Characterization and Modeling of Selected Antiandrogens and Pharmaceuticals in Highly Impacted Reaches of Grand River Watershed in Southern Ontario

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

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Abstract

Endocrine disruption and high occurrences of intersex have been observed in wild fish associated with wastewater treatment plant (WWTP) effluents in the urbanized reaches of the Grand River watershed located in southern Ontario, Canada. WWTP effluent is a complex matrix with diverse aquatic environmental contaminants and stressors. This study aimed to: (1) characterize the spatio-temporal distribution and fate of antiandrogenic personal care products (triclosan, chlorophene, and dichlorophene), along with selected pharmaceuticals (carbamazepine, ibuprofen, naproxen, and venlafaxine) and the herbicide, atrazine in the Grand River watershed and (2) model the behaviour of these contaminants in the aquatic environment. Water sampling of 29 sites which covered six municipal WWTPs and ~100 km of river length was completed during summer low flows (July 2012). Monthly samples were also collected immediately upstream and downstream of a major WWTP (Kitchener) from August to November 2012.

Many of the target pharmaceuticals and triclosan were detected in WWTP effluents in the Grand River watershed, especially those that did not nitrify (minimal treatment with high ammonia). Chlorophene was either undetected or was only found at trace levels in the effluents. Under low flow conditions, triclosan and several other pharmaceuticals exhibited a spatial pattern where concentrations increased directly downstream of the WWTPs, then decreased with distance downstream (dilution and/or degradation). Chlorophene, in contrast, was not found downstream of most of the WWTP outfalls but was first detected at a site 5 km upstream of a WWTP and then continued with relatively constant concentrations for approximately 29 km downstream. It was also only found during the summer sampling period. Atrazine was

consistently found in all sampling locations which reflected the agricultural non-point source nature of this compound.

The WASP 7.5 model (US Environmental Protection Agency) was adapted and calibrated to a reach of the Grand River associated with the Kitchener WWTP. The simulation of the fate and transport of the target compounds revealed that flow-driven transport processes (advection and dispersion) greatly influence their behaviour in the aquatic environment. However, fate mechanisms such as biodegradation and photolysis also potentially play an important role in the attenuation of most compounds. The exception was carbamazepine where it was shown to act as a conservative tracer compound for wastewater specific contaminants in the water phase. The fate model developed can be applied in the future to predict the fate of a wide variety of contaminants of emerging concern across the watershed to help define the exposure of these biologically active chemicals to sensitive ecosystems.

Acknowledgements

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I would also like to show my deep gratitude to Leslie Bragg for spending the time to teach me all the laboratory techniques I needed for my Master's. I also would like to acknowledge Mark Anderson of Grand River Conservation Authority and José Bicudo and Ryan Lacharity of the Region of Waterloo for providing me with the data I required to complete my thesis. Special thanks to Trevor Brown of the Region of Waterloo who assisted me with the Kitchener Wastewater Treatment Plant sampling. I would like to acknowledge the help of Tim Wool from the US EPA for always being available to answer all of my questions about WASP.

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Table of Contents

List of Figures

List of Tables

1 Introduction

1.1 Background

Developmental effects in fish have been associated with exposure to pharmaceuticals, personal care products, and a variety of endocrine disrupting compounds (EDCs) (Daughton and Ternes, 1999; Jobling et al., 1998; Sanchez et al., 2011). These compounds are routinely discharged from municipal wastewater treatment plants (WWTP) with treatment processes ranging from conventional activated sludge (CAS) to advanced treatment systems (Coors et al., 2004; Servos et al., 2005; Ternes et al., 1999). The effects of EDCs on aquatic species can be broad or highly specific depending on their mechanism of action (Daughton and Ternes, 1999). One of the specific physiological effects of these compounds on fish is the disruption of the endocrine system that is critical in controlling growth, development, and reproduction (Hester and Harrison, 1999). Endocrine disruption can be caused by chemicals acting as mimics (agonists) and/or blockers (antagonists) of endogenous hormones (Figure 1.1).

Trace levels of endocrine disruptors are often found in surface waters, especially in urban areas of a watershed (Writer et al., 2010). They include a diverse group of contaminants, such as steroidal hormones (endogenous and exogenous), alkylphenols (nonylphenols and octylphenols), polychlorinated biphenyls (PCBs), pesticides, and polycyclic aromatic hydrocarbons (PAHs) (Mills and Chichester, 2005). Numerous international studies have reported elevated incidence of ova-testes (intersex) in male fish exposed to wastewater effluents, suggesting exposure to EDCs (Hinfray et al., 2010; Jobling et al., 2002; Jobling et al., 2006; Larsson et al., 1999; Sanchez et al., 2011; Tetreault et al., 2011; Vajda et al., 2008). For example, fathead minnows (*Pimephales promelas*) exposed to small concentrations of 17α-ethinyl estradiol (EE2) during a whole lake

experiment demonstrated changes in histology and physiology followed by a lack of recruitment and a subsequent population collapse (Kidd et al., 2007). Furthermore, fish exposed to effluents have shown effects at the population level, although the causal linkage to specific chemicals has not been fully established (Harris et al., 2010; Mills and Chichester, 2005).

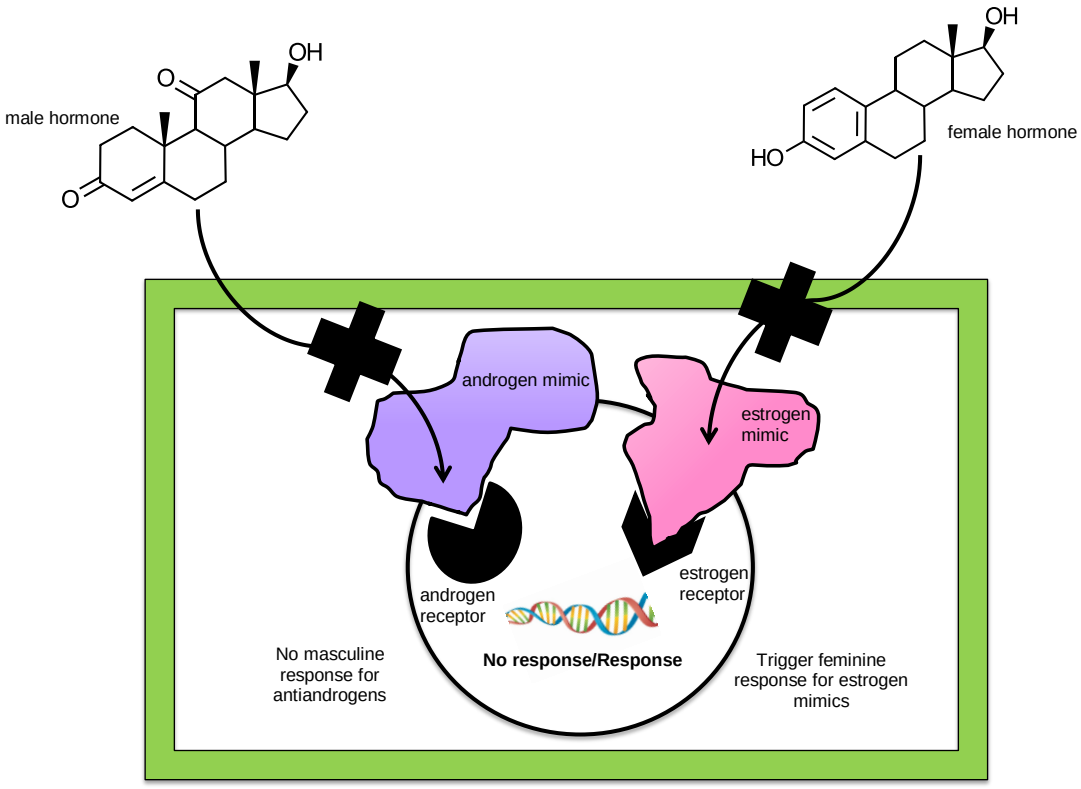

Figure 1.1. Conceptual diagram for antiandrogenic and estrogenic responses in fish. Feminization can occur by (1) blocking androgen receptor thereby preventing masculine responses and/or (2) mimicking female hormone thereby producing feminine responses. Adapted from Hester and Harrison (1999).

Endocrine disruption in wild fish populations is most commonly associated with the presence of exogenous steroidal estrogens. Jobling et al. (2006) suggested a strong correlation of wild roach (*Rutilus rutilus*) intersex to sites with high concentrations of natural and synthetic estrogens (estrone [E1], estradiol [E2], and EE2). However, due to the complex nature of

wastewater effluent mixtures, steroidal estrogens may not be the only compounds causing fish feminization and/or intersex. Antiandrogens are androgen receptor antagonists that can reduce male developmental characteristics in fish (i.e., compounds that make male fish "less male") (Baatrup and Junge, 2001). There have been several studies that have indicated antiandrogenic activity in European surface waters (Jobling et al., 2009; Johnson et al., 2007; Katsiadaki et al., 2012). For example, an assessment of the final effluents from selected wastewater treatment plants (WWTPs) in the United Kingdom (UK) has shown both antiandrogenic and estrogenic activities (Johnson et al., 2007). In addition, statistical modeling showed a strong correlation of the co-occurrence of intersex and the presence of antiandrogens and estrogens in UK WWTPs (Jobling et al., 2009). These findings led to studies that attempted to identify antiandrogenic compounds in WWTP effluents. Hill et al. (2010) and Rostkowski et al. (2011) identified the antiandrogens that accumulated in fish bile (*Oncorhynchus mykiss*) after exposure to WWTP effluents using a Yeast Androgen Screen assay. These non-steroidal antiandrogenic compounds were antimicrobial agents (chlorophene, triclosan, chloroxylenol and dichlorophene), resin acids, naphthols, oxybenzone, 4-nonylphenol, and bisphenol A (Hill et al., 2010; Rostkowski et al., 2011). Out of the compounds identified, triclosan and chlorophene comprised 51% of the antiandrogenic activity in the fish bile (Rostkowski et al., 2011).

Despite the reported prevalence of endocrine disruption in US surface waters (Barber et al., 2011; Hinck et al., 2009; Vajda et al., 2011; Woodling et al., 2006), endocrine disruption due to steroidal estrogens was considered low when the combined exposure of E1, E2, and EE2 was modeled across 12 US watersheds (Anderson et al., 2012). Also, Katsiadaki et al. (2012) investigated specific endocrine disruption biomarkers (vitellogenin and spiggin) in three-spined sticklebacks pre- and post-remediation of a UK WWTP. Vitellogenin is an egg protein precursor

often used as an in vivo biomarker to determine estrogenic activities (Kime et al., 1999). High concentrations of vitellogenin in male fish indicate exposure to estrogens. Spiggin is a biomarker for androgen exposure specific to sticklebacks (Jakobsson et al., 1999). Low levels of spiggin in female sticklebacks suggest exposure to antiandrogens. It was difficult for the researchers to compare vitellogenin induction in male fish before and after remediation due to large variability in the results collected from their control sites. However, they found an increasing trend of the female kidney spiggin downstream of the WWTP during the pre-remediation study, suggesting the presence of antiandrogens in municipal WWTP effluents (Katsiadaki et al., 2012). Their laboratory exposure of male sticklebacks to site-specific concentrations of E1 also failed to increase vitellogenin. This result suggests that the endocrine effects seen at that watershed may potentially be caused by antiandrogens since the effluent they studied did not have enough estrogenicity to produce vitellogenin induction (Katsiadaki et al., 2012). It is, however, difficult to completely account for the effects observed by the presence of steroidal estrogens or antiandrogens alone. Also, it is possible that intersex may be associated with the presence of contaminants in surface waters that cause effects independently or through interaction with a diversity of steroidal estrogens and other forms of EDCs. Additional tools may be required to better understand these biological manifestations.

1.2 Study Objectives

The goal of this thesis is to determine the occurrence and fate of antiandrogens relative to other known pharmaceuticals (ibuprofen, carbamazepine, naproxen, venlafaxine) and the pesticide atrazine through:

1. Development of an analytical method to measure the concentrations of antiandrogens in effluents and surface waters in the Grand River.

2. Prediction of the fate of selected pharmaceuticals and antiandrogens in association with a major wastewater outfall (Kitchener) in the Grand River watershed using a comprehensive surface water quality model.

The analytical method developed is primarily focused on the optimization of previously developed analytical techniques. The major goal of the surface water quality model is not based solely on maximizing the predictive accuracy of the simulations but rather on the provision of additional insights to relevant environmental conditions affecting the distribution of the compounds in the watershed. In addition, modeling is particularly necessary due to the unavailability of field data that can describe the distribution of the target compounds in watershed.

1.3 Study Scope

This thesis focuses on characterizing the distribution and behaviour of antiandrogens and selected pharmaceuticals in the Grand River watershed (agricultural and urban sites). Chapter 2 covers the large scale water survey of target compounds conducted during a summer low-flow period (July 2012). This chapter also describes the variability in the monthly samples (August-November 2012) collected at Kitchener WWTP and sites immediately upstream and downstream of this plant. Chapter 3 describes the modeling approach taken to predict the concentrations of the target contaminants.

1.3.1 Study Site

The Grand River watershed is the largest in southern Ontario entering Lake Erie (drainage area of 6,965 km^2) and receives effluents from thirty municipal WWTPs (Figure 1.2). It also receives non-point releases from agricultural lands (about 70% of the land area) and five

major urban areas (Kitchener, Waterloo, Cambridge, Guelph and Brantford) (Anderson, 2012; Cooke, 2006). Historically, most of the concerns surrounding water quality in the watershed have been related to dissolved oxygen, total phosphorus, nitrates, and ammonia. The central Grand River has been found to be the most impaired area of the watershed due to its low dissolved oxygen levels and high nutrient concentrations stemming from intensive farming activities, increasing urban development, and population growth (Cooke, 2006).

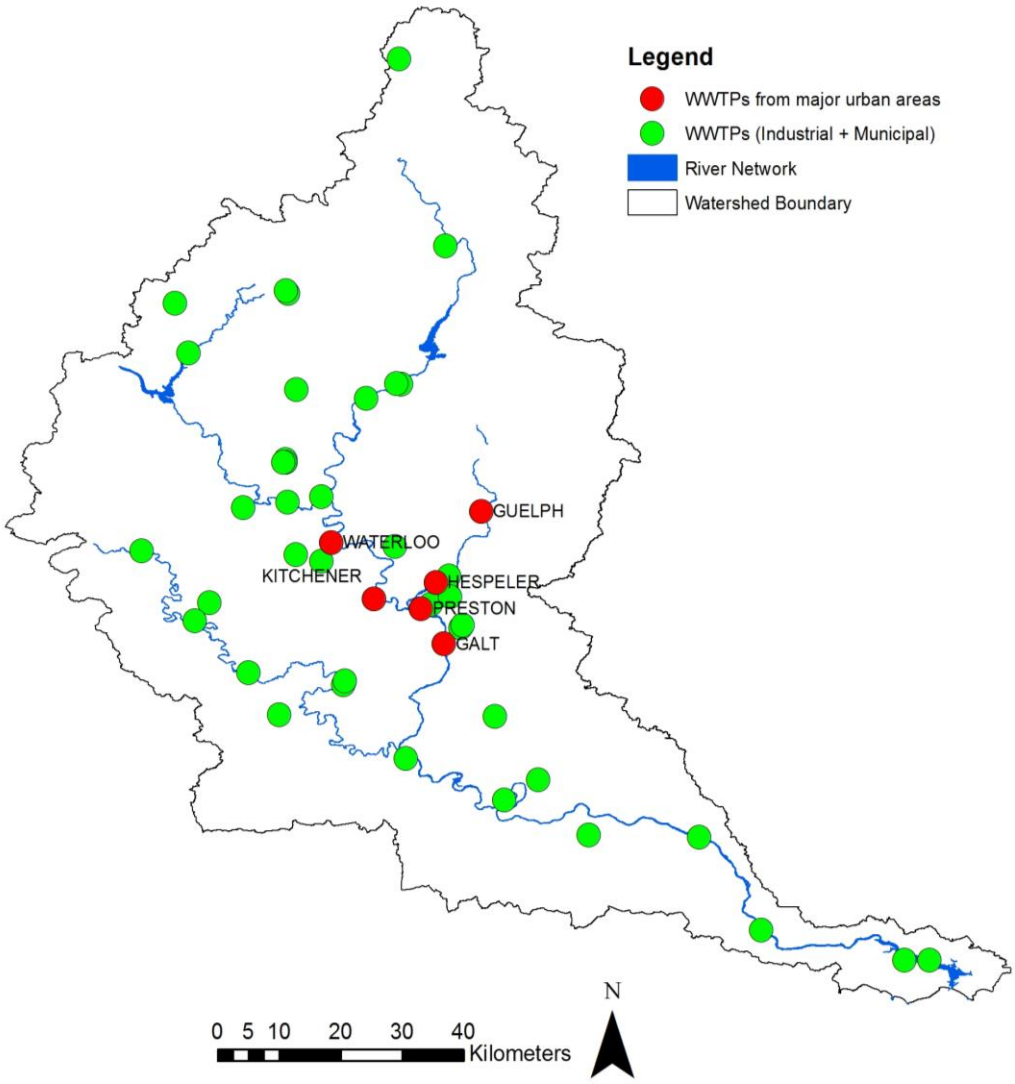

Figure 1.2. Location of WWTPs that discharge in the Grand River watershed. Vector data for the map was taken from Grand River Conservation Authority website on January 13, 2013.

1.3.2 Municipal WWTP Effluents and Watershed Water Quality

Municipal wastewater quality and quantity are dependent on the nature and characteristics of the contributing demographic areas as well as wastewater treatment types and configurations (Holeton et al., 2011). Wastewater treatment designs are predominantly based on discharge standards and management objectives set for conventional pollutants such as biological oxygen demand, ammonia, total suspended solids, and total residual chlorine (Chambers et al., 1997). There are no guidelines or standards set for pharmaceuticals, EDCs, and personal care products to date. However, there is a proposed limit of 0.02 ng L^{-1} for EE2 in Europe (Sumpter and Jobling, 2013). Despite considerable research in this area (Auriol et al., 2006; Jiang et al., 2005; Kasprzyk-Hordern et al., 2009; Liu et al., 2009), the reduction of these compounds during wastewater treatment is not currently a treatment objective but relies on the processes employed for conventional pollutants.

A conventional activated sludge (CAS) system is the most prevalent wastewater treatment type in Canada (Canadian Water and Wastewater Association, 2001). This system relies heavily on biological treatment which metabolically degrades organic contaminants in raw wastewater. CAS systems are often not effective in removing pharmaceuticals, EDCs, and personal care products (Baronti et al., 2000; Belfroid et al., 1999; Johnson et al., 2000; Ternes et al., 2004). However, it has been suggested that longer solids retention times and the addition of treatment processes that incorporate a diverse range of bacterial population (nitrifying and denitrifying systems) may enhance the removal of these compounds (Baynes et al., 2012; Fent et al., 2006; Metcalfe et al., 2003; Servos et al., 2005).

The Kitchener WWTP is a CAS plant with chemical phosphorus removal, anaerobic sludge digestion, sodium hypochlorite disinfection, and sodium bisulphite dechlorination

(Region of Waterloo, 2012). The plant services an estimated population of 226,000 and discharges an average of 65,000 m^3 of effluent per day into the Grand River (Region of Waterloo, 2012). In addition to dissolved oxygen and nutrient issues downstream of this plant, pharmaceuticals have also been detected in the downstream surface water. For instance, six antidepressants (venlafaxine, bupropion, fluoxetine, sertraline, citalopram, and paroxetine) and their metabolites have been detected in the receiving water of a WWTP within the watershed (Metcalfe et al., 2010). Water samples collected downstream of both the Waterloo and Kitchener WWTPs in spring 2010 were found to have high concentrations (ng L^{-1} range) of selected pharmaceuticals such as ibuprofen, carbamazepine, diclofenac, and venlafaxine (Tanna, 2012). Other prescription pharmaceuticals such as lipid regulators (gemfribrozil) and anti-inflammatory medications (naproxen) have also been detected in its surface waters (Lissemore et al., 2006).

A variety of pharmaceuticals have been identified in fish caged downstream of the Kitchener WWTP (Togunde et al., 2012) as well as in wild fish species present in its receiving waters (Wang et al., 2011). Studies conducted by Tetreault et al. (2011) and Tanna et al. (2013) showed an elevated frequency of intersex in wild fish downstream of the effluent discharges. The highest observed intersex was found downstream of the Kitchener WWTP but almost none was seen at the Guelph WWTP which has tertiary-treatment (Tanna et al., 2013). The Kitchener WWTP effluent was found to be estrogenic (Smith, 2013; Tanna et al., 2013) but the specific contaminants causing endocrine disruption are currently unknown. Only limited data are currently available on the distribution of EDCs in the effluents and surface water of the Grand River watershed. Thus, it is hoped that estimation of the spatial distribution of the effluent and surface water concentrations through fate and transport modeling can be used to describe the

environmental processes that are responsible for the distribution of EDCs. When environmental data are limited, water quality models can provide predictions of the behaviour of contaminants in various environmental compartments based on an understanding of their sources and fate in the environment. The following section describes water quality modeling in further detail.

1.4 Water Quality Modeling in the Grand River Watershed

As previously mentioned, very little environmental data are available for EDCs in the Grand River watershed (because of analytical or other limitations). Thus, models maybe used to predict the fate and transport of EDCs in a receiving water body. Consequently, this approach can ideally provide a prediction of fish exposure to the contaminants in the watershed where analytical data are absent or inadequate.

A wide variety of surface water quality models have been developed for different applications and they may incorporate different source types (nonpoint vs. point), phase transfer processes, and transformation mechanisms (Ramaswami et al., 2005). Water quality models are generally founded on the principle of mass balance that accounts for the movement and losses of a contaminant in each environmental compartment (Chapra, 1997; Ramaswami et al., 2005). Each model, however, will differ considerably in its complexity and ability to make predictions. For instance, some water quality models can simulate flow, fate, and transport processes in multiple dimensions. For river applications however, one-dimensionality has been used as a common and justifiable assumption. This is primarily because longitudinal movements are typically more dominant than vertical and transverse movement and well-mixed conditions are generally appropriate for these problems (Ji, 2008). Other water quality modeling assumptions exist in addition to dimensionality. Thus, prior to water quality model selection, it is important that all components of a model are understood. This enables the user to justify and account for

the uncertainties contributed by modeling assumptions (e.g., one dimensional vs. multidimensional systems). The following section aims to provide the mathematical principles behind the methods currently used in standard water quality models.

1.5 Components of a Comprehensive Water Quality Model

Conceptually, any substance traveling through a water volume in the direction of water flow is subject to various transfer and transformation mechanisms (Figure 1.3). These factors can be incorporated into a set of equations or modules solved to replicate the transport of a constituent through a surface water system. Clark (1998) suggests that a comprehensive water quality model should have the following components embedded in its implementation: (1) flow, (2) transport, and (3) fate. Flow modules describe the movement of water within the system; transport modules incorporate the processes that redistribute contaminants based on fluid motion (e.g., advection and dispersion); and fate models determine the chemical transformation of substances and the likely partitioning of compounds to different environmental systems. Detailed descriptions of each standard model component are described subsequently.

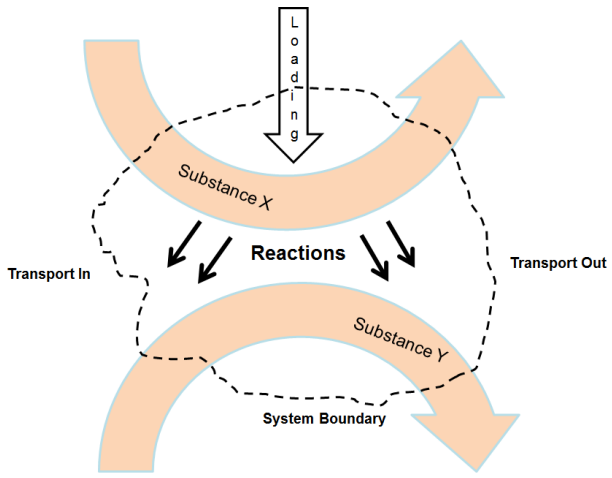

Figure 1.3. Conceptual model for contaminant fate and transport. Adapted from Chapra, 1997 (reconstructed).

1.5.1 Flow Routing

Flow routing is the prediction of the movement of water as it travels within a water body at any given time (Fread, 1993). This component is a critical part in water quality models because flows often drive mass transport and contaminant in-stream loss (Aukidy et al., 2012). For example, when the source is relatively constant, contaminant concentrations are usually low during high flow seasons due to in-stream dilution. Conversely, during low flow seasons, concentrations tend to be high due to low dilution effects (Aukidy et al., 2012). This is not always the case as the sources and removal processes may be spatially and temporally variable.

Flow routing in rivers can be categorized based on two general flow model applications: hydrologic and hydraulic (Figure 1.4). Fread (1993) defines hydrologic routing as a model that computes flow as a function of time. These flow models account for the differences in both inflows and outflows as a time rate of change of storage in that system (Fread, 1993; Martin and McCutcheon, 1999; Ramaswami et al., 2005). The simplest mass balance description for a hydrologic flow routing is presented in Equation 1.1:

$$I\ (t) - O\ (t) = \frac{dS}{dt} \qquad (1.1)$$

where I is inflow, O is outflow, S is storage, and t is time. The major limitation of this model type is its incapability in accounting for the "inherent spatial variability" of water movement that is expected in most rivers (Carpenter and Georgakakos, 2006). In other words, flow tends to vary both in space and time but hydrologic models only use average spatial characteristics of the reach it models. By contrast, hydraulic flow routing enables the user to input spatially varied parameters and computes flow as a function of both time and space (i.e., steady or unsteady with time and uniform or non-uniform with distance) (Fread, 1993). Hydraulic flow routing has been

found to be more accurate in representing the unsteady/non-uniform variations in rivers because theoretically, flow rates, velocities, and water elevations differ both in time and space (Fread, 1993).

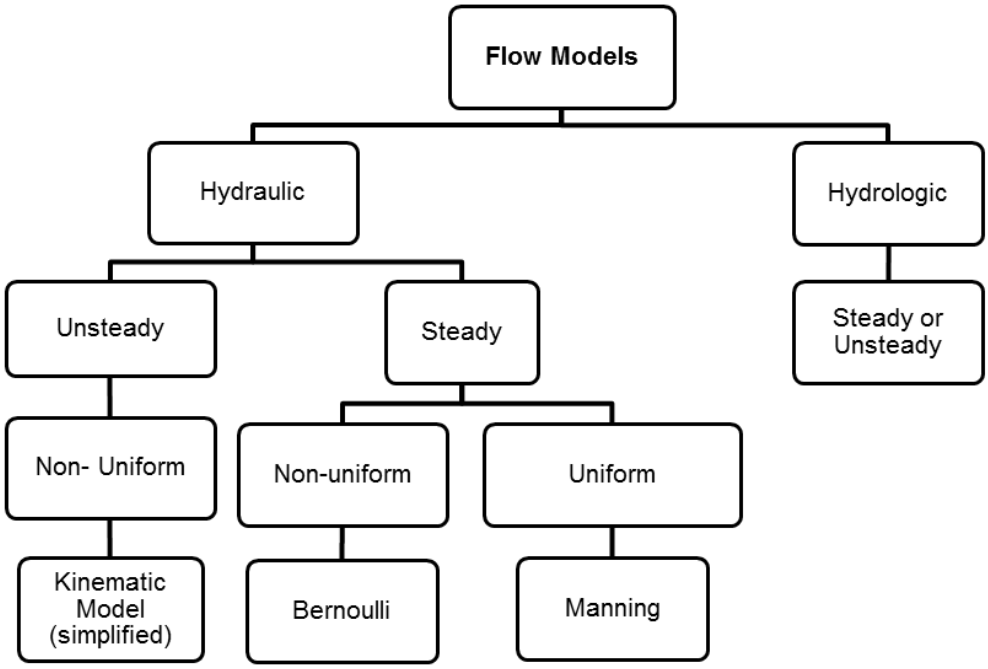

Figure 1.4. Flow model categories describing steady/unsteady and uniform/non-uniform flows. Adapted from Martin and McCutcheon (1999) (reconstructed).

Hydraulic flow routing in one dimension typically utilizes the Saint-Venant equations for continuity and momentum. This set of equations has been extensively studied over the years due to the practical utility of one-dimensional flow models in rivers and streams (Chapra, 1997; Ji, 2008; Ramaswami et al., 2005). The continuity equation is given by:

$$\frac{\partial A}{\partial t} + \frac{\partial Q}{\partial x} = q \qquad (1.\ 2)$$

where A is the cross-sectional area, t is time, Q is the volume flux or discharge, x is the downstream distance and q is the contribution of all other inflows to the control volume. The

momentum equation is described by:

$$\frac{\partial v}{\partial t} + v\frac{\partial v}{\partial x} + g\frac{\partial Y}{\partial x} - gS_o + gS_f = 0 \qquad (1.\ 3)$$

where v is the velocity in the stream, Y is the water surface elevation, S_o is the bed slope, S_f is the friction slope and g is the gravitational acceleration. Depending on the level of simplification, the momentum equation can be described as either kinematic wave, diffusion wave, or dynamic wave models (Figure 1.5).

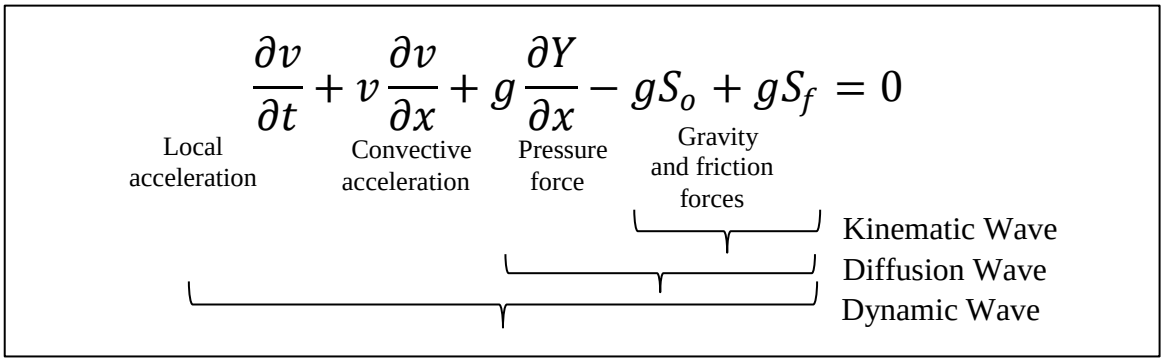

Figure 1.5. Simplifications in momentum equation. Adapted from Chapra, 1997 (reconstructed).

The kinematic wave equation has been used frequently in hydraulic flow routing due to its simplicity and practical use (Martin and McCutcheon, 1999). Equations 1.2 and 1.3 (kinematic wave simplification) can be manipulated to produce a single differential equation:

$$\frac{\partial Q}{\partial x} + \alpha\beta Q^{\beta-1}\frac{\partial Q}{\partial t} = 0 \qquad (1.\ 4)$$

where $\alpha = \left[\frac{nB^{2/3}}{S_o^{1/2}}\right]^{3/5}$, $\beta = \frac{3}{5}$ for rectangular channels, n is Manning's roughness coefficient and B is width. For channels with width-varying flows, $\beta = 0.6 + 0.4d$ and $\alpha = \left[\frac{nb^{2/3}}{S_o^{1/2}}\right]^{3/5}$. The terms d and b are exponents often expressed by the power equations developed by (Leopold and Maddock, 1953):

$$B = aQ^b \quad (1.5)$$

$$Y = cQ^d \quad (1.6)$$

$$v = kQ^m \quad (1.7)$$

where a, c, k, and m are additional empirical constants. Equation 1.4 can be solved using different numerical techniques. Often, it is necessary to divide the modeled reach into smaller segments to improve the model resolution. The segmentation constraints often depend on the contaminant and system properties as well as the choice of scales for which the water quality model is being applied.

