Reproductive steroid responses in fish exposed to pulp mill condensates

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Abstract

Since 1997, a number of approaches (artificial stream exposures, lab bioassays) have been used to identify waste-stream sources of contaminants at the Irving Pulp & Paper Ltd. (IPP) mill, in Saint John, Canada. These studies have shown that chemical recovery condensates have the greatest potential for reducing circulating and gonadal steroids in mummichog (Fundulus heteroclitus), an endemic fish species. A solid phase extraction technique was developed to isolate the hormonally–active substances from the condensates, and a toxicity identification evaluation (TIE) approach was used to gain a better understanding of the chemical characteristics of the active substances. The extract was fractionated by high performance liquid chromatography and the fractions were used in a seven-day bioassay. Mummichog were exposed in static aquaria with daily renewal to either the whole condensate extract, or one of the six fractions at 1 % v/v. Steroid reductions in recent exposures were not as pronounced as had been observed during studies which investigated the overall potency of the extract at the same concentration. As well, differences in responses were observed between the sexes. A dose-response experiment indicated that greater steroid reductions are elicited at 4% v/v in male mummichog. Therefore, the TIE was continued at 4% v/v, however, no steroid reductions were observed in any of the fractions. Some fractions induced increases in plasma testosterone, which had never been observed previously. Ongoing work is focusing on developing an understanding of the extent to which variability in the fish responses are linked to reproductive state and differences in responses between the sexes, while additional work is concentrating on variability in condensate constituents and the fractionation protocol.

Key words: mummichog, Fundulus heteroclitus, reproductive steroids, condensates, toxicity identification, bioassay

INTRODUCTION

In Canada, studies done as part of the federally-regulated Environmental Effects Monitoring (EEM) program have found a common pattern of increased condition, increased liver size and reduced gonad size (metabolic disruption) in wild fish collected from the receiving environment, relative to reference sites (Munkittrick et al., 2002). These effects are presumed to be caused by the presence of endocrine-active contaminants in the effluent; however, despite a great deal of effort, it is unclear what components are responsible. Research on characterizing the causative compounds has been hindered by the complex nature of bleached kraft mill effluents and the variability in sensitivity of different fish species. Cause and effect relationships between the components of the effluent and fish responses are difficult to determine given the range of chemicals present (e.g., wood-derived, production-derived, treatment-derived) and their potential combinations. As well, protocols in only a few fish species have been described that can link responses to waste stream sources and identify causative chemicals (Martel et al., 1997; Parrott and Wood, 2002).
Studies done at Irving Pulp and Paper Ltd. (IPP), a bleached kraft pulp mill in Saint John, New Brunswick, Canada, were some of the first to implement an investigation of cause approach (Hewitt et al., 2003) for identifying specific hormonally-active waste streams within the mill (Dubé and MacLatchy 2000a, 2001; Hewitt et al., 2002; Belknap et al., 2005; Shaughnessy et al., 2005). Since 1997, our objectives at IPP have been to: 1) identify the sources of reproductive contaminants within the mill process; 2) characterize the causative compounds within the source waste stream(s); and 3) identify potential technological solutions that improve effluent quality at end-of-pipe. We have approached these objectives in a step-wise fashion which has resulted in improved understanding of the potential of one waste stream (5th effect chemical recovery condensates) to cause reproductive endocrine changes in fish. Here, we review our investigation of cause studies at the Irving mill, including detailed updates on our most recent work.

**IRVING PULP AND PAPER (IPP)**

IPP is a bleached kraft pulp mill located at the mouth of the Saint John River in Saint John, New Brunswick, Canada. This mill produces approximately 330,000 tonnes/year of market pulp from hardwood (primarily maple and birch) and softwood (primarily spruce, fir, and pine) furnish. It discharges approximately 96,000 m$^3$/d treated and untreated effluent into the Saint John River. The bleaching technology at this mill is elemental chlorine free with a sequence specified as D$E_0$D$E_P$D (D = chlorine dioxide bleaching stage; E = lignin extraction bleaching stage with either oxygen [O] or hydrogen peroxide [P]). In the last ten years, the mill has implemented several process changes, including two-stage brownstock washing and four-stage closed screening, oxygen delignification and two-stage postoxygen washing (POW), improved foul condensate stream stripping and spill recovery, and upgraded reactors. A moving-bed bioreactor (installed in 2000 and using thermophillic bacterial action) removes biological oxygen demand (BOD) from two bleach plant effluent streams (D, $E_{OP}$) prior to final discharge.

