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Modeling the exposure of wild fish to endocrine active chemicals: Potential linkages of total estrogenicity to field-observed intersex

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ABSTRACT

Decades of studies on endocrine disruption have suggested the need to manage the release of key estrogens from municipal wastewater treatment plants (WWTP). However, the proposed thresholds are below the detection limits of most routine chemical analysis, thereby restricting the ability of watershed managers to assess the environmental exposure appropriately. In this study, we demonstrated the utility of a mechanistic model to address the data gaps on estrogen exposure. Concentrations of the prominent estrogenic contaminants in wastewaters (estrone, estradiol, and ethinylestradiol) were simulated in the Grand River in southern Ontario (Canada) for nine years, including a period when major WWTP upgrades occurred. The predicted concentrations expressed as total estrogenicity (E2 equivalent concentrations) were contrasted to a key estrogenic response (i.e., intersex) in rainbow darter (*Etheostoma caeruleum*), a wild sentinel fish species. A predicted total estrogenicity in the river of ≥10 ng/L E2 equivalents was associated with high intersex incidence and severity, whereas concentrations <0.1 ng/L E2 equivalents were associated with minimal intersex expression. Exposure to a predicted river concentration of 0.4 ng/L E2 equivalents, the environmental quality standard (EQS) proposed by the European Union for estradiol, was associated with 34% (95% CI:30–38) intersex incidence and a very low severity score of 0.6 (95% CI:0.5–0.7). This exposure is not predicted to cause adverse effects in rainbow darter. The analyses completed in this study were only based on the predicted presence of three major estrogens (E1, E2, EE2), so caution must be exercised when interpreting the results. Nevertheless, this study illustrates the use of models for exposure assessment, especially when measured data are not available.

1. Introduction

The exposure of fish to endocrine active chemicals (EACs) has been shown globally to have deleterious consequences for reproductive health (Brian et al., 2005; Kime, 1999; Nash et al., 2004; Tyler and Routledge, 1998). One of the most frequent observations is the feminization of male fish with vitellogenin induction (production of estrogen-dependent protein) and intersex (ova-testis) as examples of changes reported (Jordan et al., 2016). Progress in analytical chemistry has enabled the detection of EACs at very low concentrations (Benotti et al., 2008; Carballa et al., 2004; López-Roldán et al., 2010). However, the proposed environmental quality standards (EQS) by the European Union (EU) for some EACs such as estradiol (E2) and ethinylestradiol (EE2) are only 0.4 and 0.035 ng/L respectively (European Commission, 2012). These concentrations are below the current detection limits of most routine analytical methods. As a result, some studies have utilized biological assessments (i.e., bioassays) to quantify exposures to EACs (Busch et al., 2016; Coleman et al., 2004; Escher et al., 2013; Marinho et al., 2013; Neale et al., 2017; Ohko et al., 2002). Bioassay techniques examine the combined biological activity in a mixture and can provide an indication of the potential responses in organisms exposed to complex mixtures without identifying the specific chemicals.

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Despite the considerable chemical and bioanalytical monitoring of EACs in effluents and receiving environments worldwide (Agunbiade and Moodley, 2016; Escher et al., 2013; Leusch et al., 2014; Servos et al., 2005; Xu et al., 2007), there is still limited information to assess the spatial or temporal concentrations of EACs in receiving waters where technical challenges (e.g. detection limits) and cost are important considerations (Roig and D'Aco, 2016). In the absence of such data, the modeling of environmental systems can be used as an alternative approach to characterize fish exposure to EACs (Roig and D'Aco, 2016; Zhang et al., 2015). Models can be applied to evaluate current and future mitigation strategies for eliminating the target compounds through scenario testing (Kehrein et al., 2015) and assist in the design of effective monitoring programs (Roig and D'Aco, 2016). Furthermore, models can be employed to assess the potential relationship of stressor concentrations to observed effects in the wild (Jobling et al., 2006, 2009). Numerous models have already been developed in recent years to predict the fate and transport of emerging contaminants such as pharmaceuticals and personal care products (Arlos et al., 2014; Balaam et al., 2010; Dale et al., 2015; Grechi et al., 2016; Kehrein et al., 2015).