1.5.2 Transport Processes

Contaminants are hydro-dynamically transported as soon as they are introduced in surface water (Ji, 2008). Hydrodynamic transport can be in the form of advection, dispersion and vertical mixing/convection (Ji, 2008). Advection in rivers refers to the bulk longitudinal transport of pollutants along with water. Dispersion is the spreading of water mass caused by velocity gradients causing the movement of contaminants from an area of higher concentration to an area of low concentration. Vertical mixing and convection defines the vertical transport of water and dissolved compounds. In general, these transport processes can individually or altogether cause the movement of dissolved substances in water. Transport is often driven by the properties inherent to the system being studied. For example, river transport is typically governed by advection and dispersion while transport of contaminants in small lakes and ponds can be primarily diffusive (Ramaswami et al., 2005). This section mainly focuses on river transport mechanisms.

The transport processes in rivers involve both advective and dispersive fluxes (one-

dimension):

$$J_a = vC \tag{1. 8}$$

$$J = -D\frac{dC}{dx} \tag{1. 9}$$

where J_a is the advective flux density and depends on the concentration C and the flow velocity v. J is the dispersive mass flux density and D is the dispersion coefficient and x is the distance. Therefore, the total mass flux across a distance is:

$$m = J_a + J \tag{1. 10}$$

where m is mass, J_a is the magnitude of advective flux and A is the area (perpendicular to the flow) of the reach considered. Equation 1.10 can be incorporated into a differential mass balance to obtain the conservation of mass equation based on one-dimensional transport processes expressed as (Ji, 2008):

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + \frac{\partial C}{\partial x}\left(D\frac{\partial C}{\partial x}\right) + L + R \tag{1. 11}$$

where L is contaminant loading and R represents the reactions.

In water quality models, hydraulic flow components provide time-varying flows at different locations resulting in velocities that propagate both water and pollutants down a channel. When the kinematic wave and transport modules are applied on a conservative substance, a "dilution wave" is observed, producing an inverse pattern between contaminant concentrations and flows (Chapra, 1997).

1.5.3 Fate Processes

For non-conservative organic contaminants in rivers, dilution through transport processes is inadequate for accurately accounting for the mass distribution of these contaminants in rivers. A substance can also partition to different environmental compartments (air, water, and soil) (Ramaswami et al., 2005). This transfer is dependent on the physico-chemical properties of a substance that dictate their affinity to air, water, and soil. The major transfer processes are sorption and volatilization. Sorption is the association of compounds with solid materials (Chapra, 1997). This process is particularly important in fate modeling since sorbed substance transport is different than that of the dissolved component (Schwarzenbach, 2003). The equilibrium sorption of a compound onto solids can be described by isotherms. Isotherms can either be linear or non-linear depending on the fundamental mechanisms that influence the partitioning process. In linear isotherms, the relationship between the sorbed and dissolved components of a compound is expressed by the partitioning coefficient, K_d (Chapra, 1997):

$$K_d = \frac{C_s}{C_d} \tag{1. 12}$$

where C_s is sorbed component and C_d is the dissolved form of the compound being modeled. The fractions that are sorbed and dissolved can be expressed as (Chapra, 1997):

$$F_d = \frac{c_d}{c} = \frac{1}{1+K_d m} \tag{1. 13}$$

$$F_s = \frac{c_s}{c} = \frac{K_d m}{1+K_d m} \tag{1. 14}$$

where F_d is fraction dissolved, F_s is fraction sorbed, and m is the suspended solids concentration. Water quality models are usually developed on a framework which accounts for

the mass balances of both dissolved and sorbed contaminants.

Volatilization is the exchange of contaminants across the water and air interface. Mathematically, this process can be described by a mass transfer coefficient represented as follows (Chapra, 1997):

$$J_v = v_v \left(\frac{p_g}{H_e} - c_d\right) \quad (1.\ 15)$$

where J_v is mass flux due to volatilization, v_v is the net transfer velocity in air-water interface (m yr^{-1}), p_g is the partial pressure of gas (atm), H_e is Henry's constant (atm m^3 mol^{-1}).

Compounds can also transform into other forms through a variety of reaction mechanisms. Some organic compounds can be completely mineralized into inorganic forms or broken down into simple organic and/or inorganic constituents via an enzyme-mediated process known as biodegradation (Ji et al., 2008). Chemical processes that can be active in surface waters include hydrolysis, oxidation/reduction, and photolysis reactions. Hydrolysis involves the cleavage of bonds in a molecule followed by the formation of new bonds with the hydrogen and hydroxyl constituents of water. Oxidation/reduction occurs when electrons are either transferred from/to the molecule by either an oxidant (e.g., chlorine and ozone) or a reductant that is present in the water. Photolysis involves the transformation of a compound upon absorption of energy from sunlight (direct photolysis) or other molecules that have absorbed sunlight (indirect photolysis). The transformation reactions presented above are often mathematically represented using first-order kinetics for simplified mathematical formulation (Chapra, 1997):

$$\frac{dC}{dt} = R = -kC \quad (1.16)$$

where k is the first-order rate constant [1/T] for a given reaction. In parallel with transfer and

transformation processes that can occur in a system, a substance can be taken up by biota. Thus, it is necessary to use a model that can best predict the bioavailability of pharmaceuticals and EDCs of concern in surface water. For this study, a variety of potential models were examined based on their representation of flow, transport, and fate of contaminants. The next section describes the approach used for model selection approach.

1.6 Water Quality Model Selection

1.6.1 Models for Pharmaceutical Exposure Assessment

In the past, pharmaceuticals including EDCs have been modeled using several models including Geography-Referenced Exposure Assessment Tool for European Rivers (GREAT-ER), iSTREEM^{TM} (GIS-ROUT) and Pharmaceutical Assessment and Transport Evaluation (PhATE^{TM}) (Table 1.1). These models were specifically developed to predict the concentrations of active pharmaceutical ingredients in surface waters at a large spatial resolution (watershed or national scales) (Cunningham, 2008). They have been found to be useful for estimating the cumulative impacts of consumer chemicals in watersheds (Schwab et al., 2005; Sumpter et al., 2006) but are not capable of identifying the key environmental processes that significantly affect pollutant attenuation. For example, PhATE^{TM} uses a very simplistic stream transport equation and the fate module only utilizes a bulk in-stream decay process.

Different spatial resolutions can be employed for different modeling purposes. The watershed model PhATE^{TM} was used by Hosseini et al. (2012) to predict the concentrations of pharmaceuticals, personal care products, and EDCs in the Grand River watershed. The model

Table 1.1. Models Currently Used for Modeling Pharmaceuticals

	Flow	Transport	Fate	Other Features	Applications	Literature Sources
PhATE™	Steady state/uniform	advection only 12 US watersheds	uses a lumped degradation constant that accounts for all mass transfer and transformation processes	GIS-based; segments are considered completely mixed; watershed approach	exposure screening of pharmaceuticals in national and regional scales - U.S. Watersheds and a Canadian Watershed	Anderson et al., 2004; Cunningham, 2008; Hosseini et al., 2011
					human health risks of pharmaceuticals in US surface waters	Anderson et al., 2010
					endocrine disrupting chemicals in US surface waters	Anderson et al., 2010; Anderson et al., 2012
					trace organic compounds in WWTP sludge and biosolids	Cunningham et al., 2012
GREAT-ER[a]	Steady state/uniform	advection only 16 European Watersheds	biodegradation + river loss rates	GIS-based; segments are considered completely mixed; watershed approach	Modeling effects of mixtures of EDCs – watershed scale	Balaam et al., 2010; Sumpter et al., 2006
					Fate of β-blocker human pharmaceuticals in surface water	Alder et al., 2010
					Exposure of pharmaceuticals in European surface waters	Price et al., 2010; Schowanek and Webb, 2002
iSTREEM™[,b]	Steady state/uniform	advection only 28,000 river reaches (320,000 river km with 9,000 WWTPs in continental US)	used a lumped degradation constant that accounts for all mass transfer and transformation processes	GIS web-based	Exposure of DEET in US watersheds	Aronson et al., 2012
					Exposure of surfactants in US watersheds	Wang et al., 2005

Note. The list of the models above was adapted from the review conducted by Cunningham (2008). [a]Adapted from http://www.great-er.org/.[b]Adpated from DeLeo (2011).

defined the areas from Waterloo WWTP and Kitchener WWTP through Brantford as regions having the highest risk of exposure to these compounds (Hosseini et al., 2012) . As mentioned previously, these same areas are known to be the most impaired areas in the watershed. However, it is difficult to focus the model on smaller scale phenomena (e.g., areas downstream of Kitchener WWTP). The biological responses of concern in the watershed such as the Grand River occur over fairly short river reaches (Tanna et al., 2013). The models mentioned above generate results that are not very spatially resolved and therefore not useful in assessing the fate at the smaller reach scale of the watershed.

The model selection in this thesis was started with more general public domain models. Other than being cost-effective, public domain models are usually available in open-source packages that can be easily used by practitioners for performing various surface water quality modeling projects.

1.6.2 Public Domain Models

The models considered for study were EPD-RIV1, Qual2k, AQUATOX, Grand River Simulation Model (GRSM), Water Quality Analysis Simulation Program (WASP 7.5), and Hydrological Simulation Program – FORTRAN (HSPF). Most of the models considered are developed (and maintained) by the US Environmental Protection Agency (US EPA). As shown in Table 1.2, all the models examined are able to model flow, transport, and fate. As previously mentioned, rivers are best described by hydraulic flow routing under unsteady and non-uniform conditions. Hence, models which are incapable of modeling these flow conditions were eliminated (Qual2k and AQUATOX). All of the remaining models are able to simulate advection and dispersion transport processes. Additional models were excluded after examining the capabilities of the models to simulate fate mechanisms. EPD-RIV1 and GRSM were mainly

Table 1.2. Public domain water quality models examined in this study

Module		Flow				Transport		Fate	Spatial Dimension		
Model	**Description**	**Steady**	**Unsteady**	**Uniform**	**Non-uniform**	**Advection**	**Dispersion**		**1d**	**2d**	**3d**
EPD-RIV1	one-dimensional hydraulic and water quality model developed by US Army Engineers Waterways Association	x	x	x	X	x	x	not valid for trace organic contaminant; developed for nutrients, DO, coliform, macrophytes	x		
Qual2K	Microsoft Excel-based river and stream water quality model developed by US EPA	x			X	x	x	not valid for trace organic contaminant; developed for nutrients, DO, coliform, macrophytes	x		
AQUATOX	hydraulic and water quality model designed for ecological risk assessment developed by US EPA		x	x		x	x	nutrients + organic contaminant fate (ionization, sorption, hydrolysis, volatilization, photolysis, biodegradation)		x	
GRSM	Grand River Simulation Model: dissolved oxygen model developed for Grand River watershed. Developed by Grand River Conservation Authority	x	x	x	X	x	x	not valid for trace organic contaminants; developed for nutrients, DO, Total phosphorus, nitrates, macrophytes	x		
WASP 7.5	Water Quality Analysis Simulation Program: dynamic water quality model for surface water and underlying sediment compartment developed by US EPA	x	x	x	X	x	x	nutrients + organic contaminant fate (ionization, sorption, hydrolysis, volatilization, photolysis, biodegradation)	x	x	x
HSPF	Hydrological Simulation Program - FORTRAN: watershed model which incorporates fate and transport of contaminants developed by US Geological Survey. Covers runoff/non-point source contaminants		x	x	X	x		nutrients + organic contaminants during agricultural runoff events (sorption, plant uptake)	x		

Note. Model description of flow, transport, and fate modules were taken from the model's technical manual.

developed for conventional pollutants such as dissolved oxygen (DO) and nutrients. The mathematical theory behind modeling conventional pollutants is not directly applicable for trace organic contaminants. Hence, EPD-RIV1 and GRSM were considered not applicable for the purposes of this study.

Water Quality Analysis Simulation Program 7.5 (WASP) was the only model from the list which can simulate flow in unsteady and non-uniform cases and also model toxicant fate and transport in up to three dimensions. The model was initially developed to characterize eutrophication processes but was later modified to include toxic organic fate and transport. WASP has been used in many different organic contaminant applications including fate and transport of persistent compounds such as PCBs and the pesticide atrazine (Table 1.3).

Table 1.3. Selected WASP model applications

Application	Sources
Transport and transformation of mercury fractions in streams	Lin et al., 2011
Hydrodynamic and salinity modeling	Umgiesser and Zampato, 2001
Fate and transport of non-point source pollutants	Lai et al., 2011
Transport of polychlorinated biphenyls (PCB)	Vuksanovic et al., 1996
Nitrobenzene spill in Songhua River, China	Ren et al., 2007
Three-dimensional eutrophication model for Hamilton Harbour	Kellershohn and Tsanis, 1999
Evaluation of atrazine levels in Lake Michigan basin	Rygwelski et al., 1999
Eutrophication in Lake Winnipeg, Canada	Zhang and Rao, 2012
Eutrophication of the Neuse River Estuary, NC; eutrophication Coosa River and Reservoirs, AL; PCB pollution of the Great Lakes, eutrophication of the Potomac Estuary, kepone pollution of the James River Estuary, volatile organic pollution of the Delaware Estuary, and heavy metal pollution of the Deep River, North Carolina, mercury in the Savannah River, GA.	US EPA, retrieved from http://www.epa.gov/athens, March 2012

WASP uses the continuity equation and the kinematic wave equation when simulating one-dimensional hydraulic flows. Advection and dispersion processes and contaminant fate mechanisms are included in the modeling package. WASP is applicable to most water types (lakes, reservoirs, and rivers) but cannot handle mixing zone processes. Each segment is considered completely mixed, hence proper segmentation is required especially in areas where incomplete mixing is expected (i.e., immediately downstream of WWTP). The fate mechanisms in WASP are illustrated in Figure 1.6. In WASP, the compound first undergoes ionization which is mainly dictated by its ionization constant and the environmental pH conditions. The ionized and unionized forms undergo both transfer and/or transformation processes and each can behave differently in the environment. The major fate mechanisms represented in WASP are hydrolysis, photolysis, and biodegradation.

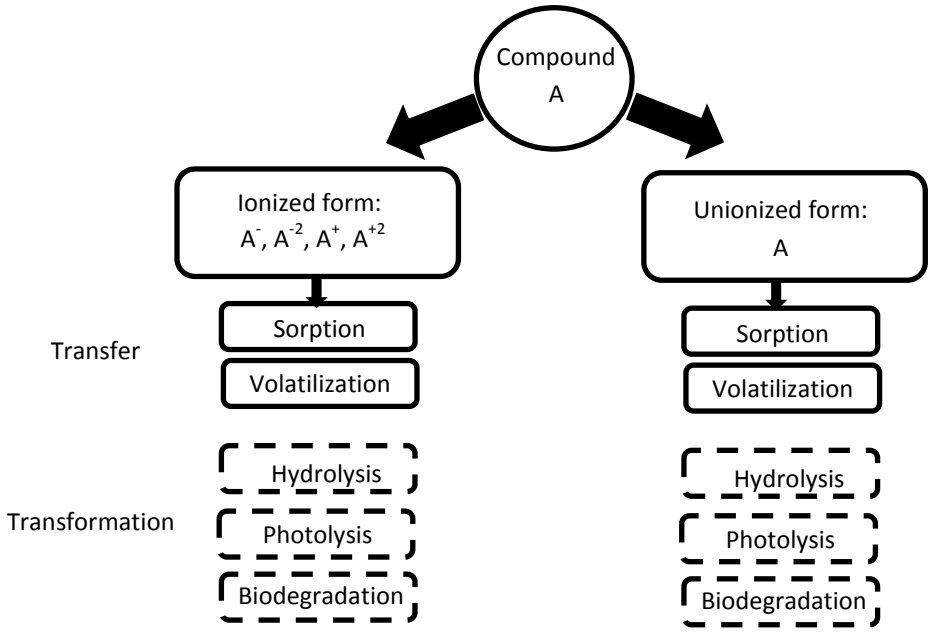

Figure 1.6. Transfer and transformation mechanisms illustrated by WASP. The compound undergoes ionization and each component are transferred and transformed separately. Adapted from Wool et al. (2002).

Overall, WASP has a modeling environment favourable for trace organic toxicant modeling in rivers. WASP has been under development for more than 30 years and is continuously subjected to modifications and improvements by its developers. As the most widely used water quality model in the US, training and technical support is also available for its users.

2 Spatial and temporal distribution of selected antiandrogens and pharmaceuticals in a highly impacted watershed

This chapter will be submitted as a manuscript to a peer-reviewed journal.

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- Leslie Bragg: Assisted with water sampling, field work, and laboratory analysis portion of this paper.
- Wayne Parker: Co-supervisor to Maricor Arlos and assisted with research direction, editing, and general advice.
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2.1 Introduction

Municipal wastewater treatment plant (WWTP) effluent is a complex matrix with diverse aquatic environmental contaminants and stressors. These include pharmaceuticals, endocrine disrupting compounds (EDCs) and personal care products (Daughton and Ternes, 1999; Kolpin et al., 2002; Schwarzenbach, 2003; Ternes et al., 2004). WWTP effluents and their receiving water bodies have been linked to endocrine disruption in male fish (Jobling et al., 1998; Purdom et al., 1994; Rodgers-Gray et al., 2001; Vajda et al., 2008). The feminization of fish in particular has generally been associated with environmental estrogens (estrogen receptor agonists) such as natural and synthetic hormones (17β-estradiol, 17α-ethinyl estradiol) and industrial chemicals such as alkylphenols (Spengler et al., 2001; Tyler and Routledge, 1998). Recently, it has been suggested that endocrine effects may be associated not only with environmental estrogens, but also antiandrogens (androgen receptor antagonists) (Jobling et al., 2009; Johnson et al., 2007; Katsiadaki et al., 2012). Grover et al. (2011) and Jobling et al. (2009) additionally suggested that the combined effects of both antiandrogenic and estrogenic compounds found in municipal WWTP effluents may also explain the expression of endocrine effects in wild fish. This hypothesis, however, has not yet been substantiated. Endocrine active contaminants are usually present at very low levels in surface waters and environmental monitoring of these compounds is very challenging (Fenlon et al., 2010).

Using bile samples collected from caged rainbow trout exposed to municipal WWTP effluents, Hill et al. (2010) and Rostkowski et al. (2011) were able to isolate a number of antiandrogens using a Yeast Androgen Screen assay (anti-YAS). The chemicals associated with the majority of the antiandrogen activity in high performance liquid chromatography (HPLC) fractions included antimicrobial agents (chlorophene, triclosan, chloroxylenol and

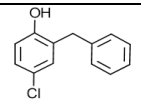
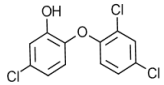
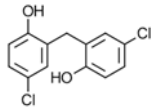
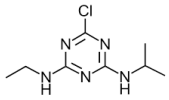
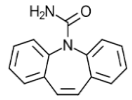
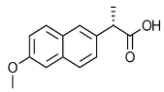
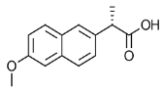
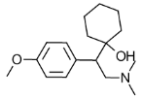
dichlorophene), resin acids, naphthols, industrial chemicals (4-nonylphenol, and bisphenol A), a sunscreen agent (oxybenzone), and a PAH metabolite (1-hydroxypyrene). Triclosan and chlorophene explained 51% of the antiandrogenic activity found in the bile of the exposed fish (Rostkowski et al., 2011).

The antiandrogens identified in fish bile are very diverse and are suspected to come from WWTP effluents. Antimicrobial agents, such as triclosan and chlorophene, are widely used ingredients in soaps and disinfectants (Miao et al., 2005; Werner et al., 1983). Triclosan has been increasingly used over the last 30 years and has already been widely detected in different environmental compartments including wastewater, surface waters, sediments and biosolids (Davis et al., 2012; Katz et al., 2013; Singer et al., 2002). Chlorophene is a common ingredient in cosmetics, cleaning solutions, and disinfectants (Swisher and Gledhill, 1973) and has been detected in wastewater effluents and associated surface waters (Kasprzyk-Hordern et al., 2008). Sources of 1-naphthol and 2-naphthol likely include domestic wastewater as they are used as ingredients in hair dyes and cosmetics (Denavarre, 1975). Personal care products, such as oxybenzone and industrial chemicals such as bisphenol A are well known contaminants in wastewater (Coronado et al., 2008; Crain et al., 2007). Although a diversity of potential antiandrogens have been identified in effluents, the level of exposure of fish to these chemicals in receiving waters, the mechanism of the responses and potential interactions with other chemicals remain poorly understood.

The Grand River in southern Ontario, Canada receives effluents from 30 wastewater treatment plants (Anderson, 2012; Cooke, 2006). A variety of effects have been reported in fish associated with wastewater effluents in the Grand River including changes in gene expression, physiology (Ings et al., 2011; Ings et al., 2012), population endpoints (Tetreault et al., 2011), and

community assemblages (Tetreault et al., 2012). Very high incidence of intersex (ova-testis) has been observed in several species of fish in areas immediately downstream of the two major WWTP outfalls (Waterloo and Kitchener) in this watershed. These effluents have been shown to be estrogenic (Tanna et al., 2013) and likely contain key environmental estrogens (Smith, 2013). However, the nature of the compounds causing endocrine disruption (e.g., antiandrogenic or estrogenic) in fish is currently unidentified. In addition, the distribution and fate of the antiandrogens in effluents and surface waters of Grand River watershed remains largely unknown. In this paper, the occurrence and distribution of several chemicals in the watershed (effluent and surface water) was examined. The survey of chemicals includes chlorophene and triclosan (Table 2.1), compounds that have been shown to have a major contribution to antiandrogenic activity in fish bile based on a previously published study (Rostkowski et al., 2011). Dichlorophene, a compound which was frequently detected by Hill et al. (2010) in the antiandrogenic fractions of fish bile samples was also included in the analysis. Some of the frequently detected pharmaceuticals (the antiepileptic drug carbamazepine, the analgesics ibuprofen and naproxen, the antidepressant venlafaxine) and the pesticide, atrazine (Table 2.1) were also examined to provide a general pattern of the spatial distribution of trace organic compounds coming from both point and nonpoint sources in the watershed. Both the spatial and temporal distribution patterns of these compounds can further enhance the overall understanding of the environmental distribution of contaminants. This information can also direct further research on the persistence of these compounds in surface waters and their adverse ecological effects on the aquatic ecosystem.

Table 2.1. Physical and chemical properties of selected contaminants included in the study

Compound	Major Use	Structure	Molecular Weight ($g\ mol^{-1}$)	Chemical Formula	pKa	log K_{ow}	Henry's Law Constant [a] ($atm\text{-}m^3\ mol^{-1}$)	Solubility[a] ($mg\ L^{-1}$)
Triclosan	Antibacterial/ Antiseptic		289.54	$C_{12}H_7Cl_3O_2$	7.9, 8.14[a]	4.76	2.1×10^{-8}	10 at 20°C
Chlorophene	Antibacterial/ Antiseptic		218.68	$C_6H_5CH_2C_6H_3OHCl$	10.8 at 20°C[b]	3.6	2.7×10^{-8}	149 at 25°C
Dichlorophene	Antibacterial/ Antiseptic		269.13	$C_{13}H_{10}Cl_2O_2l$	$pKa_1 = 7.60$ $pKa_2 = 11.60$	4.26	1.2×10^{-12}	30 at 25°C
Atrazine	Pesticide		216.54	$C_8H_{14}ClN_5$	1.9	2.61	2.6×10^{-9} at 25°C	34.7 at 26°C
Carbamazepine	Anti-epileptic drug		236.27	$C_{15}H_{12}N_2O$	13.9	2.45	1.08×10^{-7} at 25° C	18 at 25°C
Ibuprofen	Anti-inflammatory		206.28	$C_{13}\text{-}H_{18}O_2$	5.2	3.97	1.5×10^{-7}	21 at 25°C
Naproxen	Anti-inflammatory		230.26	$C_{14}H_{14}O_3$	4.15	3.18	3.39×10^{-10} at 25°C	15.9 at 25°C
Venlafaxine[1]	Antidepressant		277.4	$C_{17}H_{27}NO_2$	10.09	3.2	2.04×10^{-11} at 26°C	267 at 25°C

Note. Adapted from "Hazardous Substances Data Bank" by United States National Library of Medicine. Retrieved March 2013 from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. [a] Adapted from Perron et al.,2012.[b]"Reregistration eligibility decision: ortho-benzyl-p-chlorophenol" by United States Environmental Protection Agency, Retrieved April 2013 from http://www.epa.gov/oppsrrd1/REDs/2045red.pdf

2.2 Materials and Methods

2.2.1 Reagents and Chemicals

Chlorophene, ibuprofen, carbamazepine, venlafaxine, and chloramphenicol were purchased from Sigma-Aldrich. Lorazepam was obtained from Cerilliant Corp. (Round Rock, TX), atrazine from Chem Service (West Chester, PA), triclosan from Alfa Aesar (Wardhill, MA) and fluoxetine from Interchim (San Pedro, CA). The isotopically labeled standards (atrazine-d_5, carbamazepine-d_{10}, ibuprofen-d_3, triclosan-d_3, venlafaxine-d_6,) were purchased from CDN Isotopes Inc. (Pointe-Claire, QC, Canada). Chlorophene-d_7 was obtained from Toronto Research Chemicals (Toronto, ON). The stock solutions for all compounds were prepared in methanol (Fisher Scientific).

Acetonitrile (HPLC grade) and hydrochloric acid (10 M) were purchased from Fisher Scientific. HPLC grade methyl tert-butyl ether and ammonium acetate were obtained from Sigma-Aldrich. Ultrapure water for mobile phase preparation was obtained from a Milli-Q® system with a specific resistance of 18 MΩ cm.

2.2.2 Surface Water and Effluent Sampling

The Grand River watershed is the largest watershed in Southern Ontario with an area of 6,965 km^2 and a population of approximately one million (Anderson, 2012). The Grand River receives agricultural (approximately 70% of the total watershed area) and urban runoffs in addition to effluent discharges from WWTPs. The watershed survey conducted during the summer low flow condition (July 21, 2012) included water sample collections at 29 sampling locations (Figure 2.1) across the watershed. During summer low flows, contaminant concentrations are likely higher due to low dilution effects and thus can be detected with more

precision and accuracy. The survey covered a total of approximately 100 km of river length and altogether, the total population served by the WWTPs sampled is approximately 50% of the watershed population. The area sampled is representative of the urban and agricultural activities that are present in the watershed. Six WWTPs discharging into these reaches of the river were also included. The treatment plants also represent several different treatment processes ranging from conventional activated sludge to advanced treatment systems (Table 2.2). Nutrient data (ammonia, nitrite, nitrate) as well as chloride and field conductivity data were collected for all surface water and effluent samples. Water and effluent samples were collected for all sites in summer 2012 (July 21, 2012) (Figure 2.1). Due to other sampling constraints, this set of data is considered sufficient for the purposes of this study. This sampling program will represent the spatial distribution of compounds during a summer low flow condition, when concentrations are expected to be high. The Kitchener WWTP, one site upstream (G52), and immediately downstream within the plume (G53E) were monitored monthly during August-November 2012 to determine the temporal variability.

Grab water samples were collected in three replicates (across the river section) using 500 mL pre-cleaned amber glass bottles with Teflon® lined screw caps. For sites immediately downstream of the outfalls (G33, G53, and G54), the river was divided into two sampling locations across the river, each with three replicates to capture the incomplete mixing conditions. For wastewater samples, 125 mL pre-cleaned amber glass bottles also with Teflon® lined screw caps were used (n=3 for each WWTP site). The water samples were preserved onsite with sodium azide (200 g L^{-1}) and ascorbic acid (20 g L^{-1}). Sample bottles were stored in chilled coolers and transported to the laboratory for analysis.

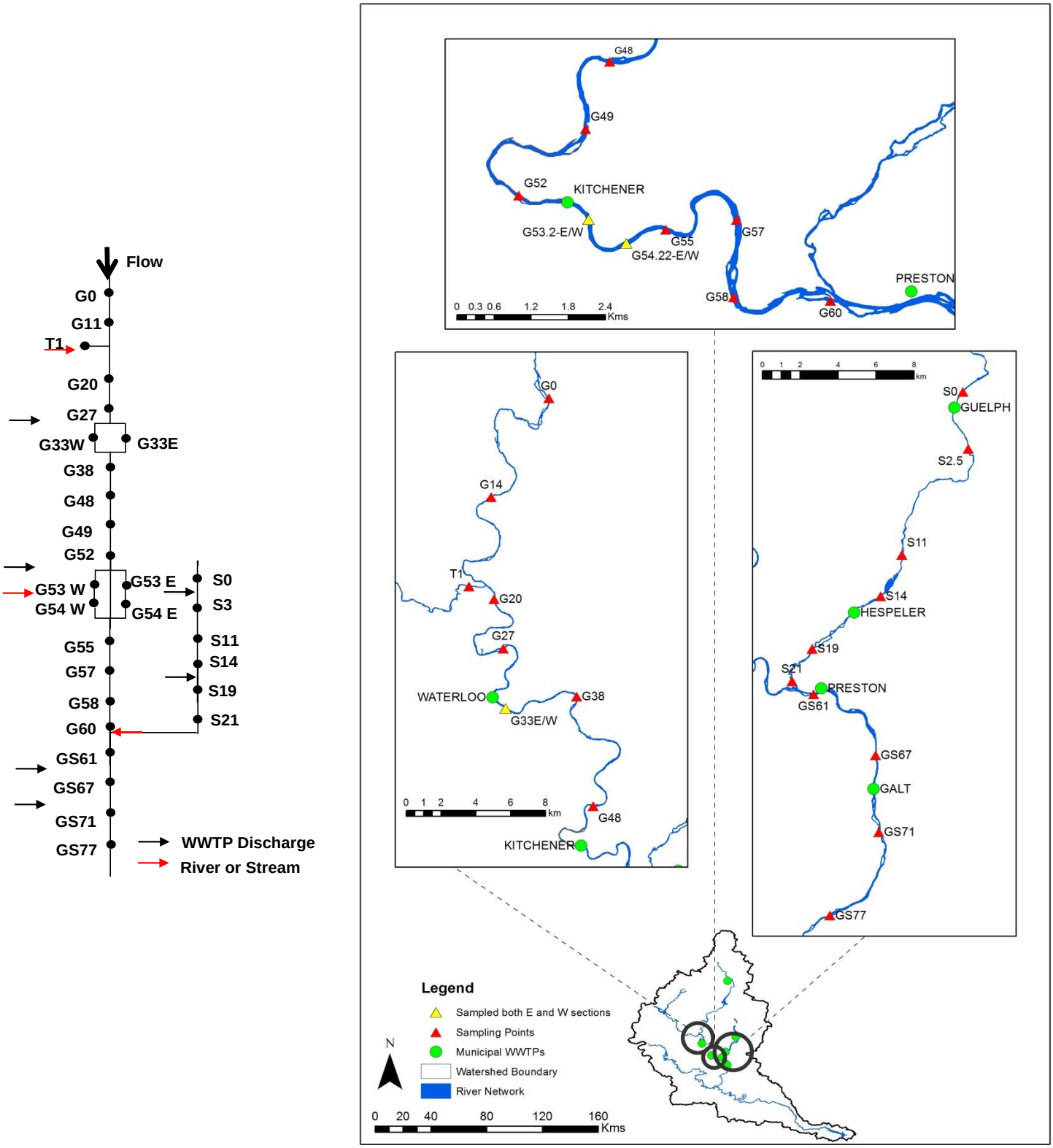


Figure 2.1. Sampling sites for July 2012 water survey. G=Grand River sites. S=Speed River sites. GS=sites in the Grand River affected by Speed River inflows. T=tributary. The number beside each letter corresponds to longitudinal distance (km) starting at 0 km for the most upstream site. GIS data source from the Grand River Conservation Authority retrieved August 2012.

