014). Persistence of ibuprofen, ketoprofen, diclofenac and clofibric acid in natural waters. Journal of Environment and Human, 1(2), 32-38.

Arlos, M. J., Bragg, L. M., Parker, W. J., & Servos, M. R. (2015). Distribution of selected antiandrogens and pharmaceuticals in a highly impacted watershed. Water Research, 72, 40-50.

Aguirre-Martínez, G. V., DelValls, A. T., & Martín-Díaz, M. L. (2015). Yes, caffeine, ibuprofen, carbamazepine, novobiocin and tamoxifen have an effect on Corbicula fluminea (Müller, 1774). Ecotoxicology and environmental safety, 120, 142-154.

Al-Habsi, A. A., Massarsky, A., & Moon, T. W. (2016). Exposure to gemfibrozil and atorvastatin affects cholesterol metabolism and steroid production in zebrafish (Danio rerio). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 199, 87-96.

Angiolillo, D. J., & Weisman, S. M. (2017). Clinical pharmacology and cardiovascular safety of naproxen. American Journal of Cardiovascular Drugs, 17, 97-107.

Aguirre-Martínez, G. V., André, C., Gagné, F., & Martín-Díaz, L. M. (2018). The effects of human drugs in Corbicula fluminea. Assessment of neurotoxicity, inflammation, gametogenic activity, and energy status. Ecotoxicology and environmental safety, 148, 652-663.

Amos Sibeko, P., Naicker, D., Mdluli, P. S., & Madikizela, L. M. (2019). Naproxen, ibuprofen, and diclofenac residues in river water, sediments and Eichhornia crassipes of Mbokodweni river in South Africa: An initial screening. Environmental Forensics, 20(2), 129-138.

Arvaniti, O. S., Frontistis, Z., Nika, M. C., Aalizadeh, R., Thomaidis, N. S., & Mantzavinos, D. (2020). Sonochemical degradation of trimethoprim in water matrices: Effect of operating conditions, identification of transformation products and toxicity assessment. Ultrasonics Sonochemistry, 67, 105139.

Adamczuk, M. (2022). Environmentally realistic concentrations of ibuprofen influence life histories but not population dynamics of Daphnia magna. Science of The Total Environment, 848, 157783.

Bantle, J. A., Finch, R. A., Burton, D. T., Fort, D. J., Dawson, D. A., Linder, G., ... & Turley, S. D. (1996). FETAX interlaboratory validation study: Phase III–Part 1 testing. Journal of applied toxicology, 16(6), 517-528.

Boyd, G. R., Reemtsma, H., Grimm, D. A., & Mitra, S. (2003). Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. Science of the total Environment, 311(1-3), 135-149.

Brain, R. A., Johnson, D. J., Richards, S. M., Hanson, M. L., Sanderson, H., Lam, M. W., Solomon, K. R. (2004). Microcosm evaluation of the effects of an eight pharmaceutical mixture to the aquatic macrophytes Lemna gibba and Myriophyllum sibiricum. Aquatic toxicology, 70(1), 23-40.

Baran, W., Sochacka, J., & Wardas, W. (2006). Toxicity and biodegradability of sulfonamides and products of their photocatalytic degradation in aqueous solutions. Chemosphere, 65(8), 1295-1299.

Blaise, C., Gagné, F., Eullaffroy, P., & Férard, J. F. (2006). Ecotoxicity of selected pharmaceuticals of urban origin discharged to the Saint-Lawrence River (Québec, Canada): a review. Brazilian Journal of Aquatic Science and Technology, 10(2), 29-51.

Buerge, I. J., Kahle, M., Buser, H. R., Müller, M. D., & Poiger, T. (2008). Nicotine derivatives in wastewater and surface waters: application as chemical markers for domestic wastewater. Environmental science & technology, 42(17), 6354-6360.

Bruton, T., Alboloushi, A., De La Garza, B., Kim, B. O., & Halden, R. U. (2010). Fate of caffeine in the environment and ecotoxicological considerations. In Contaminants of emerging concern in the environment: ecological and human health considerations (pp. 257-273). American Chemical Society.

Brausch, J. M., Connors, K. A., Brooks, B. W., & Rand, G. M. (2012). Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. Reviews of Environmental Contamination and Toxicology Volume 218, 1-99.

Bisesi Jr, J. H., Bridges, W., & Klaine, S. J. (2014). Reprint of: Effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. Aquatic toxicology, 151, 88-96.

Barreto, G. E., Iarkov, A., & Moran, V. E. (2015). Beneficial effects of nicotine, cotinine and its metabolites as potential agents for Parkinson's disease. Frontiers in aging neuroscience, 6, 123750.

Barcella, C. A., Lamberts, M., McGettigan, P., Fosbøl, E. L., Lindhardsen, J., Torp-Pedersen, C., Olsen, A. M. S., et al. (2019). Differences in cardiovascular safety with non-steroidal anti-inflammatory drug therapy—A nationwide study in patients with osteoarthritis. Basic & clinical pharmacology & toxicology, 124(5), 629-641.

Baali, H., & Cosio, C. (2022). Effects of carbamazepine in aquatic biota. Environmental Science: Processes & Impacts, 24(2), 209-220.

Blonç, M., Ruiz, N., Balasch, J. C., Llorca, M., Farré, M., Tvarijonaviciute, A., Teles, M., et al. (2023a). Effects of a chronic exposure to gemfibrozil in Carassius auratus. Journal of Hazardous Materials Advances, 12, 100376.

Blonç, M., Lima, J., Balasch, J. C., Tort, L., Gravato, C., & Teles, M. (2023b). Elucidating the Effects of the Lipids Regulators Fibrates and Statins on the Health Status of Finfish Species: A Review. Animals, 13(5), 792.