Until 1998, condensates from the 5th and 6th effect evaporators were recycled within IPP and were used as wash water in other areas of the mill. Condensates from the 5th effect evaporator and bleach plant effluents had high Microtox® toxicity, BOD, and chemical oxygen demand (COD) (Dubé and MacLatchy, 2000a). In 1998, a reverse osmosis (RO) system was installed to treat the condensates from the 5th effect evaporator prior to its reuse as wash water within the mill.

Reverse osmosis (RO) is a reverse crossflow membrane separation process that is typically used to purify and treat water and remove dissolved salts and small organic particles less than 0.001 μm in diameter. The technology has been used in the pulp and paper industry to reduce COD, total carbon, and colour of different process effluents; however, the majority of these uses were implemented on a pilot scale to explore feasibility (Knudsen et al., 1996). At IPP, condensates from the 5th effect evaporator (RO feed) are applied under pressure to force it through a semi-permeable membrane for fine filtration and removal of contaminants (approximately 4164 L/min). The permeate (clean condensates that passed through the membrane, approximately 99% of the flow) is then used as wash water at a different location within the mill, and ultimately discharged into the main chemical sewer, where it is combined with the various other waste streams generated within the mill. The concentrate (chemicals rejected by the RO membrane, approximately 1% of the flow) is burned in the bark boiler (Dubé and MacLatchy, 2000a).

**INVESTIGATION OF CAUSE APPROACH**

We define “investigation of cause” as a multi-tiered guidance framework for identification of the cause of environmental effects (Hewitt et al., 2003). The framework includes tiers to: 1) define whether there is an effect; 2) investigate individual process wastes to determine source waste streams contributing to final effluent effects; and 3) characterize and identify the chemical classes involved in causing biological responses. Our work at IPP is one of the most developed case studies in investigation of cause methodology (Table 1).

**Phase 1 (1997-2000)**

We began in the summer of 1997 with three questions: 1) could artificial stream technology be developed to assess pulp and paper effluent effects for the EEM program to gain a more controlled understanding of the effects of the effluent; 2) could particular waste streams in the pulp mill be linked
to fish reproductive responses as a mechanism to isolate where treatment technologies might be best served; and 3) could a framework be developed where the effectiveness of treatment technologies could be evaluated? The 1997 study design involved the use of mobile, field-based artificial stream systems which systematically exposed mummichog (a small-bodied, endemic saltwater minnow, *Fundulus heteroclitus*, of the local area) to multiple in-mill process wastes (condensates, post-oxygen washer filtrates and final mill effluent) at environmentally-relevant 1% concentrations (Dubé and MacLatchy, 2000a). The field-based artificial stream design was implemented in order to maximize environmental relevance. The artificial streams were used because: 1) traditional field sampling could not be carried out in the confounded estuarine environment; and 2) the system allowed the isolation and testing of several waste streams. Both males and females had reduced plasma testosterone levels at 1% final effluent. Females showed increased liver size and decreased in vitro production of plasma 17β-estradiol following exposure to the condensates, suggesting that the condensates are an important process stream which causes sublethal responses in mummichog (Dubé, 2000).

In March 1998, IPP installed the RO system; in the summer of 1998 we continued with our investigation of cause studies. Mummichog plasma testosterone levels were unaffected following exposure to final effluent (1%), a significant improvement from the previous year (Dubé and MacLatchy, 2000a). However, significant reductions in plasma testosterone were observed at 5%, indicating that the RO system reduced, but did not remove, the endocrine-disrupting properties of the final effluent. The results suggested that the chemical recovery condensates may be a source of endocrine disrupting substances (EDSs) in bleached kraft pulp mill effluent, and that RO treatment may be a successful treatment technology to improve effluent quality in regard to its effects on fish reproduction (Dubé and MacLatchy, 2000a).

