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An assessment of estrogenic organic contaminants in Canadian wastewaters

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Abstract

A suite of 30 primarily estrogenic organic wastewater contaminants was measured in several influent/effluent wastewater samples from four municipal wastewater treatment plants and effluents from one bleached kraft pulp mill (BKME) using an ultra-trace analytical method based on gas chromatography–high resolution mass spectroscopy (GC–HRMS). *In vitro* recombinant yeast assay detection of the estrogenic equivalent (EEq) on whole and solid phase extracted (SPE) and fractionated wastewater was also performed. 19-Norethindrone was the most frequently detected and abundant (26–224 ng/L) of all the synthetic estrogens/progesterones in the influent samples. 17α-Ethinylestradiol was the more frequently detected synthetic estrogen/progesterone in the effluents occurring at or below 5 ng/L with some sporadic occurrences of up to 178 ng/L. The greatest levels of steroidal estrogens in municipal effluents were $E1>E2>E3$ which were all <20 ng/L. Nonylphenol and di(2-ethylhexyl) phthalate were found to be the highest non-steroidal synthetic compounds surveyed in both municipal influent and effluent samples, both occurring at 6–7 μg/L in municipal effluents. BKME contained relatively large amounts of the plant sterol stigmasterol (4 μg/L) but low amounts of fecal sterols, and steroidal estrogens (E2 only at 6 ng/L) when compared to the municipal effluents.

In vitro EEq in the wastewater surveyed ranged from 9–106 ng E2/L and ranked from municipal influent > municipal effluent ≈ BKME, with most of the estrogenicity fractionating in the 100% methanol SPE fraction followed by a secondary amount in the diethyl ether (for municipal) or methyl-*tert* butyl ether (for BKME) SPE fractions. Most correlations between chemical and *in vitro* estrogenic equivalency were weak ($p>0.05$ in most cases). Unexpected inverse correlations between *in vitro* estrogenic activity and concentrations of the estrogenic contaminant bisphenol A were found which likely contributed to the weakness of these correlations.

A modified toxicity identification and evaluation procedure was continued with the SPE extracts from the more potent 100% methanol SPE fractions of municipal effluent. High performance liquid chromatography band elution retention times, based on *in vitro* estrogen detection, indicated that steroidal estrogens such as E2 were responsible for most of the estrogenicity of the samples. Subsequent collection and GC–MS analysis of active bands did not confirm the presence of steroidal estrogens, but expanded the possibility of phthalate esters (i.e. dibutyl phthalate) and natural sterols (i.e. β-sitosterol) contributing to the overall estrogenic load.
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Keywords: OWCs; Estrogen; *In vitro*; GC–HRMS; Wastewater

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1. Introduction

Environmental estrogens are a subset of chemicals which make up a group of environmentally important compounds known as endocrine disrupting compounds (EDCs). Potential mechanisms of action for estrogenic contaminants may involve modulation of endocrine function including aspects of biosynthesis, transport or availability, and metabolism of endogenous hormones. Additionally, the activities of hormones may be affected by estrogenic contaminants interacting with hormone receptors and signalling processes (Lister and Van Der Kraak, 2001). One of the largest bodies of evidence illustrating endocrine disruption in wild organisms due to an anthropogenic stressor is the scientific literature reporting reproductive effects in several fish species in association with municipal wastewater effluents and pulp/paper industry discharges (reviewed in CSTEE, 1999; Denton et al., 1985; Jobling et al., 1998). In North America, Nagler and colleagues (Nagler et al., 2001; Chowen and Nagler, 2004) found that a high proportion of physiologically female Chinook salmon from three naturally spawning populations in the Columbia River tested positive for a male-specific DNA marker. From these results, the authors hypothesized that endocrine disruption may be playing a role in the development of the physiological sex of these fish. Such apparent sex reversal effects have not been observed in British Columbia populations (Devlin et al., 2005), but laboratory experiments have provided some evidence for sex reversal in this species arising from exposure to municipal and kraft pulp mill effluents (Afonso et al., 2002). Thus, a possible mechanism for sex reversal of genetically male salmon involves exposure to environmental estrogens originating primarily from wastewater discharged into their habitat. The human health implications of the occurrence and levels of estrogenic contaminants among other organic wastewater contaminants (OWCs) in wastewater effluents may also be important in some locations where hydrologic route between waste and drinking water is relatively short. Ubiquitous OWCs including non-ionic surfactant metabolite nonylphenol (NP) have recently been reported in surface and drinking water at low to mid ng/L ranges (Chen et al., 2005).

In order to strengthen the relationship between potential chemical culprits and potential biological effects many researchers have used the framework of the toxicity identification and evaluation (TIE) approach (Gomes et al., 2003). TIE involves a multi-tiered procedure starting with the most crude sample manipulations (e.g. SPE, pH, EDTA, etc.) and moving to finer manipulations (e.g. HPLC fractionation, GC–MS identification) all guided by biologically relevant endpoints (e.g. recombinant yeast screen or E-screen). TIE procedures are an integral part of USEPA's toxicity reduction evaluation (TRE) process which is used to identify and reduce or eliminate sources of effluent toxicity regulated by the Clean Water Act (CWA) (USEPA, 1999). This type of procedure was used to identify many of the known estrogenic contaminants targeted in this work (Desbrow et al., 1998). Recently, Burnison et al. (2003) used a modified TIE approach to successfully identify E2, E1 and the phytoestrogen metabolite, equol as major agents responsible for the overall estrogenicity of hog manure and agricultural runoff. This type of technique may be important for identifying culprit compounds in wastewater to target for fate and reduction studies.

The issue of estrogenic contaminants in wastewater has been recognized by scientists around the world, and the ability of wastewater treatment processes to mitigate current and future environmental risks from these compounds is being investigated (Drewes et al., 2005; Shi et al., 2004; Kirk et al., 2002; Joss et al., 2004; Andersen et al., 2003). The objectives of the present study were to survey the levels, in western Canada wastewater influent and effluent samples, of typical estrogenic contaminants and other OWCs, which have been identified as likely agents of reproductive disruption in aquatic organisms in the vicinity of wastewater discharge zones. The compounds under study include industrial chemicals (e.g. nonylphenol (NP) and phthalate esters), natural and synthetic steroidal estrogens (e.g. estrone (E1), estradiol (E2), estriol (E3), 17α-ethynylestradiol (EE2) and equilenin) and progesterones (19-norethindrone and (−)-norgestrel), and various naturally occurring phytoestrogens (e.g. pinosylvin, β-sitosterol). Sample analysis included both the determination of chemical concentrations and estrogenic activity. The concentrations of these compounds were assessed using an ultra-trace analytical method based on gas chromatography–high resolution mass spectrometry (GC–HRMS). These results were complemented by an *in vitro* assay (i.e. recombinant yeast screen) to assess the estrogenic activity in the wastewater samples. Additionally, a modified TIE protocol was applied to key municipal effluents to further investigate the relationship between the chemical and *in vitro* data. A representative cross-section of wastewaters that are commonly discharged to coastal or inland waters of western Canada was selected for sampling in this study. The impacted waters include the Fraser and North Saskatchewan rivers, both of which are major fish bearing rivers in western Canada.