Table 2.2. Description of the WWTPs included during the water survey

Treatment Plant	Waterloo	Kitchener	Guelph	Hespeler	Preston	Galt
Population Served	126,029	226,106	126,000[a]	22,000[b]	22,333[b]	88,667[b]
Treatment System	Two-stage conventional activated sludge (non-nitrifying)	conventional activated sludge (non-nitrifying)	conventional and extended activated sludge (nitrifying)	conventional activated sludge (non-nitrifying)	conventional activated sludge + nitrification-denitrification	Conventional and extended activated sludge (nitrifying)
Primary Treatment	Bar screen, grit removal, primary clarifier					
Secondary Treatment	Aerobic/ anaerobic digestion	Aeration, secondary clarifier				
Advanced Treatment	phosphorus removal	phosphorus removal	nitrification, sand filters	phosphorus removal	phosphorus removal	phosphorus, nitrification, sand filters
Disinfection	UV disinfection	Sodium hypochlorite; sodium bisulphite de-chlorination	Sodium hypochlorite; sodium bisulphite de-chlorination	Sodium hypochlorite; sodium bisulphite de-chlorination	UV disinfection	UV disinfection
Current Design Flow (m^3 d^{-1})	72,730	122,745	64,000	9,320	16,860	56,800
Mean Daily Flow 2010 (m^3 d^{-1})	42,001	64,304	46,214	8,297	9,841	35,635

Note. Adapted from "Wastewater Treatment Plant Descriptions" by Region of Waterloo, 2012 and "Wastewater Treatment Plant Description" by Region of Waterloo, 2013. [a] "Wastewater Treatment Plant Annual Report", by City of Guelph, 2010 . [b] "Assessment of Future Water Quality Conditions in the Grand and Speed Rivers" by Anderson, 2012.

2.2.3 Sample Preparation and Solid Phase Extraction

The sample preparation and extraction process is summarized in Figure 2.2. Matrix effects result from different types of dissolved compounds that comprise surface water and wastewater and oftentimes cause problems in sample analysis. Matrix effects were compensated by the addition of 125 µL of a 1 mg L^{-1} solution containing isotopically labeled antiandrogen standards and 100 µL of a 100 µg L^{-1} solution containing isotopically labeled pharmaceutical standards into the samples prior to sample extraction. Bond Elut Plexa cartridges (6 cc, 500 mg, Agilent Technologies, Mississauga, ON) were used for solid phase extraction of all the water samples. A 12-port Visiprep™ manifold (Supelco, Bellefonte, PA) was used to manually extract the samples under vacuum conditions. The samples were eluted with 6 mL of methanol into 10 mL test tubes and evaporated to dryness with nitrogen using a Dionex SE 500 solvent evaporator at 30°C. Samples were reconstituted with 500 µL of methanol containing 75 µg L^{-1} of lorazepam and 75 µg L^{-1} of chloramphenicol as internal standards.

As part of the quality assurance/quality control (QA/QC) checks, solid phase extraction (SPE) recoveries were determined by spiking two samples of 475 mL ultrapure water with 125 µL each of 1.0 mg L^{-1} antiandrogen non-deuterated and deuterated solutions and 100 µL each of 100 µg L^{-1} pharmaceutical non-deuterated and deuterated solutions. Additional 475 mL ultrapure water samples were prepared as sample blanks. In general, solid phase extractions using Bond Elut Plexa cartridges were found to be effective in isolating the target analytes from surface and wastewater matrices. Mean matrix spike recoveries (July – November samples, n=18) were 79% for chlorophene, 97% for naproxen, 84% for ibuprofen, 127% for atrazine, and 99% for carbamazepine.

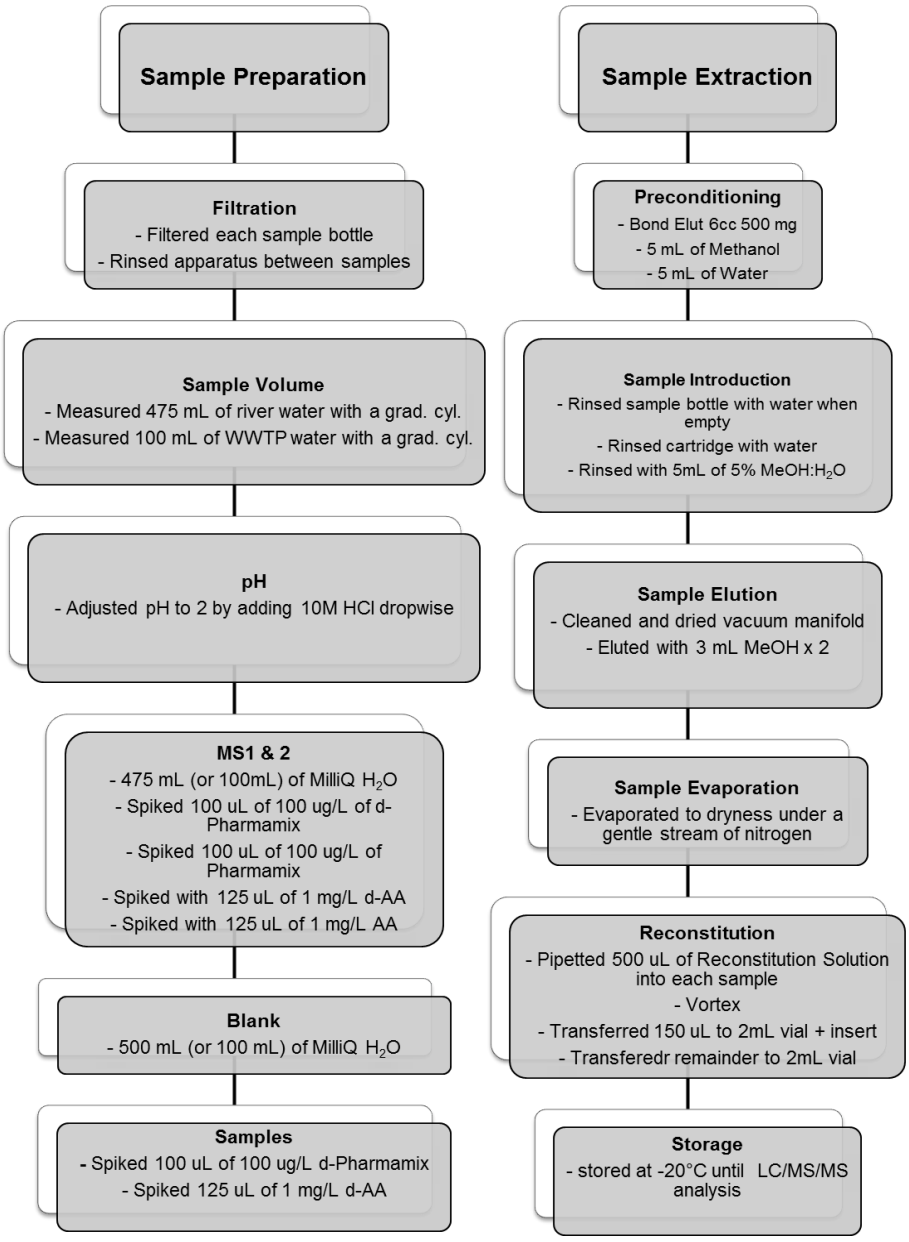


Figure 2.2. Sample preparation and extraction procedures samples collected during the survey. AA=antiandrogen; MeOH=methanol; d=deuterated; Pharmamix=pharmaceutical mixture.

2.2.4 Sample Analysis

Sample analyses for antiandrogens and selected pharmaceuticals were completed using liquid chromatography and tandem mass spectrometry (LC-MS/MS) using an Agilent 1200 HPLC coupled to an Applied Biosystems 3200 QTRAP® mass spectrometer (ABSciex,

Concord, ON, Canada) equipped with an electrospray ion (ESI) source. Positive or negative ion mode was operated using multiple-reaction monitoring (MRM) for the transition ions listed in Table 2.3. Three LC-MS/MS methods were used for analyses: one for antiandrogens (negative mode) and two for pharmaceuticals (negative and positive modes). Chromatographic separation of analytes was done in a 4.6 mm x 150 mm x 5 μm Agilent Eclipse XDB-C18 column (Mississauga, ON) for antiandrogenic analytes and 4.6 mm x 250 mm x 5 μm Agilent Eclipse XDB-C18 column (Mississauga, ON) for pharmaceuticals. Data collection and quantitation was completed using the Analyst® software version 1.4.2 (Applied Biosystems). Each analyte was quantitated using an internal standard calibration where the ratio of the analyte to the internal standards (isotopically labeled standards) was evaluated at low range calibration points (0, 0.5, 1, 10, 50, 100, 200, and 500 ug L^{-1}). The linear relationship drawn from the calibration curves were used to estimate the quantity of analytes in the samples. The linearity was evaluated using least square regression where values for coefficient of determination, R^2, were derived. The calibration curves prepared had regression correlation coefficients varying from 0.9492 – 0.9980.

For pharmaceuticals in positive mode, the mobile phase gradient began at 80% B and was ramped to 100% B over a 4.5 min period where it was held constant for 1 min. The initial negative mobile phase for pharmaceuticals gradient was 60% B which was then increased to 100% B over an 8 min period where it was held constant for 0.5 min. The initial antiandrogen mobile phase gradient was 10% B, increased to 100% B over 12 min and held constant for 5 min. The chromatographic and ionization parameters are presented in Table 2.4.

Table 2.3. Optimized values of LC-MS/MS MRM parameters used for analysis of selected antiandrogens and pharmaceuticals in this study

Analyte	Use	Q1	Q3	Polarity	DP	EP	CEP	CE	CXP
Triclosan	Antimicrobial	286.9	35.0	-	-33	-2.0	-7	-30	-3.0
Chlorophene	Antimicrobial	217.0	181.2	-	-61	-5.0	-14	-27	-2.5
Dichlorophene	Antimicrobial	268.8	128.9	-	-55	-4.0	-12	-30	0
Atrazine	Pesticide	216.2	174.3	+	67	3.8	13	27	2.4
Naproxen	Anti-inflammatory	229.0	170.0	-	-29	-1.9	-20	-25	-3.8
Carbamazepine	Antiepileptic	237.1	193.3	+	55	4.9	14	51	2.7
Ibuprofen	Anti-inflammatory	204.9	160.9	-	-41	-2.6	-19	-11	-0.5
Venlafaxine	Antidepressant	278.3	58.1	+	38	2.9	21	42	8.0
Surrogates									
Triclosan-d_3		286.9	35.0	-	-33	-2.0	-7	-30	-3.0
Chlorophene-d_7		223.9	187.1	-	-60	-10.0	-14	-26	-6.0
Atrazine-d_5		221.1	179.3	+	68	4.1	16	22	3.0
Carbamazepine-d_{10}		247.2	204.4	+	61	4.3	17	28	3.1
Naproxen-d_3		232.1	172.8	-	-15	-5.0	-10	-20	-3.0
Ibuprofen-d_3		207.9	164.1	-	-25	-7.6	-19	-10	-3.0
Venlafaxine-d_6		288.4	58.1	+	45	3.3	18	45	2.4

Note. Q1=quadrupole 1; Q3=quadropole 3; DP=declustering potential; EP=entrance potential; CEP=collision cell entrance potential; CE=collision energy; CXP=collision exit potential.

Table 2.4. Chromatographic and ionization parameters used for LC-MS/MS analysis for target analytes

	Antiandrogens	Other Pharmaceuticals	
Ionization conditions	**Negative**	**Positive**	**Negative**
Curtain Gas (psig)	10	30	10
Collision Gas (psig)	-	8	6
Ion Spray Voltage (IS)	-4500	5500	-4500
Temperature (^oC)	650	750	750
Ion Source Gas 1	70	50	60
Ion Source Gas 2	30	30	40
Chromatographic conditions			
Injection volume (μL)	20	20	20
Solvent A	water	5 mM ammonium acetate in water	5 mM ammonium acetate in water
Solvent B	acetonitrile	methanol	methanol
Flow rate (mL/min)	0.8	0.8	0.8

2.2.5 Detection Limits and Quantitation

The detection and quantification limits for the LC-MS/MS instrument (IDL and IQL) were calculated by running blanks (n=7) and a calibration curve (serially diluted standards) containing the following concentrations: 0.00625, 0.02, 0.1, 0.2, 0.5, 1.0, 10, and 50 ug L^{-1} (n=7 to 8). Using the following equations, IDL and IQL were determined:

$$IDL = \text{ any point in the cal curve with signal} \geq \bar{X}_{blanks} + 3 \times s_{blanks} \quad (2.1)$$

$$IQL = \text{ any point in the cal curve with signal} \geq \bar{X}_{blanks} + 10 \times s_{blanks} \quad (2.2)$$

where $\bar{X}$ is the average and s is the standard deviation of the blanks processed. Under the assumptions of normality, minimal matrix interferences, and constant standard deviation at low concentrations of spike, the method detection limit (MDL) was calculated using the US Environmental Protection Agency (US EPA) (1997) method:

$$MDL = s_{spiked\ aliquots} \times t_{(n-1,1-\alpha=0.01)} \quad (2.3)$$

where n is the number of aliquots processed using the analytical method developed which were then spiked at a concentration (before solid phase extraction) at least 1 to 5 times the estimated MDL, s is standard deviation of analytical results for n spiked aliquots, t is the Student's t value at n–1 degrees of freedom and 1–α (99 percent) confidence level. The Student's t value for n equals 7 and α equals 0.01 is 3.14. Equation 3 suggests that a value that is equal or greater than the MDL can be reported with 99% confidence that the analyte concentration is greater than the concentration found in blanks. Table 2.5 summarizes the instrument detection/quantification limits as well as the analytical method detection limits for each analyte. The analysis of spiked

replicates was performed for both surface water and wastewater matrices and was completed over a short period of time to minimize intraday variability in measurements.

Table 2.5. Method detection limits for methods developed for antiandrogen analysis

Compound	Surface Water MDL (ng L^{-1})	Wastewater MDL (ng L^{-1})
Triclosan	1.0	5[a]
Chlorophene	3.0	25.0
Dichlorophene	8.0	27.0
Atrazine	10.0	50[a]
Carbamazepine	1.0	5[a]
Naproxen	3.0	15[a]
Venlafaxine	1.0	5[a]
Ibuprofen	2.0	10[a]

Note. [a]Wastewater MDL was estimated to be five times the surface water MDL.

2.2.6 Statistics

Analysis of Variance (ANOVA) was used as a statistical tool to determine the presence of the significant differences in the mean concentrations of the sites sampled. A Tukey test (paired comparison) was additionally used to find the means that are significantly different from each other. The Student's t-test was used when only comparing two sites (i.e., upstream vs. downstream). All the statistical tests were performed using SigmaPlot®12.0.

2.3 Results

2.3.1 Antiandrogens in River Water and Wastewater – Summer Low Flow Conditions

Triclosan was detected in only three of the WWTPs: Waterloo, Kitchener, and Hespeler with concentrations 325 ± 89 ng L^{-1}, 960 ± 88 ng L^{-1}, and 345 ± 43 ng L^{-1} respectively. During the July 21, 2012 water survey, triclosan was detected at concentrations ranging from <3-109 ng

L^{-1} in the surface waters of the Grand River but not in its major tributary, the Speed River (Figure 2.3). Elevated concentrations of triclosan were found at sampling locations immediately downstream of the two major WWTPs: Waterloo (50 ± 13 ng L^{-1} at G33W) and Kitchener (135 ± 24 ng L^{-1} at G53E). There was a significant difference ($p=0.017$) between the average concentrations of the two groups of samples (G53E and G53W) collected across the site immediately downstream of Kitchener WWTP. This significant difference suggests an incomplete mixing of wastewater with river water downstream of this plant. Triclosan in the Grand River showed a spatial pattern expected from a point source contaminant (i.e., concentration is higher at a location directly downstream of WWTP but decreases as it travels further downstream). In addition, there is a statistical difference ($p\leq0.001$) between triclosan concentrations of sites upstream and immediately downstream of Kitchener WWTP (G52 and G53E) suggesting that WWTP effluent is a main source of this compound.

Chlorophene was only detected in two WWTPs, Preston and Galt, with concentrations of 82 ± 5 ng L^{-1} and 138 ± 8 ng L^{-1} respectively. In the Grand River, chlorophene was first detected at G48 (105 ± 27 ng L^{-1}) which is 15 km downstream of Waterloo WWTP outfall. It was also detected in all of the sites downstream of the Grand River's main branch (approximately 29 km) at concentrations ranging from 86-191 ng L^{-1}. Although there is a significant difference in the mean values among sampling locations from G48 to GS77 ($p\leq0.001$), most sites only significantly differ with G55, the sampling site 2 km downstream of Kitchener outfall (Appendix A). The difference appears to be related to one very high value from the replicates causing a large variability around the mean concentration. The rest of the sampling sites did not show differences in their mean concentrations (Appendix A), generally suggesting that the

concentrations are relatively constant with distance downstream. Dichlorophene was not detected in any of the samples both in surface water and wastewater.

2.3.2 Atrazine and Selected Pharmaceuticals in River Water and Wastewater – Summer Low Flow Conditions

The herbicide atrazine was consistently found in all sampling locations during the July 21, 2012 sampling event at concentrations ranging from 135-449 ng L^{-1}. Atrazine was only found in Waterloo (129 ± 37 ng L^{-1}) and Kitchener (207 ± 36 ng L^{-1}) WWTPs. Elevated concentration of atrazine was seen at T1, a tributary in the agricultural area of the watershed. However, there was an increasing trend in the urban sampling sites (G33E/W to G53E; G54 to G57). Other than WWTPs as potential sources, atrazine may be one of the herbicides applied in golf courses along this river reach.

All target pharmaceuticals (naproxen, ibuprofen, venlafaxine, and carbamazepine) were also detected both in surface water and wastewater samples (Figure 2.4). Carbamazepine, an antiepileptic drug, was found in relatively low concentrations in effluents (39-106 ng L^{-1}) but was persistent in downstream locations. Naproxen and venlafaxine were first seen with elevated concentrations downstream of the Waterloo WWTP, decreased with distance and increased again due to the influence of Kitchener WWTP discharge 20 km downstream of Waterloo WWTP. Both compounds persisted until GS77 (the most downstream site). Naproxen concentrations in the river ranged from <3-323 ng L^{-1} while venlafaxine ranged from <1-202 ng L^{-1}. Ibuprofen was observed throughout the watershed with concentrations generally higher than the pharmaceuticals included in this study (71-1,457 ng L^{-1}). Unlike naproxen and venlafaxine, elevated ibuprofen concentrations were first seen downstream of Kitchener WWTP. This compound persisted at high concentrations up to ~2 km downstream of Kitchener WWTP (G53

to G57, 366-1457 ng L^{-1}). The concentration dropped significantly at G58 and again, continued to persist until GS77.

In general, pharmaceutical concentrations were elevated in areas immediately downstream of WWTPs (especially Kitchener WWTP) and decreased with distance downstream of the outfalls. Significant differences in lateral concentrations (G33E vs. G33W) were seen for naproxen, venlafaxine, and ibuprofen ($p=0.021$; $p=0.004$; $p=0.005$) also signifying incomplete mixing downstream of Waterloo WWTP. Although obvious differences in lateral concentrations for naproxen, venlafaxine, and ibuprofen were observed across the site downstream of Kitchener WWTP (G53E/W), venlafaxine was the only compound that has a statistically significant difference ($p=0.008$) in lateral concentrations.

WWTPs with nitrifying secondary treatment (Guelph, Galt and Preston) have concentrations of most tested compounds lower than the non-nitrifying treatment plants (Waterloo, Kitchener, and Hespeler), with the exception of venlafaxine and carbamazepine which was found to be variable across WWTPs. In addition, differences in carbamazepine concentrations appear to be insignificant across WWTPs ($p=0.159$). Overall, the more advanced treatment plant effluent (Guelph WWTP) has lower concentrations of pharmaceuticals in its effluent compared to other treatment plants (Figure 2.5). For example, venlafaxine in other treatment plants is 2 to 5 times higher than Guelph WWTP effluent concentration. Sites downstream of Guelph WWTP (S2.5 and S11) also have the lowest concentrations of pharmaceuticals out of all the urban sites studied.

2.3.3 Monthly Concentrations in Kitchener WWTP

Triclosan was detected (100%) in Kitchener WWTP and immediately downstream (G53) but was only detected in 3 of the 4 sampling periods at the immediate upstream site (G52). In

contrast, chlorophene was infrequently detected in Kitchener WWTP and only detected once in September in the river water (both upstream and downstream). All pharmaceuticals were detected in effluent as well as river sites for all summer and fall sampling events (Table 2.6; Figure 2.6 and Figure 2.7).

There were statistical differences in monthly samples of the pharmaceuticals detected in the Kitchener WWTP effluent ($p \leq 0.001$). Ibuprofen and naproxen have elevated concentrations in July and November but remained relatively low from August to October. Carbamazepine was also detected in low ng L^{-1} range (except in October sampling event) while triclosan and venlafaxine appeared to be consistently high (Table 2.6). Atrazine in the Kitchener WWTP was detected at low concentrations relative to river concentrations (39-59 ng L^{-1}) from August to October but was undetected in November.

Table 2.6. Concentrations and detection frequencies of antiandrogens, atrazine and selected pharmaceuticals in the Kitchener WWTP and its upstream and downstream sites during August – November 2012 sampling periods

	Upstream (G52)		**Kitchener WWTP**		**Downstream (G53E)**	
	Range (mean) (ng/L)	% Freq of Detection	Range (mean) ng/L	% Freq of Detection	Range (mean) ng/L	% Freq of Detection
Triclosan	<1-106 (31)	75%	553-1,062 (832)	100%	93-197 (124)	100%
Chlorophene	<3-3.88 (0.97)	25%	<3-42 (15)	25%	<3-6 (1)	25%
Naproxen	50-166 (125)	100%	200-2,048 (731)	100%	50-320 (159)	100%
Ibuprofen	31-144 (82)	100%	33-1,463 (471)	100%	46-975 (352)	100%
Atrazine	38-151 (92)	100%	<10-59 (39)	75%	50-182 (97)	100%
Venlafaxine	36-45 (40)	100%	1,015-2,050 (1,500)	100%	102-295 (212)	100%
Carbamazepine	4-5 (4)	100%	44-507 (170)	100%	7-12 (10)	100%

Note. n=4 sampling periods (each with 3 replicates). G52=0.8 km upstream of Kitchener WWTP. G53E=0.5 km downstream of Kitchener WWTP.

Monthly samples showed temporal variability at G52 and G53E for all target compounds

except carbamazepine and venlafaxine (downstream site only, G53E) which showed almost constant values over time. The temporal pattern shown by naproxen and ibuprofen at the downstream site (G53E) agrees with the pattern seen in Kitchener WWTP (Figures 2.6 and 2.7). Naproxen and venlafaxine at the downstream site (G53E) showed elevated concentrations in July and November and also remained low from August to October. Atrazine concentrations in the river water declined over time.

2.3.4 Nitrogen Chemistry, Chloride, and Conductivity Data

As expected, high levels of ammonia were observed at non-nitrifying plants (Figure 2.7 and 2.9). Nitrate, the end-product of nitrification, is found in all nitrifying plans and was also seen throughout the watershed. Ammonia was only elevated at locations downstream of WWTPs (Waterloo, Kitchener, and Hespeler). Conductivity in WWTPs ranged from 2,060-2,650 μmho cm^{-1} while chloride ranged from 380-460 mg L^{-1}. Chloride appeared to be present in all study sites but slightly elevated concentrations were seen downstream of WWTPs.

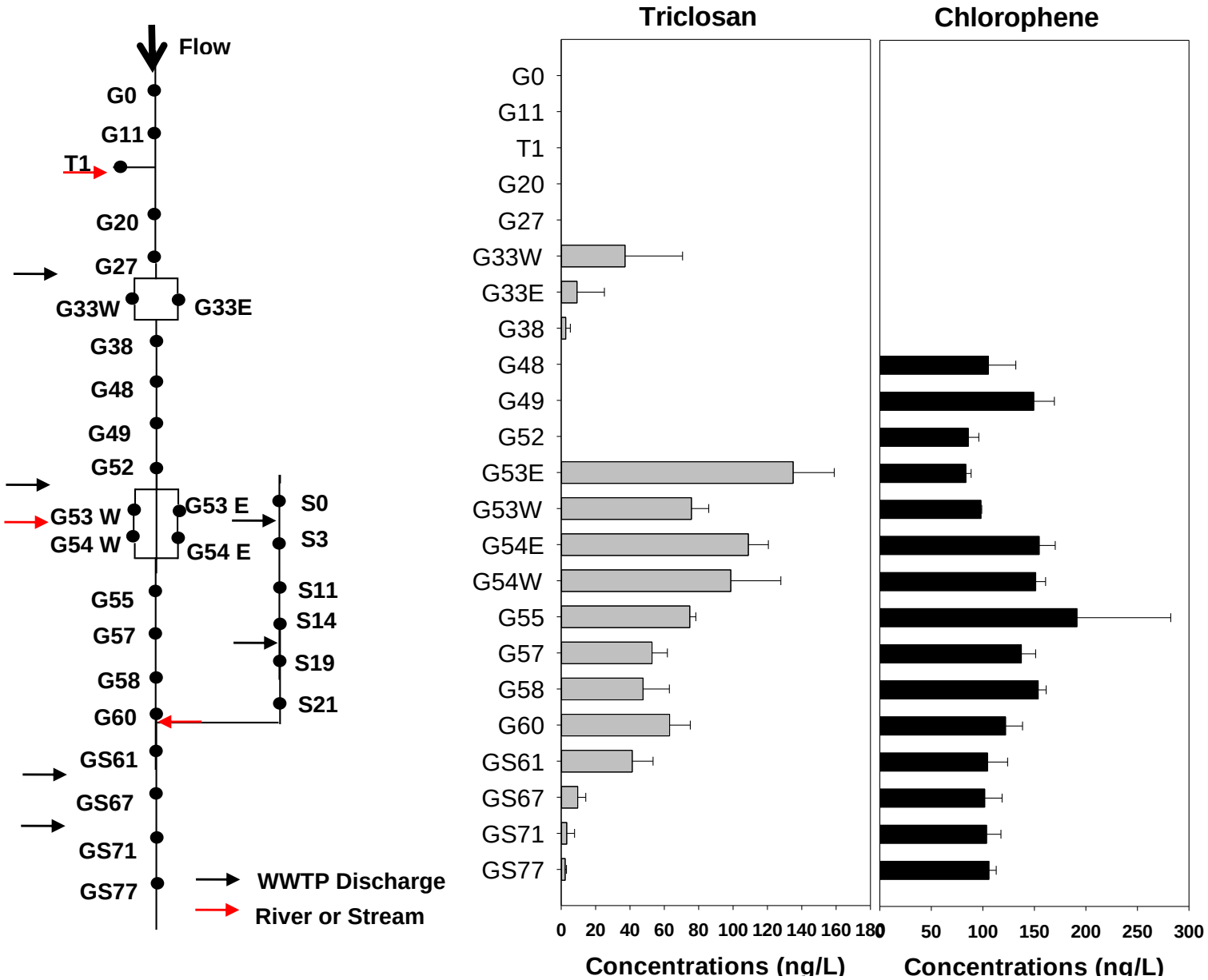


Figure 2.3. Mean concentrations of triclosan and chlorophene in the Grand River (July 2012) for sites starting at the most upstream to the most downstream location. Longitudinal distances between sites are not equal.

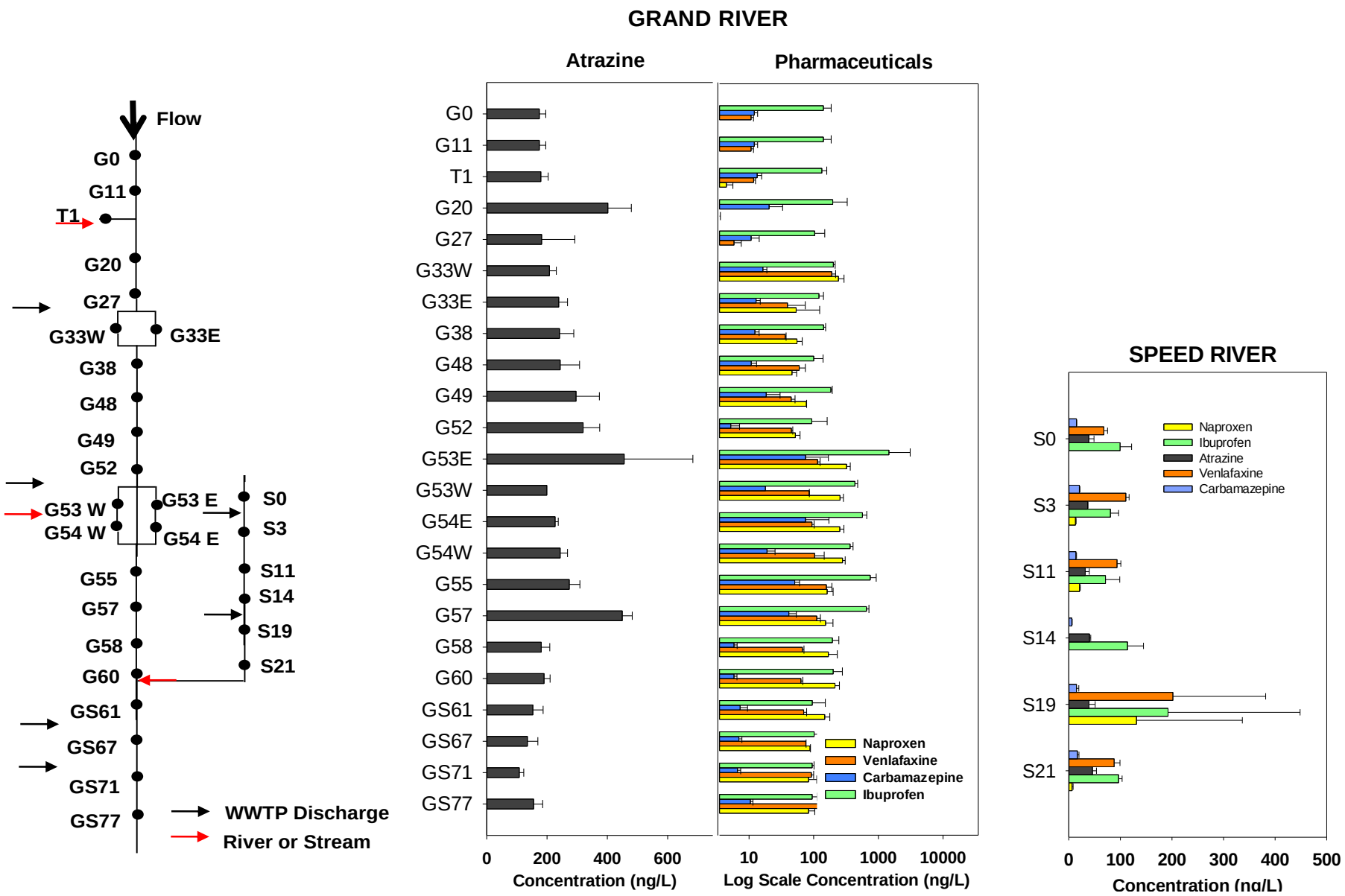


Figure 2.4. Mean concentrations of selected pharmaceuticals and atrazine in the Grand River and the Speed River (July 2012). Sites start at the most upstream to the most downstream location.

