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Evaluating the contribution to toxicity of weak black liquor in pulp mill effluents

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EVALUATING THE CONTRIBUTION TO TOXICITY OF WEAK BLACK LIQUOR IN PULP MILL EFFLUENTS

By

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ABSTRACT

Weak black liquor (WBL) losses in pulp mills may affect effluent treatment efficiencies and may be linked to aquatic toxicity observed in final mill effluents. Best management practices (BMP) for controlling losses of WBL have been effective at reducing WBL from entering the mill effluent treatment system, but it is unclear at what level WBL may contribute to increased toxicity, or whether specific chemical compounds found in WBL may be consistently responsible. The objective of this study was to evaluate the contribution of WBL in biologically-treated bleached kraft pulp mill effluents to toxicity, and to assess effluent chemical parameters that may correlate with biological responses. Weak black liquor and untreated wastewater (as it enters the biological treatment system) were collected from four bleached kraft mills along with mill-treated effluent samples. To simulate a range of potential WBL losses, various concentrations of WBL were added to untreated wastewater from each mill and treated in bench top aerobic reactors to mimic biological treatment (biotreatment). Following laboratory biotreatment, toxicity of the resulting "simulated effluents" (as well as mill-treated effluents) were evaluated using 48-h *Mytilus galloprovincialis* embryo-larval development and 7-d *Ceriodaphnia dubia* survival and reproduction chronic toxicity tests. All effluent samples were chemically characterized for pH, color, conductivity, turbidity, total suspended solids (TSS), polyphenols, hardness, alkalinity, salinity, biochemical oxygen demand (BOD), dissolved chemical oxygen demand (DCOD), dissolved organic carbon (DOC), resin acids (RAs), and phytosterols. Correlation analysis was used to determine if there were significant correlations between: 1) WBL solids and simulated effluent chemical parameters; 2) WBL solids and chronic toxicity to *M. galloprovincialis* and *C. dubia*; 3) effluent (mill-treated and simulated) chemical parameters and chronic toxicity to *M.*

galloprovincialis and *C. dubia*; and 4) between the two chronic toxicity tests. Multivariate methods including cluster analysis (hierarchical, kmeans, and non-metric Riffle) and PCA were also used to explore the data for patterns, and to identify effluent chemical parameters that might relate to WBL solids or effluent toxicity. Results were that the 48-h EC50 for *M. galloprovincialis* embryo-larval development appears to be a more sensitive endpoint than the 7-d *C. dubia* reproduction with respect to both mill-treated and simulated effluent samples. For the simulated effluent samples, color, DCOD, and polyphenols were positively correlated with WBL solids. For three out of four mills, color and polyphenols were negatively correlated with the 48-h EC50 for *M. galloprovincialis* embryo-larval development (i.e. as the EC50 decreased (toxicity increased) these chemical parameters increased)). For two out of four mills, DCOD was negatively correlated with the 48-h EC50 for *M. galloprovincialis* embryo-larval development. Significant negative correlations were also observed between the 48-h EC50 for *M. galloprovincialis* embryo-larval development and abietic acid (one mill out of four) and between the 48-h EC50 for *M. galloprovincialis* embryo-larval development conductivity (one mill out of four). None of the measured chemical parameters correlated with chronic toxicity to *C. dubia*. A significant negative correlation was also observed between the 48-h EC50 for *M. galloprovincialis* embryo-larval development, and between WBL solids and the 7-d IC25 *C. dubia* reproduction (i.e. as WBL solids increased the toxicity increased (as indicated by a decrease in EC50/IC25)). A correlation was not found between the two chronic toxicity tests. Consistent across all multivariate methods, simulated effluent samples appeared to group together based on mill rather than on the amount of WBL solids added.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
‰	parts per thousand
µg	microgram
ADST	air dry short tons
APHA	American Public Health Association
ASB	aerated stabilization basin
ASB-TPWW	aerated stabilization basin treatment pond wastewater
ASTM	American Society for Testing and Materials
BMP	best management practices
BOD	biochemical oxygen demand
CI	confidence intervals
COD	chemical oxygen demand
CuCl	copper chloride
CWA	Clean Water Act
CV	coefficient of variation
d	day
DCOD	dissolved chemical oxygen demand
DOC	dissolved organic carbon
D(X)	reactor day
ECF	elemental chlorine free
EEM	Environmental Effects Monitoring
EC50	median effective concentration
EL50	median effective loading rate
EROD	7-ethoxyresorufin-*0*-deethylase
h	hour
HSB	hypersaline brine
IC25	25% inhibition concentration
LCL	lower calibration limits
LL50	median lethal loading rate
LOEC	lowest observed effects concentration
MFO	mixed function oxygenase
NA	not available
NABF	Northwest Aquatic Biology Facility
Na_2S	sodium sulfide
NaOH	sodium hydroxide
NC	not calculated
NCASI	National Council for Air and Stream Improvement
ND	non detect
NM	not measured
NOEC	no observed effects concentration
NPDES	National Pollutant Discharge and Elimination System
NTU	nephelometric turbidity unit
PCA	principal component analysis

PCU	platinum cobalt units
PRE	proportional reduction in error
RA	resin acid
SABF	Southern Aquatic Biology Facility
SPMC	Shannon Point Marine Center
SOP	standard operating procedure
TEI	toxicity emission index
TER	toxicity emission rate
TPWW	treatment pond wastewater
TOC	total organic carbon
TPY	tons per year
TSS	total suspended solids
TU	toxic unit
v/v (%v/v)	volume/volume percentage
WBL	weak black liquor
WET	whole effluent toxicity
WCRC	West Coast Regional Center
WDOE	Washington State Department of Ecology
U.S. EPA	United States Environmental Protection Agency

[1]KEY TERMS

Acute toxicity	A discernable adverse effect (lethal or sub-lethal) induced in the test organisms within a short period of exposure to a test material (usually a few days for larger organisms).
Aerated stabilization basin (ASB)	The most commonly used type of biological (secondary) treatment system for treating effluent from pulp and paper mills.
Alkalinity	The capacity of water to neutralize acid as a measure of titration of a water sample with a dilute acid to a specific pH endpoint.
Biological treatment	Form of wastewater treatment in which bacterial or biochemical action is intensified to stabilize, oxidize, and nitrify the unstable organic matter present; a type of secondary treatment (also known as biotreatment).
Black liquor	The spent pulping liquid after the alkaline process is complete; contains dissolved organic wood materials and inorganic compounds from the wood and original alkaline cooking liquor.
Chi-squared (χ^2)	A test statistic sometimes used in assessing the fit of a model to a set of data.
Chronic toxicity	Toxicity resulting from exposure to a toxin over an extended period of time.
Cluster rules	Legislation introduced by U. S. EPA in 1988 specific to effluents from pulp and paper mills.
Concentrated black liquor	Black liquor after final evaporation or concentration process; often referred to as strong black liquor.
Confidence limits	These limits on an EC50 (or IC25) represent the upper and lower concentrations, within which the true endpoint is thought to lie, for a stated level of probability.
Control	A treatment in a toxicity test that duplicates all the conditions of the exposure treatments but contains no test materials.
Digester	A batch or continuous vessel used for pulping fibrous raw materials to remove lignin and produce pulp.
Dissolved organic carbon (DOC)	The fraction of the organic carbon pool that is dissolved in water and that passes through a 0.45 μm glass fiber filter.
Dunnett's test	A parametric, post-ANOVA test often used in the analysis of sub-lethal and chronic lethal effects data

[1]Sources consulted for key terms included Environment Canada (2005), Newman and Unger (2003), Rand (1995), Smook (1990), TAPPI (1996), and Timbrell (2000).

D(X)	Reactor day. Where X is the reactor day relative to when WBL was added to the spiked biotreatment reactors. D(-1) indicates the sample was taken one day before reactors were spiked with WBL, D(0) corresponds to the day the reactors were spiked, and D(1) corresponds to one day after the reactors were spiked.
Early life stage (ELS) test	A critical life stage test using early life stages such as embryos or larvae based on the observation that the early life stage is the most sensitive in the species life cycle.
Effluent	A discharge of pollutants into the environment (partially, or completely treated, or in its natural state); generally used in regard to discharges into waters.
Effluent chemical parameters	Chemical analysis of mill-treated and simulated effluent samples.
Ethoxyresorufin-0-deethylase (EROD) activity	Units of activity for O-deethylation of ethoxyresorufin by ethoxyresorufin O-deethylase. Used to reflect cytochrome P-450 monooxygenase activity.
Green liquor	Aqueous solution of sodium salts produced in the sulfate process by dissolving recovery boiler smelt in weak wash.
Hardwood	Wood from trees of the angiosperm class, usually with broad leaves and deciduous in temperature zones.
Inhibiting concentration (ICp)	Represents a point estimate of a concentration of test material that is estimated to cause a designated percent impairment in a quantitative biological function (where p is the percentage).
Inorganic	Pertaining to chemical compounds which do not contain carbon.
Integrated mill	A paper or board mill that produces all its own pulp.
Kraft pulping	The alkaline pulping process that uses a combination of sodium hydroxide and sodium sulfide; also known as sulfate pulping.
Laboratory biotreatment	A laboratory method used to biologically treat untreated mill effluent using 10-L bench-top aerobic reactors.
Lowest observed effect concentration (LOEC)	The lowest concentration of a material in a toxicity test that has a statistically significant different in response on the exposed population of test organisms compared with the controls.
Market pulp	Pulp sold as raw material to non-integrated paper mills.
Maximum likelihood estimation (MLE)	A parametric method used to fit dose-response or concentration-effect data to the log-normal, log-logistic, or other models; probit and logit approaches are most often applied with MLE methods.

Median effective concentration (EC50)	The concentration of material in water to which test organisms are exposed that is estimated to be effective in producing some sub-lethal response in 50% of the test organisms; usually expressed as a time dependent value (i.e. 48-h EC50).
Mill-treated effluent	Effluent that has been treated by a mill's wastewater treatment system.
Mixed function oxygenase (MFO)	The P-450 complex composed of cytochrome P450, NADPH-cytochrome P450 reductase, NADPH, and O2.
No observed effect concentration (NOEC)	The highest concentration of a material in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms compared with the controls.
Nonparametric analysis	Statistical technique that does not assume any underlying distribution for the data.
Parametric analysis	Uses a biostatistical method that considers the parameters of the population from which samples are drawn.
Phytosterols	A group of steroid alcohols, phytochemicals naturally occurring in plants.
Point estimate	A single numerical value that has been calculated or judged to represent a set of toxicity data (i.e. EC50 or IC25).
Primary treatment	The first major treatment in a wastewater treatment system; removes a substantial amount of suspended matter but little or no colloidal and dissolved matter.
Probit regression	Measures the relationship between the strength of a stimulus and the proportion of cases that exhibit a selected effect caused by the stimulus.
Pulp	General term to describe fibers after they are liberated from a fibrous raw material source such as wood chips, straw, cotton, or grasses.
Reactor control	Simulated effluent containing only ASB influent and ASB-TPWW (i.e. no added WBL)
Recovery boiler	A boiler used to recover pulping chemicals by burning off the organic material in kraft black liquor.
Resin acids	Principally monocarboxylic acids with the empirical formula C19H29COOH; can be classified into two groups (abietic or pimaric).

Secondary treatment	Wastewater treatment during which bacteria consume organic parts of the wastes; removes virtually all floating and settleable solids and approximately 90% of BOD and suspended solids.
Simulated effluents	Laboratory biotreated ASB influent, ASB-TPWW and WBL (for spiked reactors only).
Softwood	Wood from cone-bearing trees that usually retain needles or leaves for the entire year; commonly called evergreens.
Spearman-Karber	A non-parametric method to estimate the LC50, EC50, or LD50 when it is difficult or unnecessary to assume a specific model for the dose- or concentration-effect data.
Spiked reactors	Simulated effluent containing ASB influent, ASB-TPWW, and WBL.
Steel's many-one rank test	A non-parametric method, post-ANOVA test often employed in the analysis of sub-lethal and chronic lethal effects data.
Study initiation	When ASB-influent, ASB-TPWW and mill-treated effluent were collected for purposes of biotreatment, chemical analysis and toxicity tests.
Sub-lethal	Below the concentration that directly causes death.
Tertiary treatment	Wastewater treatment beyond the secondary (or biological) stage that includes removal of nutrients such as phosphorus, nitrogen, and a high percentage of suspended solids; can also be used to reduce color of effluents.
Test initiation	For toxicity tests, the start time of the exposure period.
Total organic carbon (TOC)	The sum of dissolved organic matter (DOM), particulate organic matter (POM), or suspended organic matter (SOM).
Toxicity	The inherent potential or capacity of an agent or material to cause adverse effects in a living organism when the organism is exposed to it.
WBL treatment/WBL spike level	Amount of WBL (g/L) added to spiked reactor.
Weak black liquor	Black liquor at a total concentration of about 15% dry solids.
White liquor	Aqueous solution of sodium salts derived from causticizing green liquor; main ingredients are caustic or sodium hydroxide, and sodium sulfide.
Whole effluent toxicity (WET)	The total toxic effect of an effluent measured directly with aquatic organisms in a toxicity test.

1.0 INTRODUCTION

1.1 Kraft Pulping Process and Weak Black Liquor

1.1.1 Kraft Pulping Process

Pulp and paper mill wastewater (effluent) is a complex mixture whose composition reflects primarily the contributions of a mill's pulping, bleaching and papermaking systems. The kraft pulping process (Figure 1) was developed in 1878 by C.F. Dahl and accounts for the majority of pulp produced worldwide. It can be used with any wood species and 75-80% of U.S. virgin pulp is produced by this method (Biermann 1996). Kraft pulp is used to make a variety of products, including "corrugated" boxes, grocery sacks, milk cartons and copier paper. Most kraft pulp mills in the U.S. are bleached kraft mills, and have mixed furnishes of hardwood and softwood (Kelly et al. 2004).

During the kraft pulping process, wood chips are cooked in a digester with a sodium-based alkaline solution called white liquor, which consists of sodium sulfide (Na_2S) and sodium hydroxide (NaOH; Biermann 1996). This digestion separates the wood fibers (pulp) from the aqueous solution of dissolved wood components and spent pulping chemicals, which is referred to as weak black liquor (WBL; U.S. EPA 2002a). Weak black liquor is very alkaline (pH = 11.5-13.5), and an extremely complex mixture containing most of the original inorganic cooking elements and the degraded dissolved wood substance (Biermann 1996, Kelly et al. 2004). The organic component of WBL consists of alkali lignin and the sodium salts of the polysaccharinic acids, resin acids (RAs) and fatty acids (Adams et al. 1997). The composition of WBL is variable, and depends on the type of wood being pulped, as well as the pulping process (Hodson 1997).

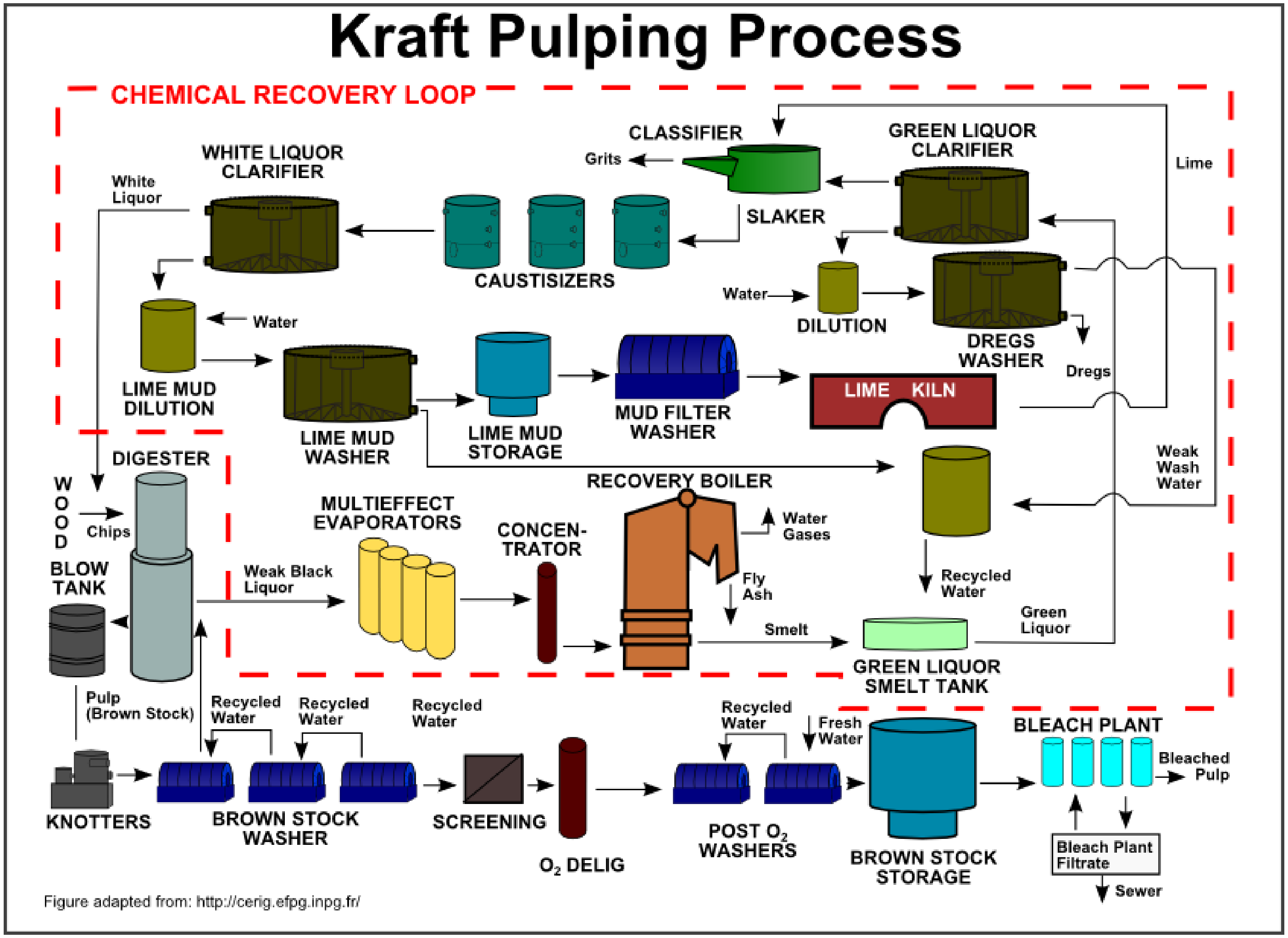


Figure 1. Kraft pulping process. Dotted red line indicates the chemical recovery loop. (Figure adapted from: http://cerig.efpg.inpg.fr/)

1.1.2 Recovery of Weak Black Liquor

A key component to the kraft pulping process is the chemical recovery of WBL. After digestion, WBL undergoes evaporation to concentrate the solids to become strong (or concentrated) black liquor. Strong black liquor is then burned in a recovery boiler which produces heat and green liquor (the partially recovered black liquor produced by dissolving the smelt from the recovery boiler in water). Further processing is used to convert the green liquor to white liquor.