In 1999, mummichog were exposed in seven- and 21-day laboratory exposures to RO feed (5th effect condensates; 1% and 3%), RO permeate (5 and 25%), bleach plant effluent (1% and 50%) and combined mill effluent (pulp mill wastes added to tissue mill wastes). The protocol used was a static exposure with daily renewal of water and effluents. Results from this study confirmed that condensates depress plasma testosterone levels in mummichog and confirmed the previous year's study that RO treatment removed the potential of the condensates to lower plasma testosterone levels in final mill effluent at an environmentally-relevant (1%) concentration (Dubé and MacLatchy, 2001). At very high concentrations (25%) RO permeate (reused as wash water in the mill) did not have testosterone-lowering properties. Other sources of endocrine disruption may be present in the processing, chemical recovery or bleaching processes as high (50%) concentrations of final effluent consistently lowered plasma testosterone. Further studies on endocrine status of laboratory-held mummichog confirmed that RO feed and RO concentrates contain endocrine-disrupting properties while RO permeate does not (MacLatchy *et al*., 2001).

In parallel studies, we showed that RO treatment of condensates also removed acute toxicity of the final effluent and reduced sublethal toxicity of the final effluent (Dubé and MacLatchy, 2000b). This was done in a mill-scale study, in which final effluents were collected for the tests with the RO either operating ("turned on") or not operating ("turned off"). Standard toxicity tests used were those regulated by the EEM program for monitoring final mill effluent quality in Atlantic Canada. Together, the IPP studies (Dubé and MacLatchy, 2000a,b; 2001) were the first direct demonstration of how toxicity (including fish reproductive endocrine status) is improved when the RO system is operating. In studies at other mills, we have successfully used standardized toxicity tests to identify waste streams that have the potential to be associated with toxicity (including endocrine disruption) and also to determine sub-lethal exposure concentrations for endocrine disruption studies (Rickwood *et al*., 2005).

Initial chemical analysis of the RO feed identified phenols and glycols as the dominate constituents, with guaicol as the main phenol constituent removed by the RO (it was not found in permeate and was concentrated 100-fold in the RO concentrate) (Dubé and MacLatchy, 2001). Manool-type alcohols from wood extractives were likewise in RO feed, not in permeate, and concentrated in the RO concentrate. The RO feed, permeate and concentrate contained little in the way of resin or fatty acids and sterols were not detected (Dubé and MacLatchy, 2001). Weak black liquor (WBL) was shown not to be entrained in the fifth effect condensate (RO feed) stream at IPP (Dubé and MacLatchy, 2001). Both chemical characterization and tracing of WBL entrainment eliminated nonvolatile, high molecular weight compounds usually found in WBL as potential compounds in the condensates responsible for endocrine depression.

It has proven difficult to identify causative compounds in pulp mill final effluent due to the complexity of final effluent and the number of unidentified chemical compounds. Furthermore, the residual lignin or “cement” of wood fibers in the tree make up a large component of effluent and these large molecules make it extremely difficult to study smaller bioactive substances. The condensates are a much less complex waste stream than the final effluent and do not contain any lignin. Therefore, we began to focus our efforts on characterizing the constituents of this waste stream linked to endocrine changes in fish. We began by confirming that the bioactivity of RO feed was found post-RO in the concentrate but not in the permeate (MacLatchy et al., 2001). Successes in this second phase were directly linked to the development of novel chemical extraction techniques and in refinement of our mummichog bioassay method for endocrine toxicity.

A solid-phase extraction (SPE) method was developed by Hewitt et al. (2002) to isolate chemical recovery IPP condensate extractives for the evaluation of their hormonal activity. Male and female mummichog were exposed (seven-day static exposures with daily renewal) to whole condensates, extracts from suspended particulates (>1 μm), fractions from SPE [SPE-1 (styrene divinylbenzene)ethyl acetate fraction, SPE-1 methanol fraction, and SPE-2 (reversible graphitized carbon) fraction, and residual condensates after SPE. The SPE-2 extracts, as well as the whole condensates, showed significant reductions in plasma testosterone following seven days of exposure. It was concluded that the SPE-2 methodology completely recovered activity from the whole condensates as there were no effects of residual condensates. Preliminary gas chromatography/mass spectrometry (GC/MS) analyses indicated that the extractives had properties consistent with lignin degradation products. This was one of the first studies to isolate chemicals derived from pulp production that were associated with reproductive endocrine effects in fish (Hewitt et al., 2002).