Bellot, M., Manen, L., Prats, E., Bedrossiantz, J., Barata, C., Gómez-Canela, C., Raldúa, D., et al. (2024). Short-term exposure to environmental levels of nicotine and cotinine impairs visual motor response in zebrafish larvae through a similar mode of action: Exploring the potential role of zebrafish α7 nAChR. Science of the Total Environment, 912, 169301.

Calleja, M. C., Persoone, G., & Geladi, P. (1994). Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Archives of Environmental Contamination and Toxicology, 26, 69-78.

Committee on Military Nutrition Research. (2001). Caffeine for the sustainment of mental task performance: formulations for military operations. National Academies Press.

Cleuvers, M. (2004). Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicology and environmental safety, 59(3), 309-315.

Csiszar, S. A., Gandhi, N., Alexy, R., Benny, D. T., Struger, J., Marvin, C., & Diamond, M. L. (2011). Aquivalence revisited—New model formulation and application to assess environmental fate of ionic pharmaceuticals in Hamilton Harbour, Lake Ontario. Environment international, 37(5), 821-828.

Coimbra, A. M., Peixoto, M. J., Coelho, I., Lacerda, R., Carvalho, A. P., Gesto, M., & Santos, M. M. (2015). Chronic effects of clofibric acid in zebrafish (Danio rerio): a multigenerational study. Aquatic Toxicology, 160, 76-86.

Chang, Y. W., Nguyen, H. P., Chang, M., Burket, S. R., Brooks, B. W., & Schug, K. A. (2015). Determination of nicotine and its metabolites accumulated in fish tissue using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. Journal of separation science, 38(14), 2414-2422.

Couperus, N. P., Pagsuyoin, S. A., Bragg, L. M., & Servos, M. R. (2016). Occurrence, distribution, and sources of antimicrobials in a mixed-use watershed. Science of the Total Environment, 541, 1581-1591.

Chabenat, A., Bellanger, C., & Knigge, T. (2021). Effects of environmental antidepressants on colour change and locomotor behaviour in juvenile shore crabs, Carcinus maenas. Aquatic Toxicology, 234, 105808.

Chen, Y., Ren, L., Li, X., & Zhou, J. L. (2024). Competitive adsorption and bioaccumulation of sulfamethoxazole and roxithromycin by sediment and zebrafish (Danio rerio) during individual and combined exposure in water. Journal of Hazardous Materials, 464, 132894.

ChemSpider. (S)-(-)-Cotinine. (2024). Retrieved from https://www.chemspider.com/Chemical-Structure.746405.html

De Lange, H. J., Noordoven, W., Murk, A. J., Lürling, M. F. L. L. W., & Peeters, E. T. H. M. (2006). Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquatic Toxicology, 78(3), 209-216.

Daughton, C. G., & Ruhoy, I. S. (2009). Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers. Environmental toxicology and chemistry, 28(12), 2495-2521. https://doi.org/10.1897/08-382.1

Dökmeci, A. H., Dökmeci, I., & Ibar, H. (2014). The determination of single and mixture toxicity at high concentrations of some acidic pharmaceuticals via Aliivibrio fischeri. Environmental Processes, 1, 95-103.

Du, J., Mei, C. F., Ying, G. G., & Xu, M. Y. (2016). Toxicity thresholds for diclofenac, acetaminophen and ibuprofen in the water flea Daphnia magna. Bulletin of environmental contamination and toxicology, 97, 84-90.

Ding, T., Lin, K., Yang, B., Yang, M., Li, J., Li, W., & Gan, J. (2017). Biodegradation of naproxen by freshwater algae Cymbella sp. and Scenedesmus quadricauda and the comparative toxicity. Bioresource technology, 238, 164-173.

Das, S. A., Karmakar, S., Chhaba, B., & Rout, S. K. (2019). Ibuprofen: its toxic effect on aquatic organisms. Journal of Experimental Zoology India, 22(2).

Desbiolles, F., Moreau, X., de Jong, L., Malleret, L., Grandet-Marchant, Q., Wong-Wah-Chung, P., & Laffont-Schwob, I. (2020). Advances and limits of two model species for ecotoxicological assessment of carbamazepine, two by-products and their mixture at environmental level in freshwater. Water research, 169, 115267.

de Oliveira Souza, H., dos Santos Costa, R., Quadra, G. R., & dos Santos Fernandez, M. A. (2021). Pharmaceutical pollution and sustainable development goals: Going the right way?. Sustainable Chemistry and Pharmacy, 21, 100428.

Duan, W., Cui, H., Jia, X., & Huang, X. (2022). Occurrence and ecotoxicity of sulfonamides in the aquatic environment: A review. Science of The Total Environment, 820, 153178.

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y. S., Goto, K., Hirata, K., & Yoshimura, H. (2004). Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere, 57(11), 1733-1738.

European Commission. (2023). Water Framework Directive. Retrieved from https://environment.ec.europa.eu/topics/water/water-framework-directive_en#implementation

Ferrari, B., Paxéus, N., Giudice, R. L., Pollio, A., & Garric, J. (2003). Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicology and environmental safety, 55(3), 359-370.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxēaus, N., Giudice, R. L., Garric, J., et al. (2004). Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?. Environmental Toxicology and Chemistry: An International Journal, 23(5), 1344-1354.

Fong, P. P., Bury, T. B., Donovan, E. E., Lambert, O. J., Palmucci, J. R., & Adamczak, S. K. (2017). Exposure to SSRI-type antidepressants increases righting time in the marine snail Ilyanassa obsoleta. Environmental Science and Pollution Research, 24, 725-731.

Fraz, S., Lee, A. H., & Wilson, J. Y. (2018). Gemfibrozil and carbamazepine decrease steroid production in zebrafish testes (Danio rerio). Aquatic Toxicology, 198, 1-9.

Falfushynska, H., Poznanskyi, D., Kasianchuk, N., Horyn, O., & Bodnar, O. (2022). Multimarker responses of Zebrafish to the effect of ibuprofen and gemfibrozil in environmentally relevant concentrations. Bulletin of Environmental Contamination and Toxicology, 109(6), 1010-1017.