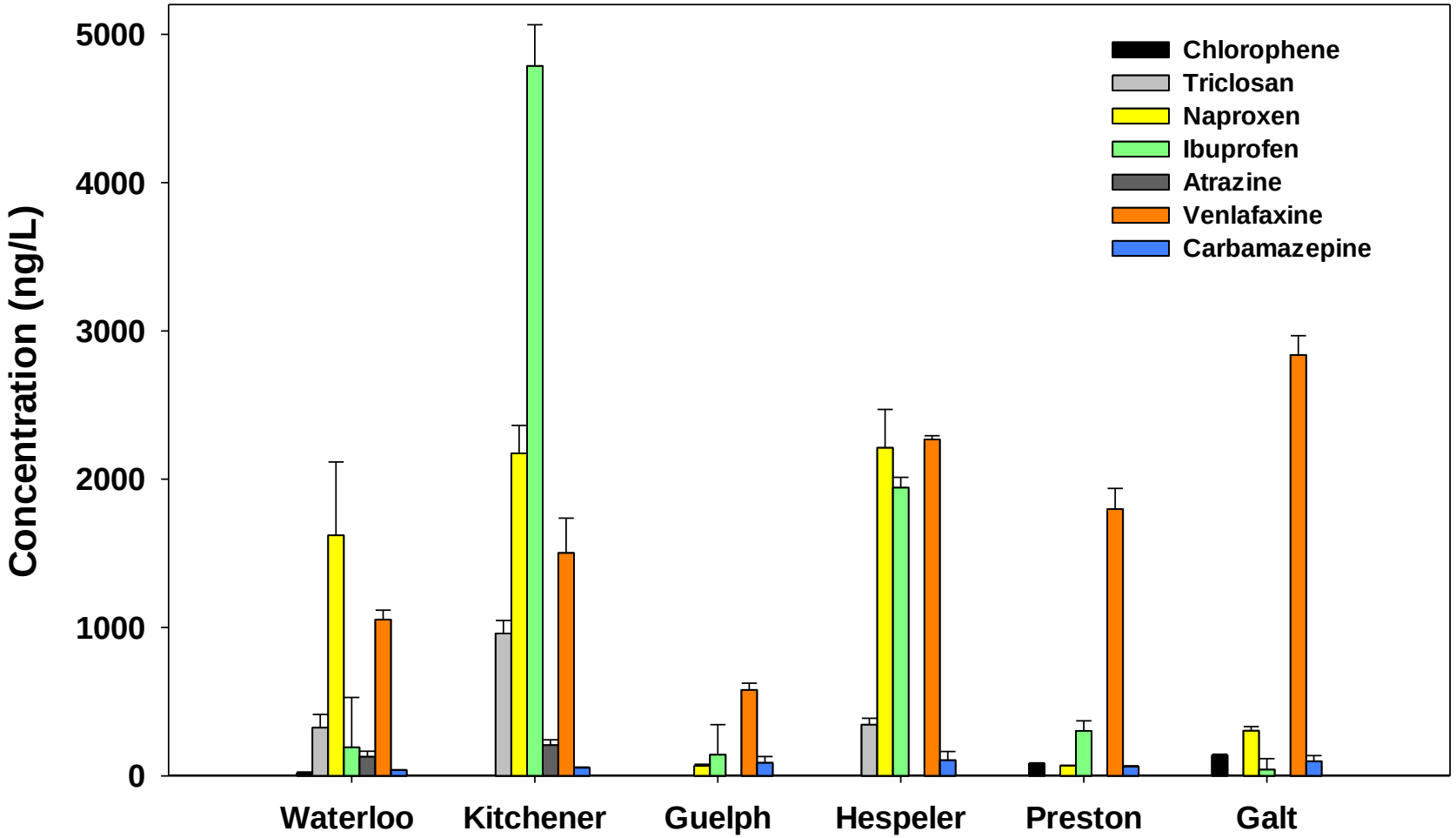


Figure 2.5. Concentrations of antiandrogens and selected pharmaceuticals in six WWTPs surveyed in July 2012. Waterloo, Kitchener, Preston and Galt WWTPs discharge in the Grand River. Guelph and Hespeler WWTPs discharge in the Speed River.

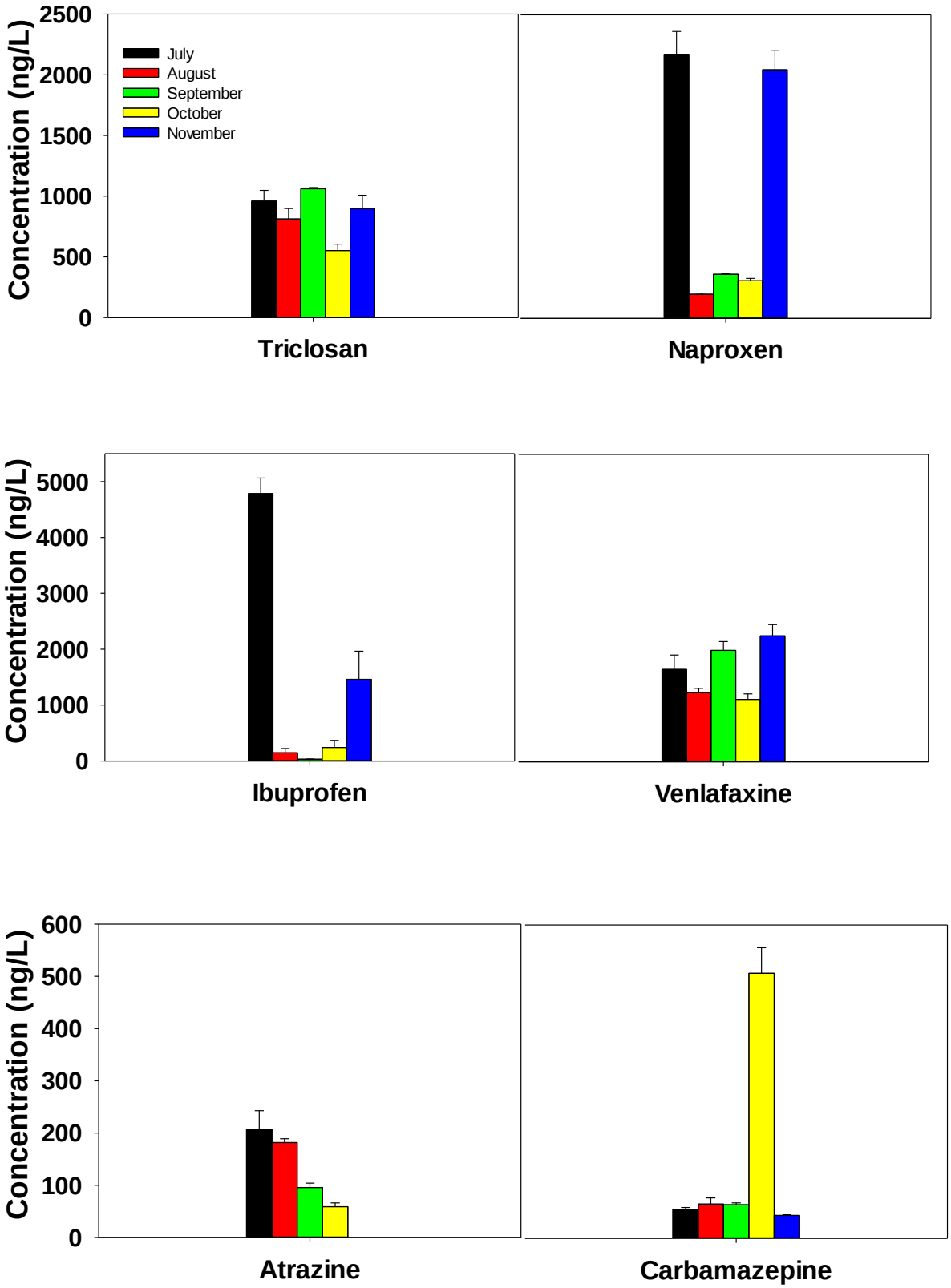


Figure 2.6. Mean concentrations of antiandrogens and selected pharmaceuticals detected in Kitchener WWTP during August-November 2012 sampling events.

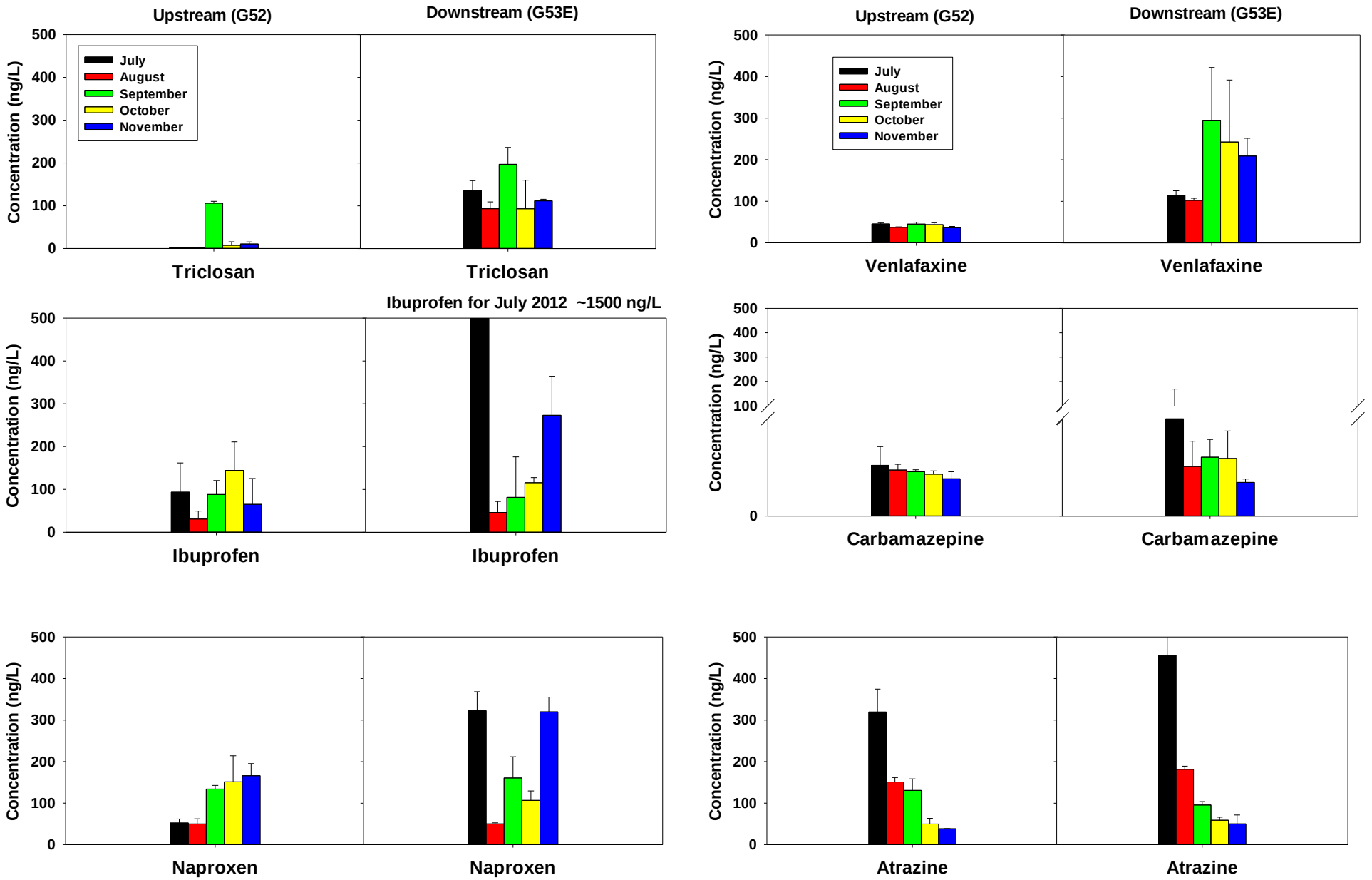


Figure 2.7. Mean concentrations of antiandrogens and selected pharmaceuticals detected in in the upstream and downstream sites during August-November 2012 sampling events.

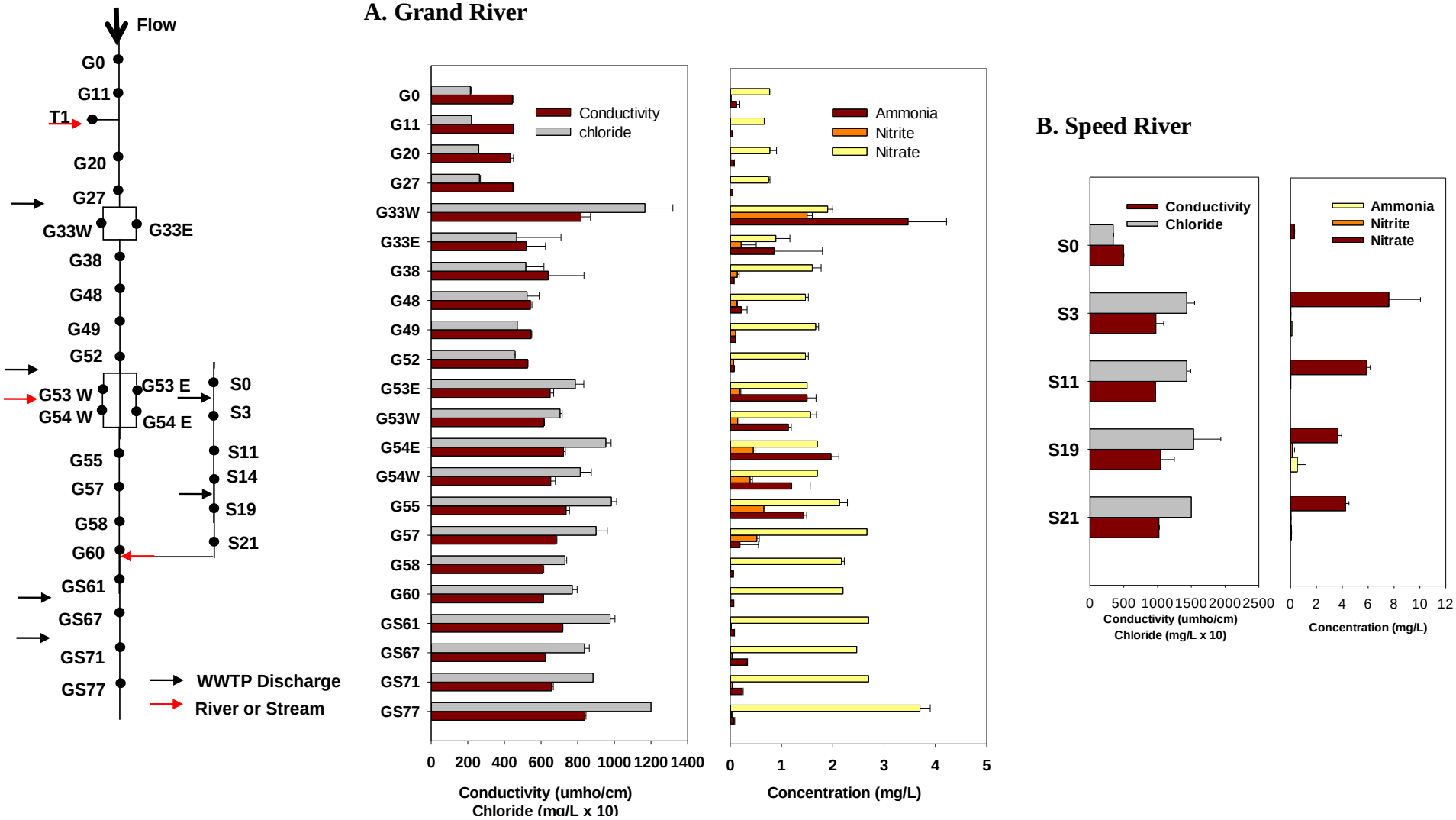


Figure 2.8. Nitrogen chemistry, chloride, and conductivity data for all the sites surveyed in July 21, 2012.

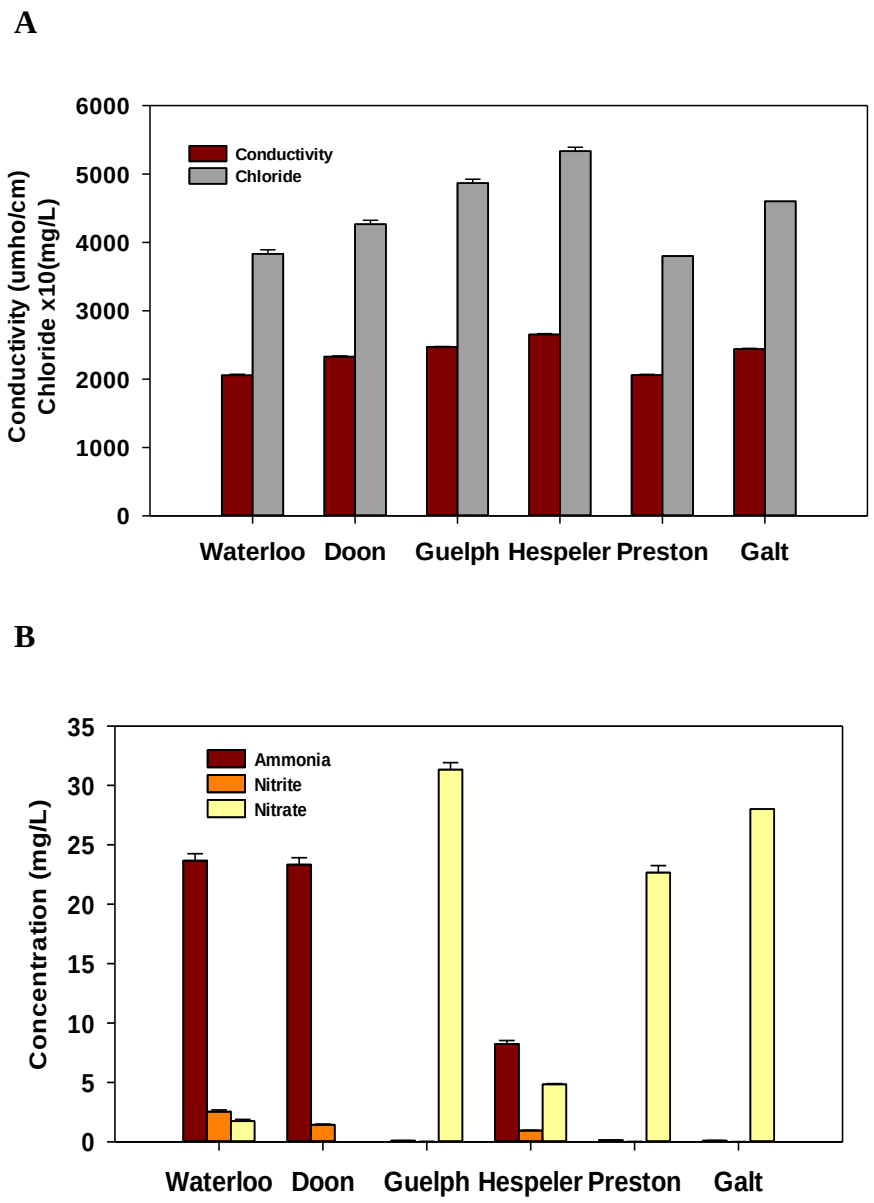


Figure 2.9. Chloride, conductivity (A) and nitrogen chemistry (B) for the WWTPs surveyed in July 21, 2012.

2.4 Discussion

Triclosan is a widely used broad-spectrum biocide that is primarily found in personal care products such as toothpaste, soap, skin care creams, and other cosmetic products (Sabaliunas et al., 2003; Singer et al., 2002). In this survey, triclosan was only found in non-nitrifying plants

(Waterloo, Kitchener, Hespeler) but not in WWTPs with more advanced treatment systems (nitrification-denitrification, phosphorus removal, and/or sand filters). This compound was consequently found in locations downstream of their respective discharge points and rapidly degraded at a fairly short distance (~5km) downstream of Waterloo. However, triclosan dissipated ~24 km downstream of Kitchener WWTP due to multiple inputs within this river reach (Speed River, Preston and Galt WWTPs). The rapid disappearance of triclosan in surface waters suggests that it is not a very persistent compound in the Grand River watershed. Many studies have already indicated photodegradation as a relevant elimination process for triclosan in surface waters (Latch et al., 2005; Sabaliunas et al., 2003), with some cases reporting ~80% contribution in total elimination of triclosan from a lake (Tixier et al., 2002). The concentrations detected in both wastewater effluents and surface waters were within the range reported by other studies. Triclosan is often found in WWTP effluents with mean concentrations ranging from 71-919 ng L^{-1} (Gómez et al., 2012; Sabaliunas et al., 2003; Zhao et al., 2010). In surface waters, other studies have reported mean concentrations ranging from 3-102 ng L^{-1} (Gómez et al., 2012; Zhao et al., 2010)

Chlorophene (o-benzyl-p-chlorophenol) is a halogenated phenolic compound often used as a disinfectant, biocide, and preservative. Although it was expected that chlorophene would be found in wastewater effluents because of its wide domestic use, the major sources of chlorophene in the Grand River were unclear. The WWTPs in the Grand River showed little to no presence of chlorophene in their effluents (Figure 2.5) except in the Preston and Galt WWTPs where chlorophene concentrations were within the range of river water concentrations found in sampling locations between G48 and GS77. Interestingly, chlorophene was only found in WWTPs located in the lower part of the study area and at concentrations similar to the river

water. None of the sites in the Speed River (a tributary), had any detection of chlorophene despite having two treatment plant outfalls. Also, the two plants where chlorophene was found both have advanced treatment. It appears that there is a source of chloroprene entering into these treatment systems that is not in the other four systems. The suspect source of chlorophene is in the upper reaches and is unlikely to be associated with the effluent discharges. Furthermore, chlorophene concentrations increase and then remain relatively constant in the area of the river where there are no municipal wastewater treatment plant outfalls (between the G55 and G58 sampling points). Also, in the July 2012 sampling, chlorophene was detected in Schneider Creek (143 ± 10ng L^{-1}), an urban stream located six km downstream of G48 and discharges into Grand River. The repeated sampling at Kitchener (effluent and surface water) also indicated that chlorophene was only detected once from August to November (Table 2.5).

In other studies, chlorophene has been detected in municipal wastewaters at concentrations in the range of 140-607 ng L^{-1} (Bueno et al., 2012; Yu et al., 2012). Kasprzyk-Hordern et al. (2009) reported a mean concentration of 12 ng L^{-1} in WWTPs and 3-4 ng L^{-1} in river water. It should be noted that chlorophene is not only used as a disinfectant but is also sold for other uses including as a cleaner for swimming pools, an algaecide for control of pond weeds, as well as being a component of other industrial and domestic products (Lanxess Corporation, 2010; United States Environmental Protection Agency, 1995). It is possible that a seasonal use of a product containing chlorophene resulted in the pattern observed in the Grand River in 2012. Municipal WWTPs are clearly not the only source of this compound in watersheds and this deserves further investigation as it may represent a risk to both drinking water and ecosystem health.

Dichlorophene, also a known bacteriocide and fungicide in personal care products, was not detected in any of the treatment plant effluents and surface water samples. A study conducted by Heidler and Rolf (2009) on the fate of organohalogens in WWTPs also reported the non-detection of dichlorophene in effluent with an MDL similar to this study (30 ng L^{-1} vs. 27 ng L^{-1}). Dichlorophene was however identified in the bile of fish exposed to WWTPs in the UK (Hill et al., 2010; Rostkowski et al., 2011)

Smith (2013) determined the total estrogen equivalence (TEQ) of Guelph, Waterloo, and Kitchener WWTPs and found an increasing TEQ trend based on WWTP treatment configuration (Kitchener > Waterloo > Guelph). There have been no studies that indicated any antiandrogenic activities in WWTP effluents in the Grand River watershed and their receiving surface waters. However, the detection of triclosan (in all sampling events) and chlorophene (in July 2012) indicates that the aquatic ecosystem in the urbanized sections of the Grand is not only exposed to estrogenic compounds but also to antiandrogens.

The concentrations of triclosan detected both in wastewater and surface water were orders of magnitude below the concentration (20 ug L^{-1}) found to induce hepatic vitellogenin production in male medaka (Ishibashi et al., 2004). Vitellogenin is a biomarker for exposure of endocrine disruptive chemicals (estrogens and/or antiandrogens). However, Orvos et al. (2002) found that triclosan can bioaccumulate in fish tissue resulting to concentrations 2,000 to 5,200 times that of the surface water concentrations when exposed to 3 ug L^{-1} and 30 ug L^{-1} nominal concentrations of triclosan at steady state conditions. Rostkowski et al. (2011) also found that antiandrogenic chemicals can bioconcentrate up to 8,600 times in fish bile. Thus, it is likely that the antiandrogens found in Grand River (chlorophene and triclosan) are also present in biota.

The consistent occurrence of atrazine in all surface water samples demonstrates the ubiquitous usage of this herbicide within the watershed, especially in corn crops where atrazine is extensively used (Lazorko-Connon and Achari, 2009). Slightly higher levels found at the location where the Conestogo River discharges were potentially due to the agricultural land use in this area. Atrazine was detected in the WWTP effluents of Waterloo and Kitchener but the sources are not known. The concentrations found in the Grand River in surface waters are within the range (<50-3,910 ng L^{-1}) determined by Byer et al. (2011) in Ontario.

Ibuprofen and naproxen belong to a group of non-steroidal anti-inflammatory drugs (NSAIDs) which treat pain, headache, colds, and flu symptoms (Hernandez et al., 2012). Ibuprofen is considered to be one of the most commonly used drugs worldwide (Hutt and Caldwell, 1983). Although it is known to have 90-99% removal in WWTPs with activated sludge systems (Martín et al., 2012; Nakada et al., 2006; Radjenovic et al., 2009), ibuprofen is still detected in the effluents at relatively high concentrations varying from 384-4,000 ng L^{-1} (Lishman et al., 2006; Metcalfe et al., 2003). Naproxen has also been shown to have high removals in WWTPs (Bueno et al., 2012; Fernandez-Fontaina et al., 2012; Kasprzyk-Hordern et al., 2009; Yu et al., 2012), but it was still detected in the Grand River watershed. Unlike ibuprofen, naproxen was present at lower concentrations in the upper section of the study area. A review on the occurrence of pharmaceutical compounds in urban wastewaters (Verlicchi et al., 2012) reported that ibuprofen and naproxen were two of the most frequently detected anti-inflammatories in WWTP influents and effluents. However, they found that ibuprofen in general has higher concentrations in raw urban wastewater than naproxen, suggesting the more frequent usage of ibuprofen.

Carbamazepine is a neuro-active compound known to treat epilepsy. In the Grand River

watershed, carbamazepine was detected in low concentrations but appeared to be persistent downstream of WWTPs. This finding suggests that carbamazepine is recalcitrant to in-stream degradation. Any in-stream loss may be due to contaminant transport processes such as advection and dispersion other than fate mechanisms such as biodegradation, photolysis, and volatilization. Clara et al. (2004) and Gasser et al. (2011) additionally proposed carbamazepine as a tracer for organic contaminants coming from WWTPs due its conservative behaviour, specificity for municipal wastewater, and high mobility in the environment. Kunkel and Radke (2012) have already used comparisons of pharmaceutical concentrations with carbamazepine concentrations to determine the elimination rates of pharmaceuticals along the river stretch.

Venlafaxine is a neuro-active medication mainly used as an antidepressant. It was found to have the same spatial pattern as naproxen (trace concentrations in the upper section of the watershed). The concentrations found in this study were consistent with the results of Metcalfe et al. (2010) (47-901 ng L^{-1}). Together with its metabolites O- and N-desmethyl venlafaxine, it persisted several kilometres downstream of the WWTPs they studied. Venlafaxine was more variable across the different WWTP treatments studied. This observation has been mentioned in other studies that reported the recalcitrant behaviour of venlafaxine in wastewater treatment as indicated by its low percent removal (~12%-40%) in WWTPs (Lajeunesse et al., 2012; Metcalfe et al., 2010).

Triclosan, ibuprofen, and naproxen have elevated concentrations in non-nitrifying plants compared to nitrifying plants. The enhancement of pharmaceuticals and personal care products removal in municipal wastewater through nitrification processes has been indicated in past studies (Fernandez-Fontaina et al., 2012; Servos et al., 2005; Suarez et al., 2010). Lower concentrations of target pharmaceuticals in Guelph, Preston, and Galt WWTPs compared to

Waterloo, Kitchener, and Hespeler WWTPs may have been contributed by the nitrification processes included in their activated sludge systems. Fernandez-Fontaina et al. (2012) suggested that the co-metabolism of pharmaceuticals with ammonium-nitrogen in nitrification systems is due to the presence of the ammonium monooxygenase produced by nitrifying bacteria responsible for oxidizing ammonium-nitrogen and a wide variety of substrates. Also, similarities in the spatial pattern (Figure 2.8) of ammonia and some pharmaceuticals (triclosan, ibuprofen, and naproxen) (Figure 2.4) indicate that ammonia may be used as a good indicator of plant performance and the removal of some biodegradable chemicals.

For most compounds, the reduction in the concentrations with distance downstream indicates that the contaminants are transformed into different by-products, transferred in other environmental compartments, and/or diluted as they move downstream. More persistent contaminants such as chlorophene, carbamazepine, and venlafaxine have concentrations that are relatively constant with distance downstream of the source. This observation is consistent with the trends in chloride seen in the Grand River watershed, suggesting that these compounds can be attenuated along the river stretch through flow-driven transport process. Samples in this study were mostly collected during periods with lower than normal summer low flow (11 $m^3 s^{-1}$) and flows were not highly variable among sampling periods (July-October) although there were periods of higher flow (Figure 2.10). The temporal variability in the presence and concentration patterns of the target compounds in the Grand River watershed are likely attributed to several factors including variable WWTP treatment effectiveness and changes in relative loadings throughout the sampling period.

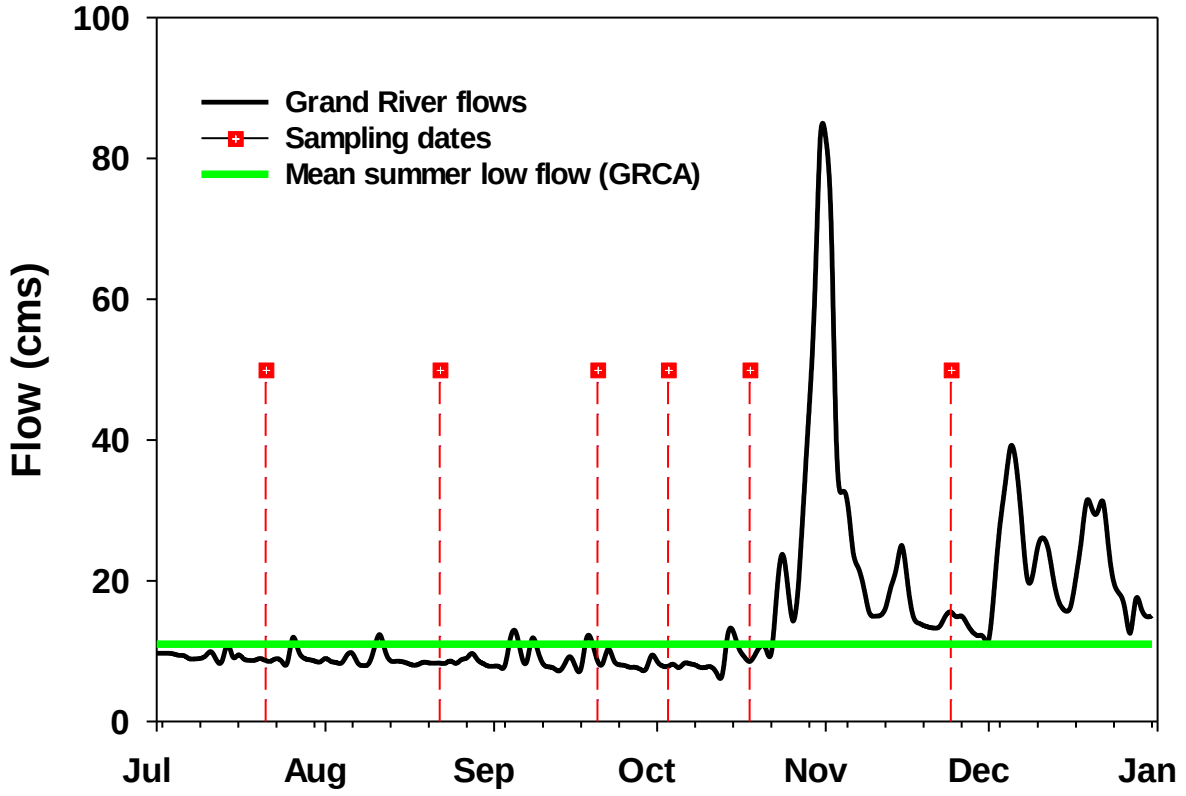


Figure 2.10. Grand River flows from July – December 2012 as measured by GRCA Doon flow gauge.

