

Inference of chemicals that cause biological effects in treated pulp and paper mill effluent using gene expression in caged fathead minnows.

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Analytical chemistry techniques can identify chemicals present in the waters of the Great Lakes areas of concern, however it remains a challenge to identify those chemicals or classes of chemicals that actually cause adverse effects. Use of caged fathead minnows (*Pimephales promelas*) for monitoring in areas of concern offers great opportunities for characterizing chemicals based on biological effects. Here, we examined the potential of transcriptomics analysis on caged fathead minnows to infer what chemicals or classes of chemicals cause biological effects. Caged fathead minnows were exposed to effluent waters of a pulp and paper mill before, during and after a shutdown event. The estrogenicity of effluent waters was examined using microarray data from pre shutdown, at day 2 and 4 of the shutdown, and on post shutdown samples for sites proximal and distal from the points of discharge. Pathway enrichment analysis showed that pre and post shutdown effluents were very estrogenic compared to the effluent during the shutdown period. These results were similar to results previously found measuring estrogenicity in water samples using an in-vitro cell bioassay with T47D-KBluc cells. Functional enrichment analysis showed increased liver toxicity during the shutdown period. Differentially expressed genes were also analyzed for potential upstream chemical association using prior knowledge from the Comparative Toxicological Database. Chemicals were identified that are related to paper mill effluent before the shutdown in addition to several endocrine disrupting chemicals such as estradiol, ethynyl estradiol, flusilazole, flutamide, and bisphenol A. A gene-metabolite-chemical association network model is being constructed using gene expression, metabolites, and chemistry data to predict gene-chemical association, which will likely improve chemical inference.