Gagnon, E. (2009). Pharmaceutical disposal programs for the public: A Canadian perspective. Ottawa, Ontario: Health Canada, Environmental Impact Initiative.

Ginebreda, A., Muñoz, I., de Alda, M. L., Brix, R., López-Doval, J., & Barceló, D. (2010). Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environment international, 36(2), 153-162.

Galus, M., Kirischian, N., Higgins, S., Purdy, J., Chow, J., Rangaranjan, S., Wilson, J. Y., et al. (2013). Chronic, low concentration exposure to pharmaceuticals impacts multiple organ systems in zebrafish. Aquatic toxicology, 132, 200-211.

Galus, M., Rangarajan, S., Lai, A., Shaya, L., Balshine, S., & Wilson, J. Y. (2014). Effects of chronic, parental pharmaceutical exposure on zebrafish (Danio rerio) offspring. Aquatic toxicology, 151, 124-134.

Ginebreda, A., Kuzmanovic, M., Guasch, H., de Alda, M. L., López-Doval, J. C., Muñoz, I., Barceló, D., et al. (2014). Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: compound prioritization, mixture characterization and relationships with biological descriptors. Science of the total environment, 468, 715-723.

Gillis, P. L., Gagné, F., McInnis, R., Hooey, T. M., Choy, E. S., André, C., Metcalfe, C. D., et al. (2014). The impact of municipal wastewater effluent on field-deployed freshwater mussels in the Grand River (Ontario, Canada). Environmental toxicology and chemistry, 33(1), 134-143.

Gray, J. P. & Hall, G.J. (2014). Cotinine. Encyclopedia of Toxicology (Third Edition), Academic Press. Pages 1050-1051. ISBN 9780123864550. https://doi.org/10.1016/B978-0-12-386454-3.00294-3.

Gheorghe, S., Petre, J., Lucaciu, I., Stoica, C., & Nita-Lazar, M. (2016). Risk screening of pharmaceutical compounds in Romanian aquatic environment. Environmental monitoring and assessment, 188, 1-16.

Gomez Cortes, L., Marinov, D., Sanseverino, I., Navarro Cuenca, A., Niegowska, M., Porcel Rodriguez, E., & Lettieri, T. (2020). Selection of substances for the 3rd Watch List under the Water Framework Directive (EUR 30297 EN). Publications Office of the European Union, Luxembourg.

Hao, C., Lissemore, L., Nguyen, B., Kleywegt, S., Yang, P., & Solomon, K. (2006). Determination of pharmaceuticals in environmental waters by liquid chromatography/electrospray ionization/tandem mass spectrometry. Analytical and bioanalytical chemistry, 384, 505-513.

Hua, W. Y., Bennett, E. R., Maio, X. S., Metcalfe, C. D., & Letcher, R. J. (2006a). Seasonality effects on pharmaceuticals and s-triazine herbicides in wastewater effluent and surface water from the Canadian side of the upper Detroit River. Environmental Toxicology and Chemistry: An International Journal, 25(9), 2356-2365.

Hua, W., Bennett, E. R., & Letcher, R. J. (2006b). Ozone treatment and the depletion of detectable pharmaceuticals and atrazine herbicide in drinking water sourced from the upper Detroit River, Ontario, Canada. Water research, 40(12), 2259-2266.

Heckmann, L. H., Callaghan, A., Hooper, H. L., Connon, R., Hutchinson, T. H., Maund, S. J., & Sibly, R. M. (2007). Chronic toxicity of ibuprofen to Daphnia magna: effects on life history traits and population dynamics. Toxicology letters, 172(3), 137-145.

Hallé, C. (2010). Biofiltration in drinking water treatment: Reduction of membrane fouling and biodegradation of organic trace contaminants.

Han, S., Choi, K., Kim, J., Ji, K., Kim, S., Ahn, B., ... & Giesy, J. P. (2010). Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (Oryzias latipes) and freshwater cladocerans Daphnia magna and Moina macrocopa. Aquatic toxicology, 98(3), 256-264.

Helm, P. A., Howell, E. T., Li, H., Metcalfe, T. L., Chomicki, K. M., & Metcalfe, C. D. (2012). Influence of nearshore dynamics on the distribution of organic wastewater-associated chemicals in Lake Ontario determined using passive samplers. Journal of Great Lakes Research, 38, 105-115.

Henriques, J. F., Almeida, A. R., Andrade, T., Koba, O., Golovko, O., Soares, A. M., & Domingues, I. (2016). Effects of the lipid regulator drug gemfibrozil: A toxicological and behavioral perspective. Aquatic Toxicology, 170, 355-364.

Huang, Q., Bu, Q., Zhong, W., Shi, K., Cao, Z., & Yu, G. (2018). Derivation of aquatic predicted no-effect concentration (PNEC) for ibuprofen and sulfamethoxazole based on various toxicity endpoints and the associated risks. Chemosphere, 193, 223-229.

Huang, X., Cui, H., & Duan, W. (2020). Ecotoxicity of chlorpyrifos to aquatic organisms: A review. Ecotoxicology and Environmental Safety, 200, 110731.

Hu, L., Ding, R., & Nie, X. (2022). Comparison of toxic effects of atorvastatin and gemfibrozil on Daphnia magna. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 252, 109224.

Isidori, M., Lavorgna, M., Nardelli, A., Parrella, A., Previtera, L., & Rubino, M. (2005). Ecotoxicity of naproxen and its phototransformation products. Science of the total environment, 348(1-3), 93-101.

Ighalo, J. O., Ajala, O. J., Umenweke, G., Ogunniyi, S., Adeyanju, C. A., Igwegbe, C. A., & Adeniyi, A. G. (2020). Mitigation of clofibric acid pollution by adsorption: a review of recent developments. Journal of Environmental Chemical Engineering, 8(5), 104264.