3 The simulation and prediction of the fate and transport of selected pharmaceuticals and a known antiandrogen in a highly impacted reach of the Grand River watershed

This chapter will be submitted as a manuscript to a peer-reviewed journal.

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- Leslie Bragg: Assisted with water sampling, field work, and laboratory analysis portion of this paper.
- Mark Servos: Co-supervisor to Maricor Arlos and assisted with field work, research direction, editing, and general advice.
- Wayne Parker: Co-supervisor to Maricor Arlos and assisted with research direction, editing, and general advice.

3.1 Introduction

In recent years, water quality research has considered several issues in addition to standard wastewater pollutants (i.e., biological oxygen demand, suspended solids, and nutrients). Studies on contaminants of emerging concern and their effects on the aquatic environment are steadily advancing (Daughton and Ternes, 1999; Petrovic et al., 2003; Richardson and Ternes, 2005). Contaminants of emerging concern collectively include pharmaceuticals, endocrine disrupting compounds (EDCs), personal care products and more recently, nanoparticles (Brar et al., 2010; Daughton, 2001; Halling-Sørensen et al., 1998; Peralta-Videa et al., 2011). Wastewater treatment is now challenged with concerns regarding the potential harmful effects of these contaminants on aquatic ecosystems (Daughton, 2004).

The Grand River is the largest watershed in southern Ontario (6,965 km^2) and receives wastewater from thirty municipal and industrial WWTPs (Anderson, 2012; Cooke, 2006). Pharmaceuticals ranging from anti-inflammatory drugs to antidepressants have already been detected both in its surface water and biota (Lissemore et al., 2006; Metcalfe et al., 2010; Oakes et al., 2010; Wang et al., 2011). These compounds are often present in low concentrations, usually in the ng L^{-1} to low μg L^{-1} range. In addition, some municipal WWTPs in the watershed are known to discharge estrogenic chemicals (Tanna et al., 2013), with the potential to disrupt the normal endocrine function of a variety of aquatic species (Colborn et al., 1994; Mills and Chichester, 2005; Purdom et al., 1994). Effects on fish, including changes in physiology and gene expression, have been found in the areas affected by wastewater effluent discharges (Ings et al., 2011; Ings et al., 2012). Changes in energy storage and allocation in fish have been reported in the urban reaches of the Grand River watershed that receive municipal wastewater (Tetreault et al., 2011). Fish collected downstream of wastewater outfalls have been found to have high

incidences of intersex (ova-testes), with 70-100% of the fish showing this condition (Tanna et al., 2013; Tetreault et al., 2011). Changes in the fish community assemblage downstream of municipal wastewater outfalls have been reported (Tetreault et al., 2013). Endocrine disruption, in particular, has been associated with municipal wastewater outfalls in the Grand River watershed. The specific chemicals responsible for the effects have yet to be identified.

Natural and synthetic estrogens have been detected in other watersheds (Belfroid et al., 1999; Kolpin et al., 2002; Kuster et al., 2008; Ying et al., 2009) and their presence has been linked to endocrine disruption (Jobling et al., 2006; Nash et al., 2004). However, male hormone receptor antagonists (antiandrogens) have also been reported to contribute to endocrine disruption in fish (Hill et al., 2010; Jobling et al., 2009; Rostkowski et al., 2011). Antiandrogens that accumulated in fish bile were identified using a Yeast Androgen Screen (YAS) assay (Hill et al., 2010; Rostkowski et al., 2011). Rostkowski et al. (2011) found that chlorophene and triclosan (antimicrobial agents) comprised 51% of the antiandrogenic activity in fish bile. These compounds are commonly found in a wide range of personal care products including disinfectants, soaps, and cosmetics (Bhargava and Leonard, 1996; Stouten and Bessems, 1998). A survey conducted in the Grand River watershed in 2012 revealed the presence of these two antiandrogens along with other pharmaceuticals (Chapter 2). High concentrations of triclosan and selected pharmaceuticals (ibuprofen, naproxen, carbamazepine, venlafaxine) were also observed immediately downstream of municipal WWTPs, further demonstrating treatment plant effluents as major sources of these compounds in the watershed. Chlorophene on one hand showed a different spatial distribution than the rest of the compounds studied, suggesting the presence of source/s other than WWTPs.

Mathematical models can be employed to understand and predict the behaviour of contaminants in aquatic environments (Martin and McCutcheon, 1999; Chapra, 1997; Ji, 2008). Water quality models have been historically used to predict concentrations of standard wastewater pollutants (Chapra, 1997). Application of water quality models has now moved beyond the prediction of conventional pollutants to cover other types of surface water stressors. For example, water quality models have been extended to predict the concentrations of industrial contaminants (such as polychlorinated biphenyls, mercury, and nitrobenzene) and more recently, contaminants of emerging concern (Vuksanovic et al., 1996, Lin et al., 2011; Ren et al., 2007, Hosseini et al., 2012; Cunningham et al., 2008).

The application of a fate and transport model for contaminants of emerging concern to the Grand River would enhance our ability to understand the distribution of selected contaminants of concern within the watershed. The prediction of the fate and transport of these compounds can serve as a supplemental tool in assessing the exposure of aquatic ecosystems to contaminants of emerging concern. In the current study, the Water Quality Simulation Program 7.5 (WASP) developed by the US Environmental Protection Agency was employed to predict the fate and understand the processes responsible for the spatial and temporal distribution of an antiandrogen (triclosan) and three selected pharmaceuticals (naproxen, venlafaxine, and carbamazepine) in the urban reaches of the Grand River watershed.

3.2 Modeling Approach

This section provides a detailed discussion on the approach used to develop a model for simulating antiandrogens and pharmaceuticals in a reach of the Grand River. The stepwise approach to transport and fate simulation as well as the manual calibration procedures used are further discussed in the following sections.

3.2.1 Target Compounds

The compounds modeled in this study were the antiandrogen, triclosan, and the pharmaceuticals, naproxen, venlafaxine, and carbamazepine. The pharmaceuticals have been frequently detected in the watershed in prior sampling events (Tanna et al. 2013; Wang et al. 2011; Chapter 2). Of the two antiandrogens detected in the watershed, only triclosan was modeled since chlorophene was only detected in one sampling campaign and the source was indeterminate (Chapter 2). The relevant physico-chemical properties of the modeled compounds are listed in Table 3.1.

Table 3.1. Selected physico-chemical properties of compounds

Compound	Molecular Weight ($g\ mol^{-1}$)	pKa	log K_{ow}	Henry's Law Constant ($atm\text{-}m^3\ mol^{-1}$)	Solubility[a] ($mg\ L^{-1}$)
Triclosan	289	7.90	4.76	2.10×10^{-8},[a]	10.0 at 20°C
Carbamazepine	236	13.90	2.45	1.08×10^{-7} at 25° C	18.0 at 25°C
Naproxen	230	4.15	3.18	3.39×10^{-10} at 25°C	15.9 at 25°C
Venlafaxine	277	10.09	3.20	2.04×10^{-11} at 26°C	267.0 at 25°C

Note: Data adapted from "Hazardous Substances Data Bank" by United States National Library of Medicine. Retrieved March 2013 from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. [a] Adapted from Perron, et al., 2012

3.2.2 Model Description

The WASP model (acquired from http://www.epa.gov/athens/wwqtsc/html/wasp.html, August 2012) is a public domain model that can simulate flow in unsteady and non-uniform cases as well as contaminant fate and transport in up to three dimensions. The model was initially developed to characterize dissolved oxygen and eutrophication processes but was later modified to include organic contaminant fate and transport. WASP has been used to simulate a variety of organic contaminants including the fate and transport of persistent compounds such as PCBs and the pesticide atrazine (Rygwelski et al., 1999; Vuksanovic et al., 1996). WASP utilizes

a “box” model approach for modeling contaminants in surface waters. The Saint-Venant equations (continuity and momentum) are employed when simulating water quality along a one-dimensional unsteady flow channel. One-dimensional transport is often assumed in river water quality modeling since longitudinal movement in rivers is more significant than vertical and transverse movements (Ji, 2008). The fundamental continuity and momentum equations are described in Equations 3.1 and 3.2 respectively:

$$\frac{\partial A}{\partial t} + \frac{\partial Q}{\partial x} = 0 \tag{3.1}$$

$$-gS_o + gS_f = 0 \tag{3.2}$$

where A is area, Q is flow, t is time, x is distance, S_o is the bed slope, S_f is the friction slope and g is gravitational constant. Equation 3.2 is the simplest form of the momentum equation (kinematic wave equation) and only considers the effects of gravity and friction on the movement of water. The kinematic wave model has been found to be practically applicable in simulating transport in rivers (Section 1.3.1; Wool et al., 2002). Equations 3.1 and 3.2 are manipulated and solved in WASP using different numerical methods (e.g., Euler, Runge-Kutta).

In the current study, the transport processes and fate mechanisms (volatilization, hydrolysis, photolysis, and biodegradation) in the river were simulated using the Organic Toxicant subroutine in WASP using the Euler solution technique. Under this subroutine, the fate and transport simulations were completed in multiple stages. Each step is further discussed in the following sections.

3.2.3 Site Selection and Segmentation

The reach modeled in this study included a portion of the river that has been found to be affected by the Kitchener WWTP (KWWTP) discharge. Historically poor water quality

conditions such as low dissolved oxygen levels, high ammonia concentrations (Anderson, 2012; Cooke, 2006) and presence of a variety of contaminants of emerging concern (Tanna et al., 2013; Wang et al., 2011) have been observed. This area has also been found to have high incidences of intersex in wild fish (Tetreault et al., 2011; Tanna et al., 2013). The total length of the reach modeled was approximately 10 km and started immediately below the Manheim Dam (~3 km upstream of the KWWTP outfall) and ended at the confluence of the Grand and Speed Rivers (~7 km downstream of the KWWTP outfall) (Figure 3.1).

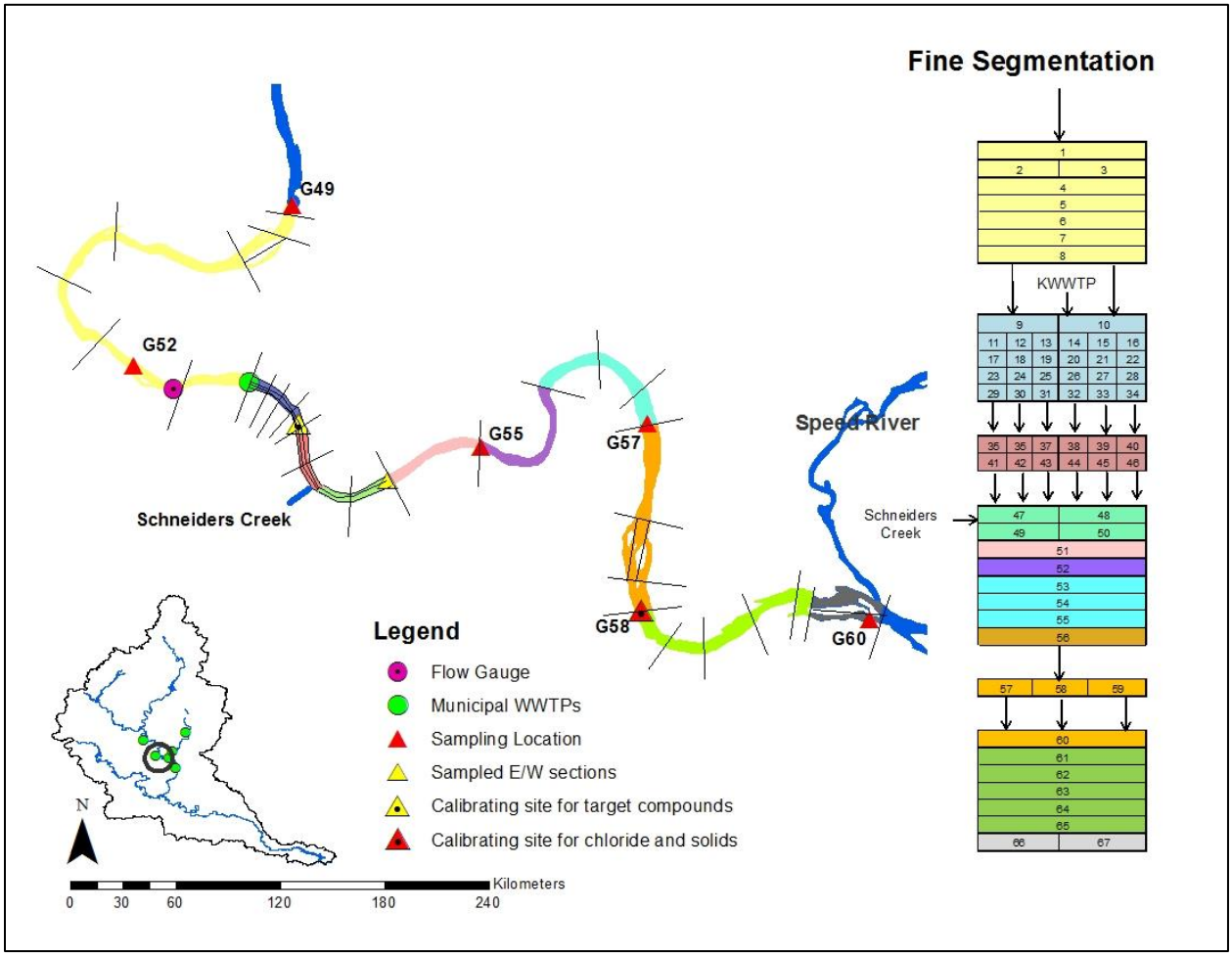

Figure 3.1. Map of the modeled segments and sampling sites. Finer segmentation of the modeled segments are also shown. Finer segments were colour-coded based on the major segments they represent.

The reach examined in this study was discretized into ten segments and the average dimensions of the segments are shown in Table 3.2. WASP treats each segment as completely mixed, so the areas known to have incomplete mixing conditions (e.g., effluent discharge and streams inputs such as Schneider Creek) were more finely segmented (Figure 3.1).

Table 3.2. Dimensions of river segments

Segment	Corresponding sampling location described in Chapter 2	Length (m)	Channel Elevation (m)	Width (m)
Manheim	G49	3678	277.44	47-81
KWWTP	KWWTP	463	273.53	52
Pioneer Tower 1 (PT1)	G53E/W	393	273.02	51
Pioneer Tower 2 (PT2)	G54E/W	532	272.66	74
Riveredge A	G55	700	272.11	48
Riveredge B	-	950	270.60	48
Edgehill	G57	1000	269.38	62-92
Blair	G58	1260	268.12	90-110
Grand River End	G61	1350	266.76	56-103
Speed Confluence	-	468	266.63	90

3.2.4 Boundary Conditions

There were three locations in the reach that required definition of boundary conditions: the upstream site (G49), the KWWTP outfall, and Schneider Creek. There are numerous wastewater treatment plant outfalls above the study area and these were considered as part of the input at the upstream site. The KWWTP outfall was considered as an extra segment acting as a tributary discharging into the Grand River. It also served as the major point source for chloride and the target compounds. Schneider Creek was a major source for chloride but not for target contaminants. It was assumed that there were no significant groundwater contributions in the study area based on the initial site reconnaissance survey.

Definition of the boundary conditions required data on (1) flow inputs and (2) concentrations of the tracer substance (chloride) and the target compounds. Daily flows for the Grand River and Schneider Creek (2009-2012) were provided by the Grand River Conservation Authority (GRCA). The KWWTP daily flows for 2009-2012 were provided by the Region of Waterloo. These time-varying flows are shown in detail in Appendix B.

Chloride concentrations for G49 and Schneider Creek were taken from the annual surface water quality monitoring reports (2009-2012) conducted by LGL Limited (requested by the Region of Waterloo) (Fausto et al., 2010; Fausto et al., 2011; Fausto et al., 2012; Fausto et al., 2013). A summary of the chloride data sets employed in this study is presented in Table 3.3. Only six values were available for the chloride concentrations in the KWWTP effluent. However, this dataset revealed that chloride concentrations were relatively consistent in the KWWTP effluent (480 ±47 mg L^{-1}). Thus, in the subsequent modeling, a constant chloride concentration discharging from KWWTP was assumed.

Table 3.3. Summary of chloride concentration data set for simulation, calibration, and validation

Boundary Location	**Number of data points**	**Year**	**Time of the year data were collected**
G49, upstream boundary condition	92	2009-2012	seasonal – winter, spring, summer, fall
Schneider Creek	93	2009-2012	seasonal – winter, spring, summer, fall
Kitchener WWTP	6	3 data points - 2008 3 data points - 2011	spring 2008, fall 2011

Unlike the chloride dataset, only limited information was available for the target organic compounds. Hence, the simulation of these substances was only conducted for a five month period as it is consistent with the sampling regime previously completed. Chapter 2 describes the

methodology that was used to measure the concentrations of the target contaminants and the resulting dataset that included monthly concentrations from July-November 2012.

3.2.5 Transport Processes

The flow-driven transport processes of advection and dispersion can have a significant effect on the movement of contaminants in rivers and streams (Ji, 2008). Advection is responsible for transporting water and its dissolved substances as the river flows downstream. This process can also contribute to the net transport of dissolved pollutants from an area of higher concentration to an area of low concentration through dispersion (Ji, 2008). Model calibration of parameters associated with advection and dispersion was first completed to ensure that these processes were properly represented in the WASP configuration. In addition to flows and concentrations entering from the boundary segments, the hydrogeometric properties of each segment were required. The information on hydrogeometric properties that was employed for the modeled segments is presented subsequently.

3.2.5.1 Advective Transport

Advective transport in WASP is dependent on the channel hydrogeometric properties (e.g., velocity, flow, depth, width, and slope). WASP represents river channel cross-sectional properties as a function of flow as described by Equations 3.3 and 3.4:

$$V = aQ^b \tag{3.3}$$

$$D = cQ^d \tag{3.4}$$

where V is velocity, Q is flow, D is depth, and a, b, c, and d are hydraulic coefficients and exponents specific to the velocity-flow and depth-flow relationships. In the kinematic wave

approach, Q is represented using the Manning's equation:

$$Q = \frac{1}{n}\frac{A^{5/3}}{B^{2/3}}S_0^{1/2} \tag{3.5}$$

where B is segment width, A is segment area and n is Manning's roughness coefficient. Values of Manning's roughness coefficient and bed slope (S_o) for each segment were taken from a hydrodynamic modeling study that was conducted by the Grand River Conservation Authority (GRCA) as part of the KWWTP assimilative capacity study (Stantec Consulting Ltd., 2010). Manning's roughness coefficients and bed slopes were not considered for calibration as these parameters had been critically parameterized during the assimilative capacity study.

WASP recommends a range of values for hydraulic coefficients and exponents but calibration to site-specific river conditions was necessary. As recommended by the WASP technical manual, a single depth exponent should first be estimated for the entire model reach while hydraulic depth coefficients for each segment should be calibrated to observed water levels. In WASP modeling practice, depth exponents are typically chosen from a range of values (0.30-0.60) based on the general river channel geometry (rectangular, U-shape, V-shape, or trapezoidal). However, for this study a depth exponent of 0.512 was chosen based on an estimate taken from a previous hydrodynamic simulation conducted by the GRCA. The water level data at the Doon Flow Gauge (Figure 3.1) from 2009-2011 were also provided by the GRCA and were employed for the calibration of the depth coefficient in each segment.

It was also observed that segmentation was a critical part of simulating advective transport. Although it requires longer model runtime, finer segmentation can be more precise and effective in approximating transport conditions in the area being studied. A step-by-step, trial-and-error approach was used. When changes were made in the segmentation, the hydraulic

depth coefficients were subsequently adjusted to fit the water level data from 2009-2011. A total of 11 iterations were required to reach the optimal segmentation. As suggested by the WASP developers, depths under average flow conditions for each segment were used as the hydraulic depth coefficient values. GRCA provided a data set of the average depths for each segment for flow profiles of 15, 20, 30, 40, 50, 70, 100, 120, and 150 $m^3 s^{-1}$ (Appendix B). Depths from a specific flow profile were tested against water levels. It was found that employing the depths described by the 40 $m^3 s^{-1}$ flow profile provided the best fit to water levels.

After the water levels were fit, chloride concentrations were simulated (2009-2011) using advective transport alone. It was observed that an additional transport process (i.e., dispersion) was necessary to accurately represent chloride responses. The initialization and calibration of dispersive transport is discussed in the following section.

3.2.5.2 Dispersive Transport

Dispersive transport in the targeted reaches was characterized upon completion of the advective transport simulation. In WASP, dispersive transport is represented by exchanges between segments (transverse and longitudinal) and is characterized by the mixing length and dispersion coefficients. Mixing lengths were estimated as half of the length (longitudinal) or width (transverse) of the smaller of the two neighbouring exchange segments as suggested by the WASP developers (Wool, 2012). Data on mixing lengths are presented in Appendix B.

A general rule-of-thumb for mixing conditions indicates that a tracer can be well-mixed at a distance of about 100-300 channel widths downstream of the source (Rutherford, 1994). At this location, longitudinal dispersion is the primary mechanism of concern. In the current study, the fully-mixed condition was expected to occur at G58 (Blair; ~7 km from the KWWTP outfall). A constant longitudinal dispersion coefficient was applied throughout all of the

segments and was calibrated against the chloride concentrations at G58. Longitudinal dispersion coefficients in rivers typically range from 10^0 to 10^2 m^2 s^{-1} (Martin, 2012). A total of 16 runs were conducted before a good fit between the simulated and measured concentrations at G58 was achieved.

Chloride datasets (May 2011 and November 2011) were available for the six segments across the G53 site (PT1) (Figure 3.1, segment nos. 35-40). These data were used to determine whether the longitudinal dispersion model was able to represent the concentration gradients across a segment that is immediately downstream of the KWWTP outfall (~0.3 km downstream). When the simulated and measured chloride concentrations were compared, it was found that transverse dispersion was necessary to represent the exchanges across the PT1 segments examined. Hence, a transverse dispersion coefficient was calibrated against the measured chloride concentrations across G53 taken from the two sampling events. Transverse dispersion coefficients for rivers typically range from 10^{-4} to 10^{-3} m^2 s^{-1} (Martin, 2012). A total of 11 (7.3 h) model runs were required to complete the calibration of the transverse dispersion coefficients.

3.2.5.3 Validation of Transport Processes

Validation of a calibrated model using an independent data set was deemed to be important to ensure that the transport conditions are simulated well. The time-varying chloride concentrations at G58 in 2012 were used to validate the calibrated transport parameters. Additionally, downstream profile concentrations involving seven sites from the October 2012 sampling event were also used to validate the spatial resolution of the model over a 7 km distance.

3.2.5.4 Additional Assessment of Transport Processes

The fit between the simulated and measured chloride concentrations at G58 (Figure 3.1) was assessed (in addition to graphical measures) using three model performance evaluators: Nash-Sutcliffe Efficiency coefficient (NSE), ratio of root mean square to observed data standard deviation ratio (RSR), and percent bias (PBIAS). There has been no agreement among water quality modelers on the use of a single performance evaluator. However, Moriasi et al. (2007) recommend the use of these three model evaluation statistics. NSE is a commonly used measure of the quality of fit between observed and predicted values (Moriasi et al., 2007). NSE values range from $-\infty$ to 1.0 with 1.0 being the optimal value. RSR is a standardized version of the error index, root mean square error (RMSE) and indicates the error in the prediction of variable of interest. Lower RSR values indicate better model simulations. PBIAS determines the tendency of the model to underpredict or overpredict the simulated concentrations (Gupta et al., 1999). According to Moriasi et al. (2007), for a model to be satisfactory, it should have an NSE > 0.5, RSR $\leq$ 0.7 and PBIAS within ±70%. The mathematical definition of each model evaluation statistic is defined in Equations 3.5-3.7.

$$NSE = 1 - \frac{\sum_{i=1}(O_i - P_i)^2}{\sum_{i=1}(O_i - \bar{O})^2} \tag{3.5}$$

$$RSR = \frac{\sqrt{\sum_{i=1}(O_i - P_i)^2}}{\sqrt{\sum_{i=1}(O_i - \bar{O})^2}} \tag{3.6}$$

$$PBIAS = \frac{\sum_{i=1}(O_i - P_i)}{\sum_{i=1} O_i} \tag{3.7}$$

where O_i and P_i referred to observed and predicted data and $\bar{O}$ is the average of observed data.

3.2.6 Suspended Solids Transport

Solids transport was included in WASP to simulate the transport of chemicals that were sorbed onto suspended solids. In the Solids Transport subroutine of WASP, three different solids types can be simulated: inorganic fines (clay and silt), sands, and biotic (organic) solids. Each solids type has its own transport and chemical properties (e.g., organic content). There were no data available to describe the contribution of each solid type to the TSS data that was provided by the GRCA. Hence, a single solids type with a particle density of 2.65 g m^{-3} (default WASP value) was employed.

In WASP, solids are transported through advection, dispersion, and solids transport processes which include settling, erosion, and sedimentation. Settling velocity was the only parameter that was calibrated to describe vertical solids transport since the advection and dispersion values had already been calibrated as previously described (Section 3.2.5). To further simplify the solids transport simulation, erosion and deposition velocities were assumed to be insignificant since the river velocities were generally within the transportation regime and were least likely to be within the sedimentation and erosion regimes based on a Hjulström curve analysis (Hjulström, 1935).

Initial and boundary concentrations for the upstream site as well as the calibration data set for G58 (Blair) were taken from the annual surface water quality monitoring program report (2009-2012) conducted by LGL Limited (Fausto et al., 2010; Fausto et al., 2011; Fausto et al., 2012; Fausto et al., 2013). TSS concentrations from the KWWTP were provided by the Region of Waterloo. These data sets are summarized in Table 3.4. The simulation period for the calibration and validation of solids transport was the same as the chloride transport simulation. Solids transport performance was evaluated using the NSE, RSR, and PBIAS metrics previously

described. A total of 6 runs were completed prior to achieving the desired fit between measured and simulated TSS concentrations at G58.

Table 3.4. Data set summary for TSS simulation, calibration, and validation

Boundary Location	Number of data points	Year	Year for calibration/validation	Time of the year data were collected
G49, upstream boundary condition	92	2009-2012	N/A	seasonal – winter, spring, summer, fall
Schneider Creek	92	2009-2012	N/A	seasonal – winter, spring, summer, fall
Kitchener WWTP	158	2009-2012	N/A	spring, fall
G58, calibration location	68 calibration 27 validation	2009-2012	2009-2011/ 2012	seasonal – winter, spring, summer, fall

Note. N/A = not applicable.

3.2.7 Simulation of Fate Mechanisms

Fate mechanisms were modeled following the calibration of the tracer and solids transport processes. Based on an initial review of the literature describing the fate of the target compounds in surface waters (i.e., laboratory and field studies found in the literature) and an examination of their physico-chemical properties (Table 3.1), sorption, photolysis, and biodegradation were considered to be the likely transfer and transformation mechanisms of contaminant in-stream loss. The behaviour of the target compounds in the aquatic environment was initially modeled starting with advection and dispersion conditions only. No transfer and transformation mechanisms were employed when the transport processes were found to be sufficient in representing the behaviour of some of the compounds in the river. However, for other target compounds, it was necessary to include transfer and transformation mechanisms to

adequately describe the observed data.

WASP requires values for reaction rate constants and partition coefficients for each fate mechanism simulation. In some cases, rate constants are calculated based on parameter inputs to the model. For instance, the sorption partitioning coefficient, K_d is estimated by WASP using the octanol-water partitioning coefficient (K_{ow}) specific to each compound and a linear relationship of K_{ow} to organic carbon partitioning coefficient, K_{oc} (Equations 3.3 and 3.4).

$$\log K_{oc} = A_1 + A_o \log K_{ow} \tag{3.3}$$

$$K_d = f_{oc} K_{oc} \tag{3.4}$$

A_1 and A_o are constants specific to log K_{oc} and log K_{ow} linear relationship and f_{oc} is the fraction of organic carbon present in suspended solids. Due to the unavailability of A_1 and A_o specific to pharmaceuticals being studied, the values 1.377 and 0.544 were used respectively. These values were taken from the empirical relationship between K_{oc} and K_{ow} developed by Kenaga and Goring (1980) for pesticides. The f_{oc} specific to the site was also not available and therefore was considered as a parameter that required calibration. The common range for f_{oc} is from 0.005 to 0.5 (Wool et al., 2002).

Biodegradation rate constants that were obtained from the literature for the target compounds (Table 3.5) were employed for the biodegradation simulation. The biodegradation rate constants were considered to be fixed for the purposes of this study. However, these values needed to be adjusted to ambient temperature conditions as described in Equation 3.5:

$$k_b = k_{b'} Q_{10}^{(T-20)/10} \tag{3.5}$$

where Q_{10} is a temperature correction coefficient that described the change in reaction rate when

temperature is increased by 10°C, k_b, is the biodegradation rate, k_b is the adjusted biodegradation rate, and T is water temperature. From Equation 3.5, Q_{10} was the parameter calibrated during the biodegradation simulation. Typical Q_{10} ranges between 1.5 and 2 (Wool et al., 2002). For compounds with rate constants that were taken from multiple biodegradation studies, the average value (arithmetic mean) was used.

Table 3.5. Literature-based biodegradation rates of target compounds in natural waters

Compound	Biodegradation Rates (d^{-1})	Reference
Triclosan	0.49-0.53	Environment Canada, 2012
Naproxen	0.0256	Grenni et al., 2013
Venlafaxine	0.0054	Gomez et al., 2013
Carbamazepine	<0.01[a]	Tixier et al., 2003

Note. [a]Refers to overall removal rate in surface water. Biodegradation studies for carbamazepine not available.

Although water temperatures varied over time, the temperatures for all segments downstream of the KWWTP were considered to be the same. Time-varying water temperatures specific to each segment were not available and a review on the temperature data from the water quality reports indicates consistent water temperatures for the segments downstream of KWWTP.