The recovery system is economical because it recovers and regenerates the chemicals used in digestion and generates energy that is used by the mill; approximately two-thirds of the energy used by the pulp and paper industry is biomass fuels (primarily black liquor; NCASI 2011). The recovery process also reduces environmental impacts by diverting the black liquor from the pulp mill effluent treatment system (Rod'ko et al. 1996).

1.1.3 Effluent Treatment and Weak Black Liquor Losses

It is estimated that 97 to 99.5% of the WBL is recovered by washing the pulp (Carey et al. 2002). Some systemic losses of WBL from the pulp mill and recovery systems occur via pulp washers, evaporators, knotting and screening systems and other processes (Figure 1). Additionally, spills due to upset conditions may result in WBL losses beyond the 0.5 to 3% from systemic losses, although most mills have collection systems in place that are designed to capture and recover concentrated spilled black liquor. Small spills of WBL are often discharged at a controlled rate into a mill's wastewater treatment system (Barkley et al. 1986).

Wastewater from pulping, bleaching, chemical recovery, pulp drying, or papermaking and other processes is treated prior to discharging into a stream, lake, or marine system. Pulp and paper mill wastewater treatment systems typically consist of pretreatment, primary treatment, and secondary (biological) treatment. Pretreatment includes screening with coarse filters, grit removal and pH adjustment. During primary treatment, settleable solids are removed from the water by allowing them to settle; the mechanical clarifier is the method most commonly used. Secondary treatment consists of biological treatment and uses oxygen and microorganisms (bacteria and fungi) to remove oxygen-consuming materials in the effluent. This process reduces the level of pollutants entering aquatic systems (Hamner 1988), and typically reduces the biochemical oxygen demand (BOD) by 80-90% or more (Biermann 1996). Effluent is typically prepared for this phase by pH balancing and adding nutrients. Aerated stabilization basins (ASB) are most commonly used in secondary treatment at kraft mills (Biermann 1996, Hanmer 1988), and have been shown to effectively remove ~90% of the toxicity, BOD, and RAs (Chandrasekaran et al. 1978). Other secondary treatment methods include oxidation basins or use of the activated sludge process (Biermann 1996, Hanmer 1988). Tertiary treatment methods are rarely used, and are often aimed at reducing the color of effluent (Biermann 1996).

In 1998 the U. S. EPA introduced new regulations called the Pulp and Paper Cluster Rules. This regulation "protects human health and the environment by reducing toxic releases to the air and water from U.S. pulp and paper mills" (U.S. EPA 2011a). One component of the Cluster Rules is a requirement that each mill develops and implements a

"Best Management Practices" (BMP) plan to control accidental discharges of black liquor (Carey et al. 2002).

1.1.4 Weak Black Liquor Contribution to Mill Effluent

Weak black liquor has a BOD approximately 100 times greater than normal mill effluent (Barkley et al. 1986) and is a primary contributor to the BOD and color load of mill effluents (Xiao 2005). Research conducted by Barkley et al. (1986) found that WBL (15% solids) increased mill effluent BOD and created an immediate oxygen demand due to the reduced sulfur. The RAs contained in WBL can induce aquatic toxicity at high concentrations (Barkley et al. 1996)

A study by Vadodaria (1999) examined the effect of black liquor on the activated sludge process. The investigators reported that black liquor affected all measured parameters of mill effluent (conductivity, COD, BOD, TOC and pH), and an addition of black liquor from 0% to 5% in total mill effluent increased chemical oxygen demand (COD) from 1000 to 7000 mg/L, while BOD increased from 250 to 1000 mg/L.

1.2 Whole Effluent Toxicity Testing

In accordance with the Clean Water Act (CWA), point source discharges into surface waters are regulated by the U.S. Environmental Protection Agency (U. S. EPA) and state agencies under the National Pollutant Discharge Elimination System (NPDES; U.S. EPA 2002a). The NPDES permitting program requires industrial dischargers to conduct routine testing, including acute and often chronic whole effluent toxicity (WET) tests to monitor the quality of their effluents and toxicity to aquatic biota (U.S. EPA 1995, 2002b, 2002c, 2002d). Whole effluent toxicity tests involve exposing specific test organisms to various concentrations of effluent to evaluate adverse biological effects with survival, reproduction, growth, and larval development, which are among the most common effects measured. Accepted methods for conducting WET tests have been published by the American Public Health Association (APHA), the American Society for Testing and Materials (ASTM) and the U.S. EPA. The frequency of WET testing depends on the NPDES permit, and is based on factors such as variability and degree of toxicity of the waste, production schedules, and process changes (U.S. EPA 2002b, 2002c, 2002d). Depending on the NPDES permit, WET test organisms may include freshwater, marine, or estuarine species.

1.2.1 Freshwater Whole Effluent Toxicity Tests

Acute freshwater WET tests include 24-96 h mortality tests with daphnids (*Ceriodaphia dubia*, *Daphnia pulex*, *D. magna*), fathead minnows (*Pimephales promelas*), and rainbow trout (*Oncorhynchus mykiss*; U.S. EPA 2002b). Freshwater WET tests for evaluating chronic toxicity of effluents include a 7-d survival and reproduction tests with *C. dubia*, 7-d survival and growth tests with *P. promelas*, and 96-h growth tests with a green algae (*Selenastrum capricornutum* Printz[2]; U.S. EPA 2002c). Currently, *C. dubia* and *P. Promelas* are the two most commonly used freshwater WET test organisms for evaluating the acute and chronic toxicity of pulp mill effluents in the U.S (U.S. EPA 2011b).

1.2.2 Marine and Estuarine Whole Effluent Toxicity Tests

Marine and estuarine WET tests for evaluating acute toxicity of effluents and receiving waters include 24-96 h mortality tests with mysid shrimp (*Americamysis bahia*), sheepshead minnow (*Cyprinodon variegatus*), and silverside (*Menidia beryllina*, *M. menidia*, and

[2]This name has been replaced by *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák

M. peninsulae; U.S. EPA 2002b). Whole effluent toxicity tests for evaluating the chronic toxicity to estuarine and marine species include 24-96 h *C. variegatus* larval survival and growth; 24-96 h *C. variegatus* embryo-larval survival and teratogenicity; 24-96 h *M. beryllina* survival and growth; 24-96 h *A.bahia* survival, growth and fecundity test; 1 h 20 min sea urchin (*Arbacia puntulata*) fertilization test; and the red algae (*Champia parvula*) sexual reproduction test (U.S. EPA 2002d).

For facilities that discharge into the Pacific Ocean, short-term estuarine and marine chronic WET tests include a 7-d topsmelt (*Atherinops affinis*) larval growth and survival test; 24-96 h mysid (*Holmesimysis costata*) survival and growth test; 48-h pacific oyster (*Crassostrea gigas*)/mussel (*Mytilus sp*.) embryo-larval development test; 72-h sea urchin (*Strongylocentrotus purpatus*/*Dendraster excentricus* larval development test; 40 minute *S. purpatus*/*D. excentricus* fertilization test; 48-h red abalone (*Haliotis refuscens*) larval development test; and 48-h giant kelp (*Macrocystis pyrifa*) germination and growth test (U.S. EPA 1995). Currently, *A. bahia*, *Menidia spp*., and *C.variegatus* are the most commonly used marine/estuarine WET test organisms for evaluating chronic and acute toxicity of pulp mill effluents in the U.S (U.S. EPA 2011b).

1.3 Literature Review: Toxicity of Weak Black Liquor

Although there are many studies examining the biotic response of exposure to pulp and paper mill effluent, studies addressing WBL specifically are relatively rare. Findings from studies examining the toxicity of WBL to aquatic organisms are summarized in the following two sections.

1.3.1 Sub-lethal Effects

Fish

Fish are the main organisms on which the toxicity of WBL has been evaluated. Weak black liquor is a potent inducer of mixed function oxygenase (MFO) activity in fish, and is one of the major sources of 7-ethoxyresorufin-*0*-deethylase (EROD) inducers in fish present in mill effluents (Carey et al. 2002, Sturm 1999).

Research by Schnell et al. (1993) examined a variety of waste streams within a bleached kraft mill to identify which mill effluent waste streams were responsible for MFO/EROD activity in fish. Results were that untreated WBL at 0.045% (v/v) showed an 8-fold average level of induction in juvenile *O. mykiss*. Aerobic biological treatment, however, reduced the EROD induction potency to biologically insignificant levels.

Experiments by Hodson et al. (1997) using WBL from six bleached kraft mills demonstrated that untreated WBL at low concentrations caused increased MFO activity, with significant increased EROD activity of *O. mykiss* at concentrations ranging from 0.001 to 0.01% (v/v). The authors also found that potency appeared the highest for black liquors derived from hardwood pulping versus softwood. When EROD activity was normalized to dissolved organic carbon (DOC), however, the differences in potency were reduced. Results support the hypothesis that EROD-inducing agents in black liquor are wood extractives (rather than the breakdown products of lignin, cellulose, or hemicellulose), and that natural components of wood can cause MFO induction in fish (Hodson et al. 1997). In another study, Sturm et al. (1999) exposed juvenile *O. mykiss* to 0.01% untreated black liquor for 4 to 8 d in the laboratory and found a two fold increase in EROD induction relative to the controls.

Although it is unknown which compounds in black liquor induce MFO activity, Burnison et al. (1996) used a Toxicity Identification and Evaluation (TIE) approach to identify which effluent fraction causes MFO induction in fish. Results were that the majority of the EROD activity were found in the moderately non-polar region of the chromatogram (K_{ow} = 4.6 to 5.1).

Studies have also been conducted to examine the link between WBL and patterns of endocrine disruption and other organ-level responses in fish. A study published by Zacharewski et al. (1995) used *in vitro* recombinant receptor/reporter gene assays to examine untreated pulp and paper mill black liquor for estrogenic, dioxin-like, and anti-estrogenic activity. Results were that black liquor contains estrogenic and dioxin-like ligands. The researchers concluded that further studies are required to identify the compounds, and to determine if these ligands are responsible for adverse effects observed in fish exposed mill effluents.

Environment Canada’s Environmental Effects Monitoring (EEM) program is a regulatory program in which the response of aquatic biota to pulp and paper mill effluent exposure is assessed through field sampling and laboratory testing in three-year cycles (Lowell et al. 2005). In a review paper by Carey et al. (2002), research conducted by McCubbin in 2000 was highlighted that examined the relationship between estimated WBL losses (where WBL losses were estimated based on mass balances provided by mill personnel) and sub-lethal toxicity to *C. dubia* for five bleached kraft mill in Ontario using Cycle 2 data from the EEM program (Section 1.3.1 – *C. dubia*). Carey et al. (2002) employed a similar approach using Cycle 2 EEM data, but instead focused on bleached kraft mills located in British Columbia to examine relationships between estimated WBL losses and sub-lethal and lethal effects to aquatic organisms (fish, daphnids, and algae). Using Cycle 2 EEM data from six bleached kraft mills in British Columbia, Carey et al. (2002) reports a trend of increasing sub-lethal toxicity to fish (i.e. decrease in IC25) with increasing estimated WBL losses (R^2 = 0.1843; p-value not reported; Figure 2). Additional results by Carey et al. (2002) using the Cycle 2 EEM data are discussed in Section 1.3.1 – *C. dubia* and in Section 1.3.2.

A field study in 2008 examined the exposure of fish populations after a black liquor spill using RA analysis of bile from two species of wild fish, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*). Results showed elevated concentrations of RAs in the bile in the weeks following the spill (Meriläinen and Oikari 2008).

A literature review to examine the roles of various compounds of altered fish reproduction found that reduced use of molecular chlorine, improved condensate handling, and increased WBL spill control improved reproductive performance (Hewitt et al. 2008). Unfortunately this improvement could not be attributed to a specific process modification, as the mills studied performed multiple modifications simultaneously.

Ceriodaphnia dubia

As mentioned previously, a review by Carey et al. (2002) evaluated an analysis conducted in 2000 by McCubbin which explored the relationship between estimated WBL losses and *C. dubia* sub-lethal toxicity using Cycle 2 EEM data from five bleached kraft mills in Ontario. The review of McCubbin's data by Carey et al. (2002) indicated a general trend of increased sub-lethal toxicity to *C. dubia* (i.e. decreased IC25) with increased estimated WBL losses (R^2 = 0.8864; p-value not reported; Figure 3). Carey et al. (2002) further explored the relationship between estimated WBL losses and sub-lethal toxicity to *C. dubia* using Cycle 2 EEM data from six bleached kraft mills in British Columbia. Results found a trend of increased sub-lethal toxicity to *C. dubia* (i.e. decreased IC25) with increased estimated WBL losses (R^2 = 0.6121; p-value not reported; Figure 2).

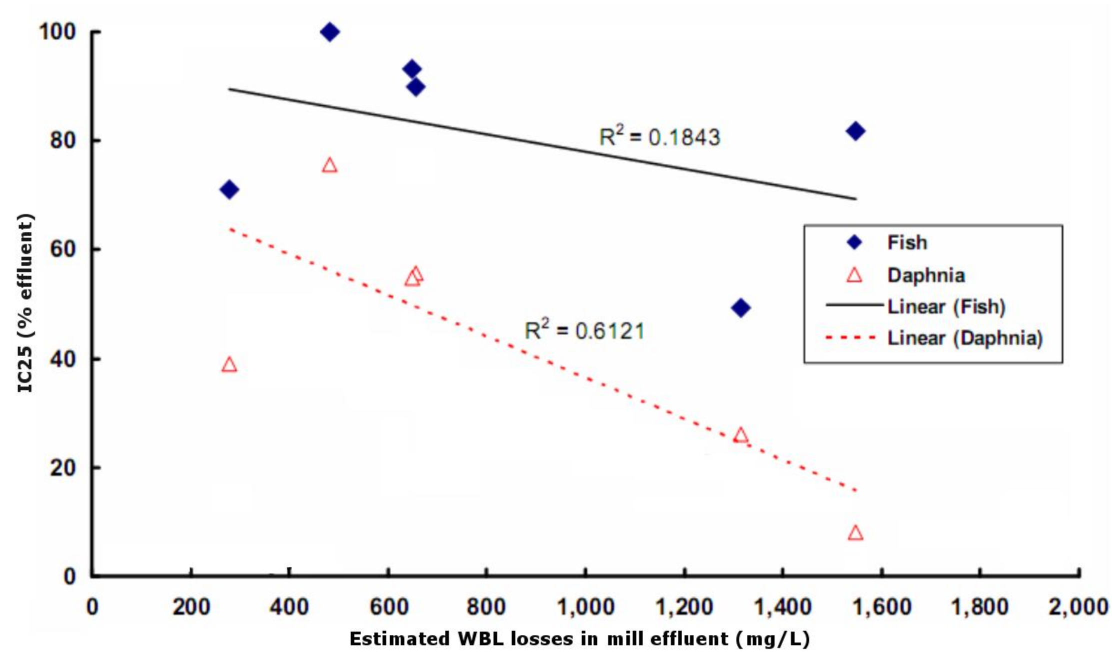


Figure 2. Analysis by Carey et al. (2002) examining relationship between estimated weak black liquor losses and sub-lethal toxicity to fish and daphnia using Cycle 2 Environmental Effects Monitoring data from six bleached kraft mills in British Columbia, Canada. (Figure adapted from Carey et al. 2002).

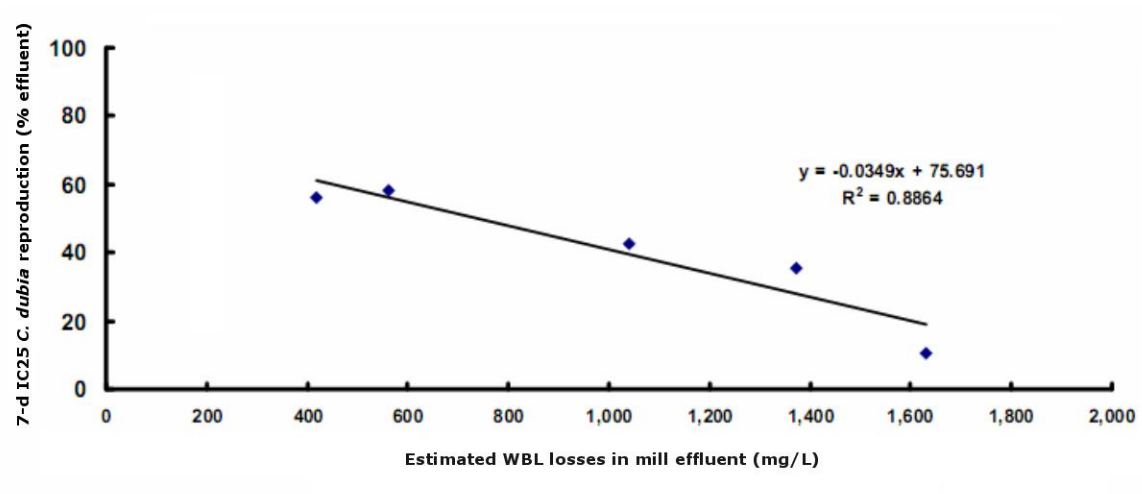


Figure 3. Analysis conducted by McCubbin in 2000 examining relationship between estimated weak black liquor losses and sub-lethal toxicity to *Ceriodaphnia dubia* using Cycle 2 Environmental Effects Monitoring data from five bleached kraft mills in Ontario, Canada. (Figure adapted from Carey et al. 2002).