Jasim, S. Y., Irabelli, A., Yang, P., Ahmed, S., & Schweitzer, L. (2006). Presence of pharmaceuticals and pesticides in Detroit river water and the effect of ozone on removal. Ozone: Science and Engineering, 28(6), 415-423.

Johnson, A. C., Dumont, E., Williams, R. J., Oldenkamp, R., Cisowska, I., & Sumpter, J. P. (2013). Do concentrations of ethinylestradiol, estradiol, and diclofenac in European rivers exceed proposed EU environmental quality standards?. Environmental science & technology, 47(21), 12297-12304.

Jarvis, A. L., Bernot, M. J., & Bernot, R. J. (2014). The effects of the pharmaceutical carbamazepine on life history characteristics of flat-headed mayflies (Heptageniidae) and aquatic resource interactions. Ecotoxicology, 23, 1701-1712.

Ji, Y., Xie, W., Fan, Y., Shi, Y., Kong, D., & Lu, J. (2016). Degradation of trimethoprim by thermo-activated persulfate oxidation: Reaction kinetics and transformation mechanisms. Chemical engineering journal, 286, 16-24.

Juhel, G., Bayen, S., Goh, C., Lee, W. K., & Kelly, B. C. (2017). Use of a suite of biomarkers to assess the effects of carbamazepine, bisphenol A, atrazine, and their mixtures on green mussels, Perna viridis. Environmental toxicology and chemistry, 36(2), 429-441.

Jan-Roblero, J., & Cruz-Maya, J. A. (2023). Ibuprofen: Toxicology and Biodegradation of an Emerging Contaminant. Molecules, 28(5), 2097.

Kim, J. W., Ishibashi, H., Yamauchi, R., Ichikawa, N., Takao, Y., Hirano, M., & Arizono, K. (2009). Acute toxicity of pharmaceutical and personal care products on freshwater crustacean (Thamnocephalus platyurus) and fish (Oryzias latipes). The Journal of toxicological sciences, 34(2), 227-232.

Kleywegt, S., Pileggi, V., Yang, P., Hao, C., Zhao, X., Rocks, C., Whitehead, B., et al. (2011). Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada—occurrence and treatment efficiency. Science of the Total Environment, 409(8), 1481-1488.

Kelvin K. Ogilvie & Art Eggleton, P.C. (2014). Prescription Pharmaceuticals in Canada: Unintended Consequences. Standing Senate Committee on Social Affairs S&T.

Kalasekar, S. M., Zacharia, E., Kessler, N., Ducharme, N. A., Gustafsson, J. Å., Kakadiaris, I. A., & Bondesson, M. (2015). Identification of environmental chemicals that induce yolk malabsorption in zebrafish using automated image segmentation. Reproductive Toxicology, 55, 20-29.

Kleywegt, S., Pileggi, V., Lam, Y. M., Elises, A., Puddicomb, A., Purba, G., Fletcher, T., et al. (2016). The contribution of pharmaceutically active compounds from healthcare facilities to a receiving sewage treatment plant in Canada. Environmental Toxicology and Chemistry, 35(4), 850-862. https://doi.org/10.1002/etc.3124

Kwak, K., Ji, K., Kho, Y., Kim, P., Lee, J., Ryu, J., & Choi, K. (2018). Chronic toxicity and endocrine disruption of naproxen in freshwater waterfleas and fish, and steroidogenic alteration using H295R cell assay. Chemosphere, 204, 156-162.

Kleywegt, S., Payne, M., Ng, F., & Fletcher, T. (2019). Environmental loadings of active pharmaceutical ingredients from manufacturing facilities in Canada. Science of the Total Environment, 646, 257-264. https://doi.org/10.1016/j.scitotenv.2018.07.240

Korekar, G., Kumar, A., & Ugale, C. (2020). Occurrence, fate, persistence and remediation of caffeine: a review. Environmental Science and Pollution Research, 27, 34715-34733.

Kovalakova, P., Cizmas, L., McDonald, T. J., Marsalek, B., Feng, M., & Sharma, V. K. (2020). Occurrence and toxicity of antibiotics in the aquatic environment: A review. Chemosphere, 251, 126351.

Lawrence, J. R., Swerhone, G. D., Wassenaar, L. I., & Neu, T. R. (2005). Effects of selected pharmaceuticals on riverine biofilm communities. Canadian journal of microbiology, 51(8), 655-669.

Lissemore, L., Hao, C., Yang, P., Sibley, P. K., Mabury, S., & Solomon, K. R. (2006). An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. Chemosphere, 64(5), 717-729.

Li, H., Helm, P. A., & Metcalfe, C. D. (2010). Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. Environmental Toxicology and Chemistry: An International Journal, 29(4), 751-762.

Luo, Y., Xu, L., Rysz, M., Wang, Y., Zhang, H., & Alvarez, P. J. (2011). Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environmental science & technology, 45(5), 1827-1833.

Li, Z., Lu, G., Yang, X., & Wang, C. (2012). Single and combined effects of selected pharmaceuticals at sublethal concentrations on multiple biomarkers in Carassius auratus. Ecotoxicology, 21, 353-361.

Lawrence, J. R., Zhu, B., Swerhone, G. D., Roy, J., Tumber, V., Waiser, M. J., Korber, D. R. (2012). Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities. Environmental Toxicology and Chemistry, 31(3), 508-517.

Lu, G., Li, Z., & Liu, J. (2013). Effects of selected pharmaceuticals on growth, reproduction and feeding of Daphnia Magna. Fresenius Environmental Bulletin, 22(09), 2588-2594.

Li, M. H. (2013). Acute toxicity of 30 pharmaceutically active compounds to freshwater planarians, Dugesia japonica. Toxicological & Environmental Chemistry, 95(7), 1157-1170.