The WASP model provides several options to describe photolysis processes. In the current study, a reference photolysis was specified and then adjusted for site-specific conditions. For example, the photolysis rate was adjusted to the latitude of the study site from the latitude for which the reference photolysis rate was measured. Other parameters used to adjust the photolysis rates were cloud cover, fraction daylight, and predicted water levels from the transport simulation. The equations used for this option are summarized in Table 3.6. Photolysis studies have not been reported for the Grand River watershed. Thus, as suggested by the WASP technical manual, the parameters associated with site specific light conditions such as normalized

Table 3.6. Parameters used for photolysis simulation in WASP

Equation	Parameters	Units	Description	Typical Range[a]	Site or compound specific	Triclosan	Naproxen	Venlafaxine	Carbamazepine
$k_{ai} = k_{ari} I'_o (\frac{I_G}{I_o})(1 - 0.056C) X_L$	k_{ai}	d^{-1}	adjusted photolysis rate	-	compound	calculated by WASP			
	k_{ari}	d^{-1}	reference photolysis rate	-	compound	3.327,62[c]	11.88,23.76[d]	0.326[e]	0.145[f]
	I'_o	-	normalized light intensity function	0-10	site	required calibration			
	$\frac{I_G}{I_o}$	-	average light intensity attenuation	-	site	calculated by WASP			
	C	tenths	cloud cover	0-10	site	0.36			
	X_L	-	latitude correction factor	-	site	calculated by WASP			
$\frac{I_G}{I_o} = \frac{1 - e^{-dK_e D}}{dK_e D}$	D	m	segment depth	0.1-10	site	calculated by WASP			
	K_e	m^{-1}	extinction coefficient	0.1-5.0	site	required calibration			
	d	cm cm^{-1}	optical depth	1.19[b]	site	1.19			
$X_L = \frac{191969.65 + 87054.63\cos(0.039L)}{191969.65 + 87054.63\cos(0.039L_r)}$	L	degrees	study site latitude	0-90	site	43	43	43	43
	L_r	degrees	reference site latitude	0-90	site	47[c]	34,45[d]	50[e]	-

[a]Adapted from Watershed & Water Quality Modeling Technical Support Center US EPA. Retrieved from http://www.epa.gov/athens/wwqtsc/courses/ wasp7/index.html. August 2012.
[b]WASP recommended/default value.
[c] Adapted from (Latch et al., 2005; Tixier et al., 2002).
[d]Adapted from Lin and Reinhard, 2005; Packer et al., 2003.
[e]Combined direct and indirect photodegradation. Adapted from Gomez et al., 2013.
[f]Adapted from (Lam and Mabury, 2005) .

light intensity (NLI) and light extinction coefficient (LEC) required calibration (Table 3.6). NLI and LEC are site specific parameters and were considered constant during the simulation of each target compound. NLI is the ratio of light intensity of the reference conditions to the light intensity of the study site. LEC (m^{-1}) defines the attenuation of light in a water column. It is a function of chlorophyll *a*, dissolved organic carbon, and inorganic solids concentrations in the water. These parameters were not available for the study site, so a lumped LEC was specified and adjusted during the calibration. Values for LEC usually range from 0.1-9 m^{-1} while values for NLI range from 0-10 (Wool et al. 2002).

It was difficult to simultaneously calibrate all of the fate model parameters for all target compounds. Hence, triclosan was chosen to calibrate light intensity and light extinction coefficients. Triclosan, along with diclofenac, have previously been considered as model compounds for photodegradation of pharmaceuticals and personal care products in natural waters (Boreen et al., 2003). The LEC and NLI values were subsequently verified by using these values to simulate the concentrations of venlafaxine and naproxen. Ideally, if LEC and NLI were successfully applied to venlafaxine and naproxen, under the assumption that the measured photolysis rates were applicable to the Grand River study site, then there was no need for further calibration of these parameters in the subsequent simulations. For naproxen where direct photolysis rates were derived from different field studies, averaging the rates was not a direct approach since photolysis rates are highly dependent on the field conditions during the experiments (e.g., averaging the latitudes from several experiments is not straightforward). As a result, each rate was applied separately and was adjusted to site specific conditions. The reference photolysis rate that provided the best fit after the necessary adjustments was chosen. If in situations where none of the reference photolysis rates was considered applicable, then a

constant photolysis rate was employed and eventually calibrated (but unadjusted to any site-specific conditions).

The addition of the fate mechanisms to the model was done in a systematic fashion (i.e., stepwise fitting). The mechanism considered to be the least important was added first. This procedure determined whether the mechanism was indeed insignificant even after reaching the minimum and maximum limits of the possible parameter values specific to each mechanism. Due to the slight to moderate hydrophobicity of the target compounds and relatively low TSS simulated concentrations, sorption was considered first. The typical range for biodegradation rates (0.01-0.5 d^{-1}) was much lower than the range for photolysis rates (1-10 d^{-1}). Thus, biodegradation was considered after sorption was evaluated. Photolysis was the last fate mechanism added in the simulation.

For a complex model such as WASP, this step-by-step approach was considered helpful in identifying the most accurate fate model setup for each target compound because it could help verify the accuracy of the conceptual model implemented (e.g., was sorption relevant or not?). This procedure was also helpful when conducting a manual calibration (i.e., trial-and-error approach) since simultaneous fitting of all the parameters from different mechanisms can lead to ambiguous results. The manual calibration was completed in a process-based approach with simplifications derived from conceptually realistic judgment.

The simulation period for the trace organic contaminants spanned from July to December 2012. The simulated concentrations were compared with the measured concentrations collected in G53E (Figure 3.1) for calibration. When the temporal simulation and the measured concentrations showed a good fit, the model performance was tested against the downstream concentration profile for samples collected in October 2012. In occasions when the simulations

did not match this data set, the model was refined by characterizing additional mechanisms. The total number of trial and error simulations (transport and fate) completed to achieve a good fit between the measured and simulated values were 88 for triclosan, 48 for venlafaxine, and 31 for naproxen.

3.2.8 Fate Mechanism Model Sensitivity Analysis

A sensitivity analysis was conducted to obtain insight into the uncertainty associated with the calibrated model predictions. The sensitivity of the model predictions to changes in the fate parameter values was assessed by changing the parameter values one-at-a-time and comparing the outputs to the baseline model results. The parameters were perturbed ±50% and the sensitivity percentage was computed using Equation 3.6 (Kim et al., 2004):

$$Sensitivity\ \% = \frac{(C-C_B)/C_B}{(M-M_B)/M_B} \times 100 \qquad (3.6)$$

where C is the output value after perturbation, C_B is the baseline output value, M is the adjusted parameter value and M_B is the baseline parameter value. Equation 3.6 measures the ratio of relative change in the output value after a relative change in parameter value has been applied. The calibrated parameter values (photolysis rate, LEC, NLI, biodegradation rates, and Q_{10}) were considered the baseline conditions for the sensitivity analysis.

3.3 Results and Discussion

This section summarizes the modeling results after the calibration of the parameters associated with chloride transport, solids transport, and fate simulation. This section also provides a detailed discussion of the results and evaluates the approach used for modeling of the

transport and fate processes. Finally, this section describes the overall robustness of the model, including its limitations and potential future applications in the Grand River watershed.

3.3.1 Segment Hydrogeometry and Tracer Transport

Uncertainties in geometric characterization (e.g., lengths, widths, and bed slopes) are difficult to estimate but errors in the inputs of hydrogeometric properties can easily prevent the accurate simulations of water levels (Martin and McCutcheon, 1999). This becomes more apparent when sufficient data are available for testing. In this advective transport simulation, the predicted water levels (2009-2011) just above the KWWTP were found to agree with the water levels measured by the flow gauge at that location (Figure 3.2A). Hence, it was concluded that the segmentation, calibrated hydrogeometric parameters (Table 3.7), and other input parameters primarily taken from the hydrodynamic modeling conducted by the GRCA were adequate in representing the advective transport in the modeled reach. In addition to the graphical evaluation of the model fit, NSE, RSR, and PBIAS for water levels simulation as shown in Table 3.8 were found to be within the satisfactory criteria further suggesting that the segment hydrogeometry and advection are well represented in the model.

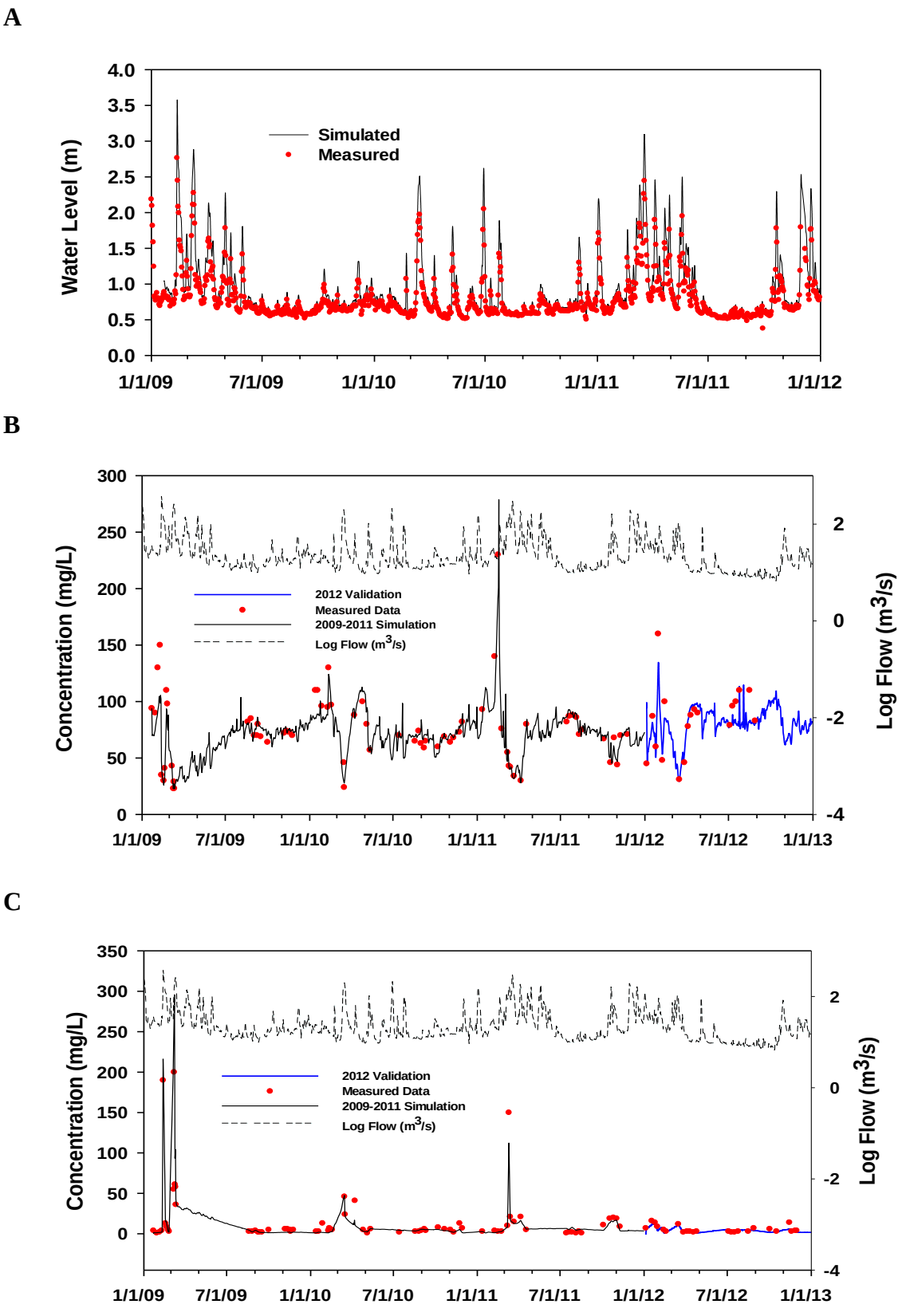

Figure 3.2. Simulated values for (A) water level, (B) chloride (tracer) concentration, and (C) solids concentration. Water levels were measured at 0.5 km upstream of WWTP. Tracer and solids transport were evaluated at G58, 7 km downstream of KWWTP.

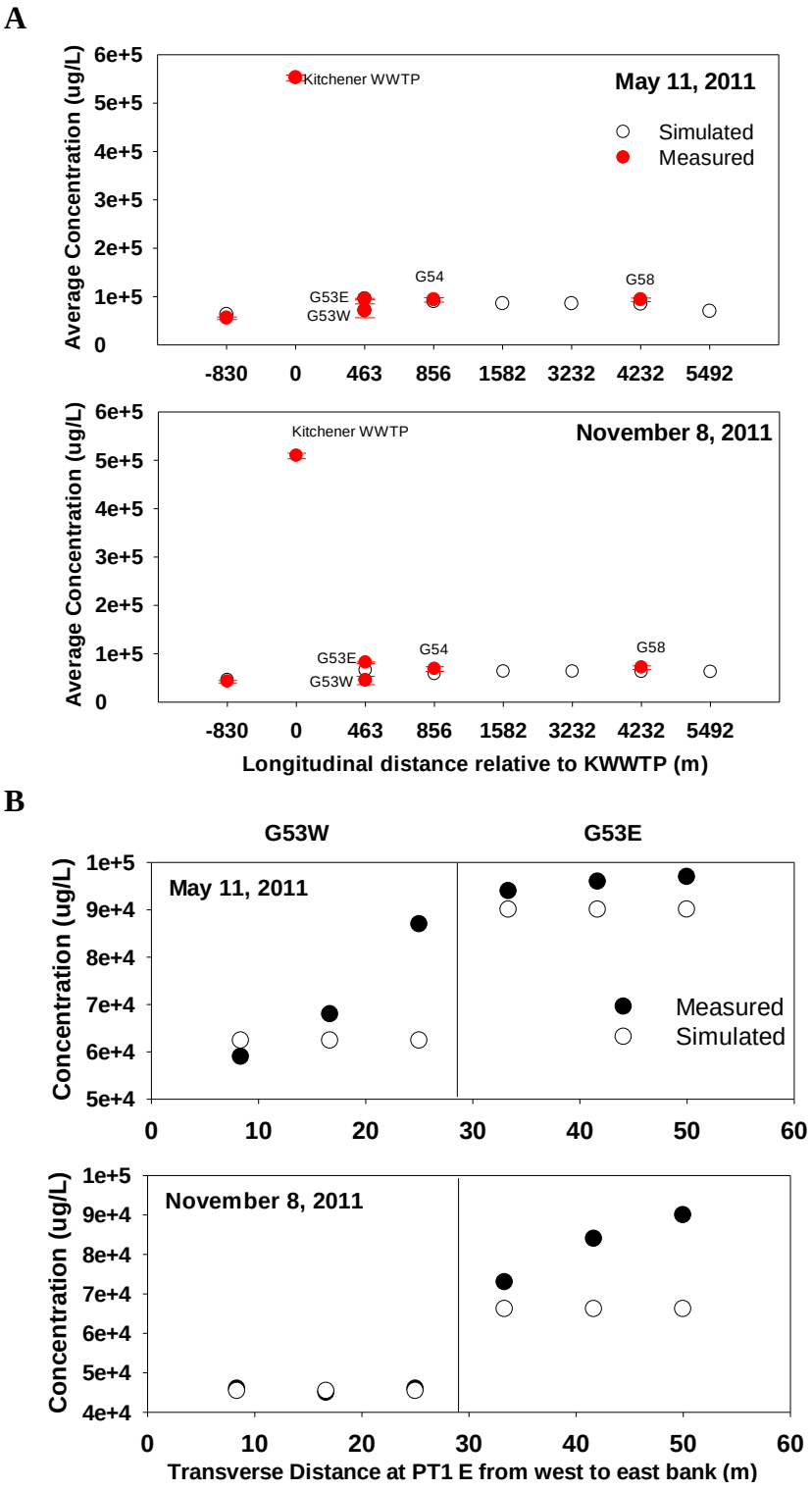

Figure 3.3. Validation of the downstream concentrations for (A) May and November, 2011 data set. (B) The six measurements collected across G53E (divided in west and east sections) illustrate incomplete mixing at that site. WASP can only resolve two distinct concentrations across G53.

Table 3.7. Calibrated parameters for transport simulation

Transport		
Advection	**Segment**	**Depth Coefficient, c**
	Manheim	1.272
	KWWTP	1.170
	PT1	0.850
	PT2	1.153
	Riveredge A	1.340
	Riveredge B	1.340
	Edgehill	0.553
	Blair	1.965
	Grand River End	1.200
Segmentation	from 10 segments to 67 segments – KWWTP and PT1E were finely segmented	
Dispersion Coefficients $m^2 s^{-1}$	longitudinal	10
	transverse, KWWTP	2.0×10^{-2}
	transverse, PT1	1.4×10^{-2}
	transverse, PT2	3.0×10^{-3}

WASP also successfully simulated the transport of the tracer substance (chloride) as depicted by a good agreement of time-varying simulated and measured concentrations at G58 (Blair), suggesting that the dispersion coefficients were adequately calibrated (G58) (Figure 3.2B). In addition, the model was able to reflect the sporadic extremes in chloride concentrations during the simulation period (2009-2011). The model also successfully replicated the time-varying chloride data set used for the validation period (Figure 3.2B) as well as the data set used to validate the spatial variation in the downstream concentrations (Figure 3.3A, Table 3.8). All of the model performance evaluators for chloride simulation (calibration and validation) were considered well within the satisfactory performance rating range (Table 3.8). The consistent performance of the advective and dispersive transport process simulations suggests that the hydrodynamics of the modeled reach were well-represented by the one-dimensional assumption and the kinematic wave simplification (provided that a fine segmentation and good estimation of the hydrogeometric properties were completed). It also suggests that the boundary concentrations

were measured/estimated with sufficient precision and were not considered as significant sources of uncertainties in the model.

Table 3.8. Performance evaluators for the temporal simulation of water level, chloride, and TSS

	Water Level	**Chloride**		**TSS**		**Satisfactory Criteria**
		Calibration	Validation	Calibration	Validation	
NSE	0.685	0.724	0.827	0.556	0.569	>0.5
RSR	0.561	0.525	0.416	0.666	0.656	≤0.7
PBIAS	-15%	+9%	+1.3%	+18%	+18%	±70%

Note. Temporal = model evaluation for time-varying concentration. Spatial = model evaluation for spatially-varying concentrations (for samples collected in October 2012). Positive PBIAS = underprediction. Negative PBIAS = overprediction.

There were, however, some aspects of the observed chloride data set that were not reflected in the calibrated model. For instance, an incomplete mixing condition was identified at G53 (Figure 3.1, segment nos. 35-40) as illustrated by the concentration gradient observed across the river during the May and November 2011 sampling event (Figure 3.3B). The model was not able to capture this incomplete mixing condition well. The segmentation employed for this location only produced two different concentrations in the western and eastern portions of G53 (Figure 3.3B). This result was considered a model limitation as WASP was not developed to represent poorly mixed conditions. Thus, concentration gradients in the mixing zone cannot be reliably simulated. This result suggests that there may be other transport conditions occurring within this segment that can further explain the observed concentration gradients. For instance, vertical mixing could possibly explain this observation. This process can be represented in WASP by adding segments in the vertical direction and providing exchange paths via vertical dispersion coefficients. However, measured chloride data to support this modification is not available. In addition, the concentrations that WASP simulated were in agreement with the

overall average concentrations in the eastern and western sections of G53 (Figure 3.3B). Hence, it was decided that transverse and longitudinal exchanges are sufficient processes in representing mixing at G53.

There were, however, some aspects of the observed chloride data set that were not reflected in the calibrated model. For instance, an incomplete mixing condition was identified at G53 (Figure 3.1, segment nos. 35-40) as illustrated by the concentration gradient observed across the river during the May and November 2011 sampling event (Figure 3.3B). The model was not able to capture this incomplete mixing condition well. The segmentation employed for this location only produced two different concentrations in the western and eastern portions of G53 (Figure 3.3B). This result was considered a model limitation as WASP was not developed to represent poorly mixed conditions. Thus, concentration gradients in the mixing zone cannot be reliably simulated. This result suggests that there may be other transport conditions occurring within this segment that can further explain the observed concentration gradients. For instance, vertical mixing could possibly explain this observation. This process can be represented in WASP by adding segments in the vertical direction and providing exchange paths via vertical dispersion coefficients. However, measured chloride data to support this modification is not available. In addition, the concentrations that WASP simulated were in agreement with the overall average concentrations in the eastern and western sections of G53 (Figure 3.3B). Hence, it was decided that transverse and longitudinal exchanges are sufficient processes in representing mixing at G53.

Effective dispersion in water quality models where numerical approximations are used to solve differential equations is influenced by numerical dispersion (Chapra, 1997). Numerical dispersion is an unavoidable artificial mixing induced by finite difference methods such as the

Euler solution technique employed by WASP. It should be noted that effective dispersion is essentially a combination of calibrated dispersion coefficients and the numerical dispersion. However, Chapra (1997) and Martin (2012) suggest that dispersion is oftentimes less relevant in rivers and streams than advection. Estimation of this parameter can be relaxed without undermining the accuracy in the solution technique.

Two observations were generalized from the calibrated chloride transport simulation. First is the inverse relationship between flow and chloride concentrations (Figure 3.2B). This relationship can be attributed to higher volume of water in each segment resulting in a more dilute concentration. Daily dilution factors (Appendix D) were determined using a simple dilution model (van Leeuwen and Vermeire, 2007) that calculates the ratio of the concentration in the effluent and the concentration at G53 (PT1, 0.3 km downstream of KWWTP) while accounting for the effects of the upstream background concentration (G49). The dilution factors range from 11 to 232 with high values corresponding to high flow conditions (Appendix D). This also suggests that chloride concentrations are controlled by a point-source loading (i.e., KWWTP) rather than non-point sources. If chloride were to have come from non-point sources such as storm run-off events, high chloride concentrations would have been observed during high flows when these contaminants are transported into the river during a rain event. This, however, was not the case for the reach studied.

Second is the relatively constant chloride concentration from G54 (PT2) to G60 (Grand/Speed River confluence) during the May and November 2011 sampling events (Figure 3.3A). This observation indicates that a complete mixing condition may have already been achieved at a distance that was approximately 1.0 to 1.5 km from the KWWTP outfall. It is likely that advective transport is dominant at this location and dispersive transport could be ignored.

Overall, these findings provide a useful and conservative basis on the behaviour of a point source contaminant in the Grand River after its release from KWWTP.

3.3.2 Suspended Solids Transport Simulation

Solids transport was also well simulated as shown by a good fit between the time-varying simulated TSS and measured observations (Figure 3.2C, Table 3.8). The results suggest that the transport conditions (advection, dispersion, and settling) used to simulate solids transport were adequate in explaining the TSS concentrations at Blair (G58). Settling velocities for TSS usually range from 0.2-30 m d^{-1} (Chapra, 1997). The calibrated settling velocity was 0.5 m d^{-1} and hence was in the lower end of the range. This result suggests that most of the suspended solids are made of fine particles that settle slowly. However, Chapra (1997) argues that one-dimensional models tend to use lower settling velocities to compensate for other mechanisms (e.g., upwelling) that are omitted in these model types. These mechanisms reduce settling effects and are oftentimes inadequately expressed in one-dimensional models. Thus, the certainty surrounding the use of the calibrated settling velocity is difficult to evaluate unless a field estimate has been completed. However, the simple solids transport generated by WASP was able to simulate the sporadic extremes in TSS concentrations during the calibration and validation period (Figure 3.2C) and was considered satisfactory for the purposes of this study. Unfortunately, downstream concentrations similar to that of chloride were not available to further validate this model.

The TSS concentration simulated over the four-year period ranged from 0.2 to 295 mg L^{-1} with an average concentration of 9 ± 20 mg L^{-1}. TSS play a role in the sorption mechanism and this simulation is re-examined in the analysis of the fate mechanisms described in the following section.

3.3.3 Transport Simulation of Target Compounds

The behaviour of the target compounds in the aquatic environment was initially simulated considering only transport processes. Figure 3.4A-D present the observed and predicted values for the target compounds at G53E when contaminant transport only was modeled. The flow in the river at this location is also presented. From Figure 3.4A-D, it can be observed that transport only modeling was able to adequately simulate (i.e., well within the satisfactory criteria used) the temporal behaviour of the target compounds at this location in the Grand River. However, when the model was tested against the downstream concentration profile of samples collected in October 2012, the graphical fit, NSE, and RSR for triclosan, venlafaxine, and naproxen were unsatisfactory (Figure 3.5A-D) and thus required further model development to predict the downstream concentrations well. The downstream concentrations were found to be overestimated suggesting that additional mechanisms other than transport processes may be needed to explain the removal of these compounds as they move downstream of the source . As a result, fate mechanisms (sorption, biodegradation, and photolysis) were introduced in the simulation to further improve the model predictions (both temporal and spatial). The results for the fate simulation are also shown in Figures 3.4A-D and 3.4A-D which are further explained in the next section.

The transport-only modeling supports the reported persistence of carbamazepine in surface waters. Carbamazepine has been shown to be resistant to in-stream degradation processes in previous studies (Clara et al., 2004; Gasser et al., 2011; Kunkel and Radke, 2012). Furthermore, a review conducted by Zhang et al. (2008) on the removal of carbamazepine in different WWTP configurations showed that most removal efficiencies were below 10%. This

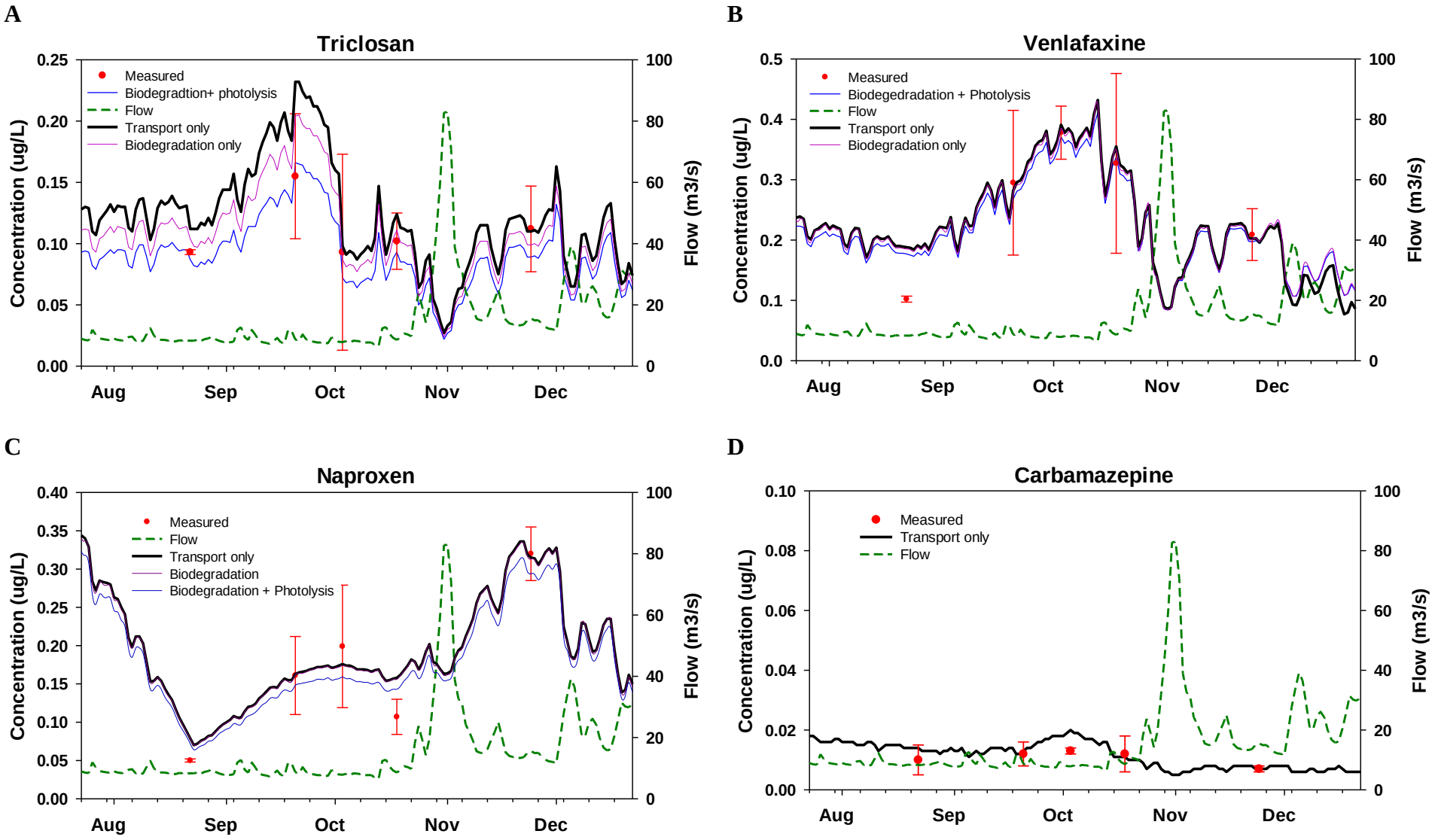

Figure 3.4. Time-varying simulated and measured concentrations for (A) triclosan, (B) venlafaxine, (C) naproxen and (D) carbamazepine at G53E.

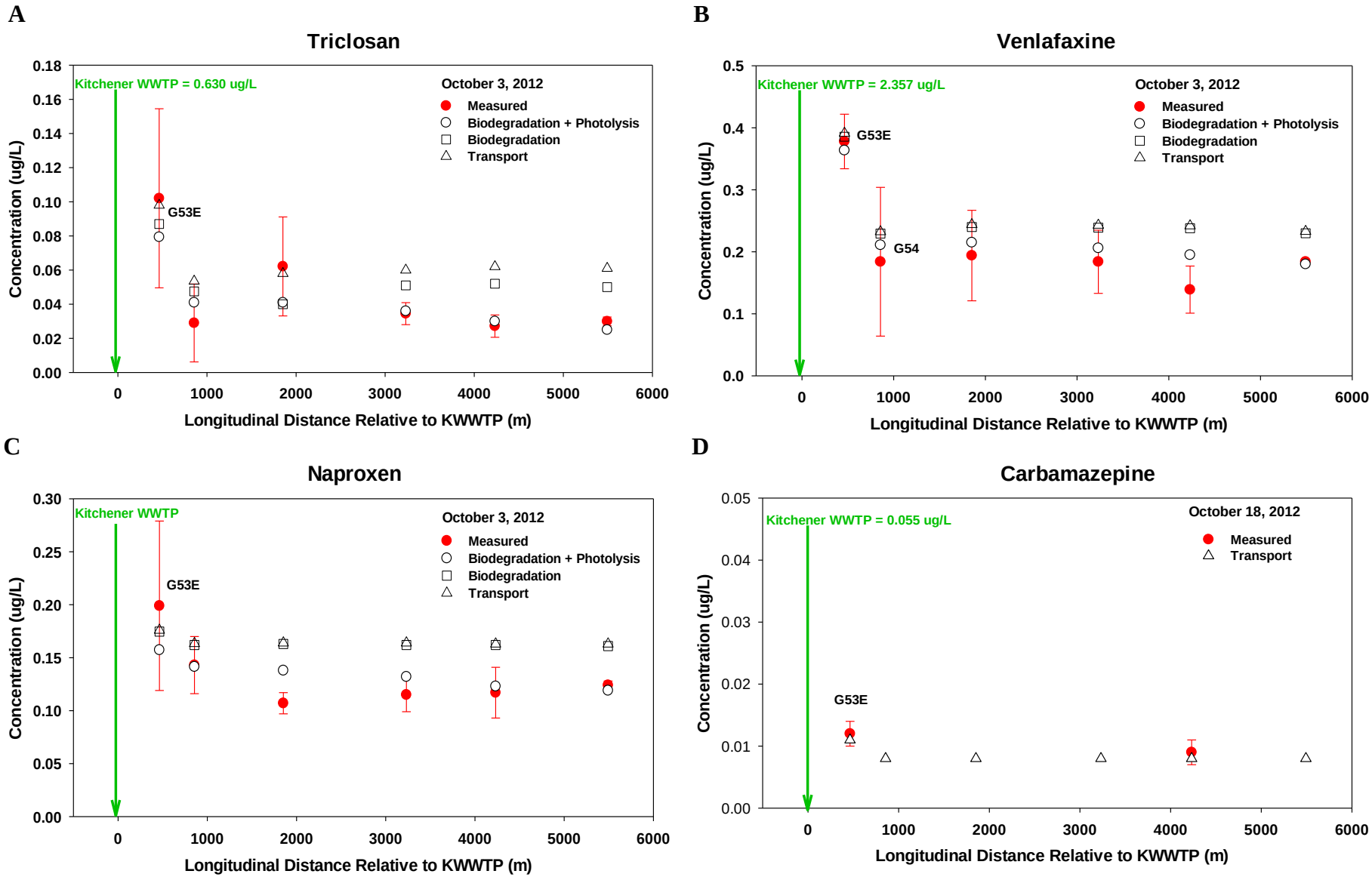

Figure 3.5. Simulated and measured downstream concentrations for (A) triclosan, (B) venlafaxine, (C) naproxen, and (D) carbamazepine relative to KWWTP. October 3, 2012 river flow = 7.84 m^3s^{-1}. WWTP effluent flow = 0.71 m^3s^{-1} (contributes 8% of the total river flow).

poor removal was attributed to its resistance to biodegradation (Joss et al., 2006; Stamatelatou et al., 2003) and poor sorption onto sludge (Ternes et al., 2004). Biodegradation and sorption likely occur at even slower rates in surface waters due to the much lower concentrations of microorganisms and solids that would contribute to in-stream removal. Also, there have been studies indicating that carbamazepine can be photodegraded when exposed to sunlight but only at a low rate (6.93×10^{-3} d^{-1} in double distilled water) (Andreozzi et al., 2003). Thus, flow-driven transport processes, as depicted by the modeling results in this study, can capture the temporal and spatial trend of carbamazepine given its poor removal by different fate mechanisms (biotic and abiotic).