1.3.2 Lethal Effects

A number of studies have been conducted evaluating the lethal effects of WBL exposure on aquatic biota, with most of these studies conducted on effluents from Canadian mills. An eight-month study at a mill in British Columbia examined the effectiveness of an aerated lagoon in detoxifying kraft pulp mill effluent using acute toxicity tests with sockeye fry (*Oncorhynchus nerka*). Results were that aerated lagoons were generally capable of detoxifying effluent; there were, however, some instances where black liquor spills were responsible for substandard detoxification. These black liquor spills sometimes disrupted treatment system performance, which resulted in final effluent toxicity (Servizi and Gordon 1973). Similarly, a one-month study of a bleached kraft mill in British Columbia examined the effectiveness of an ASB in removing toxicity using 96-h tests with *O. mykiss* (Chandrasekaran et al. 1978). Examination of the bioassay results were that toxic compounds in the mill effluent were biodegradable and treated effectively in an ASB, peak black liquor losses, however, coincided with peak values of both toxicity emission rate (TER) and toxicity emission index (TEI).

Using Cycle 2 EEM data from nine bleached kraft mills in British Columbia (as described in Section 1.3.1), Carey et al. (2002) examined the relationship between lethal effects to fish and estimated WBL losses. Results from this analysis do not suggest that there is a relationship between WBL losses and acute toxicity to fish.

Kelley et al. (2004) studied the acute toxicity of untreated strong black liquor from an elemental chlorine free (ECF) kraft mill, where strong black liquor is 50-70% solids compared to 15% solids in WBL. Tests were conducted to determine the 48-h median lethal loading rate (LL50) for *D.magna* and the 96-h LL50 for *P. promela*s (where loading rate is defined as the total amount of substance added to water). Results were that the LL50 for both organisms was >1000 mg/L strong black liquor (Kelly et al. 2004).

1.4 Project Overview and Research Objectives

The primary objectives of this study were to evaluate the contribution of WBL in biologically treated kraft mill effluents to chronic effluent toxicity, and to assess effluent chemical parameters that may correlate with biological responses and increased WBL solids. The following sections (1.4.1 through 1.4.3) provide a brief overview of project and research objectives with more detailed descriptions of individual test methods in Section 2.

1.4.1 Laboratory Biotreatment

To simulate a range of systemic WBL losses and WBL spills at a pulp mill, different volumes of WBL (i.e. WBL treatments) were added to untreated wastewater (influent) from four U.S. bleached kraft pulp mills. This resulted in a range of WBL concentrations, in addition to a control where no WBL was added, that were biologically treated in benchtop laboratory bioreactors. The objective of the laboratory biotreatment was to mimic the secondary treatment of pulp mill effluent, and produce a series of simulated effluents in which varying levels of WBL losses had occurred.

1.4.2 Toxicity Testing

48-h *Mytilus galloprovincialis* Embryo-Larval Development Test

Following laboratory biotreatment, the chronic toxicity of the resulting simulated effluents were evaluated using 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests. In addition to toxicity tests conducted with the simulated effluent samples, tests were also conducted using mill-treated effluents without added WBL. The objective of these tests was to determine if toxicity increased as the amount of WBL solids in the simulated effluent samples increased.

Mytilus galloprovincialis were selected as the primary test organism for this study due to their sensitivity to toxicants, ease of use for conducting multiple chronic WET tests concurrently, and the availability of quality test organisms from a local supplier. Additionally, tests with *Mytilus spp.* are cost effective; do not require expensive equipment; and are short term assays, taking hours or days rather than weeks for completion (Johnson 1988).

7-d *Ceriodaphnia. dubia* Survival and Reproduction

Although *C. dubia* are commonly used by pulp mills for the purpose of meeting NPDES WET testing requirements (U.S. EPA 2011b), it was not logistically feasible to conduct toxicity tests using *C. dubia* on all of the simulated effluents concurrently for this project. As such, a sub-set of the simulated effluent samples were selected for 7-d *C. dubia* survival and reproduction tests conducted by researchers at the National Council for Air and Stream

Improvement (NCASI; see Section 1.4.4). All of the mill-treated effluents samples were also evaluated using the 7-d *C. dubia* survival and reproduction tests.

The objective of these tests was to determine if toxicity increased as the amount of WBL solids in the simulated effluent samples increased. Results from the *C. dubia* toxicity tests were also compared to results of *M. galloprovincialis* toxicity tests to evaluate the relative sensitivities of these two test organisms with respect to WBL, and to determine if there was a correlation in toxicity response between these two test methods.

1.4.3 Chemical Analysis

Upon completion of laboratory biotreatment, all mill-treated and simulated effluent samples were analyzed for the following chemical parameters: pH, color, conductivity, turbidity, total suspended solids (TSS), polyphenols, hardness, alkalinity, salinity, BOD, dissolved chemical oxygen demand (DCOD), DOC, phytosterols, and RAs. The purpose of these analyses was to see if any of the chemical parameters correlated with: 1) toxicity to *C. dubia*; 2) toxicity to *M. galloprovincialis*; and 3) increased WBL solids. It should be noted that if there is good laboratory biotreatment efficiency, then the concentrations of some of the chemical parameters will likely not correlate with increased WBL solids.

1.4.4 Collaboration with National Council for Air and Stream Improvement

Research for this project was supported by NCASI, and is part of a larger on-going study examining the effects of biologically treated WBL to aquatic organisms. The author of this thesis is a researcher at the NCASI Northwest Aquatic Biology Facility (NABF), and other NCASI laboratories assisting with research for this project are shown in Table 1. Some of the laboratory work and results presented in this report were conducted solely by NCASI researchers without the author's assistance, and are noted as such in the Table 2 and in the Methods section (Section 2). All toxicity tests with *M. galloprovincialis* were carried out at the NCASI NABF with NCASI researchers assisting the author with test set-up and termination. Basic chemical analysis of all effluent samples was conducted at the NCASI NABF laboratory, with some laboratory assistance from NCASI researchers.

Table 1. List of National Council for air and Stream Improvment facilities contributing to research efforts.

NCASI Laboratory	Location	Expertise
Northwest Aquatic Biology Facility (NABF)	Anacortes, WA	Aquatic Biology
Southern Aquatic Biology Facility (SABF)	New Bern, NC	Aquatic Biology
West Coast Regional Center (WCRC)	Corvallis, OR	Chemistry

Table 2. Laboratory activities conducted solely by National Council for Air and Stream Improvement researchers. SABF: Southern Aquatic Biology Facility; WCRC: West Coast Regional Center; WBL: weak black liquor; ASB-TPWW: aerated stabilization basin treatment pond wastewater; ASB-influent: aerated stabilization basin influent.

NCASI Facility	Parameter	Sample
SABF	7-d *C. dubia* survival and reproduction toxicity tests	mill-treated effluents, simulated effluents
	Laboratory biotreatment (Mill D)	simulated effluents
WCRC	Biochemical oxygen demand (BOD)	WBL
	Chemical oxygen demand (COD)	WBL, ASB-influent, ASB-TPWW
	Conductivity	ASB-influent
	Dissolved chemical oxygen demand (DCOD)	mill-treated effluents, simulated effluents, WBL, ASB-influent, ASB-TPWW
	Dissolved organic carbon (DOC)	mill-treated effluent, simulated effluents, WBL, ASB-influent, ABS-TPWW
	Laboratory biotreatment (Mills A, B, C)	simulated effluents
	Resin acids (pimaric acid, sandrocopimaric acid, isopimaric acid, palustric acid, dehydroabietic acid, abietic acid, neoabietic acid)	mill-treated effluents, simulated effluents
	Solids analysis	WBL
	Sterols (campesterol, stigmastanol, beta-sitosterol, stigmasterol)	mill-treated effluents, simulated effluents
	Total organic carbon (TOC)	WBL, ASB-TPWW
	Total suspended solids (TSS)	ASB-TPWW, ASB-influent, ASB-TPWW

1.4.5 Research Contribution

Results from this study will benefit the pulp and paper industry by helping mill managers further identify mill effluent components that may be contributing to final chronic effluent toxicity. This will allow them to implement BMP necessary to reduce WBL losses and potential adverse impacts to the aquatic environment.

2.0 METHODS

2.1 Mill Selection

Four bleached kraft pulp mills were selected by NCASI researchers for this study using Fisher-Solve™, a database containing information about every operating pulp and paper mill in the world making over 50 tons per day. The selection criteria for the four mills were as follows: 1) bleached kraft pulp mills; 2) two mills from the pacific northwest region and two from the southeast region of the United States; 3) wood furnishes within each region having a similar mix of species; 4) all mills having low WBL losses when operating under normal conditions; 5) mills with ASB biotreatment systems (i.e. no activated sludge plants); and 6) mills with either integrated paper making or market pulp mills (Steve Stratton, Regional Manager, NCASI, personal communication). An integrated mill is a mill where pulp, pulp and paper, or pulp and paperboard are produced, whereas a non-integrated mill is one where paper or paperboard are manufactured but pulp is not produced on-site (Hanmer 1988). In the case of integrated mills, a further criterion was that purchased + recycled fiber should be < 20% total with a minimum of 80% of the pulp made on site. A summary of mill processes and furnish types for the four selected mills for this study are shown in Table 3

Table 3. Summary of mill process type and furnish type using data from Fisher-Solve™. TPY: tons of pulp per year; ADST: air dry short tons; PNW: pacific northwest; SE: southeast.

				% TPY (pulp)			
Mill	Region	Site Type	TPY All Pulp by Site (ADST)	Fir	Pine	Oak	Gum
Mill A	PNW	Integrated*	217,350	100			
Mill B	PNW	Integrated	549,780	90	10		
Mill C	SE	Integrated*	367,500		100		
Mill D	SE	Integrated	331,508		71	15	15

*Pulp only

2.2 Sample Collection

The week prior to study initiation, sample bottles were shipped to each of the four mills to collect WBL. This WBL sample was used to conduct solids and other analytical measurements (BOD, COD, DCOD, DOC, and TOC) by NCASI researchers at the WCRC in advance of study initiation, and was used to determine appropriate WBL spiking concentrations for the simulated effluents (Table 2).

One week later (study initiation), the following samples were collected from each of the four mills: 60 L of ASB influent, 10 L of mill-treated effluent, 10 L of ASB treatment pond wastewater (ASB-TPWW; liquid taken from the ASB near the influent end to provide a source of active biological seed), and 1 L of WBL. For mills A and D, NCASI researchers collected the samples on-site. Samples for Mills B and C were collected by mill personnel. In all cases, samples were either transported in person or shipped on ice priority overnight to the appropriate NCASI laboratory. Sample collection dates are shown in Table 4.

Table 4. Sample collection dates at study initiation for mill-treated effluent, aerated stabilization basin influent, and aerated stabilization basin treatement pond wastewater. NCASI: National Council for Air and Stream Improvement.

Mill	Sampling Collection Date	Collected By
A	08/31/2010	NCASI researchers
B	10/19/2010	Mill personnel
C	11/02/2010	Mill personnel
D	02/08/2011	NCASI researchers

Mill-treated effluent samples were sent from each mill directly to: 1) NCASI NABF laboratory for chemical analysis and 48-h *M. galloprovincialis* embryo-larval development toxicity tests, 2) NCASI SABF for 7-d *C. dubia* survival and reproduction toxicity tests, and 3) NCASI WCRC for chemical analysis (Figure 4).

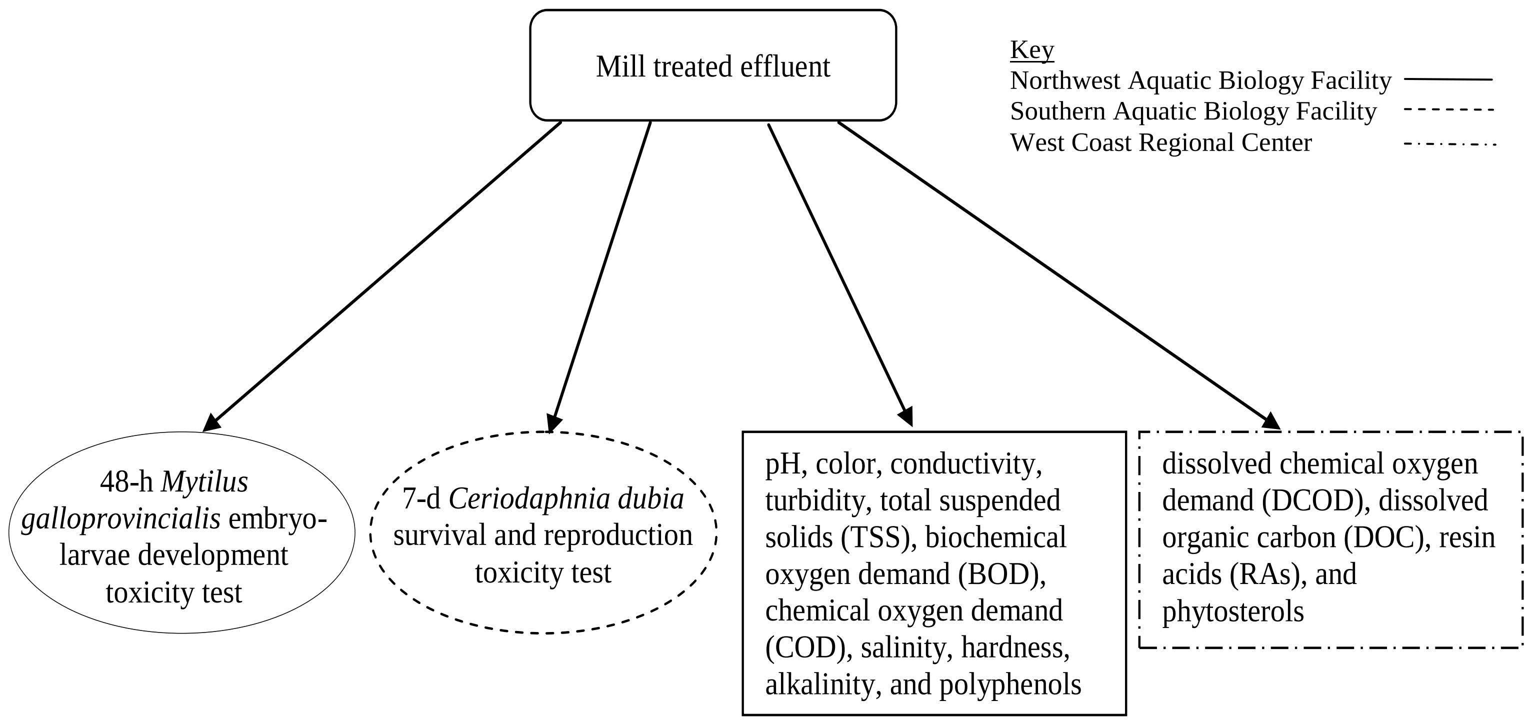


Figure 4. Mill-treated effluent sample analysis. Circles indicate toxicity tests, squares indicate chemical analysis, line type indicates National Council for Air and Stream Improvement laboratory

The ASB influent, ASB-TPWW, and WBL were sent to either the NCASI WCRC (Mills A, B, and C) or the NCASI SABF (Mill D) for laboratory biotreatment. After laboratory biotreatment, these simulated effluent samples were sent to: 1) NCASI NABF laboratory for chemical analysis and 48-h *M. galloprovincialis* embryo-larval development toxicity tests, 2) NCASI SABF for 7-d *C. dubia* survival and reproduction toxicity tests, and 3) NCASI WCRC for chemical analysis (Figure 5). Samples of the ASB influent and ASB-TPWW were also sent to the WCRC for analysis of COD, conductivity, DCOD, DOC, TOC, and TSS (Table 2).