Lin, T., Yu, S., Chen, Y., & Chen, W. (2014). Integrated biomarker responses in zebrafish exposed to sulfonamides. Environmental Toxicology and Pharmacology, 38(2), 444-452.

Lacaze, E., Pédelucq, J., Fortier, M., Brousseau, P., Auffret, M., Budzinski, H., & Fournier, M. (2015). Genotoxic and immunotoxic potential effects of selected psychotropic drugs and antibiotics on blue mussel (Mytilus edulis) hemocytes. Environmental Pollution, 202, 177-186.

Lucero, G. M. A., Marcela, G. M., Sandra, G. M., & Manuel, G. O. L. (2015). Naproxen-enriched artificial sediment induces oxidative stress and genotoxicity in Hyalella azteca. Water, Air, & Soil Pollution, 226, 1-10.

Li, Q., Wang, P., Chen, L., Gao, H., & Wu, L. (2016). Acute toxicity and histopathological effects of naproxen in zebrafish (Danio rerio) early life stages. Environmental Science and Pollution Research, 23, 18832-18841.

Lian, L., Yan, S., Yao, B., Chan, S. A., & Song, W. (2017). Photochemical transformation of nicotine in wastewater effluent. Environmental Science & Technology, 51(20), 11718-11730.

Limbu, S. M., Zhou, L., Sun, S. X., Zhang, M. L., & Du, Z. Y. (2018). Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environment international, 115, 205-219.

Lin, X., Xu, J., Keller, A. A., He, L., Gu, Y., Zheng, W., Li, G., et al. (2020). Occurrence and risk assessment of emerging contaminants in a water reclamation and ecological reuse project. Science of the Total Environment, 744, 140977.

Li, S., He, B., Wang, J., Liu, J., & Hu, X. (2020). Risks of caffeine residues in the environment: necessity for a targeted ecopharmacovigilance program. Chemosphere, 243, 125343.

Liu, Q., Wang, L., Xu, X., Yan, S., Zha, J., Wang, D., & Zhu, D. (2023). Antiepileptic drugs in aquatic environments: Occurrence, toxicity, transformation mechanisms and fate. Critical Reviews in Environmental Science and Technology, 1-25.

Littlejohn, C., Renaud, J. B., Sabourin, L., Lapen, D. R., Pappas, J. J., Tuteja, B., Sumarah, M. W., et al. (2023). Environmental Concentrations of the Type 2 Diabetes Medication Metformin and Its

Transformation Product Guanylurea in Surface Water and Sediment in Ontario and Quebec, Canada. Environmental Toxicology and Chemistry, 42(8), 1709-1720

Metcalfe, C. D., Koenig, B. G., Bennie, D. T., Servos, M., Ternes, T. A., & Hirsch, R. (2003). Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environmental Toxicology and Chemistry: An International Journal, 22(12), 2872-2880.

Mimeault, C., Woodhouse, A. J., Miao, X. S., Metcalfe, C. D., Moon, T. W., & Trudeau, V. L. (2005). The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, Carassius auratus. Aquatic toxicology, 73(1), 44-54.

Metcalfe, C. D., Chu, S., Judt, C., Li, H., Oakes, K. D., Servos, M. R., & Andrews, D. M. (2010). Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. Environmental Toxicology and Chemistry, 29(1), 79-89.

Martinez Gomez, D. A. (2012). A Survey Of Selected Pharmaceuticals And Personal Care Products In A Binational River And Their Effects On A Member Of Its Zooplankton Community, Plationus patulus (Rotifera).

Minguez, L., Farcy, E., Ballandonne, C., Lepailleur, A., Serpentini, A., Lebel, J. M., Halm-Lemeille, M. P., et al. (2014). Acute toxicity of 8 antidepressants: what are their modes of action?. Chemosphere, 108, 314-319.

Moermond, C. T., & Smit, C. E. (2016). Derivation of water quality standards for carbamazepine, metoprolol, and metformin and comparison with monitoring data. Environmental Toxicology and Chemistry, 35(4), 882-888.

Metcalfe, C. D., Sultana, T., Li, H., & Helm, P. A. (2016). Current-use pesticides in urban watersheds and receiving waters of western Lake Ontario measured using polar organic chemical integrative samplers (POCIS). Journal of Great Lakes Research, 42(6), 1432-1442.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., & Halm-Lemeille, M. P. (2016). Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environmental Science and Pollution Research, 23, 4992-5001.

Madikizela, L. M., & Chimuka, L. (2016). Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction. Journal of pharmaceutical and biomedical analysis, 128, 210-215.

Melvin, S. D. (2017). Effect of antidepressants on circadian rhythms in fish: Insights and implications regarding the design of behavioural toxicity tests. Aquatic toxicology, 182, 20-30.

Minguez, L., Bureau, R., & Halm-Lemeille, M. P. (2018). Joint effects of nine antidepressants on Raphidocelis subcapitata and Skeletonema marinoi: A matter of amine functional groups. Aquatic toxicology, 196, 117-123.

Mezzelani, M., Gorbi, S., Fattorini, D., d'Errico, G., Consolandi, G., Milan, M., Regoli, F., et al. (2018). Long-term exposure of Mytilus galloprovincialis to diclofenac, Ibuprofen and Ketoprofen: Insights into bioavailability, biomarkers and transcriptomic changes. Chemosphere, 198, 238-248.

Martyniuk, V., Gylytė, B., Matskiv, T., Khoma, V., Tulaidan, H., Gnatyshyna, L., Stoliar, O. et al. (2022). Stress responses of bivalve mollusc Unio tumidus from two areas to ibuprofen, microplastic and their mixture. Ecotoxicology, 31(9), 1369-1381.

Nieto, E., Blasco, J., González-Ortegón, E., Drake, P., & Hampel, M. (2013). Is Atyaephyra desmarestii a useful candidate for lethal and sub-lethal toxicity tests on pharmaceutical compounds?. Journal of hazardous materials, 263, 256-265.