Although the predicted temporal and spatial concentrations for triclosan, venlafaxine, and naproxen were overestimated using transport processes alone, these predictions could perhaps be used as conservative estimates of pharmaceutical concentrations in a watershed. There are a few studies that have suggested this. For instance, Kolpin et al. (2004) recommended the use of flow conditions as predictors of contaminant concentrations. Others (Loraine and Pettigrove, 2006; Osorio et al., 2012) further demonstrated the dependency of pharmaceutical concentrations on seasonality, where maximum concentrations corresponded to low flow conditions. This finding is also evident in the transport simulations of the compounds being modeled where an increase in flows corresponded to a decrease in concentrations (Figure 3.4A-D). A modeling study conducted by Wang et al. (2012) showed that mass transport due to advection was the most significant process in reducing the concentrations of phenolic compounds (major components of personal care products and industrial chemicals) in a riverine environment. Thus, a transport model of pharmaceuticals in the Grand River watershed can provide a conservative

representation of contaminant behaviour when information on fate mechanisms is difficult to obtain.

3.3.4 Fate Simulation of Target Compounds

Triclosan, venlafaxine, and naproxen required further improvements in the model in order to successfully reproduce downstream concentration profiles (Figure 3.5). Sorption was first added but was found to be an irrelevant mechanism for all the target compounds. Chapra (1997) examined the sorption of various organic chemicals with a wide range of log K_{ow}'s (1-10). It was observed that under different environmentally relevant suspended solids concentration (1 – 50 mg L^{-1}) and f_{oc} of 0.05, most chemicals with log K_{ow} < 4 to 5 are mostly in the dissolved form. The compounds studied here have log K_{ow}'s < 5 (Table 3.1) and the simulated suspended solids concentrations in this part of the Grand River was relatively low (average concentration of 9 mg L^{-1}; Section 3.2.6). This combination of system and chemical properties does not allow for significant uptake of the target compounds through sorption.

When biodegradation was considered in the simulation of each target compound, only triclosan had a slight improvement in the prediction of the downstream concentration profile (Figure 3.5A) but the model was still considered unsatisfactory (Table 3.9). Adjustments in Q_{10}, the single parameter required for the calibration of the biodegradation process for all target compounds, was found to be inadequate (i.e., satisfactory criteria were not achieved). This result was primarily due to the low biodegradation rates that rendered the model insensitive to any changes made to biodegradation parameter values. The addition of biodegradation to the simulation of the temporal and spatial data sets for venlafaxine resulted in even poorer model performance (NSE and RSR were unsatisfactory, see Table 3.9), suggesting that biodegradation may be an irrelevant mechanism for venlafaxine removal in surface waters. This is consistent

with a field study conducted by Gomz et al. (2013) who found that biodegradation of venlafaxine in a river occurred at a very low rate (Table 3.5) and can be considered as an insignificant fate mechanism. Naproxen was also found to be non-biodegradable in WWTPs (Jones et al., 2002; Richardson and Bowron, 1985). Although triclosan has been observed to be biodegradable under aerobic conditions, it has been found to be only partially removed in WWTPs (Bedoux et al., 2012). Thus, simulation with biodegradation alone could not explain the downstream concentration profile (Figure 3.5A) in spite of the adjustments made in Q_{10}.

Table 3.9. Model performance evaluation for different modeling setups in both temporal and spatial simulations

Compound	Mechanism	NSE		RSR		PBIAS (%)		Rating	
		TPL	SPL	TPL	SPL	TPL	SPL	TPL	SPL
Satisfactory Criteria		>0.5	>0.5	≤0.7	≤0.7	±70%	±70%		
Triclosan	T	0.678	±70%	0.567	0.885	-11	-38	S	US
	TB	0.752	0.468	0.498	0.729	3	6	S	US
	TBP	0.559	0.672	0.664	0.573	17	5	S	S
Venlafaxine	T	0.520	0.394	0.691	0.778	-5	-26	S	US
	TB	0.421	0.449	0.761	0.742	-13	-24	US	US
	TP	0.572	0.859	0.654	0.375	-8	-9	S	S
Naproxen	T	0.917	-0.760	0.288	1.300	-2	-23	S	US
	TB	0.920	-0.675	0.292	1.294	-1	-22	S	US
	TP	0.910	0.504	0.300	0.704	5	-1	S	S
Carbamazepine	T	0.780	0.660	0.469	0.578	-22	-28	S	S
	TB	-	-	-	-	-	-	-	-
	TBP	-	-	-	-	-	-	-	-

Note. TPL=temporal. SPL=spatial. T=transport only. TB=transport and biodegradation. TP=transport and photolysis. TBP=transport, biodegradation, and photolysis. US=unsatisfactory. " –" = not applicable since fate mechanisms were not simulated for this compound.

The predictions improved (both graphical and statistical performance evaluation) for venlafaxine, naproxen, and triclosan when photolysis was added to the fate simulation. As previously mentioned, the photolysis rates found in the literature were extrapolated to ambient conditions by inputting the site-specific parameters such as water levels and cloud cover, and calibrating the light-specific parameters (LEC and NLI). In the simplified calibration process, it

was assumed that the light intensity associated with the reference photolysis rates and the study site was similar. Hence, an NLI of 1 was used. The photolysis calibration process then only required LEC to be fit. An LEC of 8 m^{-1} was found to be the optimal value that provided the best fit between the measured and simulated concentrations for both the temporal and spatial simulations for triclosan (Figure 3.4A-D; Figure 3.5A-D; Table 3.9).

Venlafaxine concentrations downstream were well represented by the transport and the calibrated site-specific photolysis conditions (LEC and NLI). This result suggests that the LEC and NLI values were appropriate for the conditions of the study site (Figure 3.5B). Naproxen concentrations, however, were not reproduced successfully when photolysis rates from two different field studies were adjusted to the calibrated site-specific conditions. This suggested that the measured photolysis rates taken from the literature could not be extrapolated to the study site. Due to the lack of data to simulate photolysis through other available options in WASP, a constant photolysis rate was input and calibrated assuming that this rate was more representative for the study site. It was found that this option was more successful in reproducing the downstream concentrations of naproxen (Figure 3.5C). The optimal photolysis rate for naproxen (under manual calibration) was found to be 1.1 d^{-1}.

Based on the stepwise fate simulation and calibration approach, photolysis was deemed a more significant fate mechanism for the target compounds than biodegradation. The calculated biodegradation rates were always lower than the calculated photolysis rates in the simulations as seen in Figure 3.6A-C. It was also noticed that biodegradation rates decreased over time as the water temperatures dropped (Figure 3.6A-C). Although photolysis rates were also shown to decrease over the period of the study, the rates were still large enough to cause considerable reductions in concentrations (Figure 3.4A-D and 3.5A-D). An experimental study conducted by

Latch et al. (2005) also found that photolysis is a significant process for triclosan fate is surface water (lake) and comprised about 80% of the total loss.

For triclosan, the reductions in concentrations after the addition of fate mechanisms were pronounced at the beginning of simulation when temperatures were higher and daylight hours were longer (Figure 3.4A). Photolysis rates were also enhanced when water levels were low (Figure 3.6A). In WASP, the adjustments in direct photolysis rates consider the effect of water levels. In addition, light extinction in surface waters is quantified using the Beer-Lambert law which is dependent on segment depth (Wool et al., 2002). Photolysis rates in some cases can be inversely proportional to depth (Zepp and Cline, 1977). With shallow water depths, the transmission of sunlight in the water body is increased, rendering contaminants more exposed to solar radiation. Direct photodegradation can also be significant for compounds with maximum absorption wavelengths falling within the visible and ultraviolet portions (290-600 nm) of the solar energy spectrum (Chapra, 1997). In this fate simulation, photolysis had the largest effect on triclosan and to some extent on naproxen due to their more photolabile properties. Previous studies have shown that direct photodegradation is a relevant elimination process for triclosan (Latch et al., 2005; Sabaliunas et al., 2003; Tixier et al., 2002) and naproxen (Lin and Reinhard, 2005; Packer et al., 2003) while indirect photodegradation is significant for venlafaxine (Rúa-Gómez and Püttmann, 2012). Kunkel and Radke (2013) conducted an experiment of pharmaceutical attenuation during (1) sunny/dry periods and (2) high flow conditions after a heavy rainfall. They found that overall pharmaceutical elimination was higher during sunny periods than high flow conditions. They attributed the removals during sunny/dry periods to longer residence time in river segments. They argued that low water velocities during these periods resulted in longer residence time, providing more time for compound elimination (such

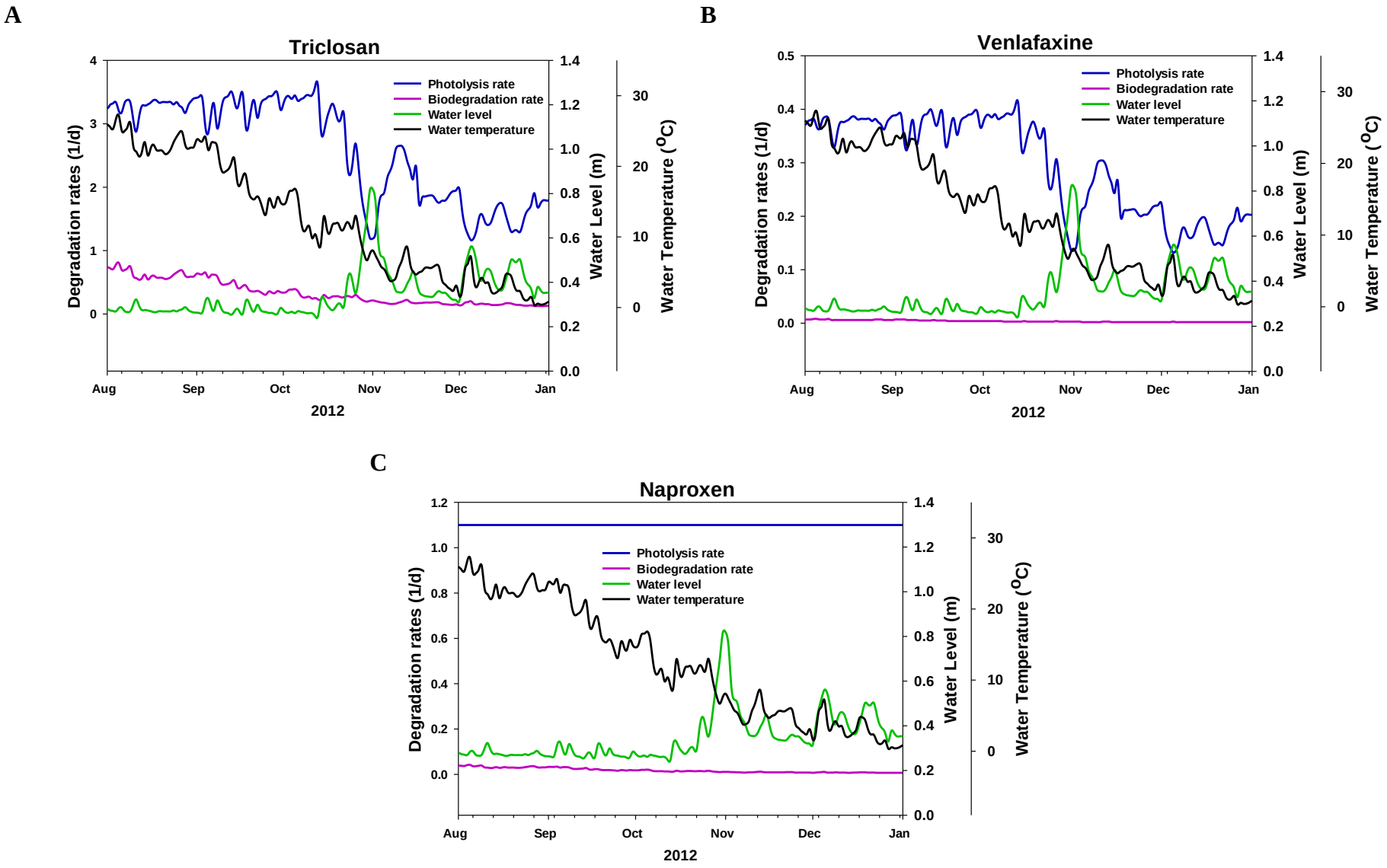

Figure 3.6. Calculated biodegradation and photolysis rates for the target compounds at G53E. Water levels and surface water temperatures are also plotted. Photolysis option 2 was used for triclosan and venlafaxine. A constant photolysis rate was entered and adjusted for naproxen.

as photolysis) in river segments. The reduction of triclosan concentrations through photolysis in the areas modeled suggests that the Grand River watershed provides conditions conducive for effective photolytic degradation.

In order to illustrate the contribution of each rate at different locations downstream of the KWWTP, plots (Figure 3.7) of biodegradation and photolysis rates for triclosan at two different temperature (low vs. high temperature) and two flow conditions (low vs. high flow) were completed. As mentioned previously, water temperatures were assumed to be constant for all modeled segments downstream of KWWTP and only varied with time (Section 3.2.7). As a result, the biodegradation rate was constant for each downstream segment at a given time period. It was observed Figure 3.7 that photolysis was still the dominant mechanism at any location regardless of the flow and temperature conditions. Also, high temperature and low flow conditions favoured high photolysis and biodegradation rates.

In general, relatively high reductions in concentrations over a 3 km distance downstream of the wastewater discharge indicate favourable environmental conditions for effective in-stream removal by fate and transport mechanisms (Figure 3.5A-D). However, these compounds continued to persist at a distance of ~7 km from KWWTP (8 to ~200 ng L^{-1}). Venlafaxine for example persisted with a concentration ~200 ng L^{-1} near Grand River and Speed River confluence (7 km). The Grand River at this point cannot fully assimilate pharmaceutical loadings. The river is faced with more water quality challenges as it receives water from additional wastewater outfalls downstream and inputs from its tributaries (e.g., Speed River).

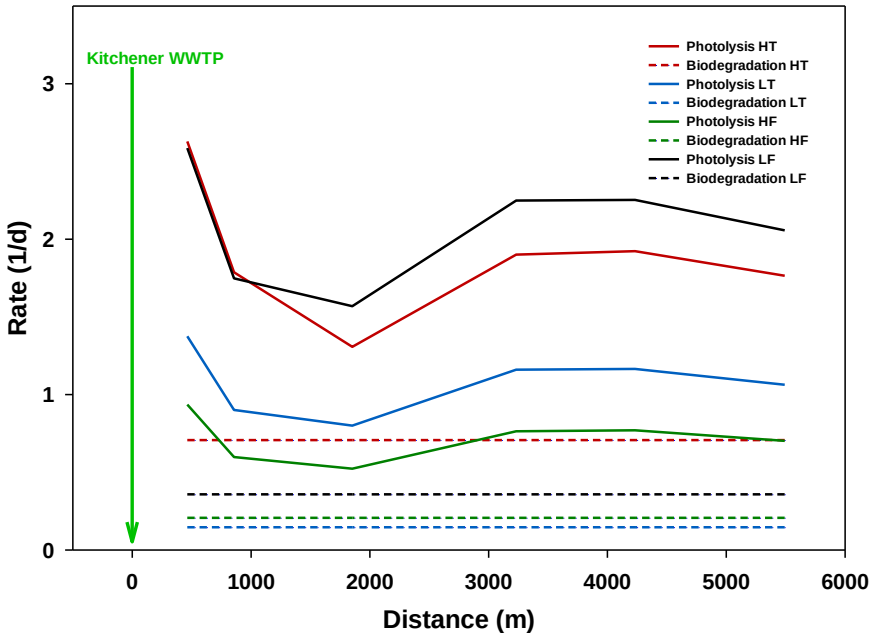

Figure 3.7. Photolysis and biodegradation rates for the sites downstream at different flow and temperature conditions. HT = dry/high temperature (August 4, 2012); LT = dry/low temperature (December 15, 2012); HF = high flow (October 31, 2012); LF = low flow (September 20, 2012).

3.3.5 Sensitivity Analysis

The results of the sensitivity analysis that was conducted in this study are presented in Figure 3.8A-C. The fate simulation of triclosan, venlafaxine, and naproxen strongly depended on the first-order photolysis rates, LEC, and NLI. The biodegradation parameters did not affect the concentrations of the target compounds. With the low initial biodegradation rates, the impact of perturbations to these constants was minimal. Overall, the sensitivity analysis showed that parameters associated with photolysis are very influential when simulating the fate of triclosan, venlafaxine, and naproxen. Thus, it may be beneficial to conduct photolysis experiments in the Grand River watershed to reduce output uncertainties for these target compounds. The model also behaved as expected when the input parameters were varied. For example, the overall model output decreased when photolysis and biodegradation rates were increased (Figure 3.8A-C).

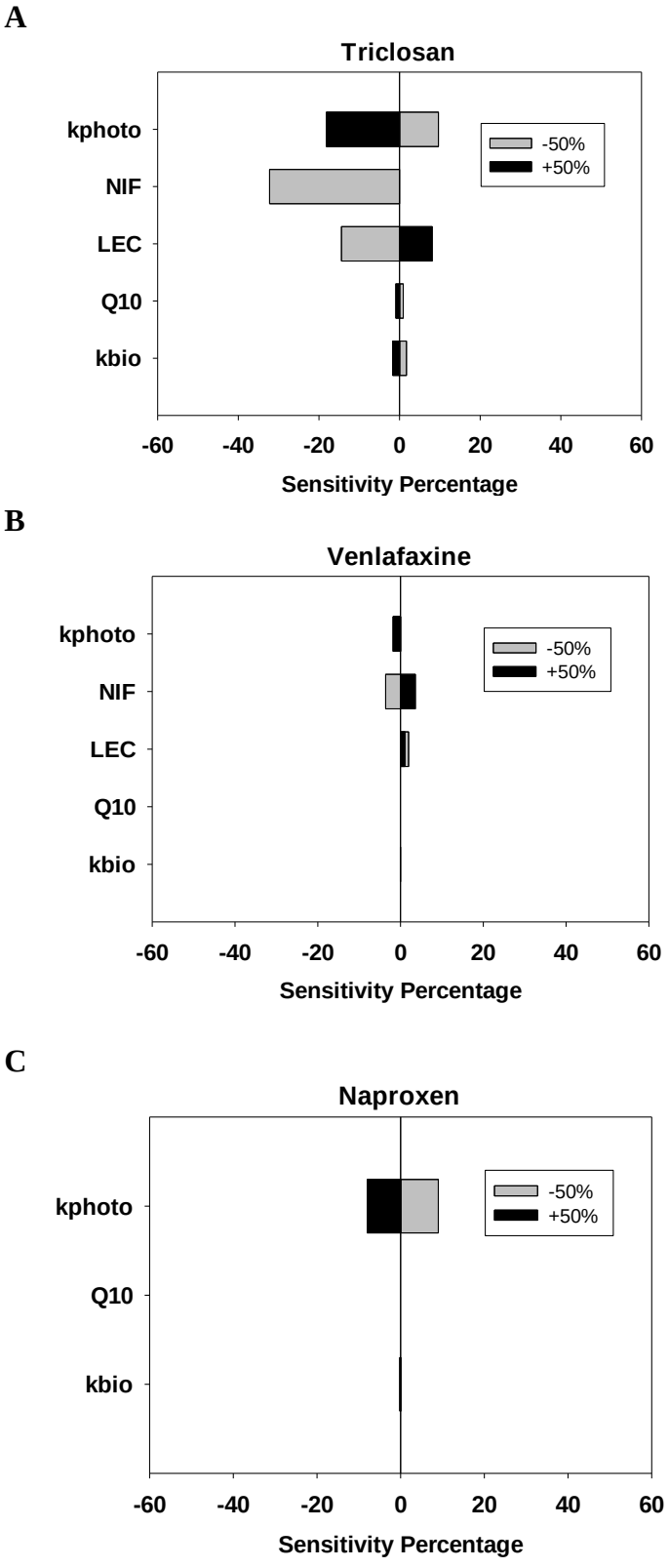

Figure 3.8. Sensitivity analysis results for the mechanisms responsible for the fate of the compounds modeled. k_{photo}=photolysis rate; NIF=normalized intensity function; LEC=light extinction coefficient; Q_{10}=temperature correction factor; k_{bio}=biodegradation rate. NIF and LEC perturbations were not applied for naproxen (used a different photolysis option).

4 Conclusions

This study demonstrates that selected antiandrogens (triclosan and chlorophene), along with other pharmaceuticals (naproxen, ibuprofen, carbamazepine, and venlafaxine), are present in the Grand River watershed. Triclosan was present in the effluents but was observed to degrade quickly in the aquatic receiving environment. Although chlorophene was very persistent in surface waters it likely comes from a source other than WWTPs and its occurrence appears to be affected by the seasonal use of this compound. Although dichlorophene is another antiandrogen previously associated with WWTPs, it was not found in any of the samples collected in the Grand River watershed including wastewater effluents. Additional information regarding the concentrations of antiandrogens in fish tissues (e.g., bile) is required to assess the potential exposure and bioaccumulation of antiandrogens in fish and to evaluate the potential endocrine disruptive effects caused by these compounds.

Overall, triclosan and pharmaceuticals have elevated concentrations in the central Grand River. Reductions in concentrations were observed as these compounds move downstream, except for carbamazepine where relatively constant concentrations were seen in the sampling sites downstream of WWTPs. All target pharmaceuticals were present in the Speed River but no antiandrogenic compounds were detected in its associated sampling sites. The herbicide atrazine was found in all sampling sites suggesting the ubiquitous usage of this compound in both agricultural and urban sections of the watershed.

The distribution of the contaminants was highly dependent on the treatment process and effluent quality. Nitrifying plants were found to have lower concentrations of the more biodegradable compounds (triclosan and ibuprofen) than non-nitrifying plants. Other compounds such as venlafaxine and carbamazepine were persistent in the effluents and in surface waters.

Ammonia and nitrate (an indication of the degree of treatment) may serve as good indicators of performance of WWTP in the removals of pharmaceuticals, personal care products, and endocrine disrupting compounds

The results of the above-mentioned study were incorporated in a fate and transport model and considerably assisted in the understanding of the behaviour of these compounds in the area of concern. The simulated results indicated that the water quality model used (WASP) was calibrated well. The modeling completed provided more insights regarding the environmental conditions necessary for contaminant attenuation in the reach modeled. For instance, the simulation of triclosan and selected pharmaceuticals showed that the fate and transport of these compounds are mainly driven by flow-driven transport processes. Carbamazepine especially was well simulated by modeling this compound as a tracer contaminant. The transformation mechanisms such as photolysis and biodegradation may also play a role in the attenuation of these compounds in the Grand River. Photolysis had a major effect on predicted concentrations of triclosan and naproxen. Venlafaxine was persistent over ~7 km travel distance from the KWWTP. This study further showed that the model formulation was consistent with the scientific information required to describe the behaviour of contaminants in the area of concern.

In the future, it is beneficial for the study of EDCs in the watershed to include a survey of estrogenic compounds (i.e., steroidal hormones) similar to what was conducted in this study. This allows for a more comprehensive exposure assessment of the co-occurrence of both estrogenic and antiandrogenic compounds in the watershed and can potentially suggest the relation (if any) of the chemical distribution to endocrine disruptive effects observed in the watershed. The model can also be further extended to include tributaries and WWTPs upstream and/or downstream of the reach examined in this study.

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Appendix A. ANOVA-Chlorophene concentrations in Grand River

This appendix presents the results of the ANOVA test conducted for chlorophene concentrations during the summer 2012 sampling event. The analysis was conducted through SigmaPlot® 12.0. ANOVA was used to detect any statistical differences among samples. Tukey test was completed to determine which of the samples are different from each other.

One Way Analysis of Variance Friday, May 24, 2013, 1:00:40 PM

Data source: Chlorophene in July 2012 STATS v.1.JNB

Group Name	N	Missing	Mean	Std Dev	SEM
GS77	3	0	105.933	7.044	4.067
GS71	3	0	103.633	13.911	8.031
GS67	3	0	101.767	16.925	9.772
GS61	3	0	104.500	19.539	11.281
G60	3	0	122.000	16.523	9.539
G58	3	0	153.667	7.767	4.485
G57	3	0	137.333	13.868	8.007
G55	3	0	191.333	90.941	52.505
G54W	3	0	151.333	9.504	5.487
G54E	3	0	154.667	15.535	8.969
G53W	3	0	98.100	0.849	0.490
G53E	3	0	83.467	5.169	2.985
G52	3	0	85.867	10.187	5.881
G49	3	0	149.333	20.033	11.566
G48	3	0	105.333	26.603	15.359

Source of Variation	DF	SS	MS	F	P
Between Groups	14	40943.866	2924.562	3.889	<0.001
Residual	30	22558.560	751.952		
Total	44	63502.426			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.961

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
G55 vs. G53E	107.867	15	6.813	0.003	Yes
G55 vs. G52	105.467	15	6.662	0.004	Yes
G55 vs. G53 W	93.233	15	5.889	0.016	Yes
G55 vs. GS67	89.567	15	5.657	0.024	Yes
G55 vs. GS71	87.700	15	5.539	0.029	Yes
G55 vs. GS61	86.833	15	5.485	0.032	Yes
G55 vs. G48	86.000	15	5.432	0.035	Yes
G55 vs. GS77	85.400	15	5.394	0.037	Yes
G55 vs. G60	69.333	15	4.379	0.175	No

G55 vs. G57	54.000	15	3.411	0.525	Do Not Test
G55 vs. G49	42.000	15	2.653	0.847	Do Not Test
G55 vs. G54 W	40.000	15	2.527	0.886	Do Not Test
G55 vs. G58	37.667	15	2.379	0.923	Do Not Test
G55 vs. G54 E	36.667	15	2.316	0.936	Do Not Test
G54 E vs. G53E	71.200	15	4.497	0.149	No
G54 E vs. G52	68.800	15	4.346	0.184	Do Not Test
G54 E vs. G53 W	56.567	15	3.573	0.453	Do Not Test
G54 E vs. GS67	52.900	15	3.341	0.557	Do Not Test
G54 E vs. GS71	51.033	15	3.223	0.611	Do Not Test
G54 E vs. GS61	50.167	15	3.169	0.637	Do Not Test
G54 E vs. G48	49.333	15	3.116	0.661	Do Not Test
G54 E vs. GS77	48.733	15	3.078	0.678	Do Not Test
G54 E vs. G60	32.667	15	2.063	0.973	Do Not Test
G54 E vs. G57	17.333	15	1.095	1.000	Do Not Test
G54 E vs. G49	5.333	15	0.337	1.000	Do Not Test
G54 E vs. G54 W	3.333	15	0.211	1.000	Do Not Test
G54 E vs. G58	1.000	15	0.0632	1.000	Do Not Test
G58 vs. G53E	70.200	15	4.434	0.163	Do Not Test
G58 vs. G52	67.800	15	4.282	0.200	Do Not Test
G58 vs. G53 W	55.567	15	3.510	0.481	Do Not Test
G58 vs. GS67	51.900	15	3.278	0.586	Do Not Test
G58 vs. GS71	50.033	15	3.160	0.640	Do Not Test
G58 vs. GS61	49.167	15	3.106	0.665	Do Not Test
G58 vs. G48	48.333	15	3.053	0.689	Do Not Test
G58 vs. GS77	47.733	15	3.015	0.706	Do Not Test
G58 vs. G60	31.667	15	2.000	0.979	Do Not Test
G58 vs. G57	16.333	15	1.032	1.000	Do Not Test
G58 vs. G49	4.333	15	0.274	1.000	Do Not Test
G58 vs. G54 W	2.333	15	0.147	1.000	Do Not Test
G54 W vs. G53E	67.867	15	4.287	0.199	Do Not Test
G54 W vs. G52	65.467	15	4.135	0.242	Do Not Test
G54 W vs. G53 W	53.233	15	3.362	0.547	Do Not Test
G54 W vs. GS67	49.567	15	3.131	0.654	Do Not Test
G54 W vs. GS71	47.700	15	3.013	0.706	Do Not Test
G54 W vs. GS61	46.833	15	2.958	0.730	Do Not Test
G54 W vs. G48	46.000	15	2.906	0.752	Do Not Test
G54 W vs. GS77	45.400	15	2.868	0.768	Do Not Test
G54 W vs. G60	29.333	15	1.853	0.989	Do Not Test
G54 W vs. G57	14.000	15	0.884	1.000	Do Not Test
G54 W vs. G49	2.000	15	0.126	1.000	Do Not Test
G49 vs. G53E	65.867	15	4.160	0.234	Do Not Test
G49 vs. G52	63.467	15	4.009	0.282	Do Not Test
G49 vs. G53 W	51.233	15	3.236	0.606	Do Not Test
G49 vs. GS67	47.567	15	3.004	0.710	Do Not Test
G49 vs. GS71	45.700	15	2.887	0.760	Do Not Test
G49 vs. GS61	44.833	15	2.832	0.782	Do Not Test
G49 vs. G48	44.000	15	2.779	0.802	Do Not Test
G49 vs. GS77	43.400	15	2.741	0.816	Do Not Test
G49 vs. G60	27.333	15	1.726	0.994	Do Not Test
G49 vs. G57	12.000	15	0.758	1.000	Do Not Test
G57 vs. G53E	53.867	15	3.402	0.529	Do Not Test
G57 vs. G52	51.467	15	3.251	0.599	Do Not Test
G57 vs. G53 W	39.233	15	2.478	0.899	Do Not Test
G57 vs. GS67	35.567	15	2.247	0.948	Do Not Test
G57 vs. GS71	33.700	15	2.129	0.966	Do Not Test

G57 vs. GS61	32.833	15	2.074	0.972	Do Not Test
G57 vs. G48	32.000	15	2.021	0.977	Do Not Test
G57 vs. GS77	31.400	15	1.983	0.981	Do Not Test
G57 vs. G60	15.333	15	0.969	1.000	Do Not Test
G60 vs. G53E	38.533	15	2.434	0.910	Do Not Test
G60 vs. G52	36.133	15	2.282	0.942	Do Not Test
G60 vs. G53 W	23.900	15	1.510	0.999	Do Not Test
G60 vs. GS67	20.233	15	1.278	1.000	Do Not Test
G60 vs. GS71	18.367	15	1.160	1.000	Do Not Test
G60 vs. GS61	17.500	15	1.105	1.000	Do Not Test
G60 vs. G48	16.667	15	1.053	1.000	Do Not Test
G60 vs. GS77	16.067	15	1.015	1.000	Do Not Test
GS77 vs. G53E	22.467	15	1.419	0.999	Do Not Test
GS77 vs. G52	20.067	15	1.267	1.000	Do Not Test
GS77 vs. G53 W	7.833	15	0.495	1.000	Do Not Test
GS77 vs. GS67	4.167	15	0.263	1.000	Do Not Test
GS77 vs. GS71	2.300	15	0.145	1.000	Do Not Test
GS77 vs. GS61	1.433	15	0.0905	1.000	Do Not Test
GS77 vs. G48	0.600	15	0.0379	1.000	Do Not Test
G48 vs. G53E	21.867	15	1.381	0.999	Do Not Test
G48 vs. G52	19.467	15	1.230	1.000	Do Not Test
G48 vs. G53 W	7.233	15	0.457	1.000	Do Not Test
G48 vs. GS67	3.567	15	0.225	1.000	Do Not Test
G48 vs. GS71	1.700	15	0.107	1.000	Do Not Test
G48 vs. GS61	0.833	15	0.0526	1.000	Do Not Test
GS61 vs. G53E	21.033	15	1.329	1.000	Do Not Test
GS61 vs. G52	18.633	15	1.177	1.000	Do Not Test
GS61 vs. G53 W	6.400	15	0.404	1.000	Do Not Test
GS61 vs. GS67	2.733	15	0.173	1.000	Do Not Test
GS61 vs. GS71	0.867	15	0.0547	1.000	Do Not Test
GS71 vs. G53E	20.167	15	1.274	1.000	Do Not Test
GS71 vs. G52	17.767	15	1.122	1.000	Do Not Test
GS71 vs. G53 W	5.533	15	0.350	1.000	Do Not Test
GS71 vs. GS67	1.867	15	0.118	1.000	Do Not Test
GS67 vs. G53E	18.300	15	1.156	1.000	Do Not Test
GS67 vs. G52	15.900	15	1.004	1.000	Do Not Test
GS67 vs. G53 W	3.667	15	0.232	1.000	Do Not Test
G53 W vs. G53E	14.633	15	0.924	1.000	Do Not Test
G53 W vs. G52	12.233	15	0.773	1.000	Do Not Test
G52 vs. G53E	2.400	15	0.152	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Appendix B. Detailed Hydraulic Geometry and Transport Parameters

This appendix describes all the physical parameters required for all the 69 segments in WASP including the (B1) vertical elevations of the major reaches, (B2) hydraulic geometry, (B3 and B4) dispersion/exchanges in segments. Schneider Creek and Kitchener WWTP were considered as "tributaries" described by flow and boundary concentrations, and (B4) the average flow depths from different flow regimes provided by the Grand River Conservation Authority.