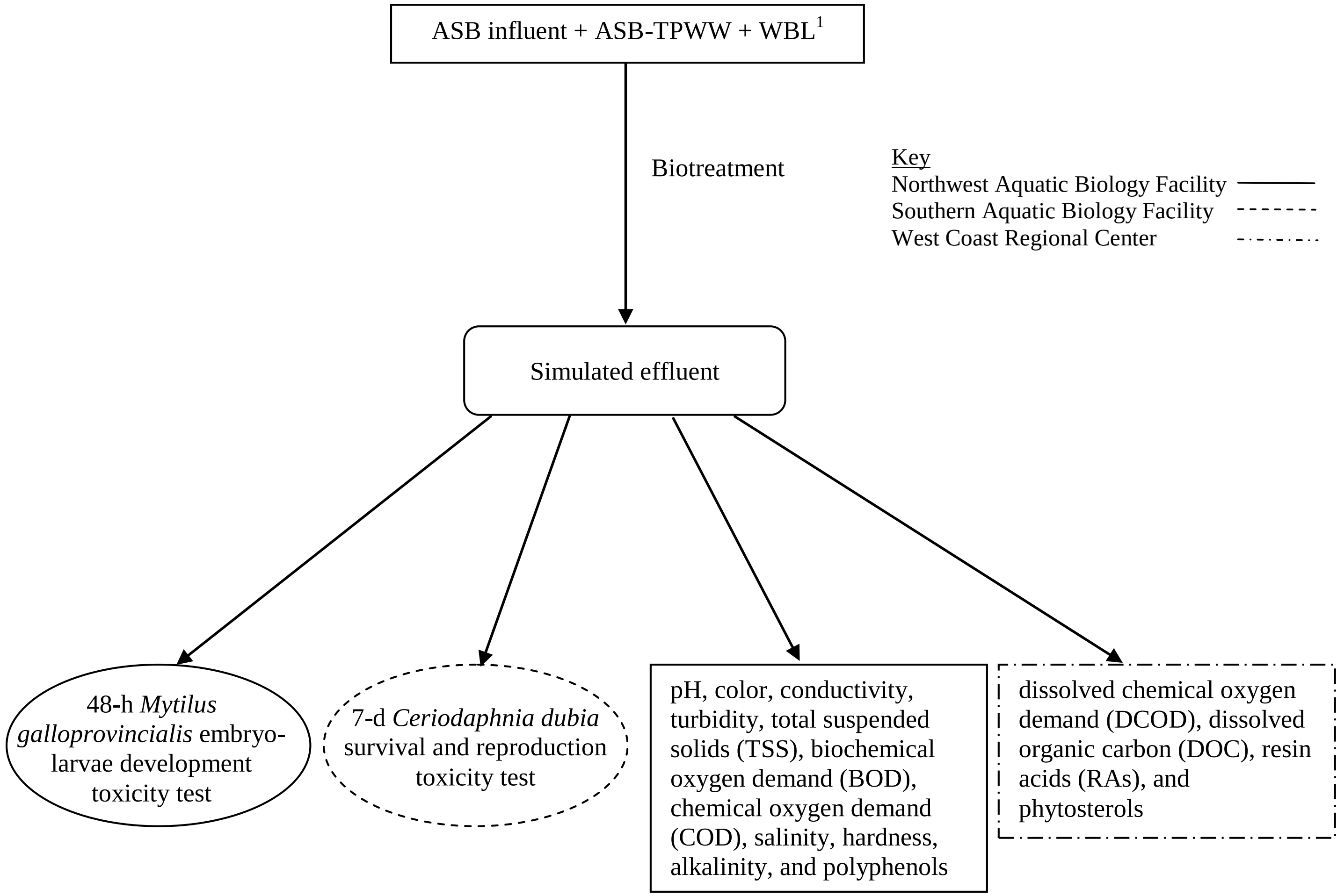


Figure 5. Simulated effluent sample analysis, where simulated effluent is laboratory biotreated aerated stabilization influent, aerated stabilization basin treatment pond wastewater, and weak black liquor (where relevant) collected from the mill. Circles indicate toxicity tests, squares indicate chemical analysis, line type indicates National Council for Air and Stream Improvement laboratory. [1]Weak black liquor was not added to one simulated effluent sample per mill, and is identified in this report as the reactor control. *Ceriodaphnia dubia* tests conducted for a subset of simulated effluent samples.

2.3 Laboratory Biotreatment

For each of the four mills, a series of simulated effluents were created in the laboratory using bench top aerobic reactors (Figure 6). Laboratory biotreatment of simulated effluents was carried out by NCASI researchers at the WCRC or the SABF. Biotreatment at the WCRC was conducted using six bench top aerobic reactors for Mills A, B and C, while biotreatment at SABF used five bench top aerobic rectors for Mill D. Biotreatment reactors were prepared using 8.5 L of ASB-influent and 1.5 L of ASB-TPWW collected at the front end of the treatment pond to ensure the reactors were sufficiently biologically active. Dissolved oxygen content was monitored throughout the study to verify that the system did not become oxygen deficient and pH and temperature were monitored to ensure they were consistent with typical treatment system processes. The standard operating procedure (SOP) used at the NCASI WCRC and the NCASI SABF for biotreatment of simulated effluent samples can be found in Appendix A.

To represent a range of WBL losses at a mill, six simulated effluents were created for mills A, B, and C, and five simulated effluents were created for Mill D. Appropriate spiking concentrations were determined by NCASI researchers at the WCRC. Factors considered in calculating WBL spiking levels included: 1) the solids content, COD, DCOD, and DOC of WBL collected one week prior to test initiation; 2) the COD, DCOD, and DOC analysis of ASB-influent, and ASB-TPWW collected at test initiation; and 3) the COD, DCOD, TSS, and DOC analysis of the unspiked reactor control measured on the day before and on the day WBL was added to the spiked reactors (Table 2). Analytical test methods can be found in Section 2.6 (COD, DCOD, DOC, TSS, and WBL solids). Ultimately, WBL spiking concentrations were based primarily on COD normalized on a WBL solid weight basis. Weak black liquor spike levels (i.e. WBL treatments) for simulated effluents were chosen to cover a range of systemic WBL losses, with the highest spiked reactor containing enough WBL to equal approximately two times the background COD concentration measured in unspiked reactor control and the lowest spiked reactor containing enough WBL so that the COD spike was significantly greater than background COD levels in the reactor control. A summary of the simulated effluents for each of the four mills, along with the chosen WBL spiking levels are shown in Table 5.

This simulated effluent served as a control, hereafter referred to as "reactor control", as no WBL was added. The purpose of the reactor control was to establish the baseline toxicity and concentrations of chemical parameters for the simulated effluent samples. The remaining reactors contained ASB influent, ASB-TPWW, and different additions of WBL (hereafter referred to as "spiked reactors"). The reactor control and spiked reactors will be referred to as simulated effluents when no distinction between the two is needed.

Every 1-3 d, a small aliquot was taken from each reactor for DCOD analysis by WCRC or SABF researchers. The reactors were run until a stable DCOD was reached in all of the reactors, at which point the simulated effluents were considered fully treated and ready for chronic toxicity testing (with *M. galloprovincialis* and *C. dubia*) and chemical analysis (Figure 5). For Mill B, Mill C, and Mill D, simulated effluent samples were allowed to settle before they were sampled. Because Mill A samples were not settled, for Mill B and Mill C, a side by side comparison of settled and unsettled samples taken from the highest spiked reactor (WBL spike 5) were analyzed to determine if the toxicity results were comparable (see Section 3.3.1).

Figure 6. West Coast Regional Center laboratory biotreatment of Mill A simulated effluent samples. The reactor on the left contains only aerated stabilization basin influent and aerated stabilization basin treatment pond wastewater (reactor control), and the reactor on the right contains aerated stabilization basin influent, aerated stabilization basin treatment pond wastewater, and the highest spike level of weak black liquor (WBL spike 5).

Table 5. Summary of simulated mill effluents for each of the four mills (WBL: weak black liquor).

Mill	Description	Effluent Sample Code	Spiking Level (WBL solids added (g/L))
A	Reactor control	A-RWW-Ctrl	0.000
	WBL spike 1	A-RWBL-1	0.097
	WBL spike 2	A-RWBL-2	0.193
	WBL spike 3	A-RWBL-3	0.290
	WBL spike 4	A-RWBL-4	0.386
	WBL spike 5	A-RWBL-5	0.483
B	Reactor control	B-RWW-Ctrl	0.000
	WBL spike 1	B-RWBL-1	0.092
	WBL spike 2	B-RWBL-2	0.185
	WBL spike 3	B-RWBL-3	0.277
	WBL spike 4	B-RWBL-4	0.370
	WBL spike 5	B-RWBL-5	0.462
C	Reactor control	C-RWW-Ctrl	0.000
	WBL spike 1	C-RWBL-1	0.083
	WBL spike 2	C-RWBL-2	0.167
	WBL spike 3	C-RWBL-3	0.250
	WBL spike 4	C-RWBL-4	0.334
	WBL spike 5	C-RWBL-5	0.417
D	Reactor control	D-RWW-Ctrl	0.000
	WBL spike 1	D-RWBL-1	0.103
	WBL spike 2	D-RWBL-2	0.206
	WBL spike 4	D-RWBL-3	0.309
	WBL spike 5	D-RWBL-5	0.515

2.4 48-h *Mytilus galloprovincialis* Embryo-larval Development Tests

2.4.1 Test Method

For each of the four mills, 48-h *M. galloprovincialis* embryo-larval development toxicity tests were conducted with mill-treated effluent and each of the simulated effluents. A list of *M. galloprovincialis* toxicity test methods and endpoints are shown in Table 6. All toxicity tests with *M. galloprovincialis* were carried out at the NCASI NABF in Anacortes, WA and followed U.S. EPA methods (1995) with modifications described in Washington State Department of Ecology (WDOE 2008). For each mill, toxicity tests with the mill-treated effluent were conducted prior to toxicity tests with the simulated mill effluents (Table 7). A dilution series with seven concentrations of effluent were used, with filtered seawater the dilution water, for tests with mill-treated and simulated effluents. An individual effluent concentration in a dilution series for each effluent sample is hereafter referred to as "toxicity test treatment," and is designated as toxicity test treatment 1-7 (with 1 having the lowest percent effluent concentration and 7 having the highest percent effluent concentration). Each toxicity test treatment contained four replicates (designated A, B, C, and D).

Effluent samples were held at 4˚C until testing, which occurred 1-4 d after sampling of mill-treated effluent or simulated effluents (Table 7). For each set of toxicity tests, a reference toxicant test with copper chloride (CuCl) was run concurrently following methods described by the U.S. EPA (1995).

Table 6. 48-h *Mytilus galloprovincialis* toxicity test descriptions, test methods, endpoints and contribution to research. WBL: weak black liquor.

Sample		Test Method	Endpoint	Contribution to research
Mill-treated effluent		U.S. EPA 1995, WDOE 2008	EC50	Determine if mill-treated effluent is toxic to *M. galloprovincialis*.
Reference toxicant (CuCl)[1]		U.S. EPA 1995, WDOE 2008	EC50	Determine quality and sensitivity of test organisms.
Simulated effluent	Reactor control	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with no added WBL is toxic to *M. galloprovincialis*.
	WBL spike 1	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with added WBL is toxic to *M. galloprovincialis*.
	WBL spike 2	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with added WBL is toxic to *M. galloprovincialis*.
	WBL spike 3	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with added WBL is toxic to *M. galloprovincialis*.
	WBL spike 4	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with added WBL is toxic to *M. galloprovincialis*.
	WBL spike 5	U.S. EPA 1995, WDOE 2008	EC50	Determine if simulated effluent with added WBL is toxic to *M. galloprovincialis*.

[1]CuCl reference toxicant test run concurrently with mill-treated effluent toxicity test and with simulated effluent toxicity tests.

Table 7. Summary of *Mytilus galloprovincialis* toxicity tests. WBL: weak black liquor.

Mill	Sample Description	Sample Code	Sampling/ Collection Date	Test Initiation	Test Termination
A	Mill effluent	A-Effluent	8/31/2010	9/1/2010	9/3/2010
	Reference toxicant: CuCl	A-RT-Effluent		9/1/2010	9/3/2010
	Unspiked reactor control	A-RWW-Ctrl	9/8/2010	9/9/2010	9/11/2010
	WBL spike 1	A-RWBL-1	9/8/2010	9/9/2010	9/11/2010
	WBL spike 2	A-RWBL-2	9/8/2010	9/9/2010	9/11/2010
	WBL spike 3	A-RWBL-3	9/8/2010	9/9/2010	9/11/2010
	WBL spike 4	A-RWBL-4	9/8/2010	9/9/2010	9/11/2010
	WBL spike 5	A-RWBL-5	9/8/2010	9/9/2010	9/11/2010
	Reference toxicant: CuCl	A-RT-Reactors		9/9/2010	9/11/2010
B	Mill effluent	B-Effluent	10/19/2010	10/22/2010	10/24/2010
	Reference toxicant: CuCl	B-RT-Effluent		10/22/2010	10/24/2010
	Unspiked reactor control	B-RWW-Ctrl	10/28/2010	11/1/2010	11/3/2010
	WBL spike 1	B-RWBL-1	10/28/2010	11/1/2010	11/3/2010
	WBL spike 2	B-RWBL-2	10/28/2010	11/1/2010	11/3/2010
	WBL spike 3	B-RWBL-3	10/28/2010	11/1/2010	11/3/2010
	WBL spike 4	B-RWBL-4	10/28/2010	11/1/2010	11/3/2010
	WBL spike 5	B-RWBL-5	10/28/2010	11/1/2010	11/3/2010
	WBL spike 5 (unsettled)	B-RWBL-5	10/28/2010	11/1/2010	11/3/2010
	Reference toxicant: CuCl	B-RT-Reactors		11/1/2010	11/3/2010
C	Mill effluent	C-Effluent	11/2/2010	11/4/2010	11/6/2010
	Reference toxicant: CuCl	C-RT-Effluent		11/4/2010	11/6/2010
	Unspiked reactor control	C-RWW-Ctrl	11/15/2010	11/17/2010	11/19/2010
	WBL spike 1	C-RWBL-1	11/15/2010	11/17/2010	11/19/2010
	WBL spike 2	C-RWBL-2	11/15/2010	11/17/2010	11/19/2010
	WBL spike 3	C-RWBL-3	11/15/2010	11/17/2010	11/19/2010
	WBL spike 4	C-RWBL-4	11/15/2010	11/17/2010	11/19/2010
	WBL spike 5	C-RWBL-5	11/15/2010	11/17/2010	11/19/2010
	WBL spike 5 (unsettled)	B-RWBL-5	10/28/2010	11/1/2010	11/3/2010
	Reference toxicant: CuCl	C-RT-Reactors		11/17/2010	11/19/2010
D	Mill effluent	D-Effluent	2/8/2011	2/9/2011	2/11/2011
	Reference toxicant: CuCl	D-RT-Effluent		2/9/2011	2/11/2011
	Unspiked reactor control	D-RWW-Ctrl	2/17/2011	2/21/2011	2/23/2011
	WBL spike 1	D-RWBL-1	2/17/2011	2/21/2011	2/23/2011
	WBL spike 2	D-RWBL-2	2/17/2011	2/21/2011	2/23/2011
	WBL spike 3	D-RWBL-3	2/17/2011	2/21/2011	2/23/2011
	WBL spike 5	D-RWBL-5	2/17/2011	2/21/2011	2/23/2011
	Reference toxicant: CuCl	D-RT-Reactors		2/21/2011	2/23/2011

2.4.2 Test Organisms

Mytilus galloprovincialis (Figure 7) is a test species approved by the U.S. EPA for chronic WET testing under the NPDES permitting program. Test organisms raised in Totten Bay Inlet near Shelton, WA were obtained from Taylor Shellfish Farm, Bellingham, WA, and housed at Shannon Point Marine Center (SPMC) in Anacortes, WA in flow-through seawater tanks until test initiation.

Figure 7. *Mytilus galloprovincialis*. (Source: http://nas.er.usgs.gov/)

2.4.3 Brine Preparation

A few days prior to the test, hypersaline brine (HSB) for use in adjusting the salinity of test solutions was prepared by filtering seawater collected from SPMC. Seawater was collected from the spigots supplying seawater to the test organisms using 1-gal glass bottles. Sample bottles were rinsed thoroughly with seawater from the spigot prior to collection. At the

NABF laboratory, seawater was filtered using a 10-µm Nitex cloth placed over a plastic funnel. The plastic funnel was placed into a 1-L Erlenmeyer flask. Once filtered, approximately 3 L of filtered seawater was poured into a 1-gal Cubitainer® and placed in a freezer for 24 to 36 h, or until frozen solid. A day in advance of the test, the Cubitainer® was removed from the freezer and placed onto the laboratory bench. The spigot was opened, and as the seawater melted, the liquid was collected in a 1-L beaker. The salinity of the melting seawater was checked periodically using a refractometer until the brine reached 100 parts per thousand (‰). Once the brine reached 100 ‰, it was covered with plastic wrap and stored at 4°C until test day.

2.4.4 Filtered Seawater Preparation

The day before the test, seawater was collected from SPMC to prepare filtered seawater for use in test solutions. Seawater filtering and collection methods followed those described previously. Once filtered, seawater was poured into 1-L glass beakers, covered with plastic wrap, and placed into an environmental chamber adjusted to 18 ± 1°C. A non-mercury glass thermometer was used to check the temperature of the environmental chamber.

2.4.5 Test Chambers

The day before the test, test chambers (shell vials: 7-dram, 29.35 x 65 mm) were labeled with the test treatment (C for control, BC for brine control, and #1-7 for the seven effluent dilutions) and replicate (A-D). An additional replicate (E) was added for the purpose of measuring chemistry in the test chamber upon test termination. Additionally, six vials were labeled for stocking densities. For the reference toxicant test, there were six test Cu treatments (test treatments #1-6) and a control (test treatment C) with five replicates (A-E), and six vials labeled for stocking densities. Test chambers were placed into glass baking dishes and arranged with rows of replicates of the same concentration, and in increasing concentration for ease of distributing test solutions into the vials (Figure 8). Each baking dish was labeled with the appropriate sample code (Table 7). The glass baking dishes were covered with plastic wrap until test initiation (Figure 9).