Ortiz de García, S. A., Pinto Pinto, G., García-Encina, P. A., & Irusta-Mata, R. (2014). Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. Ecotoxicology, 23, 1517-1533.

OCDE (2019), Pharmaceutical Residues in Freshwater : Hazards and Policy Responses, OECD Studies on Water, Éditions OCDE, Paris, https://doi.org/10.1787/c936f42d-en

Oliveira, C., Lima, D. L., Silva, C. P., Calisto, V., Otero, M., & Esteves, V. I. (2019). Photodegradation of sulfamethoxazole in environmental samples: The role of pH, organic matter and salinity. Science of the total environment, 648, 1403-1410.

O. Reg. 298/12: COLLECTION OF PHARMACEUTICALS AND SHARPS - RESPONSIBILITIES OF PRODUCERS. https://www.ontario.ca/laws/regulation/120298

Pounds, N., Maclean, S., Webley, M., Pascoe, D., & Hutchinson, T. (2008). Acute and chronic effects of ibuprofen in the mollusc Planorbis carinatus (Gastropoda: Planorbidae). Ecotoxicology and environmental Safety, 70(1), 47-52.

Painter, M. M., Buerkley, M. A., Julius, M. L., Vajda, A. M., Norris, D. O., Barber, L. B., Schoenfuss, H. L. et al. (2009). Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas). Environmental Toxicology and Chemistry, 28(12), 2677-2684.

Parolini, M., Binelli, A., & Provini, A. (2011). Chronic effects induced by ibuprofen on the freshwater bivalve Dreissena polymorpha. Ecotoxicology and Environmental Safety, 74(6), 1586-1594.

Parrott, J. L., & Metcalfe, C. D. (2017). Assessing the effects of the antidepressant venlafaxine to fathead minnows exposed to environmentally relevant concentrations over a full life cycle. Environmental pollution, 229, 403-411.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chemical reviews, 119(6), 3510-3673.

Parolini, M. (2020). Toxicity of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: A review. Science of the Total Environment, 740, 140043.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Moher, D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. International journal of surgery, 88, 105906.

Quinn, B., Gagné, F., Blaise, C., 2008. The effects of pharmaceuticals on the regeneration of the cnidarian, Hydra attenuata. Sci. Total Environ. 402 (1), 62–69.

Qiang, L., Cheng, J., Yi, J., Rotchell, J. M., Zhu, X., & Zhou, J. (2016). Environmental concentration of carbamazepine accelerates fish embryonic development and disturbs larvae behavior. Ecotoxicology, 25, 1426-1437.

Qu, H., Ma, R., Wang, B., Yang, J., Duan, L., & Yu, G. (2019). Enantiospecific toxicity, distribution and bioaccumulation of chiral antidepressant venlafaxine and its metabolite in loach (*Misgurnus anguillicaudatus*) co-exposed to microplastic and the drugs. Journal of hazardous materials, 370, 203-211.

Runnalls, T. J., Hala, D. N., & Sumpter, J. P. (2007). Preliminary studies into the effects of the human pharmaceutical Clofibric acid on sperm parameters in adult Fathead minnow. Aquatic toxicology, 84(1), 111-118.

Raldúa, D., André, M., & Babin, P. J. (2008). Clofibrate and gemfibrozil induce an embryonic malabsorption syndrome in zebrafish. Toxicology and applied pharmacology, 228(3), 301-314.

Rosal, R., Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Gonzalo, S., & Petre, A. (2010). Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms. Environmental Science and Pollution Research, 17, 135-144.

Rhee, J. S., Kim, B. M., Jeong, C. B., Park, H. G., Leung, K. M. Y., Lee, Y. M., & Lee, J. S. (2013). Effect of pharmaceuticals exposure on acetylcholinesterase (AchE) activity and on the expression of AchE gene in the monogonont rotifer, Brachionus koreanus. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 158(4), 216-224.

Rúa-Gómez, P. C., & Püttmann, W. (2013). Degradation of lidocaine, tramadol, venlafaxine and the metabolites O-desmethyltramadol and O-desmethylvenlafaxine in surface waters. Chemosphere, 90(6), 1952-1959.

Rodríguez-Gil, J. L., Cáceres, N., Dafouz, R., & Valcárcel, Y. (2018). Caffeine and paraxanthine in aquatic systems: Global exposure distributions and probabilistic risk assessment. Science of the total environment, 612, 1058-1071.

Rebelo, D., Correia, A. T., & Nunes, B. (2020). Acute and chronic effects of environmental realistic concentrations of clofibric acid in Danio rerio: Behaviour, oxidative stress, biotransformation and lipid peroxidation endpoints. Environmental Toxicology and Pharmacology, 80, 103468.

Ribeiro, O., Félix, L., Ribeiro, C., Castro, B., Tiritan, M. E., Monteiro, S. M., & Carrola, J. S. (2022). Enantioselective ecotoxicity of venlafaxine in aquatic organisms: daphnia and zebrafish. Environmental Toxicology and Chemistry, 41(8), 1851-1864.

Smith, G. R., & Burgett, A. A. (2005). Effects of three organic wastewater contaminants on American toad, Bufo americanus, tadpoles. Ecotoxicology, 14, 477-482.

Servos, M. R., Smith, M., McInnis, R., Burnison, B. K., Lee, B. H., Seto, P., & Backus, S. (2007). The Presence of Selected Pharmaceuticals and the Antimicrobial Triclosan in Drinking Water in Ontario, Canada. Water Quality Research Journal of Canada (Canadian Association on Water Quality), 42(2). https://doi.org/10.2166/wqrj.2007.016

Straub, J. O., & Stewart, K. M. (2007). Deterministic and probabilistic acute-based environmental risk assessment for naproxen for Western Europe. Environmental Toxicology and Chemistry: An International Journal, 26(4), 795-806.

Santos, L. H., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2010). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. Journal of hazardous materials, 175(1-3), 45-95.