Since 1999 we have been actively working on the standardization of short-term exposure bioassays with mummichog, including validation of effects from model endocrine disruptors (MacLatchy et al., 2003; Sharpe et al., 2004). In brief, mummichog are collected from clean reference estuaries in New Brunswick and transported to laboratory facilities at UNB Saint John, where they were acclimated for at least two weeks prior to experimentation. Fish are maintained at a natural photoperiod in 16 ppt salinity seawater and dissolved oxygen (> 80%) in a flow-through system. Fish are fed daily with commercial trout pellets and water quality measurements are conducted daily in order to maintain standardized conditions. Subsequent to laboratory acclimation, three or four adult mummichog (minimum 65 mm) of each sex are weighed and randomly allocated to glass aquaria. Fish are held in the experimental aquaria for one week prior to the commencement of treatments. Throughout the acclimation period and exposure, fish are kept at a 14-h L:10-h D photoperiod (late spring conditions), and fed commercial trout pellets daily. Each tank is aerated and water quality parameters are recorded. Temperature is approximately 16°C for each exposure. Water is completely renewed and extracts/effluents are re-administered every 24 hours for seven days. Extracts are dissolved in methanol; reference tanks received equivalent doses of methanol (0.0125% v/v) for control purposes.

To the end of 2002, the investigation of cause methodology had progressed from initial identification of a particular waste stream associated with endocrine responses in fish (Phase 1) to: development of an optimized SPE method that completely recovers chemicals that reduced testosterone levels in mummichog; characterizations of active fractions by GC-MS that revealed compounds possessing functionalities consistent with lignin degradation products; and development and refinement of a short-term reproductive bioassay for the mummichog to determine biological activity of pulp mill effluent constituents.

Phase 3: 2003-2005

The primary objectives of this phase of our studies were to: 1) develop chemical methods for HPLC (high pressure liquid chromatography) fractionation techniques of the hormonally active SPE-2 eluate that could be employed on a preparative scale for testing using mummichog in the seven-day static renewal assay; 2) isolate HPLC fractions responsible for plasma testosterone depression in mummichog and identify causative compounds; and 3) identify candidate plasma testosterone depressing substances in condensate extracts, in lieu of definitive bioassay finding. As part of method development, we also evaluated the chemical stability, consistency, and composition of the hormonally-active condensate extract over time and under various production conditions at the mill.
Chemical recovery condensates were collected from the 5th effect evaporator feed and processed through the dual SPE process (Hewitt et al., 2002). The eluates from the second SPE cartridge (graphitized carbon) in series were evaporated and the extract was injected on the HPLC system and optimization of separation conditions was achieved through modification of elution conditions. Initially, six fractions were collected and evaporated to just-dryness. Fractions were then reconstituted in methanol to original volume and reinjected on the HPLC. Recoveries were determined for each of the detected peaks by comparison of the fraction peak areas to the original chromatogram peak area. Fractions were then analyzed by GC-MS. Detailed methodology was described in Belknap et al. (2004).

Fractions were subjected to GC-MS and displayed many similarities in the presence of mass spectral unknowns identifiable by their apparent molecular ions. Variability in HPLC fractions remained low and consistent; the average standard deviation across all peak averages for temporally-separate sampling periods was 1.54% (Belknap et al., 2004). The compounds tentatively identified by GC-MS represent those expected from kraft pulping: terpenoids, lignins and associated degradation products. GC-MS results showed Bis(2-ethylhexyl) phthalate and bis 1-1-(1,2-ethanediyl) benzene spectra in all blank fractions. Unknowns in HPLC fractions 1 and 2 displayed mass spectra indicative of phenolic as well as cyclic alkane and alkene compounds. Fractions 3 and 4 contained unknowns with straight chain alkane and cyclic amine functionalities. Fractions 5 and 6 contained unknowns with tentative evidence of substituted naphthalene and molecular sulphur (S8) (Belknap et al., 2004). The detection of free phenolic, as well as aromatic carboxylic acids and alcohols, are in agreement with terpenoid functionalities previously identified (Hewitt et al., 2002).

The preparative method was determined to be adequate for bioassay testing using the mummichog in vivo seven-day assay. The objective of the first toxicity identification evaluation (TIE) was to determine which fractions of the SPE-2 bioactive condensate extract caused significant depressions in plasma testosterone at 1% v/v. Our previous studies showed that 1% SPE-2 significantly reduced plasma testosterone in both sexes (Hewitt et al., 2002). The exposures consisted of a positive control (total SPE-2), a reference (distilled water processed through SPE and dissolved in methanol), and the six HPLC-generated fractions (mixed with methanol). Responses in fish exposed to the whole extract at 1% v/v were not as pronounced as had been observed previously (female plasma testosterone was reduced by 16% in the current study compared to 75% previously). Additionally, a follow-up exposure at 1.5% v/v did not yield consistent effects. In total, there were little and/or inconsistent effects of the fractions between exposures and between sexes (Shaughnessy et al., 2005).