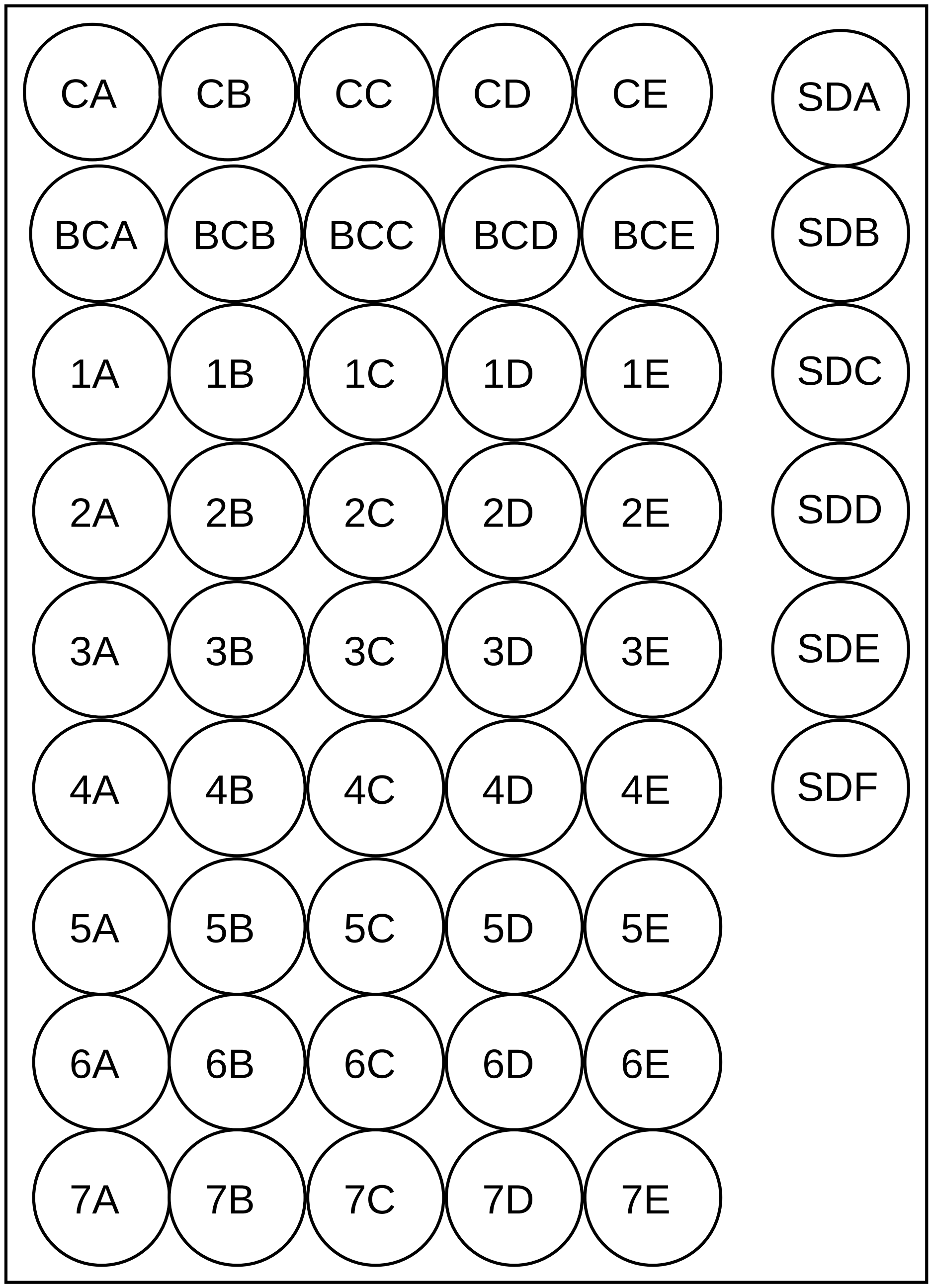


Figure 8. Diagram of a single 48-h *Mytilus galloprovincialis* effluent toxicity test set-up showing vials labeled to include all replicate tests concentrations, controls, and stocking densities (SD).

Figure 9. Test chambers for *Mytilus galloprovincialis* 48-h embryo-larval development toxicity tests.

2.4.6 Toxicity Test Solution Preparation

All toxicity test solutions, controls for effluent (mill-treated and simulated), and reference toxicant tests were made using the filtered seawater stored in the environmental chamber at 18 ± 1°C and the HSB stored at 4°C. For each toxicity test concentration, 70 ml of test solution was mixed in 25 x 200 mm KIMAX borosilicate glass culture tubes or in 250-ml glass beakers.

In addition to a seawater control, a brine control was used for all effluent (simulated and mill-treated) toxicity tests. The brine control was prepared by mixing HSB and Barnstead DI water to achieve the same volume of brine found in the highest effluent toxicity test concentration tested using brine (i.e. if 70% effluent is the highest effluent concentration tested, 21 ml HSB was used per 70 ml test solution; Table 8). Once prepared, the brine control was poured into a 1-L glass beaker, covered with plastic wrap, and placed into an environmental chamber adjusted to 18 ± 1°C.

Toxicity test treatments for mill-treated and simulated effluents included: a control (filtered seawater), brine control (HSB + Barnstead DI water), and seven effluent concentrations for Mills A and B (1.09, 2.19, 4.38, 8.75, 17.5, 35, and 70% effluent) and for Mills C and D (1.09, 2.19, 3.28, 4.38, 8.75, 17.5, and 35 effluent; Table 8). The target test salinity for the effluent toxicity tests was 30 ± 2 ‰. Effluent test concentrations and volumes are shown in Table 8.

Table 8. Mixing chart for mill-treated and simulated effluent toxicity test solutions for exposure concentrations in *Mytilus galloprovincialis* tests.

Effluent concentration (%)	Total Volume required (ml)	Effluent (ml)	Brine (ml)	Filtered Seawater (ml)
[a]70.00	70	49.00	21.00	0.00
35.00	70	24.50	10.50	35.00
17.50	70	12.25	5.25	52.50
8.75	70	6.13	2.63	61.25
4.38	70	3.06	1.31	65.63
[b]3.28	70	2.30	0.98	66.72
2.19	70	1.53	0.66	67.81
1.09	70	0.77	0.33	68.91

[a] Not used for weak black liquor spike 5 for Mills C and D
[b] Used only for weak black liquor spike 5 for Mills C and D

A reference toxicant test using CuCl was run concurrently with each set of mill-treated and simulated effluent toxicity tests according to U.S. EPA methods (U.S EPA 1995). Test treatments for all reference toxicant tests included: a control (filtered sweater) and six CuCl test concentrations (3.0, 4.5, 6.6, 9.6, 13.8, & 20.4 μg Cu/L as CuCl) with five replicates per treatment and six stocking densities. Barnstead DI water was used to make the copper stock solutions (2000 mg/L) and sub-stock solution (3 mg/L).

After test solutions were mixed, salinity was checked to ensure that all test solutions were 30 ‰ ± 2. Each test chamber was then filled with 10 ml of appropriate test solution using a 10-ml automatic pipette. Stocking density test chambers (n=6 per test) were filled with 10 ml of filtered seawater. Once filled, test chambers were randomized within each baking dish, covered with plastic wrap, and placed in the environmental chamber set at 18 ± 1°C until test initiation.

The remaining 20 ml of test solution in the culture tubes/beakers (extra solution left over after filling the test chambers) were covered with plastic wrap and placed in the environmental chamber set at 18 ± 1°C and used later to determine pH and temperature at test initiation (Appendix B).

2.4.7 Spawning

The morning of the test, 18 adult *M. galloprovincialis* were collected from the SPMC holding tanks along with seawater to be used for spawning. Seawater was collected from the spigots using several 1-gal glass bottles and 1-gal Cubitainers®. At the NABF, mussels were placed in a glass baking dish containing unfiltered seawater and placed into an environmental chamber set at 12°C to prevent the mussels from spawning prematurely.

To induce spawning, mussels were placed in a glass baking dish and covered with unfiltered seawater heated to 20°C (Figure 10). Algae concentrate (Algae Diet C-5, Hilton's Coast Seafoods Company), was also used to promote spawning. A dilute mixture of algae was prepared by adding a small amount of algae concentrate with a disposable plastic pipette into a plastic specimen cup filled with 20°C seawater. The diluted algal mixture was then added to the glass baking dish containing the mussels to induce the mussels to pump.

Individuals that began to spawn were quickly removed from the baking dish, rinsed with unfiltered seawater to remove any live sperm, and placed into 250-ml spawning beakers containing 20°C unfiltered seawater to collect the gametes. Each spawning beaker was labeled with the time spawning began and the sex of the gametes (Figure 11). Spawning information was also recorded on the *Bivalve Spawning Data Sheet* (Appendix C).

Figure 10. *Mytilus galloprovincialis* prior to test spawning.

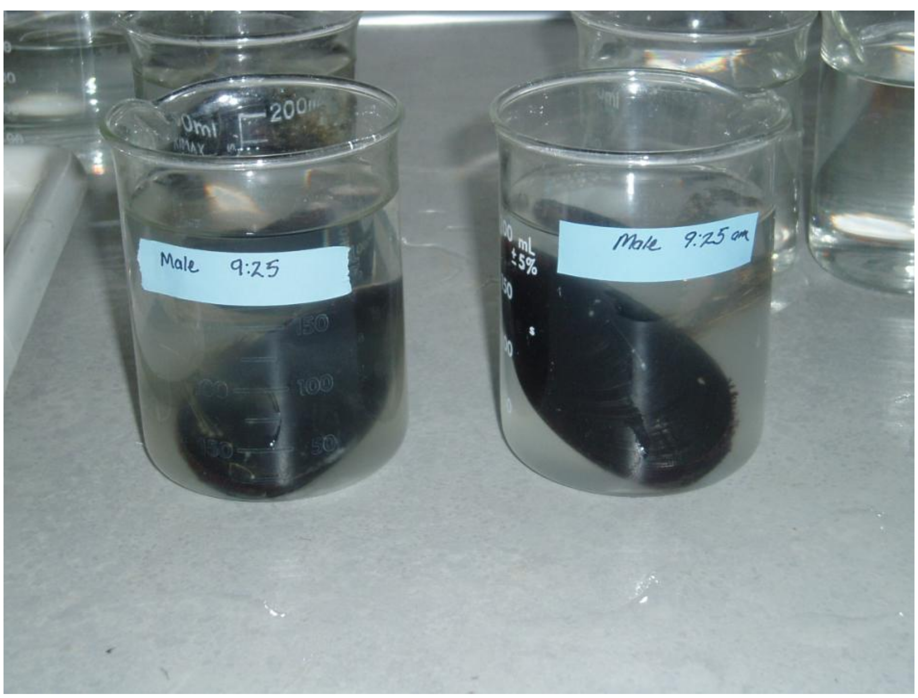


Figure 11. Spawning beakers with male *Mytilus galloprovincialis*.

2.4.8 Selection of Gametes

Samples of gametes were examined under an inverted microscope to determine quality. Only the highest quality gametes (highly active sperm and full round eggs) were selected for use. Gametes from at least two males and two females were pooled and used on each test day. A clean transfer pipette was used to combine equal amounts of eggs from each female in a 400-ml glass beaker containing approximately 200 ml of filtered 18°C seawater and labeled as "egg stock solution." Sperm were pooled by pouring an equal volume of liquid from each male spawning beaker into a 400-ml beaker labeled, "sperm stock solution."

2.4.9 Fertilization

Three embryo solutions were prepared by carefully pouring equal volumes of the egg stock solution into three 250-ml beakers. Each beaker was diluted to approximately 200 ml using 18°C filtered seawater; the volume of dilution water added was dependent on the concentration and volume of the original egg stock solution. Each beaker was diluted to achieve a density of approximately 150 eggs per 10 ml; this was accomplished by adding dilution water to the three beakers until print could be read through the beakers. A manual pipette was used to add 100, 300, or 700 µl of sperm stock solution to the three beakers. The three embryo solutions were mixed gently with glass Pasteur pipettes, and time of fertilization was recorded on the *Bivalve Spawning Data Sheet* (Appendix C).

Embryo solutions were kept in suspension by frequent agitation with glass Pasteur pipettes. Approximately 1-h after fertilization, fertilization success was checked in each of the three embryo solutions. After gentle stirring with a Pasteur pipette, a 20 µl aliquot of each embryo solution was placed onto a glass microscope slide and examined using an inverted microscope. Percent fertilization was determined by counting the number of fertilized and unfertilized eggs in each sperm dilution. Fertilization was determined by: 1) the presence of a polar body; or 2) cell division. Of the three embryo solutions, the solution with > 90% fertilization and with the lowest volume of sperm added was selected. Once the appropriate embryo solution was selected, three 20-ml aliquots were counted to calculate mean percent fertilization and to determine the appropriate embryo stocking volume. The embryo stocking volume was calculated so that approximately 150 embryos were stocked into each test chamber. Embryos from the same spawn were used when multiple tests were run concurrently.

2.4.10 Test Initiation

Fertilized embryos were added to each test chamber within 4 h of fertilization using a micropipette. Embryos were kept in suspension by gentle stirring with a glass Pasteur pipette during stocking. After embryos were added to each test chamber, the stocking density test chambers for each test were removed and fixed by adding 100 μl of formaldehyde to each vial; these were later used to determine initial stocking density of test organisms (Section 2.4.12). The glass baking dish was covered with plastic wrap and placed into the environmental chamber set at 18 ± 1°C with a the photoperiod set at 16 h light/8 h dark (Figure 12). Because multiple tests were run simultaneously, baking dishes were arranged randomly within the environmental chamber to minimize any potential light or temperature gradients. Onset Stow Away TidbiT Temp Loggers in the environmental chamber were used to monitor temperature continuously throughout the duration of the tests for Mill B, Mill C and Mill D (Appendix D). Two non-mercury glass thermometers were used to measure temperature twice per day in the environmental chambers during the tests for Mill A.

For each effluent (mill-treated or simulated effluent) or reference toxicant test, pH and temperature were measured in both the lowest and highest toxicity test treatment at test initiation using the 20 ml of test solution left over after dispensing test solutions to the test chambers. Additionally, pH and temperature of the control (filtered seawater) and brine control (HSB + Barnstead DI) were measured in the test solution left over after dispensing test solutions to the test chambers. Methods for measuring pH and temperature can be found in Section 2.6.

Figure 12. Environmental chamber at initiation of *48-h Mytilus galloprovincialis* test.

2.4.11 Test Termination

After 48 h, all replicates labeled, “E,” were removed from the glass baking dishes. All remaining replicates (A, B, C, D) were terminated by adding 100 μl of formaldehyde to each vial. Each test chamber was capped securely with a plastic lid until embryos could be enumerated.

Using the unpreserved test chambers (replicate E), pH, dissolved oxygen (DO) and salinity were checked in lowest and highest test concentrations for all effluent and reference toxicant tests (Appendix B). Salinity, pH, and DO were also measured on a sub-set (approximately half) of the replicate E controls (filtered seawater) and brine controls (HSB + Barnstead DI). Chemistry measurements for the sub-set of control and brine control test chambers were selected at random and included at a minimum 50% of the available control chambers. Dissolved oxygen (in mg/L) was measured with a YSI 55 meter. Details for measuring pH and salinity can be found in Section 2.6.

2.4.12 Stocking Densities

To calculate a stocking density for each test, the number of fertilized eggs was counted in each stocking density vial. The mean of the six replicates was calculated to determine the mean stocking density for each test. This number was used later as the number added to each test chamber for the percent survival calculations in the controls.

2.4.13 Enumeration of Larvae

Preserved larvae in each test chamber were counted and classified as normal or abnormal using an inverted microscope. Normal larvae were those with a clearly defined D-shaped shell (Figure 13), while abnormal larvae were veligers with an incomplete shell or trochophore larvae (Figure 14). For each test, the D-shaped cell criterion was recalibrated using the control larvae as a reference. Meatless larvae with completed D-shaped cells were counted as normal. For each test, all “A” replicates were counted first in order of increasing test concentration followed by “B” replicates (i.e. all four replicates of a test concentration were not counted in succession). If 100% abnormal larvae were observed in a test concentration, only the next highest test concentration was counted (higher test concentrations were not). If this pattern remained consistent while counting across replicates (i.e. all four replicates had two consecutive test concentrations with 100 % abnormality) then enumeration of the higher test concentrations was determined to be unnecessary. If only some of the replicates in a test concentration showed 100% abnormality, then the next highest test concentration was counted (i.e. enumeration was not terminated until there were two consecutive test concentrations showing 100% abnormality in all replicates). All larval counts were recorded on the *Bivalve Bioassay Data Sheet* (Appendix E).


Figure 13. Normal D-shaped shell of *Mytilus galloprovincialis* larvae

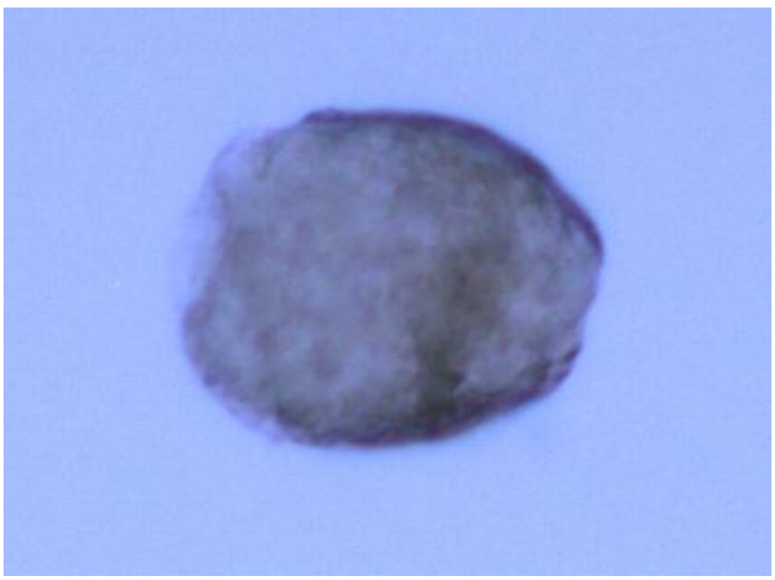


Figure 14. Abnormal *Mytils galloprovincialis* larvae

2.4.14 Quality Control

Control performance (survival and normality) and stocking density coefficient of variation (CV) were calculated for each test. The target criterion for stocking density CV was ≤ 15 and the target criterion for the controls was ≥ 70% survival compared to the stocking densities and ≥ 70% normal shell development (based on survivors; WDOE 2008).

2.4.15 Calculation of Endpoints

All endpoints for the 48-h *M. galloprovincialis* embryo-larval development toxicity tests were calculated using ToxCalc statistical software (Version 5.0.231; Tidepool Scientific Software, McKinleyville, CA, USA). The no observed effects concentration (NOEC) and lowest observed effects concentration (LOEC) values were calculated using a hypothesis testing approach such as Dunnett's Procedure or Steel's Many-one Rank Test. The EC50s were calculated using Probit regression. If data did not meet the requirements for Probit regression, the Spearman-Karber or the trimmed Spearman-Karber method was used as described by U.S. EPA (1995) and Environment Canada (2005). For each reference toxicant test with CuCl, the EC50 was calculated and plotted on a control chart maintained at the NCASI NABF.

