

## Abstract

### Assessment of Androgenic Response Potential of Effluents using *In Vitro* and *In Vivo* Methods

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The androgenic potential of a New Zealand pulp mill effluent (PME) and a Canadian PME was assessed along with a New Zealand sewage treatment plant effluent (STP) using a combination of *in vivo* and *in vitro* methods. The *in vitro* methods included: 1) a fish-based androgen receptor binding assay, 2) a fish-based aromatase inhibition activity assay, and 3) an analysis of gonadal sex steroid levels in exposed female mosquitofish (*Gambusia affinis*) ovaries by radioimmunoassay. The *in vivo* method included a quantifiable analysis of anal fin ray length for female mosquitofish exposed to the effluents.

Effluent extracts for the Canadian PME and New Zealand STP were found to have low *in vitro* androgenic potential compared to upstream reference extracts. All effluent extracts (Canadian PME, New Zealand PME and STP) showed a low degree of *in vitro* aromatase inhibition potential compared to upstream reference extracts. *In vivo* analysis showed no androgenic potential of the New Zealand PME and STP. The *in vitro* androgen receptor assay and *in vivo* mosquitofish bioassay did show androgenic responses for androstenedione (AD) and 1,4-androsta-diene-3,17-dione (ADD), which are two products of the microbial conversion of  $\beta$ -sitosterol (a plant sterol commonly found in PME) by *Mycobacterium smegmatis*. Also, the potential of the mosquitofish bioassay to determine anti-androgenic effects in effluents was demonstrated.