

Forensic Ecotoxicology: In search for Endocrine Disrupting Compounds in Municipal Wastewater

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Municipal wastewater is one of the largest sources of pollution by volume that is discharged to surface water bodies in Canada. The 3,500 facilities that collect and release waste range from providing no treatment at all, to simple screening, to state of the art, high-tech plants. The total treated effluent discharged from these plants amounts to more than 3 trillion litres every year. A new Canada-wide strategy for controlling the quality and quantity of effluent from municipal and community wastewater treatment plants is being proposed by the CCME. Of particular concern is the discharge of toxic as well as endocrine disrupting compounds. One common observation that has been reported is the feminization of male fish downstream of municipal treatment plants. We present a bioassay directed forensic approach to isolate and identify endocrine disrupting compounds present in treated municipal effluent.

Biological screening for endocrine disrupting compounds was performed using yeast cells (yeast estrogen screen, YES and yeast androgen screen, YAS) as well as the Fathead minnow. Endpoints monitored following exposure to Fathead minnow included: total plasma protein, vitellogenin induction (in males), gonad somatic index and secondary sexual characteristics.

Municipal effluent samples were collected from the Gold Bar Wastewater treatment plant on two occasions and were found to illicit a estrogenic response using the YES and Fathead minnow assays. In order to identify the causative agents for such responses a forensic approach was applied. This involved passage of municipal effluent through solid phase extraction cartridges. Following extraction of 2 – 100 L of effluent, the cartridges were stripped with water alcohol mixtures. These mixtures were subjected to bioassay testing for endocrine responses.

An estrogenic response was observed following extraction of treated wastewater using solid phase extraction and elution of the t-C18 extraction cartridge with 50% methanol in water. This fraction was subjected to chemical analyses using gas chromatography/mass spectrometry after several chemical derivatizations. A unique series of homologues typified by m/z 207 and 251 were observed. Analyses using high resolution mass spectrometry (exact mass measurement) as well as liquid chromatography/tandem mass spectrometry identified these compounds to be nonylphenol carboxylates. These compounds are degradation products of commonly used nonylphenol ethoxylate surfactants. Details of our forensic approach will be described.