2.5 7-d *Ceriodaphnia dubia* Survival and Reproduction Tests

A series of 7-d *Ceriodaphnia dubia* survival and reproduction chronic toxicity tests were conducted by NCASI researchers at the SABF following U.S. EPA WET test methods (U.S EPA 2002c). *Ceriodaphnia dubia* toxicity tests were conducted using the mill-treated effluent, the reactor control, and the two of the simulated effluents (WBL spike 2 and WBL spike 5) for all four mills. A summary of 7-d *C. dubia* survival and reproduction toxicity tests, endpoints, and sampling dates are shown in Table 9 and Table 10.

Test organisms, *C. dubia* neonates (≤ 24 hours old at test initiation), were obtained from in-house cultures maintained at the SABF. For all tests, synthetic moderately hard water was used as the dilution water (U.S. EPA 2002c) to create a standard 0.5 dilution series with four test concentrations (100, 50, 25 and 12.5% effluent) with five replicates per test concentration and one test organism per replicate. All endpoints for the *C. dubia* 7-d survival and reproduction toxicity tests (NOEC, LOEC, and 25% inhibition concentration [IC25[3]]) were calculated using the ICPIN statistical program. Linear interpolation was used to calculate IC25s.

Table 9. 7-d *Ceriodaphnia dubia* survival and reproduction toxicity test descriptions, test methods, endpoints and contribution to research. WBL: weak black liquor.

Sample		Test Method	Endpoint	Contribution to research
Mill-treated effluent		U.S. EPA 2002c	IC25	Determine if mill-treated effluent is toxic to *C. dubia*.
Simulated effluent	Reactor control	U.S. EPA 2002c	IC25	Determine if simulated effluent with no added WBL is toxic to *C. dubia*.
	WBL spike 2	U.S. EPA 2002c	IC25	Determine if simulated effluent with added WBL is toxic to *C. dubia*.
	WBL spike 5	U.S. EPA 2002c	IC25	Determine if simulated effluent with added WBL is toxic to *C. dubia*.

[3]The IC25 is reported here and throughout this document (though it should be identified as the LC25) because the EPA recommends the IC25 for regulatory use (den Besten 2005, U.S. EPA 2009).

Table 10. 7-d *Ceriodaphnia dubia* survival and reproduction tests and sampling dates

Mill	Sample Description	Sample Code	Sampling/ Collection Date	Test Initiation	Test Termination
A	Mill effluent	A-Effluent	8/31/2010	9/8/2010	9/14/2010
	Unspiked reactor control	A-RWW-Ctrl	9/8/2010	9/14/2010	9/20/2010
	WBL spike 2	A-RWBL-2	9/8/2010	9/14/2010	9/20/2010
	WBL spike 5	A-RWBL-5	9/8/2010	9/9/2010	9/11/2010
B	Mill effluent	B-Effluent	10/19/2010	10/25/2010	10/31/2010
	Unspiked reactor control	B-RWW-Ctrl	10/28/2010	11/2/2010	11/8/2010
	WBL spike 2	B-RWBL-2	10/28/2010	11/2/2010	11/8/2010
	WBL spike 5	B-RWBL-5	10/28/2010	11/2/2010	11/8/2010
C	Mill effluent	C-Effluent	11/2/2010	11/11/2010	11/17/2010
	Unspiked reactor control	C-RWW-Ctrl	11/15/2010	11/17/2010	11/23/2010
	WBL spike 2	C-RWBL-2	11/15/2010	11/17/2010	11/23/2010
	WBL spike 5	C-RWBL-5	11/15/2010	11/17/2010	11/23/2010
D	Mill effluent	D-Effluent	2/8/2011	2/11/2011	2/18/2011
	Unspiked reactor control	D-RWW-Ctrl	2/17/2011	2/21/2011	2/27/2011
	WBL spike 2	D-RWBL-2	2/17/2011	2/21/2011	2/27/2011
	WBL spike 5	D-RWBL-5	2/17/2011	2/21/2011	2/27/2011

2.6 Chemical Analysis

2.6.1 Basic Chemical Analysis of Mill-Treated Effluents and Simulated Effluents

Mill-treated effluent and simulated effluents (sampled after completion of laboratory biotreatment) were analyzed for pH, color, conductivity, turbidity, TSS, BOD, COD, salinity, hardness, alkalinity, and polyphenols at the NCASI NABF laboratory. Unless otherwise noted: 1) all samples were stored at 4°C and measurements were made as soon as possible (within 24 h of sampling); 2) reagent grade water (Barnstead DI) was used for preparation of all standards and effluent dilutions; 3) reagent grade chemicals were used for the preparations of all standards; 4) all samples were measured in duplicate, and the readings were averaged to provide a single number; and 5) control charts maintained at NABF were used to check the performance of standards and duplicates for all test parameters. The control charting method used at NABF follows instructions provided by the WDOE; control chart criteria for standards and duplicates as well as method detection limits (MDLs) for the chemical parameters listed in this section (2.6.1) can be found in Appendix F.

pH

pH was measured using a Hach sensION™2 pH meter and methods were based on the Electrometric Method (4500-H^+; APHA 1998). The pH meter was calibrated each time it was used with a three-point calibration (pH 7.0, pH 10.0, and pH 4.01). After calibration, the pH meter was checked with an 8.0 buffer standard.

Color

Color measurements were based on NCASI (2000). This method was developed to provide a technique for color measurement in pulping wastewaters and their receiving waters. A spectrophotometer (Hach DR/2010) was used to measure the absorbance of a sample at 465 ηm once the sample was adjusted to pH 7.6 and filtered through a 0.8 µm membrane filter to remove turbidity. Color was determined by comparing the absorbance of the sample to the absorbance of a platinum cobalt color standard. Prior to each use, the spectrophotometer was checked with a 500 platinum cobalt stock standard (Hach #1414-53).

Conductivity

Conductivity was measured using a Hach Model 44600 conductivity meter and followed methods by Hach (2003). The conductivity meter was checked with 1000 µs/cm and 1990 µs/cm conductivity standards prior to each use.

Turbidity

Nephelometric turbidity was measured using a Hach Model 2100P turbidimeter based on APHA (1998) with modifications following Hach (2003). The turbidimeter was checked prior to each use with a StablCal 20.0 nephelometric turbidity unit (NTU) standard (Hach #26594-05).

Total Suspended Solids

Total suspended solids were measured following APHA (1998). A 20 mg/L TSS standard (using Sigmacell® cellulose) was prepared in conjunction with each batch of samples.

Biochemical Oxygen Demand

The BOD method followed APHA (1998). Dissolved oxygen of the BOD samples were measured using a YSI model 58 DO meter with self-stirring BOD probe (YSI model 5905). Prior to each set of tests, the DO probe was calibrated using the BOD dilution water. Dissolved oxygen of the BOD dilution water was determined following Hach (2003). The BOD seed source was primary effluent provided by the Anacortes Wastewater Treatment Plant (Anacortes, WA) and each batch of tests included two seed controls (6 ml and 9 ml). For each set of tests, a dilution water blank and standard solution of 198 mg/L glucose-glutamic acid standard (North Central Laboratories) was run alongside the effluent samples. For each effluent sample, a dilution series consisting of four effluent concentrations was used to determine the final BOD.

Salinity

A salinity refractometer (Reichert Precision Salinity Tester: Model 13104190) was used to measure salinity. This unit is automatically temperature compensated to provide accurate readings at temperatures from 16˚C to 38˚C.

Hardness

Hardness measurements were based on methods by APHA (1998) with modifications following Hach (2003) using a Hach digital titrator. Alongside each batch of samples, a 100 mg/L hardness standard was prepared using a 10,000 mg/L $CaCO_3$ stock standard solution (Hach #2187-10).

Alkalinity

Alkalinity was measured using a Hach digital titrator and pH meter (Hach sensION™2 pH meter), and followed methods by Hach (2003). Samples were titrated with 1.600 N sulfuric acid (H_2SO_4; Hach #14389-01) until a pH of 4.5 was reached. Alongside each batch of samples, a 100 mg/L alkalinity standard was prepared using a 25,000 mg/L $CaCO_3$ stock standard solution (Hach #14278-10).

Polyphenols

Polyphenol samples were analyzed using a spectrophotometer (Hach DR/2010) with the wavelength set to 700 ηm, and followed Hach (2003). An 8 mg/L tannic acid standard (Sigma-Aldrich #403040-50G) was prepared and analyzed alongside each batch of samples. A linear regression equation derived from an eight point calibration curve conducted and maintained at NCASI NABF was used to calculate polyphenol concentrations (in mg/L; Appendix G).

2.6.2 National Council for Air and Stream Improvement Chemical Analysis of Mill-Treated Effluents and Simulated Effluents

Analysis of DCOD, DOC, RAs, and phytosterols were conducted by NCASI researchers at the WCRC. Methods for DOC and DCOD analysis were based on Hach (2003). Resin acids and phytosterols were analyzed following methods by NCASI (1997). QA/QC criteria lower calibration limits (LCLs) for phytosterols and RAs are summarized in Appendix H.

2.6.3 Background Chemical Analysis by National Council for Air and Stream Improvement

Preliminary chemical analysis of WBL, ASB-influent, ASB-TPWW, and the unspiked reactor control measured on the day before and on the day WBL was added to the spiked reactors were conducted by NCASI researchers at the WCRC and the SABF. Parameters measured included: BOD, COD, DCOD, DOC, TSS, and WBL solids. Methods for measuring BOD and TSS followed APHA (1998); COD, DCOD, and DOC measurements followed Hach (2003); and WBL solids measurements followed TAPPI (2009).

2.7 Statistical Analysis

All statistical analyses were calculated using R statistical software version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria) unless noted otherwise.

2.7.1 Assumptions

For correlation analysis and multivariate methods, Shapiro-Wilk’s tests were used to determine if data fit a normal distribution for parametric testing. If data did not meet the assumptions for parametric testing, non-parametric methods were selected. Non-parametric methods were also used when data contained censored data (i.e. values below detection limit or IC25/EC50s > 100%).

2.7.2 Toxicity Tests

For the *M. galloprovincialis* toxicity tests, endpoints used for statistical analysis were calculated as described in section 2.4.15. *Ceridaphnia dubia* endpoints used in statistical analysis were calculated as described in section 2.5. For all toxicity tests, significant differences between samples were indicated by the 95% confidence intervals (CI), with no overlap in CIs indicating a significant difference.

2.7.3 Correlation Analysis

Correlation analysis was used to determine if there were significant correlations between: 1) WBL solids and simulated effluent chemical parameters, as described in Section 1.4.3; 2) WBL solids and chronic toxicity to *M. galloprovincialis* and *C. dubia*; 3) effluent (mill-treated and simulated) chemical parameters and chronic toxicity to *M. galloprovincialis* and *C. dubia*; and 4) between the two chronic toxicity tests. In cases where both variables met the assumptions for parametric testing, Pearson's r was used with the p-value set at 0.05. In cases where one or both parameters did not meet the assumptions for parametric testing, Spearman's rho was used with p-value set at 0.01. The conservative p-value for the non-parametric tests was used because the rank-based Spearman's rho cannot calculate an exact p-value when there are tied values (Crawley 2007).

2.7.4 Multivariate Analysis

Multivariate analysis was also used to examine the relationships between toxicity, WBL solids, and chemical parameters. Multivariate methods are a powerful tool for exploring data to find patterns that might not be apparent using confirmatory statistical methods such as correlation analysis. For all multivariate tests, non-parametric Spearman's rho correlation analysis was used to identify if there were redundant variables that should be excluded from the multivariate analysis.

Clustering Methods

Three clustering methods, hierarchical clustering, kmeans clustering, and nonmetric Riffle clustering, were used to explore how the simulated effluent samples grouped together based on the chemical analysis. The clustering methods used for this study are considered uninformed, which means that known groups (such as mill or WBL treatment) were not identified during cluster analysis. For all clustering methods, a technique called association analysis was used after cluster analysis to measure the degree of association between generated clusters and known groups (i.e. mill or WBL treatment; Matthews et al. 1995). Association analysis uses Pearson's χ^2 tests to determine if clustering with samples defined by a known group is random (with the null hypothesis that clustering with samples defined by group is random).

One of the most commonly used clustering methods is hierarchical clustering (Balcan and Gupta 2010). This method calculates the distances between all of the points and groups them together. With hierarchical clustering, there are multiple distance and clustering methods available. The selection of the appropriate distance and clustering method is dependent upon the data, as certain combinations are better for handling outliers, and heteroscedasticity.

Kmeans clustering is a divisive clustering method that uses the within groups sum of squares to group the samples together to minimize distance between points (Crawley 2007). This method can be sensitive to heteroscedastic variance because it relies on the within groups sum of squares. Kmeans clusters are based on iterations; therefore, repeated kmeans clustering can produce different results (Torres et al. 2009). For this reason, repeated kmeans clustering was used to verify that clusters were stable.

Riffle clustering is a nonparametric and nonmetric (does not use *n*-dimensional distance metrics to define similarity distance metrics) clustering method (Matthews 2011, Matthews and Hearne 1991). Riffle is a robust clustering method that works well with heteroscedastic data and is able to identify variables that don't contribute to clustering and exclude them (Matthews 2011, Matthews and Hearne 1991).

Principal Component Analysis

Principal component analysis (PCA) was used to explore which chemical parameters might explain: 1) the differences in toxicity between samples, and 2) the differences in WBL solids between samples. Principal component analysis is a linear model that uses all variables to find combinations of variables that explain the most variance in the data (Crawley 2007). These different combinations of variables are called principal components (PC), and the most variation is explained by PC1, followed by PC2, and so on. A successful ordination will explain most of the variance in the first three principal components.

3.0 RESULTS

3.1 Biotreatment

A summary table of laboratory biotreatment of simulated effluent samples for each of the four mills including test days, volume, and duration is shown in Table 11. In this section, reactor days (D) are labeled in the format D(X), where X is the reactor day relative to when WBL was added to the spiked biotreatment reactors. D(-1) indicates the sample was taken one day before reactors were spiked with WBL, D(0) corresponds to the day the reactors were spiked, and D(1) corresponds to one day after the reactors were spiked, and so on (i.e. D(10) indicates 10 days after reactors were spiked). Results of the initial chemical analysis of WBL, ASB-influent, ASB-TPWW, and unspiked reactor control samples are shown in Table 12 - Table 15. The WBL spike levels used for the simulated effluents are shown in Table 5.

Table 11. Summary of laboratory biotreatment including test days, volume, and duration. `ASB: aerated stabilization basin; TPWW: treatment pond wastewater; WBL: weak black liquor.

	Mill A	**Mill B**	**Mill C**	**Mill D**
Reactor Set-up	8/31/2010	10/22/2010	11/4/2010	2/8/2011
ASB Influent (L)	8.5	8.5	8.5	8.5
ASB-TPWW (L)	1.5	1.5	1.5	1.5
Total volume (L)	10	10	10	10
WBL spike date	9/1/2010	10/23/2010	11/5/2010	2/9/2011
End date	9/8/2010	10/28/2010	11/15/2010	2/17/2011
Reactor days	7	5	10	8

Table 12. Chemical analysis of weak black liquor samples collected from four bleached kraft mills. COD: chemical oxygen demand; DCOD: dissolved chemical oxygen demand; TOC: total organic carbon; DOC: dissolved organic carbon; BOD: biochemical oxygen demand. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center.

Parameter (units)	Mill A	Mill B	Mill C	Mill D
Solids content (% w/w basis)	17.2	14.6	16.1	18.87
Density (g/ml)	1.123	1.055	1.036	1.091
WBL solids (g/ml)	0.19	0.15	0.17	0.21
COD solids basis (mg/g)	958	1051	1067	1022
COD (mg/L)	1.85E+05	1.62E+05	1.78E+05	2.10E+05
DCOD (mg/L)	NM	1.56E+05	1.75E+05	2.11E+05
TOC (mg/L)	5.38E+04	NM	5.68E+04	NM
DOC (mg/L)	5.08E+04	4.90E+04	5.49E+04	NM
BOD (mg/L)	2.27E+04	2.81E+04	2.90E+04	NM

NM: Not Measured

Table 13. Chemical analysis of aerated stabilization basin influent samples collected from four bleached kraft mills. COD: chemical oxygen demand; DCOD: dissolved chemical oxygen demand; TSS: total suspended solids; DOC: dissolved organic carbon. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center.

Parameter (units)	Mill A	Mill B	Mill C	Mill D
COD (mg/L)	1,037	741	1297	437
DCOD (mg/L)	NM	677	1229	412
TSS (mg/L)	NM	76.1	92.9	22.5
DOC (mg/L)	NM	234	454	127
Conductivity (us)	NM	1432	435	NM

NM: Not Measured

Table 14. Chemical analysis of aerated stabilization basin treatment pond wastewater samples collected from four bleached kraft mills. COD: chemical oxygen demand; DCOD: dissolved chemical oxygen demand; TSS: total suspended solids; TOC: total organic carbon; DOC: dissolved organic carbon. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center.

Parameter (units)	Mill A	Mill B	Mill C	Mill D
COD (mg/L)	524	472	853	367
DCOD (mg/L)	NM	358	720	308
TSS (mg/L)	NM	88.3	187	51.9
TOC (mg/L)	NM	NM	257	NM
DOC (mg/L)	NM	110	220	104

NM: Not Measured

Table 15. Chemical analysis of unspiked reactor control before and after overnight aeration (D(-1) and D(0), respectively). COD: chemical oxygen demand; DCOD: dissolved chemical oxygen demand; TSS: total suspended solids; DOC: dissolved organic carbon. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center or Southern Aquatic Biology Facility

	Parameter	Mil A	Mill B	Mill C	Mill D
Before overnight aeration D(-1)	COD (mg/L)	NM	NM	1220	NM
	DCOD (mg/L)	415	629	1106	NM
After overnight aeration D(0)	COD (mg/L)	NM	682	1069	680
	DCOD (mg/L)	NM	NM	887	663
	TSS (mg/L)	NM	105	164	NM
	DOC (mg/L)	NM	166	NM	NM
	Conductivity (µS)	NM	1516	NM	NM

NM: Not Measured

Biotreatment efficiency was monitored by regular analysis of DCOD (Table 16 - Table 19; Figure 15 - Figure 18). Overall DCOD reductions during biotreatment of simulated effluents were variable between mills in terms of the level of DCOD reduction and the time for biotreatment to occur. Mill B showed the lowest percent reduction in DCOD (31-37%), while Mill D showed the greatest with percent reduction ranging from 37-65%. Reduction in DCOD was similar for Mills A (41-53%) and C (44-47%). Although biotreatment efficiencies between mills were different, the % DCOD reductions in the spiked reactors within each mill were similar to those in the reactor control indicating good treatment efficiency of WBL. Only Mill D showed declining treatment efficiency in relation to increasing WBL spike levels (e.g. control 65% DCOD reduction compared with 37% in Spike level 5).