Field investigations on the incidence and severity of intersex in male rainbow darter (*Etheostoma caeruleum*) in the Grand River watershed (southern Ontario) have been ongoing since 2007 (Hicks et al., 2017). The presence of severe intersex in rainbow darter has been linked to poor reproductive success (Fuzzen et al., 2015) with potential negative impacts on the fish population. However, a direct link between the exposure to specific compounds and intersex is very difficult to establish as the effluent composition and fate of EACs in the receiving environments are complex. The potential of natural estrogens (E2 and estrone [E1]) and synthetic estrogens (EE2) to cause endocrine disruption in fish has dominated many laboratory and field studies in recent years (Corcoran et al., 2010; Desbrow et al., 1998; Jobling et al., 2006; Kidd et al., 2007; Palace et al., 2009). The effects directed analysis (EDA) of the two major WWTP effluents in the Grand River suggested that the total estrogenicity was mainly contributed by E1, E2, and EE2 based on a receptor agonist screen assay (yeast estrogen screen [YES]) (Arlos et al., 2018). However, there are many other EACs entering the receiving environment (e.g., estrogens from diffuse sources) that can interfere with the endocrine function in fish. Some responses including intersex may also be caused by androgen antagonists (Jobling et al., 2009) or chemicals such as metformin (antidiabetic) that may work through mechanisms other than receptor binding (Niemuth and Klaper, 2015). Also, the fate of other EACs may be correlated with the estrogen exposure, making it difficult to generate direct cause-and-effect relationships.

The current modeling work is focused on three major estrogens (E1, E2, and EE2) identified in the prior EDA as important contributors to the total estrogenicity in the effluents. In this study, the concentrations of E1, E2, and EE2 were simulated along the Grand River where the widespread presence of pharmaceuticals and personal care products has been documented (Arlos et al., 2015). The modeled reach also includes areas that were previously predicted (via models) to have elevated levels of estrogens (Grill et al., 2016; Hosseini et al., 2012). A major upgrade in one of the treatment plants (Kitchener WWTP) has resulted in major effluent quality changes during the study period but minimal data in effluents were available, especially during the pre-upgrade period when the environmental exposure to municipal wastewater-derived estrogens was likely at its peak. This scenario additionally provides a unique opportunity to apply models that can help assess the efficiency of WWTP upgrades. The overall goals of this study were to estimate the concentrations of select EACs (E1, E2, and EE2) in the Grand River through mechanistic water quality modeling and to determine whether the exposure to these key estrogens is consistent with the observed responses (intersex) in wild fish.

2. Methodology

2.1. Study site

The Grand River watershed in southern Ontario (~6,800 km^2) drains into Lake Erie and is inhabited by close to 1 million people. In addition to the non-point sources from numerous agricultural activities (~70% of total land use), the watershed also receives inputs from 30 WWTPs. The Grand River has also been extensively investigated for several biological effect endpoints on fish health since the late 2000s (Bahamonde et al., 2014; Fuzzen et al., 2015, 2016; Tanna et al., 2013; Tetreault et al., 2011, 2013). In this study, ~80 km of the Grand River was modeled starting below a regulated water reservoir (Shand Dam) to an area that is ~2 km above the Grand and Speed River confluence (Fig. 1a). This section captures both agriculture and urban gradients in the watershed and incorporates the inputs from two major (Waterloo and Kitchener) and two smaller (Elora and Fergus) WWTPs (Table S1). In 2012, Kitchener WWTP underwent major process upgrades including improved aeration, nitrification, and replacement of chlorination/de-chlorination with UV effluent disinfection.

2.2. Modeling strategy

The water quality modeling included three separate components: (1) source, (2) transport and fate, and (3) effects as outlined in Fig. S1. The source modeling predicted the effluent concentrations from the target WWTPs and was completed as detailed in Arlos et al. (2018). The transport and fate component simulated the distribution of target EACs in the study area and was completed using a mechanistic water quality model. Finally, the effects component evaluated the potential relationship between the predicted river concentrations derived from the transport and fate model component and field-recorded intersex conditions. Due to their relatively high site fidelity (Hicks and Servos, 2017) and constant exposure to WWTP effluents throughout their life cycle, data on rainbow darter were considered suitable for quantifying the exposure impacts. The intersex data for rainbow darter at nine sites in the Grand River watershed (2007–2015) were based on the same samples compiled by Hicks et al. (2017) and were used in the concentration-response regression analysis (see section 2.5). The selection of these sites is also described in detail in Hicks et al. (2017).

A similar approach to Arlos et al. (2014) was employed to simulate estrogen concentrations in the Grand River. The Water Quality Simulation Program developed by the US Environmental Protection Agency (WASP version 7.3) was used as the model platform. This model was employed in a recent study to describe the distribution of frequently detected pharmaceuticals with varying physical-chemical properties downstream of the Kitchener WWTP (10-km reach) (Arlos et al., 2014). The model has already been calibrated for compounds that spanned the properties of those examined in the current study and was found to provide robust mechanistic predictions of pharmaceutical fate and transport (Arlos et al., 2014).

The following major steps were completed to predict the river concentrations: discretization of the river network; simulation of river transport mechanisms (i.e., advection); testing of the transport processes using a tracer compound (chloride); and integration of organic compound modeling through the addition of significant in-river fate mechanisms (e.g., biodegradation and photolysis). The first three steps were iterative in nature and were deemed as