B.1 Vertical Elevations and slopes for the major reaches modeled.

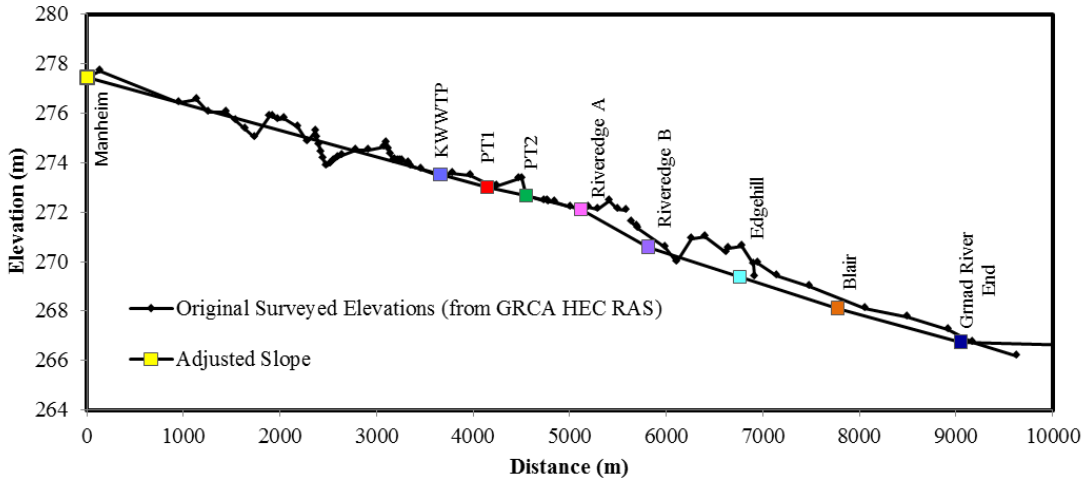


Figure A.1 Vertical elevations of the reaches modeled

B.2 Hydraulic Geometry for all the segments in WASP.

River Segments	Segment Number WASP	Length (m)	Width (m)	Depth (m)	Slope (m m^{-1})	Roughness
Manheim_1	1	303.03	81.33	1.272	0.00097	0.037
Manheim_2_W	2	351	41.56	1.272	0.00097	0.037
Manheim_2_E	3	351	29.17	1.272	0.00097	0.037
Manheim_3	4	605.95	58.31	1.272	0.00097	0.037
Manheim_4	5	359	46.67	1.272	0.00097	0.037
Manheim_5	6	849.76	47.16	1.272	0.00097	0.037
Manheim_6	7	680	48.82	1.272	0.00097	0.037
Manheim_7	8	513.18	50.95	1.272	0.00097	0.045
KWWTP_1DS_W	9	106.91	26.035	1.17	0.00109	0.045
KWWTP_1DS_E	10	106.91	26.035	1.17	0.00109	0.045
KWWTP_2DS_W_a	11	138.89	8.323	1.17	0.00109	0.045
KWWTP_2DS_W_b	12	138.89	8.323	1.17	0.00109	0.045
KWWTP_2DS_W_c	13	138.89	8.323	1.17	0.00109	0.045
KWWTP_2DS_E_a	14	138.89	8.323	1.17	0.00109	0.045
KWWTP_2DS_E_b	15	138.89	8.323	1.17	0.00109	0.045
KWWTP_2DS_E_c	16	138.89	8.323	1.17	0.00109	0.045
KWWTP_3DS_W_a	17	92.64	7.995	1.17	0.00109	0.045

KWWTP_3DS_W_b	18	92.64	7.995	1.17	0.00109	0.045
KWWTP_3DS_W_c	19	92.64	7.995	1.17	0.00109	0.045
KWWTP_3DS_E_a	20	92.64	7.995	1.17	0.00109	0.045
KWWTP_3DS_E_b	21	92.64	7.995	1.17	0.00109	0.045
KWWTP_3DS_E_c	22	92.64	7.995	1.17	0.00109	0.045
KWWTP_4DS_W_a	23	73.78	12.34	1.17	0.00109	0.045
KWWTP_4DS_W_b	24	73.78	12.34	1.17	0.00109	0.045
KWWTP_4DS_W_c	25	73.78	12.34	1.17	0.00109	0.045
KWWTP_4DS_E_a	26	73.78	12.34	1.17	0.00109	0.045
KWWTP_4DS_E_b	27	73.78	12.34	1.17	0.00109	0.045
KWWTP_4DS_E_c	28	73.78	12.340	1.17	0.00109	0.045
KWWTP_5DS_W_a	29	70.3	10.728	1.17	0.00109	0.045
KWWTP_5DS_W_b	30	70.3	10.728	1.17	0.00109	0.045
KWWTP_5DS_W_c	31	70.3	10.728	1.17	0.00109	0.045
KWWTP_5DS_E_a	32	70.3	10.728	1.17	0.00109	0.045
KWWTP_5DS_E_b	33	70.3	10.728	1.17	0.00109	0.045
KWWTP_5DS_E_c	34	70.3	10.728	1.17	0.00109	0.045
PT1_1W_a	35	227.36	7.833	0.85	0.00118	0.045
PT1_1W_b	36	227.36	7.833	0.85	0.00118	0.045
PT1_1W_c	37	227.36	7.833	0.85	0.00118	0.045
PT1_1E_a	38	227.36	7.833	0.85	0.00118	0.045
PT1_1E_b	39	227.36	7.833	0.85	0.00118	0.045
PT1_1E_c	40	227.36	7.833	0.85	0.00118	0.045
PT1_2W_a	41	174.75	11.167	0.85	0.00118	0.045
PT1_2W_b	42	174.75	11.167	0.85	0.00118	0.045
PT1_2W_c	43	174.75	11.167	0.85	0.00118	0.045
PT1_2E_a	44	174.75	11.167	0.85	0.00118	0.045
PT1_2E_b	45	174.75	11.167	0.85	0.00118	0.045
PT1_2E_c	46	174.75	11.167	0.85	0.00118	0.045
PT2_1_W	47	283	37.06	1.153	0.00118	0.045
PT2_1_E	48	283	37.06	1.153	0.00118	0.045
PT2_2_W	49	283	27.975	1.153	0.00118	0.045
PT2_2_E	50	283	27.975	1.153	0.00118	0.045
Riveredge_1	51	700	47.58	1.34	0.00099	0.045
Riverdege_2	52	950	47.87	1.34	0.00099	0.045
Edgehill_1	53	421.25	61.76	0.553	0.00099	0.045
Edgehill_2	54	327.78	61.76	0.553	0.00099	0.045
Edgehill_3	55	263.57	92.15	0.553	0.00099	0.045
Blair_1	56	639.54	90	1.965	0.00099	0.045
Blair_2_W	57	399	30	1.965	0.00099	0.045
Blair_2_C	58	399	69	1.965	0.00099	0.045
Blair_2_E	59	399	20.5	1.965	0.00099	0.045
Blair_3	60	235.3	111.15	1.965	0.00099	0.045
Speed_1	61	176.35	82.52	1.2	0.00099	0.045
Speed_2	62	336.58	56.62	1.2	0.00099	0.045
Speed_3	63	220	68.34	1.2	0.00099	0.045
Speed_4	64	198.41	103.86	1.2	0.00099	0.045

Speed_5	65	321.36	65	1.2	0.00099	0.045
Speed_confluence_W	66	465.71	63.94	1	0.00099	0.045
Speed_confluence_E	67	465.71	27.05	1	0.00099	0.045
Schneider_Creek	68	465.71	27.05	1	1.00099	0.045
Kitchener_WWTP	69	465.71	27.05	1	2.00099	0.045

B.3 Longitudinal Dispersion/Exchanges data required for WASP

Segment 1	Segment 2	Area (m^2)	Distance (m)
1	2	53	152
1	3	37	152
2	4	53	176
3	4	37	176
4	5	59	180
5	6	60	180
6	7	60	340
7	8	62	257
8	9	30	53
8	10	30	53
9	11	10	53
9	12	10	53
9	13	10	53
10	14	10	53
10	15	10	53
10	16	10	53
11	17	9	46
12	18	9	46
13	19	9	46
14	20	9	46
15	21	9	46
16	22	9	46
17	23	9	37
18	24	9	37
19	25	9	37
20	26	9	37
21	27	9	37
22	28	9	37
23	29	14	35
24	30	14	35
25	31	14	35
26	32	14	35
27	33	14	35
28	34	14	35
29	35	13	35

30	36	13	35
31	37	13	35
32	38	13	35
33	39	13	35
34	40	13	35
35	41	7	87
36	42	7	87
37	43	7	87
38	44	7	87
39	45	7	87
40	46	7	87
41	47	9	87
42	47	9	87
43	47	9	87
44	48	9	142
45	48	9	142
46	48	9	142
47	49	43	142
48	50	326	142
49	51	32	142
50	51	32	142
51	52	64	350
52	53	64	211
53	54	34	164
54	55	34	132
55	56	51	132
56	57	59	200
56	58	136	200
56	59	40	200
57	60	59	118
58	60	99	118
59	60	68	118
60	61	218	88
61	62	99	88
62	63	68	110
63	64	82	99
64	65	125	99
65	66	64	233
65	67	27	233

B.4 Transverse Dispersion/Exchanges data required for WASP.

KWWTP

Segment 1	Segment 2	Area (m^2)	Distance (m)
9	10	125	13
11	12	1156	13
12	13	1156	4
13	14	1156	4
14	15	1156	4
15	16	1156	4
17	18	108	4
18	19	108	4
19	20	108	4
20	21	108	4
21	22	108	4
23	24	86	6
24	25	86	6
25	26	86	6
26	27	86	6
27	28	86	6
29	30	82	5
30	31	82	5
31	32	82	5
32	33	82	5
33	34	82	5

PT1

Segment 1	Segment 2	Area (m^2)	Distance (m)
35	36	193	4
36	37	193	4
37	38	193	4
38	39	193	4
39	40	193	4
41	42	149	6
42	43	149	6
43	44	149	6
44	45	149	6
45	46	149	6

PT2

Segment 1	Segment 2	Area (m^2)	Distance (m)
47	48	326	19
49	50	326	14

B.5 Average depths under different flow profiles for each segment provided by GRCA

Flow Profile ($m^3 s^{-1}$)	Average Depth (m)	Flow Profile ($m^3 s^{-1}$)	Average Depth (m)
Manheim		**KWWTP**	
0.0	0.001	0.0	0.000
5.0	0.680	5.0	0.429
10.0	0.802	10.0	0.613
15.0	0.901	15.0	0.744
20.0	0.992	20.0	0.853
30.0	1.144	30.0	1.028
40.0	1.273	40.0	1.173
50.0	1.388	50.0	1.298
70.0	1.593	70.0	1.508
100.0	1.854	100.0	1.759
120.0	2.004	120.0	1.896
150.0	2.209	150.0	2.069
PT1		**PT2**	
0	-0.004	0	0.002
5	0.320	5	0.437
10	0.392	10	0.564
15	0.466	15	0.688
20	0.524	20	0.804
30	0.702	30	0.995
40	**0.865**	**40**	**1.153**
50	1.005	50	1.286
70	1.240	70	1.506
100	1.509	100	1.762
120	1.650	120	1.900
150	1.827	150	2.076
Riveredge A		**Riveredge B**	
0	0.000	0	0.000
5	0.629	5	0.286
10	0.821	10	0.378
15	0.950	15	0.446
20	1.067	20	0.503
30	1.228	30	0.597
40	**1.340**	**40**	**0.463**
50	1.442	50	0.553
70	1.611	70	0.711
100	1.822	100	0.899
120	1.940	120	1.001
150	2.094	150	1.122

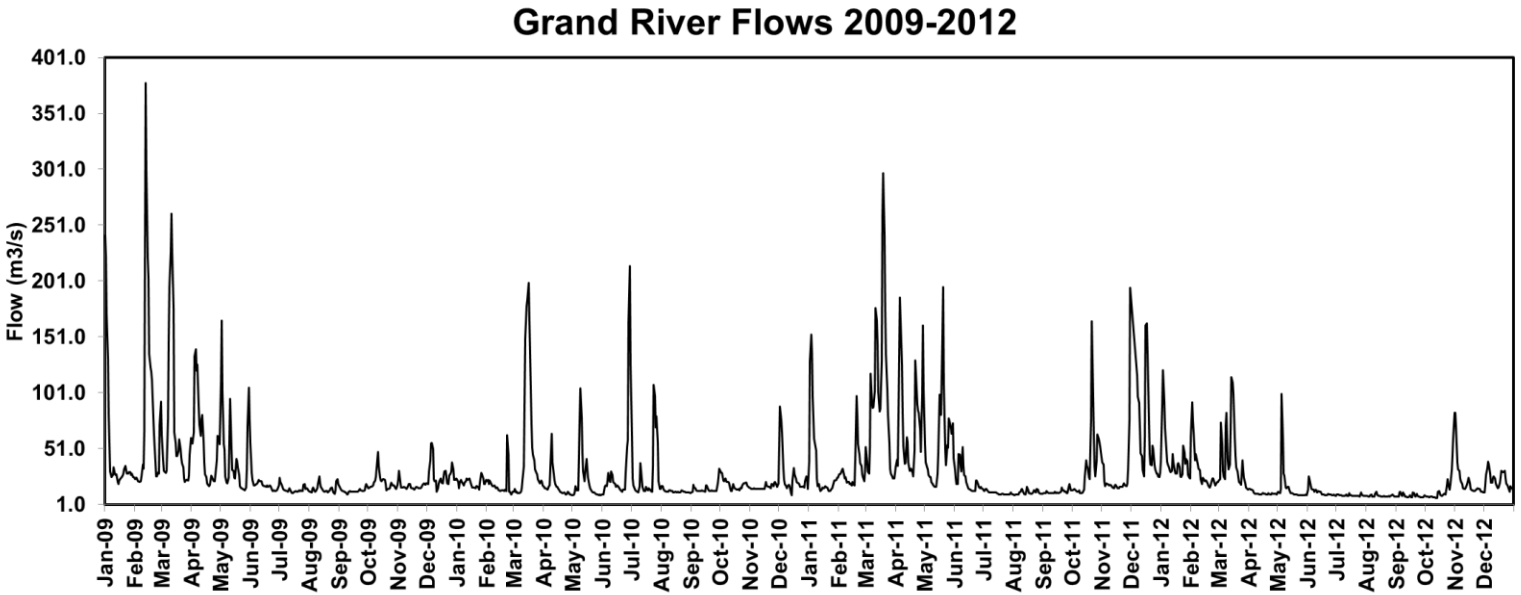
Edgehill		**Blair**	
0	0.000	0	0.000
5	1.061	5	1.061
10	1.281	10	1.281
15	1.441	15	1.441
20	1.582	20	1.582
30	1.799	30	1.799
40	**1.966**	**40**	**1.966**
50	2.102	50	2.102
70	2.290	70	2.290
100	2.431	100	2.431
120	2.441	120	2.441
150	2.365	150	2.365

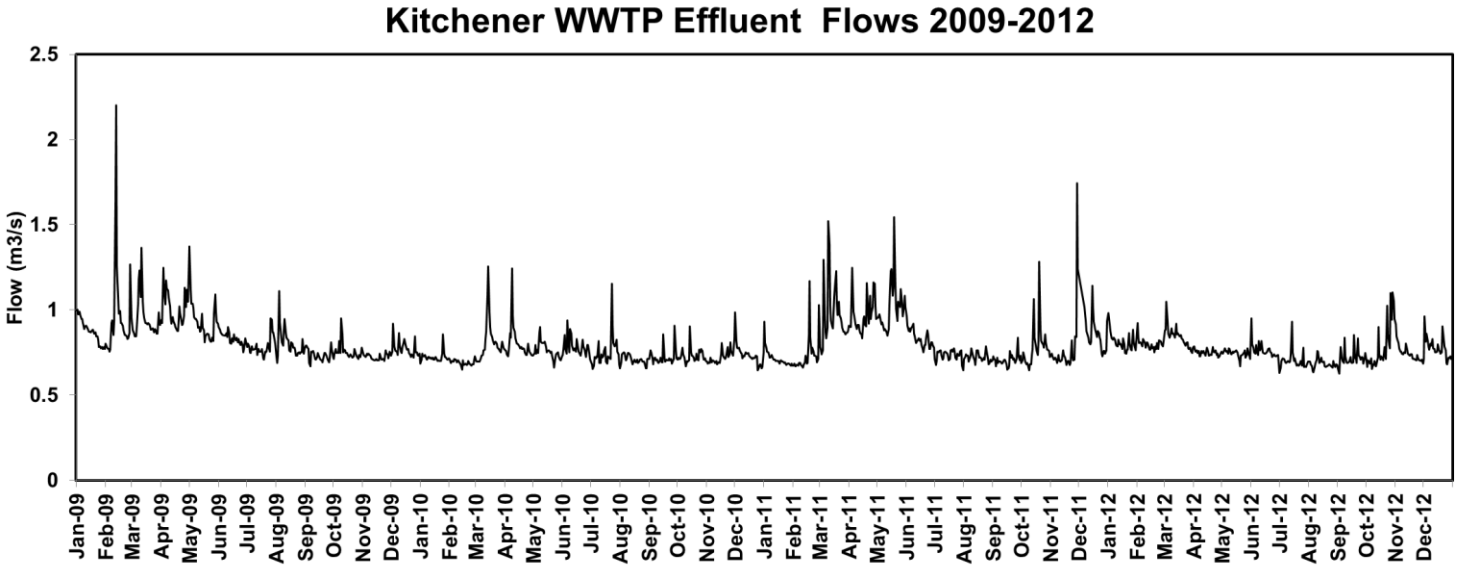
Grand River End	
0	0.001
5	0.913
10	1.163
15	1.342
20	1.491
30	1.689
40	1.858
50	1.997
70	2.155
100	2.240
120	2.170
150	1.886

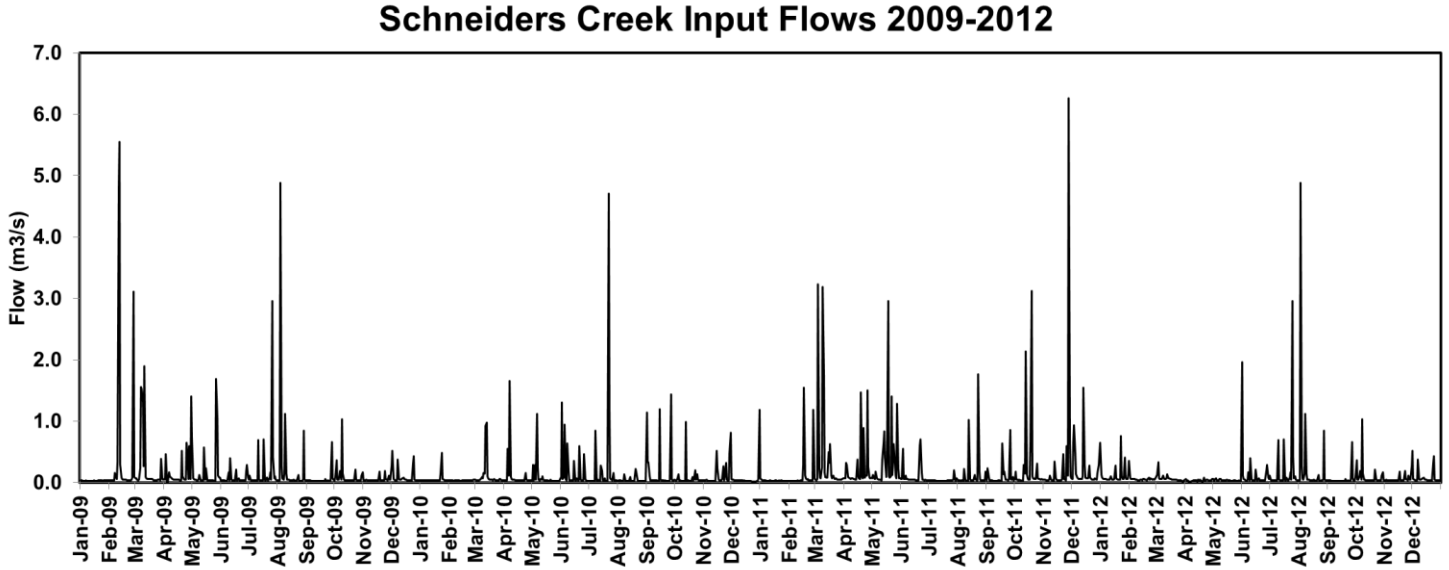
Appendix C. Time function inputs for WASP

This appendix describes the time function inputs for WASP including (B1) annual hydrographs for Grand River, Kitchener WWTP, and Schneider Creek, (B2) temperature and (B3) pH in Grand River. The hydrographs show that peak flows occur around spring. Water temperatures in 2012 ranged from -0.37 to 29.52°C while pH ranged from 7.39 to 9.26.

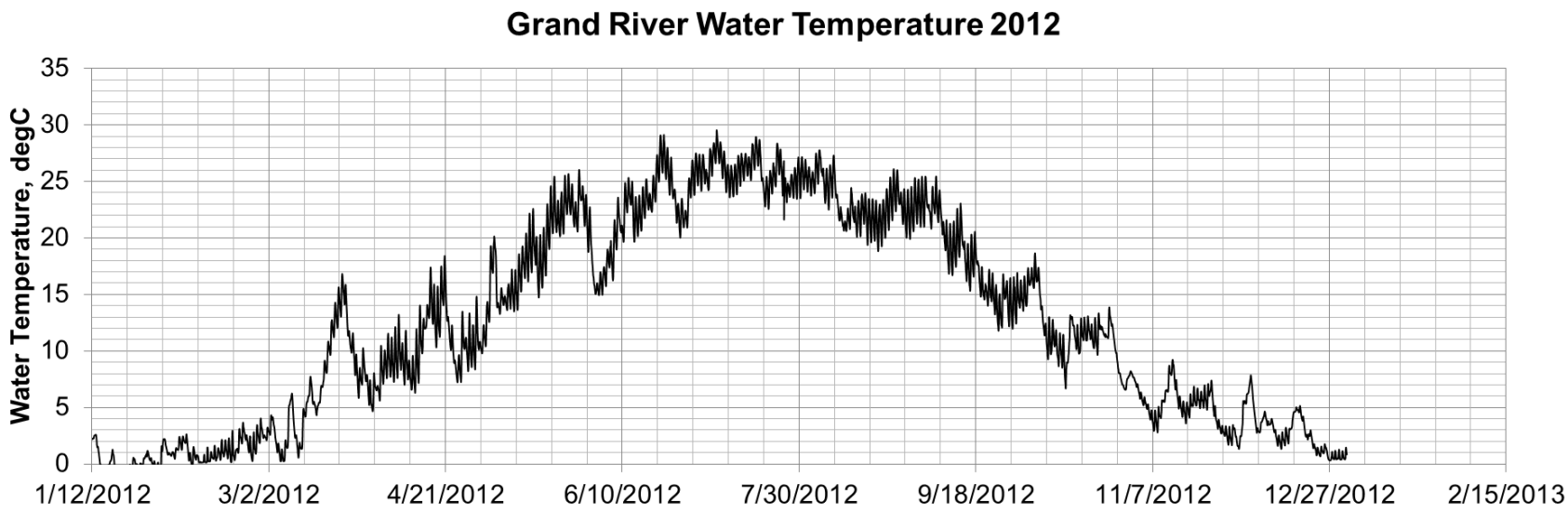
C1. Flow Functions



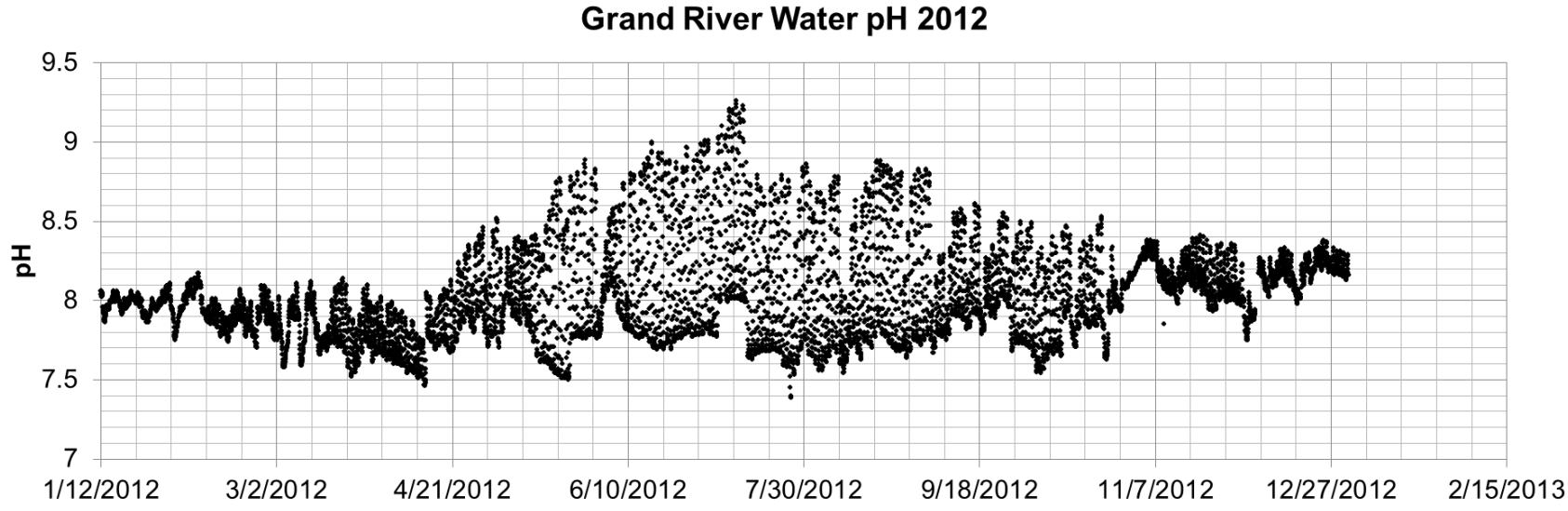

Kitchener WWTP Effluent Flows 2009-2012
Flow (m3/s)
2.5
2
1.5
1
0.5
0
Jan-09 Feb-09 Mar-09 Apr-09 May-09 Jun-09 Jul-09 Aug-09 Sep-09 Oct-09 Nov-09 Dec-09 Jan-10 Feb-10 Mar-10 Apr-10 May-10 Jun-10 Jul-10 Aug-10 Sep-10 Oct-10 Nov-10 Dec-10 Jan-11 Feb-11 Mar-11 Apr-11 May-11 Jun-11 Jul-11 Aug-11 Sep-11 Oct-11 Nov-11 Dec-11 Jan-12 Feb-12 Mar-12 Apr-12 May-12 Jun-12 Jul-12 Aug-12 Sep-12 Oct-12 Nov-12 Dec-12


Schneiders Creek Input Flows 2009-2012
Flow (m3/s)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Jan-09 Feb-09 Mar-09 Apr-09 May-09 Jun-09 Jul-09 Aug-09 Sep-09 Oct-09 Nov-09 Dec-09 Jan-10 Feb-10 Mar-10 Apr-10 May-10 Jun-10 Jul-10 Aug-10 Sep-10 Oct-10 Nov-10 Dec-10 Jan-11 Feb-11 Mar-11 Apr-11 May-11 Jun-11 Jul-11 Aug-11 Sep-11 Oct-11 Nov-11 Dec-11 Jan-12 Feb-12 Mar-12 Apr-12 May-12 Jun-12 Jul-12 Aug-12 Sep-12 Oct-12 Nov-12 Dec-12

C2. Temperature Time Function



C2. pH Time Function



Appendix D. Dilution Factors at PT1

Dilution factors were calculated using the following equation:

$$DF = \frac{C_e - C_u}{C_p - C_u} \qquad \text{AC.1}$$

where DF is dilution factor, C_e is the chloride concentration in the effluent, C_u is the chloride concentration at the upstream site, and C_p is the chloride concentration at point of interest. Conversely, the contribution of the wastewater flows in total stream flow can be calculated as the reciprocal of the dilution factor. Sample calculations are shown below. A plot of DFs versus time coincide with the flows over time.

Dilution factor at PT1 in June 30, 2010 (maximum dilution factor):

$C_u = 47 \frac{mg}{L}$ (at G49)

$C_p = 70 \frac{mg}{L}$ (at G53E)

$C_e = 480 \frac{mg}{L}$ (KWWTP)

$$DF = \frac{480 - 47}{70 - 47} = 222$$