Skolness, S. Y., Durhan, E. J., Jensen, K. M., Kahl, M. D., Makynen, E. A., Villeneuve, D. L., & Ankley, G. T. (2012). Effects of gemfibrozil on lipid metabolism, steroidogenesis, and reproduction in the fathead minnow (Pimephales promelas). Environmental toxicology and chemistry, 31(11), 2615-2624.

Selderslaghs, I. W., Blust, R., & Witters, H. E. (2012). Feasibility study of the zebrafish assay as an alternative method to screen for developmental toxicity and embryotoxicity using a training set of 27 compounds. Reproductive toxicology, 33(2), 142-154.

Stancová, V., Ziková, A., Svobodová, Z., & Kloas, W. (2015). Effects of the non-steroidal anti-inflammatory drug (NSAID) naproxen on gene expression of antioxidant enzymes in zebrafish (Danio rerio). Environmental toxicology and pharmacology, 40(2), 343-348.

Staley, Z. R., Grabuski, J., Sverko, E., & Edge, T. A. (2016). Comparison of microbial and chemical source tracking markers to identify fecal contamination sources in the Humber River (Toronto, Ontario, Canada) and associated storm water outfalls. Applied and Environmental Microbiology, 82(21), 6357-6366.

Streets, S., & Dobbins, L. (2017). Minnesota's Aquatic Toxicity Profiles. https://www.pca.state.mn.us/sites/default/files/wq-cec2-02.pdf

Sehonova, P., Plhalova, L., Blahova, J., Doubkova, V., Prokes, M., Tichy, F., Svobodova, Z., et al. (2017). Toxicity of naproxen sodium and its mixture with tramadol hydrochloride on fish early life stages. Chemosphere, 188, 414-423.

Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., & Faggio, C. (2018). Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Science of the Total Environment, 631, 789-794.

Santos-Silva, T. G., Montagner, C. C., & Martinez, C. B. (2018). Evaluation of caffeine effects on biochemical and genotoxic biomarkers in the neotropical freshwater teleost Prochilodus lineatus. Environmental toxicology and pharmacology, 58, 237-242.

Steele, W. B., Mole, R. A., & Brooks, B. W. (2018). Experimental protocol for examining behavioral response profiles in larval fish: application to the neuro-stimulant caffeine. JoVE (Journal of Visualized Experiments), (137), e57938.

Shi, C., He, Y., Liu, J., Lu, Y., Fan, Y., Liang, Y., & Xu, Y. (2019). Ecotoxicological effect of single and combined exposure of carbamazepine and cadmium on female Danio rerio: A multibiomarker study. Applied Sciences, 9(7), 1362.

Schwartz, H., Marushka, L., Chan, H. M., Batal, M., Sadik, T., Fediuk, K., & Tikhonov, C. (2021). Pharmaceuticals in source waters of 95 First Nations in Canada. Canadian Journal of Public Health, 112(1), 133-153.

Świacka, K., Michnowska, A., Maculewicz, J., Caban, M., & Smolarz, K. (2021). Toxic effects of NSAIDs in non-target species: a review from the perspective of the aquatic environment. Environmental Pollution, 273, 115891.

Sultana, T., & Metcalfe, C. D. (2022). Calibration and field validation of POCIS passive samplers for tracking artificial sweeteners as indicators of municipal wastewater contamination in surface waters. Environmental Monitoring and Assessment, 194(8), 564.

Singh, A., Saidulu, D., Gupta, A. K., & Kubsad, V. (2022). Occurrence and fate of antidepressants in the aquatic environment: Insights into toxicological effects on the aquatic life, analytical methods, and removal techniques. Journal of Environmental Chemical Engineering, 10(6), 109012.

Tabe, S., Jamal, T., Seth, R., Yue, C., Yang, P., Zhao, X., & Schweitzer, L. (2009). PPCPs and EDCs: Occurrence in the Detroit River and Their Removal by Ozonation. Water Research Foundation.

Trovó, A. G., Nogueira, R. F., Agüera, A., Sirtori, C., & Fernández-Alba, A. R. (2009). Photodegradation of sulfamethoxazole in various aqueous media: persistence, toxicity and photoproducts assessment. Chemosphere, 77(10), 1292-1298.

Tanna, R. N., Tetreault, G. R., Bennett, C. J., Smith, B. M., Bragg, L. M., Oakes, K. D., Servos, M. R., et al. (2013). Occurrence and degree of intersex (testis–ova) in darters (Etheostoma SPP.) across an urban gradient in the Grand River, Ontario, Canada. Environmental Toxicology and Chemistry, 32(9), 1981-1991.

Trombini, C., Hampel, M., & Blasco, J. (2016). Evaluation of acute effects of four pharmaceuticals and their mixtures on the copepod Tisbe battagliai. Chemosphere, 155, 319-328.

Thompson, W. A., Arnold, V. I., & Vijayan, M. M. (2017). Venlafaxine in embryos stimulates neurogenesis and disrupts larval behavior in zebrafish. Environmental science & technology, 51(21), 12889-12897.

Tan, X., Vrana, K., & Ding, Z. M. (2021). Cotinine: pharmacologically active metabolite of nicotine and neural mechanisms for its actions. Frontiers in Behavioral Neuroscience, 15, 758252.

Thompson, W. A., & Vijayan, M. M. (2021). Zygotic exposure to venlafaxine disrupts cortisol stress axis activity in multiple generations of zebrafish. Environmental Pollution, 274, 116535.

Thompson, W. A., Shvartsburd, Z., & Vijayan, M. M. (2022). The antidepressant venlafaxine perturbs cardiac development and function in larval zebrafish. Aquatic Toxicology, 242, 106041.

Trognon, J., Albasi, C., & Choubert, J. M. (2023). A critical review on the pathways of carbamazepine transformation products in oxidative wastewater treatment processes. Science of The Total Environment, 169040.