Because responses in plasma testosterone in the two TIE experiments were minimal when compared to responses observed by Hewitt et al. (2002), an exposure was carried out to determine the responses in plasma testosterone following exposure to 1% and 4% RO feed (Shaughnessy et al., 2005). A secondary objective was to determine if potential compound degradation throughout the duration of the seven-day exposure would have an impact on responses observed. Therefore, for both 1% and 4% treatments, RO feed was collected once from the mill, and the same stock of effluent was kept at room temperature and used throughout the exposure (batch treatments). As well, 1% and 4% RO feed were collected daily and administered to the aquarium such that a new stock of feed was used each day (daily treatments). It was determined that 4% v/v was required to elicit significant plasma steroid responses in males, while females did not respond to either concentration. Significant decreases in circulating testosterone were observed in male mummichog exposed to 4% RO feed whether collections were made daily or only once at the beginning of the exposure, demonstrating consistency and stability of the effluent over time (Shaughnessy et al., 2005).

One question remaining from the TIE exposures was whether SPE-2 is an accurate representation of the RO feed. Therefore, we exposed mummichog to 0.5%, 1%, 2%, and 4% SPE-2 (reference group received methanol) to compare results to the previous 1% and 4% RO exposure (Shaughnessy et al., 2005). Again, female mummichog did not show any responses in circulating testosterone levels for any treatment. Male fish exposed to 4% SPE-2 showed the greatest depression in plasma testosterone relative to the other treatments when compared to control values (p=0.015). Therefore, it was concluded that the SPE-2 fraction is a good representation of the RO feed as consistent effects were found in both females and males (with males responding in both experiments at 4% concentration (Shaughnessy et al., 2005).

A third TIE exposure, carried out in April 2004, was done to determine the responses in plasma testosterone following exposure to the previously-tested HPLC fractions at 4% v/v. SPE-2 treatments consisted of fractions 1 and 2 combined, fractions 3 and 4 combined, fraction 5, and fraction 6 for a
total of four treatment groups (treatment groups were reduced to allow increased replication within treatments). Reference tanks received the processed blank and whole condensates (RO feed) were a positive control. No significant responses in plasma testosterone levels were observed in females (Shaughnessy et al., 2005). Male fish showed significant increases in plasma testosterone for fraction 3.4 (p = 0.032) and fraction 6 (p=0.045). Plasma testosterone was significantly reduced compared to reference fish in males exposed to 4% RO feed (p<0.001), the positive control treatment (Shaughnessy et al., 2005).

The reduced potency of the condensates to alter steroid profiles in fish as compared to previous work (Dubé and MacLatchy, 2001; Hewitt et al., 2002) was a significant finding of the exposures in Phase 3. However, these changes could not be linked to mill operations since no known changes in processes have been made since 2000 (D. Muir, Environmental Manager, IPP, personal communication). The TIE experiments also demonstrated that the extract fractions were highly variable in their effects on plasma testosterone; the April 2004 experiment was the first in which increases in plasma testosterone due to condensate exposure have been found. Relatively consistent differences in response by sex were also found. Therefore, an analysis of all exposures carried out at the University of New Brunswick between 1997 and 2004 in which mummichog were exposed to model compounds or complex effluents suspected of possessing hormonal activity was done (Shaughnessy, 2005). A primary objective of the analysis was to determine if differences in reproductive state influence mummichog sensitivity to contaminant exposure. The study revealed that mummichog are reproductively active from mid-March until mid-August and that lunar cues appear to influence circulating testosterone levels in male mummichog, such that peak levels correlate with the full moon. Moon phase did not play a role in regulating gonad size for either sex during the spawning season. Mummichog with large gonads responded to contaminants less often than fish with smaller gonad sizes, indicating that mummichog sensitivity to exposure can change depending on the reproductive state of the fish. Results from the analysis are being used to develop study designs that take into account sensitivity of the bioassay for detecting contaminant effects at different parts of the reproductive cycle.