For Mill B and Mill D, RAs were measured in the control and the highest spiked reactor (WBL spike 5) before and after biotreatment to estimate treatment efficiencies. Results were that estimated treatment efficiencies ranged from 96.1 to 100% for the highest spiked reactor (WBL spike 5) and from 67.2 to 100% for the reactor control.

The time required for complete biotreatment also varied between mills. Biotreatment occurred most quickly in Mill B with DCOD stabilizing after 5 days. Heavy foaming and high solids content that caused clogging of pumps during the biotreatment of Mill C resulted in DCOD that did not stabilize until D(10). Biotreatment in Mill A and D were completed after 7 and 8 days, respectively.

Other chemical parameters monitored during laboratory biotreatment included conductivity, DOC, COD, temperature and pH. Results of those analyses can be found in Table 16 - Table 19.

Table 16. Mill A reactor chemistry as measured during 7 day laboratory biotreatment. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center. DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon

Description	WBL Solids (g)	DCOD (mg/L) D(0)	D(1)	D(2)	D(4)	D(6)	D(7)	Conductivity (µS) D(3)-D(4)	DOC (mg/L) D(0)	D(7)	Temp(ºC) Mean	pH Mean
Reactor control	0	267	178	150	132	132	125	829	78	36	29.7	7.9
WBL spike 1	0.966	312	221	189	178	166	162	964	84	42	NM	NM
WBL spike 2	1.932	392	308	232	219	194	198	1049	108	48	NM	NM
WBL spike 3	2.897	435	337	273	278	267	257	1108	114	69	NM	NM
WBL spike 4	3.863	522	403	333	317	294	289	1193	120	72	NM	NM
WBL spike 5	4.829	600	465	369	369	310	314	1287	143	78	29.9	8.2

NM: Not Measured

Table 17. Mill B reactor chemistry as measured during 5 day laboratory biotreatment. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center. DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon

Description	WBL Solids (g)	DCOD (mg/L) D(0)	D(2)	D(4)	D(5)	Conductivity (µS) D(5)	DOC (mg/L) D(5)	Temp(ºC) Mean	pH Mean
Reactor control	0.000	486	342	314	310	1597	109	29.9	30.5
WBL spike 1	0.924	572	399	367	360	1662	122	NM	NM
WBL spike 2	1.849	629	463	442	429	1739	150	NM	NM
WBL spike 3	2.773	702	522	499	486	1787	160	NM	NM
WBL spike 4	3.698	798	595	556	549	1848	168	NM	NM
WBL spike 5	4.622	910	670	634	613	1910	175	8.3	8.4

NM: Not Measured

Table 18. Mill C reactor chemistry as measured during 10 day laboratory biotreatment. Analysis conducted by National Council for Air and Stream Improvement researchers at the West Coast Regional Center. DCOD: dissolved chemical oxygen demand; COD: chemical oxygen demand; DOC: dissolved organic carbon.

Description	WBL Solids (g)	DCOD (mg/L)						COD (mg/L)		Conductivity (µS)	DOC (mg/L)		Temp (ºC)	pH
		D(0)	D(3)	D(4)	D(5)	D(7)	D(10)	D(0)	D(10)	D(10)	D(0)	D(10)	Mean	Mean
Reactor control	0.000	887	604	566	551	498	467	1069	562	3480	297	157	29.4	8.2
WBL spike 1	0.834	962	638	614	614	546	519	NM	584	3550	325	163	NM	NM
WBL spike 2	1.668	1012	675	643	634	582	544	NM	575	3660	349	174	NM	NM
WBL spike 3	2.502	1069	698	668	634	600	571	NM	731	3680	370	191	NM	NM
WBL spike 4	3.336	1167	759	736	704	695	630	NM	797	3740	410	200	NM	NM
WBL spike 5	4.170	1207	802	777	765	695	673	1476	817	3780	423	211	29.8	8.2

NM: Not Measured

Table 19. Mill D reactor chemistry as measured during 8 day laboratory biotreatment. Analysis conducted by National Council for Air and Stream Improvement researchers at the Southern Aquatic Biology Facility. DCOD: dissolved chemical oxygen demand; COD: chemical oxygen demand; DOC: dissolved organic carbon.

Description	WBL Solids (g)	DCOD (mg/L)						COD (mg/L)		Conductivity (µS)	DOC (mg/L)	Temp (ºC)	pH
		D(0)	D(2)	D(5)	D(6)	D(7)	D(8)	D(0)	D(8)	D(8)	D(8)	Mean	Mean
Reactor control	0	663	314	315	248	246	230	680	257	1773	70	29.8	8.4
WBL spike 1	0.966	739	332	316	299	276	279	764	382	1830	75	NM	NM
WBL spike 2	1.932	758	370	372	380	341	332	835	342	1908	104	NM	NM
WBL spike 3	2.897	775	465	574	371	370	433	882	473	1979	110	NM	NM
WBL spike 5	4.829	878	549	589	491	508	550	1001	567	2140	127	29.7	8.5

NM: Not Measured

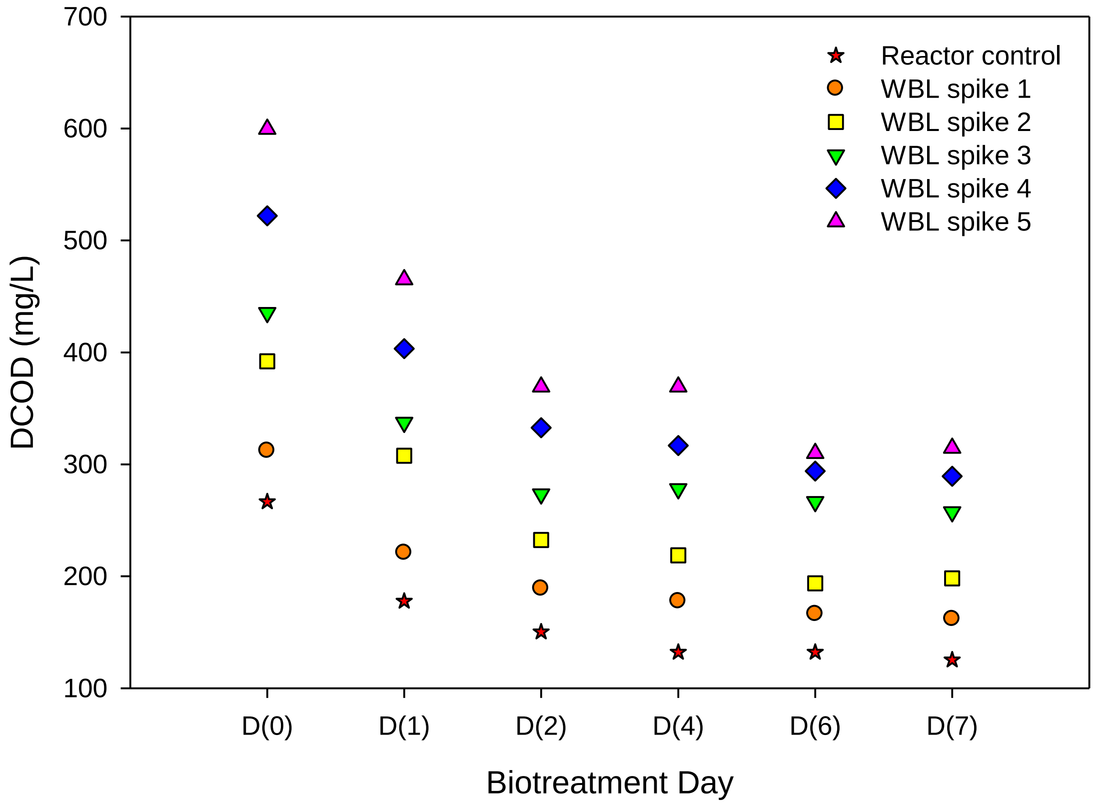


Figure 15. Mill A dissolved chemical oxygen demand of simulated effluent samples measured during laboratory biotreatment.

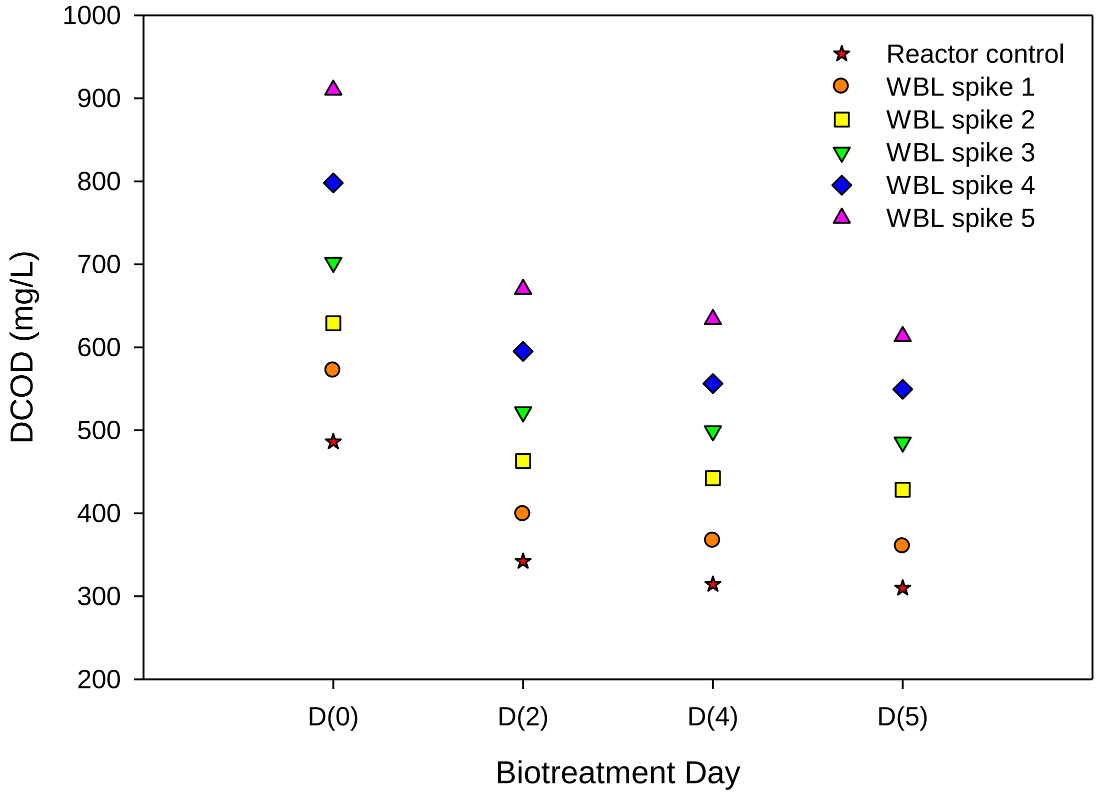


Figure 16. Mill B dissolved chemical oxygen demand of simulated effluent samples measured during laboratory biotreatment.

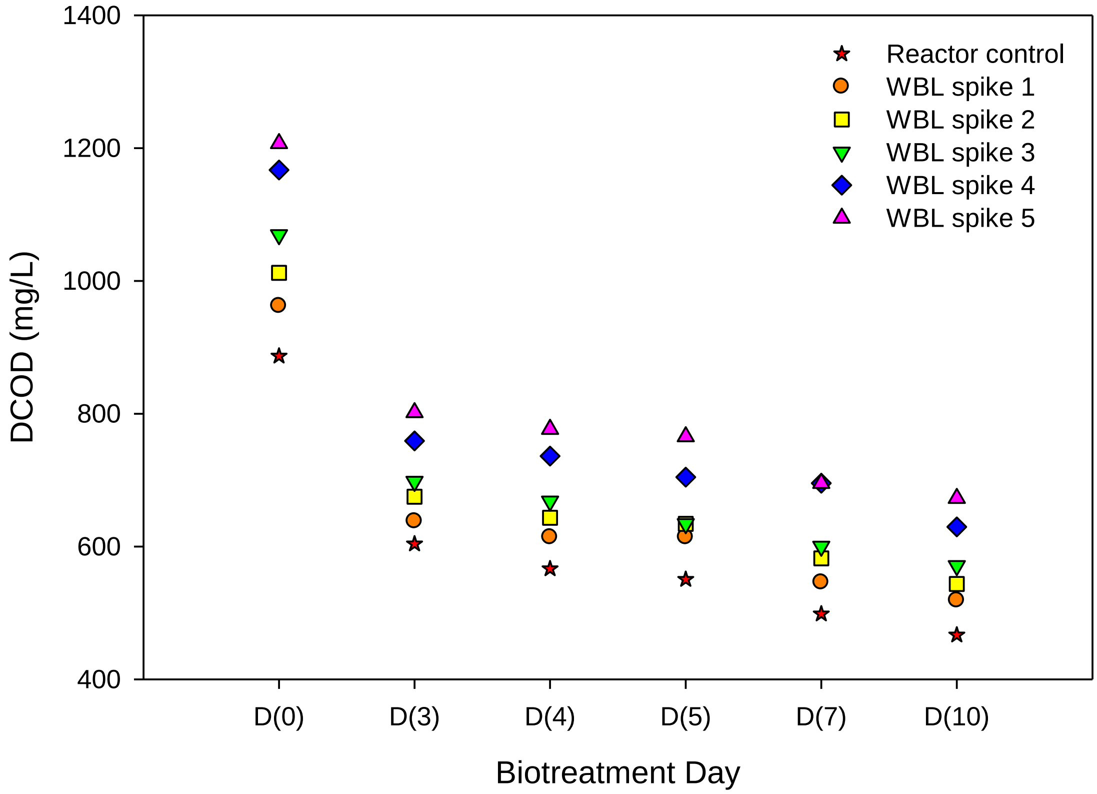


Figure 17. Mill C dissolved chemical oxygen demand of simulated effluent samples measured during laboratory biotreatment.

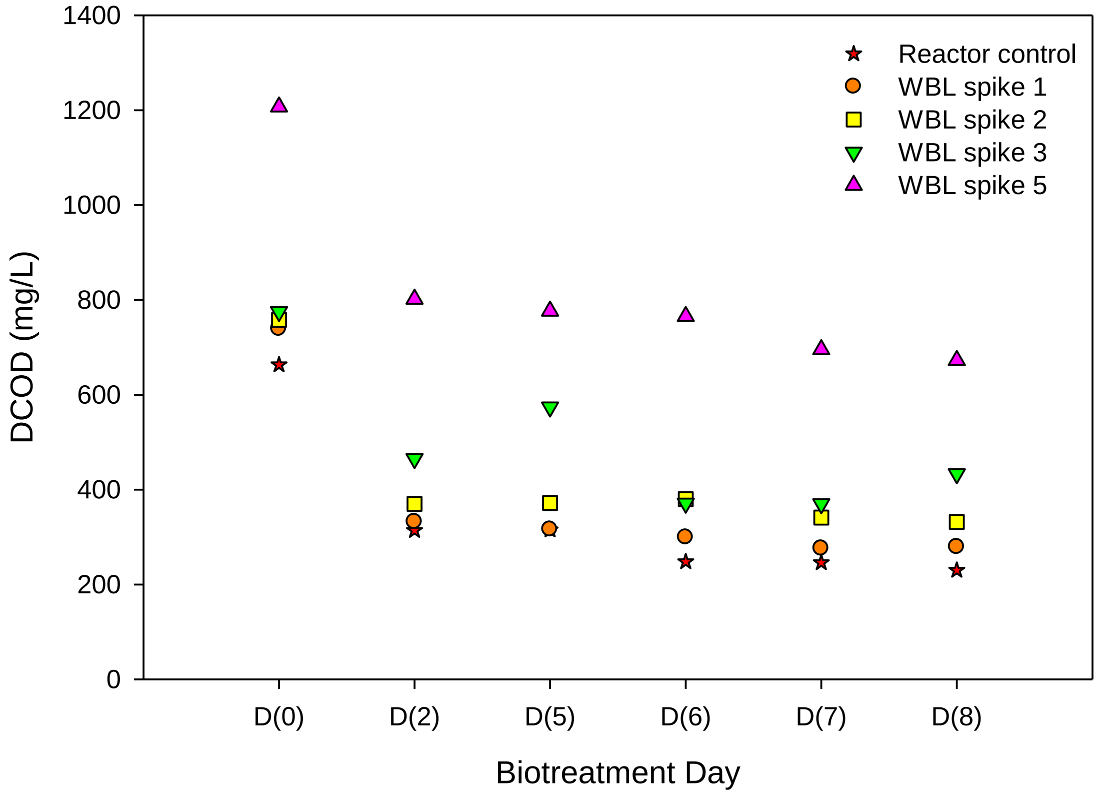


Figure 18. Mill D dissolved chemical oxygen demand of simulated effluent samples measured during laboratory biotreatment.

3.2 Chemical Analysis of Mill-Treated Effluents and Simulated Effluents

Results of chemical analyses of the simulated effluent samples (after completion of laboratory biotreatment) and mill-treated effluents samples can be found in Sections 3.2.1 through 3.2.3. Because efficient laboratory biotreatment may reduce some of these chemical parameters, patterns with increased WBL solids may not always be observed. For all samples except for Mill A, the reactors solids were settled prior to sampling the simulated effluents.