Uslu, M. O., Jasim, S., Arvai, A., Bewtra, J., & Biswas, N. (2013). A survey of occurrence and risk assessment of pharmaceutical substances in the Great Lakes Basin. Ozone: science & engineering, 35(4), 249-262.

Valcárcel, Y., Alonso, S. G., Rodríguez-Gil, J. L., Gil, A., & Catalá, M. (2011). Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. Chemosphere, 84(10), 1336-1348.

Water Framework Directive 2000/60/EC of the European Parliament and of the Council. https://eur-lex.europa.eu/eli/dir/2000/60/oj

Weston, A., Caminada, D., Galicia, H., & Fent, K. (2009). Effects of lipid-lowering pharmaceuticals bezafibrate and clofibric acid on lipid metabolism in fathead minnow (Pimephales promelas). Environmental toxicology and chemistry, 28(12), 2648-2655.

Waiser, M. J., Humphries, D., Tumber, V., & Holm, J. (2011). Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. Environmental Toxicology and Chemistry, 30(2), 508-519.

Wang, L., Peng, Y., Nie, X., Pan, B., Ku, P., & Bao, S. (2016). Gene response of CYP360A, CYP314, and GST and whole-organism changes in Daphnia magna exposed to ibuprofen. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 179, 49-56.

Wojcieszyńska, D., & Guzik, U. (2020). Naproxen in the environment: its occurrence, toxicity to nontarget organisms and biodegradation. Applied Microbiology and Biotechnology, 104, 1849-1857.

Wilkinson, J. L., Boxall, A. B., Kolpin, D. W., Leung, K. M., Lai, R. W., Galbán-Malagón, C., Teta, C., et al. (2022). Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences, 119(8), e2113947119.

Xiong, J. Q., Kim, S. J., Kurade, M. B., Govindwar, S., Abou-Shanab, R. A., Kim, J. R., Jeon, B. H., et al. (2019). Combined effects of sulfamethazine and sulfamethoxazole on a freshwater microalga, Scenedesmus obliquus: toxicity, biodegradation, and metabolic fate. Journal of hazardous materials, 370, 138-146.

Xu, C., Niu, L., Guo, H., Sun, X., Chen, L., Tu, W., Liu, J., et al. (2019). Long-term exposure to the non-steroidal anti-inflammatory drug (NSAID) naproxen causes thyroid disruption in zebrafish at environmentally relevant concentrations. Science of the total environment, 676, 387-395.

Yang, L. H., Ying, G. G., Su, H. C., Stauber, J. L., Adams, M. S., & Binet, M. T. (2008). Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry: An International Journal, 27(5), 1201-1208.

Yamindago, A., Lee, N., Woo, S., & Yum, S. (2019). Transcriptomic profiling of Hydra magnipapillata after exposure to naproxen. Environmental Toxicology and Pharmacology, 71, 103215.

Yuan, X., Li, S., Hu, J., Yu, M., Li, Y., & Wang, Z. (2019). Experiments and numerical simulation on the degradation processes of carbamazepine and triclosan in surface water: A case study for the Shahe Stream, South China. Science of the Total Environment, 655, 1125-1138.

Yang, X., Xu, X., Wei, X., Wan, J., & Zhang, Y. (2019). Biomarker effects in carassius auratus exposure to ofloxacin, sulfamethoxazole and ibuprofen. International journal of environmental research and public health, 16(9), 1628.

Yang, D., Yu, W., Qu, J., Shen, Y., Yu, J., Meng, R., Zhao, M., et al. (2023). Environmentally Relevant Exposure to Cotinine Induces Neurobehavioral Toxicity in Zebrafish (Danio Rerio): A Study Using Neurobehavioral and Metabolomic Approaches. Available at SSRN 4664808.

Zurita, J. L., Repetto, G., Jos, Á., Salguero, M., López-Artíguez, M., & Cameán, A. M. (2007). Toxicological effects of the lipid regulator gemfibrozil in four aquatic systems. Aquatic toxicology, 81(1), 106-115.

Zhang, Y., Geißen, S. U., & Gal, C. (2008). Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere, 73(8), 1151-1161.

Zarrelli, A., DellaGreca, M., Iesce, M. R., Lavorgna, M., Temussi, F., Schiavone, L., Isidori, M. (2014). Ecotoxicological evaluation of caffeine and its derivatives from a simulated chlorination step. Science of the total environment, 470, 453-458.

Zhang, R., Yang, Y., Huang, C. H., Li, N., Liu, H., Zhao, L., & Sun, P. (2016). UV/H2O2 and UV/PDS treatment of trimethoprim and sulfamethoxazole in synthetic human urine: transformation products and toxicity. Environmental science & technology, 50(5), 2573-2583.

Zhou, L. J., Wu, Q. L., Zhang, B. B., Zhao, Y. G., & Zhao, B. Y. (2016). Occurrence, spatiotemporal distribution, mass balance and ecological risks of antibiotics in subtropical shallow Lake Taihu, China. Environmental Science: Processes & Impacts, 18(4), 500-513.

Zhang, X., Liu, Z., Kong, Q., Liu, G., Lv, W., Li, F., & Lin, X. (2018). Aquatic photodegradation of clofibric acid under simulated sunlight irradiation: kinetics and mechanism analysis. RSC advances, 8(49), 27796-27804.

Zhao, H., Wang, Y., Mu, M., Guo, M., Yu, H., & Xing, M. (2020). Lycopene alleviates sulfamethoxazole-induced hepatotoxicity in grass carp (Ctenopharyngodon idellus) via suppression of oxidative stress, inflammation and apoptosis. Food & function, 11(10), 8547-8559.

Zeeshan Qadar, S. M., Gordon, M., Mph, T., & Haworth-Brockman Msc, M. (2021). A Call to Action: An Evidence Review on Pharmaceutical Disposal in the Context of Antimicrobial Resistance in Canada (Issue January). www.nccid.ca