In order to assess sources of inconsistencies in fish effects that may be arising due to the fractionation process, analytical methodologies for fraction preparations were evaluated. Condensates were collected over a six-month period, extracted by SPE, and analyzed by GC-MS and gas chromatography-flame photometric detection (GC-FPD). Concentrations of confirmed condensate extractives were consistent in all samples collected. However, spiking experiments of confirmed extractives did reveal substantial losses following HPLC fractionation and three different methods of fraction preparation (Belknap et al., 2005). During these studies, all unique extractives were cataloged by mass spectra, peak area, and retention time. Nine compounds were confirmed and quantified against authentic standards. Confirmed components included a range of phenolic guaiacyl-based lignin degradation products, sulfur (S$_2$), three diterpenoids, and a dimethoxy pinosylvin stilbene (Belknap et al., 2005).

In the absence of advancement in identifying causative compounds through the TIE protocol using the fractions, it was still possible to make progress in identifying the chemical classes associated with biological activity. We proceeded to identify unique condensate components in bioactive and inactive waste streams associated with the RO. Candidates were identified from SPE-2 extracts of RO feed collected at IPP in July 2004 and were selected following a set of predetermined criteria (Belknap et al., 2005). These criteria were based on previous observations of steroid disruptions whereby RO feed and RO concentrate depressed testosterone, while RO permeate had no effect in males and a 25% (v/v) exposure was required to induce a depression in females (Dubé et al., 2001; MacLatchy et al., 2001).

As a first step, unique components in RO feed were compared to RO concentrate and RO permeate SPE-2 extracts. All unique extractives that were higher in RO feed than RO permeate were accepted for the next step of selection. Previous studies had demonstrated SPE-1 ethyl acetate showed no activity, SPE-1 methanol showed some activity, and SPE-2 contained most activity (Hewitt et al., 2002). Therefore, those components higher in SPE-2 than SPE-1 ethyl acetate were selected as final candidates. In order to account for a margin of error ($\leq$ 20%) in bioassay evaluations and chemical measurements, sample components in SPE-2 that were 80-100% as high as in SPE-1 were designated as ‘moderate candidates’. Out of 39 unique components in bioactive SPE extracts of condensates, six were associated with hormonal activity. Mass spectral interpretation indicated hydroxylated diterpenoids, sesquiterpenoids, and a lignin-derived stilbene as classes of chemicals associated with steroid depressions (Belknap et al., 2005; Hewitt et al., 2006).
We have always been interested in the applicability of the findings at IPP to other bleached kraft mills. To determine the comparability of the condensates at the Saint John mill with other similar condensate streams, an evaluation of concentrations of candidate compounds of softwood condensates from the Saint John mill with condensates from a mill in Ontario, Canada, was carried out (Belknap et al., 2005). Semi-quantitative concentrations of candidate compounds were consistent in extracts of softwood condensates at both mills with the exception of some diterpenoids. Concentrations of these diterpenoids (acyl C20 diterpenoid alcohol and acyl C20 diterpenoid diol) were the highest of all candidates in the Ontario mill’s condensates and were substantially lower in Saint John (Belknap et al., 2005). Caparratriene was the only candidate present in Saint John softwood condensates that was not observed in the Ontario condensates.

FUTURE STUDIES

Within the investigation of cause framework, our studies into the identities of endocrine-active compounds in pulp mill effluent are following several lines of enquiry. We are investigating the changes in potency of the condensates to determine if these are linked to changes in the effluent quality (changes in mill processes) or with sensitivity of the mummichog in our laboratory exposures. As discussed, some variability in mummichog sensitivity to endocrine disruptors has been noted according to the time of the year and closeness to the new moon in the timing of the exposures. Refinement of the bioassay protocol will lessen within- and among-experiment variability.

We are also committed to revisiting the TIE process using the fractionation protocol described here once we have addressed the challenges in the fraction preparation techniques. To this end, we are working to eliminate volatilization issues occurring in the preparation of the fractions. We are also working to minimize the degradation of thermally labile compounds. Experiments are planned to determine recoveries of model extractives specifically associated with nitrogen evaporation. It is hypothesized that chemical losses are occurring primarily during solvent evaporation and reconstitution of HPLC fractions for exposure and GC-MS processes.

Refinement of chemical fractionation and bioassay techniques should enable us to identify the hormone-active compounds in the condensates at IPP. Once the compounds at IPP are identified, the compounds can be tracked through to final effluent to determine their contribution to the endocrine toxicity of final effluent (as well as the efficacy and importance of the RO for removing the compounds from final effluent). The potential of RO to improve effluent quality in the industry can best be assessed once similarities between condensate and final effluent compositions are confirmed at a number of kraft mills in addition to IPP.

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REFERENCES


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