3.2.1 Basic Chemical Analysis of Mill-Treated Effluents and Simulated Effluents

pH of mill-treated and simulated effluent samples ranged from 7.67 (Mill B mill effluent) to 8.69 (Mill C WBL spike 3; Table 20). pH did not appear to change predictably with increased WBL solids. Because pH was held between 7 and 8.5 during laboratory biotreatment, this would explain why there was little difference in pH among the reactors.

Color was variable across mills and effluents ranging from 2.74 (Mill A reactor control) to 1905 PCU (Mill C WBL spike 5; Table 20). Among effluent types (mill-treated and simulated effluents) and WBL spiking levels (WBL spike 1 through WBL spike 5) Mill C effluents had the highest color. For all four mills, color increased with increasing WBL spike levels with the lowest color was observed in the reactor control effluent samples.

Conductivity ranged from 918 µs/cm (Mill A reactor control) to 3900 µs/cm (Mill C WBL spike 5; Table 20). For all four mills, conductivity increased as WBL solids increased. The Mill C reactor control effluent, however, had higher conductivity compared to the lowest WBL spiked Mill C effluent (WBL spike 1; Table 20). Across all effluent types and WBL spikes, Mill C had the highest conductivity.

Turbidity ranged from 3.7 ntu (Mill D reactor control) to 94.4 ntu (Mill A WBL spike 5; Table 20). For 5 out of the 7 effluents types, Mill B effluents had the highest turbidity; for the remaining two effluent types (WBL spike 4 and WBL spike 5) Mill A effluent samples had the highest turbidity (Table 20).

Total suspended solids ranged from 6.4 mg/L (Mill D reactor control) to 113.4 mg/L (Mill A WBL spike 5). For all of the WBL spiked effluents, Mill A had the highest TSS; for the mill-treated effluent and reactor control effluents, Mill B had the highest. It is likely that the higher levels of TSS observed for the Mill A spiked effluents were due to the fact that the samples were collected prior to settling. Across all mills, TSS did not appear to show any clear trend with increased WBL solids.

Polyphenols ranged from 11.72 mg/L (Mill D reactor control) to 78.86 mg/L (Mill B WBL spike 5; Table 20). For all four mills, polyphenol concentrations increased with increasing WBL spike levels with the lowest polyphenol concentrations observed in the reactor control effluent samples.

Salinity ranged from 2 to 4 ‰, with the highest salinity observed in the Mill D WBL spike 3 and Mill D WBL spike 5 effluent samples (Table 20). Salinity showed no clear trend with increasing WBL solids.

Hardness ranged from 40.1 mg/L (Mill A WBL spike 1) to 214 mg/L (Mill C mill effluent). Across all effluent types and WBL spiking levels, Mill C effluents had the highest hardness, while Mill A had the lowest hardness Hardness did not show any clear trend with increasing WBL solids.

Alkalinity ranged from 144 mg/L (Mill A reactor control) to 473 mg/L (Mill C mill effluent). Across all effluent types and WBL spiking levels, Mill C effluents had the highest alkalinity. For all of the simulated effluents (i.e. the reactor control and all WBL spiked effluents) Mill A had the lowest alkalinities. Of the mill-treated effluents, Mill B had the lowest alkalinity (Table 20).

Biochemical oxygen demand ranged from 4.8 mg/L (Mill C WBL spike 3) to 27.2 mg/L (Mill B mill effluent). For all of the WBL spiked reactors, Mill D had the highest BODs. Biochemical oxygen demand was not measured for the mill-treated effluent from Mill A. For Mill B and Mill C, the mill-treated effluents had higher BODs compared to the simulated effluents. Biochemical oxygen demand showed no clear trend with increased WBL solids.

Dissolved chemical oxygen demand ranged from 125 mg/L (Mill A reactor control) to 673 mg/L (Mill C WBL spike 5; Table 20). Across all effluent types (mill-treated and simulated effluents) and WBL spiking levels, DCOD was the highest in Mill C samples and the lowest in Mill A samples. For all four mills, DCOD increased with increasing WBL spike levels and the lowest DCOD levels were observed in the reactor control effluent samples.

Dissolved organic carbon ranged from 36 mg/L (Mill A WBL spike 2) to 211 mg/L (Mill C WBL spike 5; Table 20). For Mill C and Mill D, DOC increased as WBL solids increased; this trend, however, was not observed for effluent samples from mills A and B.

Table 20. Results for basic chemical analysis of mill-treated and simulated effluent samples from four bleached kraft mills. Simulated effluent samples analyzed after completion of laboratory biotreatment. WBL: weak black liquor; TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Mill	Sample	pH	Color (PCU)	Conductivity (µs/cm)	Turbidity (ntu)	TSS (mg/L)	Polyphenols (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	Salinity (ppt)	BOD (mg/L)	DCOD (mg/L)	DOC (mg/L)
A	Mill effluent	7.80	611	1977	9.6	11.7	23.95	94	374	3	NM	342	102
	Reactor control	8.18	274	918	42.9	56.0	15.45	43	144	2	7.2	125	72
	WBL Spike 1	8.16	422	990	52.6	77.5	27.42	40	156	2	7.0	162	78
	WBL Spike 2	7.87	604	1077	66.8	88.5	38.68	46	165	2	9.4	198	36
	WBL Spike 3	7.81	816	1157	72.0	87.5	51.11	42	169	2	8.2	257	42
	WBL Spike 4	7.99	923	1232	82.3	95.0	58.70	43	191	2	9.5	289	48
	WBL Spike 5	7.77	975	1318	94.4	113.4	71.73	48	192	2	11.2	314	69
B	Mill effluent	7.67	732	1650	44.6	36.5	34.29	102	258	2	27.2	356	115
	Reactor control	8.33	623	1630	87.0	79.0	25.72	149	268	3	15.3	310	168
	WBL Spike 1	8.20	776	1696	72.4	67.4	37.20	158	288	2	12.7	360	175
	WBL Spike 2	8.45	999	1769	70.6	57.7	46.73	145	304	2	14.5	429	109
	WBL Spike 3	8.27	1233	1830	83.3	70.0	59.17	151	328	2	12.2	486	122
	WBL Spike 4	8.24	1440	1885	65.9	55.0	68.33	147	343	2	10.9	549	150
	WBL Spike 5	8.14	1641	1948	74.0	61.7	78.86	145	369	2	17.8	613	160
C	Mill effluent	7.89	1170	3445	18.5	21.0	35.40	214	473	3	19.3	490	154
	Reactor control	8.44	1068	3610	14.1	27.0	26.70	207	379	2	18.3	467	157
	WBL Spike 1	8.52	1181	3595	15.2	21.5	34.43	208	388	2	7.9	519	163
	WBL Spike 2	8.51	1342	3720	14.0	18.7	38.03	192	400	2	7.2	544	174
	WBL Spike 3	8.69	1475	3785	18.4	23.0	46.27	200	411	2	4.8	571	191
	WBL Spike 4	8.52	1599	3830	25.0	27.8	56.68	208	419	2	9.1	630	200
	WBL Spike 5	8.66	1905	3900	39.4	48.8	65.51	204	439	2	14.6	673	211
D	Mill effluent	7.89	584	2300	25.1	21.0	35.80	158	413	2	23.9	385	127
	Reactor control	8.43	308	1735	3.7	6.4	11.72	128	269	3	6.2	230	70
	WBL Spike 1	8.47	407	1841	10.4	10.3	20.68	144	315	3	26.4	279	75
	WBL Spike 2	8.44	522	1916	13.0	11.0	27.82	128	307	3	15.9	332	104
	WBL Spike 3	8.18	614	1998	24.7	41.7	36.98	132	294	4	16.3	433	110
	WBL Spike 5	8.56	821	2180	31.5	23.0	52.82	132	364	4	25.0	550	127

NM = not measured

3.2.2 Phytosterols

The four main phytosterols in pulp and paper mill effluent (campesterol, stigmastanol, beta-sitosterol, and stigmasterol) were measured in mill-treated and simulated effluents. Among the four phytosterols measured, beta-sitosterol was found in the greatest concentration in most effluents. Campesterol concentrations ranged from 1.19 (Mill D reactor control) to 80.12 μg/L (Mill A WBL spike 5; Table 21). Across all effluent types (mill-treated and simulated effluents) and WBL spiking levels, campesterol levels were the highest in Mill A effluent samples, while Mill D had the lowest. Stigmastanol ranged from 5.35 (Mill D WBL spike 2) to 138.00 μg/L (Mill C WBL spike 3; Table 21). For all but one of the simulated effluents (WBL spike 5), Mill C effluents showed the highest stigmastanol concentrations while Mill D had the lowest. No trend, however, was observed between stigmastanol concentrations and WBL solids following biotreatment.

Beta-sitosterol ranged from 10.63 (Mill D reactor control) to 315 μg/L (Mill C WBL spike 3; Table 21). For all but one of the simulated effluents (WBL spike 3), Mill A effluent samples had the highest beta-sitosterol concentrations. For all but one simulated effluent (WBL spike 1) the lowest concentrations of beta-sitosterol were in Mill D. Stigmasterol ranged from 2.67 (Mill D WBL spike 5) to 40.64 μg/L (Mill A WBL spike 3). For all of the WBL spiked effluent samples, Mill A had the highest stigmasterol concentrations. For all of the simulated effluents (including the reactor control effluents), Mill D had the lowest stigmasterol concentrations. Stigmasterol showed no clear trend with WBL solids following biotreatment.

Table 21. Results of phytosterol analysis of mill-treated and simulated effluent samples. Simulated effluent samples analyzed after completion of laboratory biotreatment. WBL: weak black liquor.

Mill	Sample	Campesterol (µg/L)	Stigmastanol (µg/L)	Beta-sitosterol (µg/L)	Stigmasterol (µg/L)
A	Mill effluent	25.8	26.7	80.8	35.5
	Reactor control	11.5	12.6	38.5	7.69
	WBL spike 1	19.6	10.8	67.2	15.3
	WBL spike 2	67.2	26.8	179.8	35.6
	WBL spike 3	67.9	55.4	214.0	40.6
	WBL spike 4	56.5	32.2	162.7	32.1
	WBL spike 5	80.1	56.8	184.1	30.3
B	Mill effluent	10.6	23.9	34.1	16.7
	Reactor control	8.71	39.3	32.5	15.9
	WBL spike 1	6.47	27.0	25.4	12.8
	WBL spike 2	10.6	53.4	41.1	18.8
	WBL spike 3	12.5	49.4	41.2	18.6
	WBL spike 4	9.68	41.1	34.1	15.6
	WBL spike 5	5.45	58.8	37.2	15.8
C	Mill effluent	1.87	5.92	21.2	8.00
	Reactor control	2.21	76.2	11.6	5.95
	WBL spike 1	2.18	70.2	12.5	6.11
	WBL spike 2	2.84	84.1	15.7	7.86
	WBL spike 3	10.4	138.0	315.0	10.2
	WBL spike 4	2.10	64.7	32.2	7.06
	WBL spike 5	2.34	51.8	55.2	6.86
D	Mill effluent	11.1	14.7	86.6	14.5
	Reactor control	1.19	6.30	10.6	2.81
	WBL spike 1	1.51	6.31	13.9	3.31
	WBL spike 2	2.04	5.35	15.6	3.37
	WBL spike 3	1.94	6.41	14.3	3.25
	WBL spike 5	1.39	5.97	11.4	2.67

3.2.3 Resin Acids

The concentrations of seven RAs were measured in simulated effluent samples after laboratory biotreatment as well as in the mill-treated effluent samples. In addition, for Mill B and Mill D, RAs were measured in the reactor control and in the highest spiked reactor (WBL spike 5) before and after laboratory biotreatment to estimate treatment efficiencies (Section 3.1). A comparison of RA concentrations for Mill B and Mill D found that estimated treatment efficiencies ranged from 96.1 to 100% for the highest spiked reactor (WBL spike 5) and from 67.2 to 100% for the reactor control. Resin acid levels in simulated effluent samples after laboratory biotreatment were variable and appeared unrelated to WBL spike levels (Table 22). Because RAs are typically reduced during biotreatment, a clear trend between WBL solids and RAs after laboratory biotreatment would not be expected with the WBL spiking levels used for this study.

Several of the effluent samples tested for RAs were below detection limits. Only two RAs (dehydroabietic acid and abietic acid) were present and above the detection limit in all samples. Overall, Mill A had the most non-detects (35 out of 49). The only RA that appeared to increase with WBL solids was abietic acid; this relationship was later examined using correlation analysis (Section 3.4.1). For Mills C and D, the highest concentrations of RAs were observed in the mill-treated effluents compared to simulated effluents. The higher levels in the Mill C and Mill D mill-treated effluents could be due to better treatment efficiency achieved in the laboratory compared to the mill (though this could not be verified using results from this study), or they could be due to higher levels of RAs in the untreated effluent prior to mill treatment (compared to RAs in the untreated effluent prior to laboratory biotreatment). For Mills A and B, the highest concentrations of RAs were generally observed in the highest spiked reactor (WBL spike 5).

Table 22. Results of resin acid analysis of mill-treated and simulated effluent samples. Simulated effluent samples were analyzed after completion of laboratory biotreatment. WBL: weak black liquor; ND: non-detect.

Mill	Sample	Pimaric acid (μg/L)	Sandracopimaric acid (μg/L)	Isopimaric acid (μg/L)	Palustric acid (μg/L)	Dehydroabietic acid (μg/L)	Abietic acid (μg/L)	Neoabietic acid (μg/L)
A	Mill effluent	ND	ND	ND	ND	1.33	2.88	ND
	Reactor control	ND	ND	ND	ND	1.75	1.99	ND
	WBL spike 1	ND	ND	ND	ND	2.28	2.54	ND
	WBL spike 2	ND	ND	ND	ND	2.66	3.56	ND
	WBL spike 3	ND	ND	ND	ND	2.96	3.83	ND
	WBL spike 4	ND	ND	ND	ND	3.00	4.44	ND
	WBL spike 5	ND	ND	ND	ND	3.56	5.54	ND
B	Mill effluent	4.36	ND	4.47	2.05	4.96	7.27	1.49
	Reactor control	2.40	ND	5.38	2.45	5.46	11.7	1.58
	WBL spike 1	ND	ND	5.04	2.06	5.64	10.2	2.00
	WBL spike 2	ND	ND	4.56	2.50	4.34	8.92	1.84
	WBL spike 3	ND	ND	5.62	2.96	5.56	11.7	2.06
	WBL spike 4	ND	ND	5.32	3.16	5.66	11.3	1.72
	WBL spike 5	ND	ND	6.08	2.96	7.08	13.2	2.46
C	Mill effluent	29.4	7.12	27.2	19.8	133.0	128.2	15.4
	Reactor control	ND	ND	1.34	ND	6.78	7.80	ND
	WBL spike 1	2.98	ND	2.58	3.16	9.90	12.1	1.50
	WBL spike 2	2.44	ND	2.62	3.42	8.52	11.4	1.46
	WBL spike 3	3.44	ND	3.62	2.48	12.7	14.4	2.12
	WBL spike 4	5.12	ND	5.26	4.24	19.7	21.6	3.40
	WBL spike 5	4.10	ND	3.82	4.76	16.3	19.1	2.62
D	Mill effluent	165.7	22.8	60.2	25.4	242.8	526.4	30.1
	Reactor control	1.27	ND	ND	0.99	4.57	4.01	ND
	WBL spike 1	2.79	ND	1.45	1.37	9.18	7.74	1.47
	WBL spike 2	2.72	ND	1.38	1.48	9.14	8.54	ND
	WBL spike 3	3.19	ND	1.41	1.31	10.9	7.09	1.20
	WBL spike 5	4.83	ND	2.42	2.74	15.8	25.4	2.81

3.3 Toxicity Tests

Results of 48-h *M. galloprovincialis* embryo-larval development and 7-d *C. dubia* survival and reproduction toxicity tests can be found in Sections 3.3.1 through 3.3.2.

A significant difference between samples is noted when there was no overlap in the 95% CIs.

3.3.1 48-h *Mytilus galloprovincialis* Embryo-larval Development

Results of 48-h *M. galloprovincialis* embryo-larval development toxicity tests are shown in Table 23 and in Figure 19, with concentration-response curves found in Appendix I. For all four mills, the lowest toxicity (i.e. the highest EC50) was observed in the reactor control effluent samples and the highest toxicity (i.e. the lowest EC50) was observed in the WBL spike 5 effluent samples. For Mill A and Mill B, toxicity of the mill-treated effluent fell within levels between those observed in WBL spike 1 and WBL spike 2. The Mill C and Mill D mill-treated effluents showed very similar toxicity to the WBL spike 1 effluent samples.

For Mill A and C, significant differences in toxicity were observed between all simulated effluent samples, except between WBL spike 4 and WBL spike 5 (Table 23, Figure 19). Significant differences were observed between all Mill B simulated effluent samples as well as between all Mill D simulated effluent samples. For the simulated effluent samples from all four mills, as WBL solids increased, EC50s decreased (i.e. toxicity increased).

Table 23. Results of 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests for four bleached kraft mils. Units for NOEC, LOEC and EC50 are % Effluent. Bold and italicized EC50s indicate spiked effluent samples that were significantly different from the reactor control. CI: Confidence Interval; WBL: weak black liquor.

Mill	Sample	Test date	WBL Solids (g/L)	NOEC	LOEC	EC50	95% CI EC50
A	Mill effluent	9/1/2010	NA	4.4	8.75	7.1	6.9-7.3
	Reactor control	9/9/2010	0.000	8.75	17.5	20.6	20.0-21.2
	WBL spike 1	9/9/2010	0.097	4.4	8.75	***9.8***	9.5-10.1
	WBL spike 2	9/9/2010	0.193	2.2	4.4	***6.1***	5.9-6.2
	WBL spike 3	9/9/2010	0.290	2.2	4.4	***4.6***	4.5-4.8
	WBL spike 4	9/9/2010	0.386	1.1	2.2	***2.9***	2.7-3.0
	WBL spike 5	9/9/2010	0.483	1.1	2.2	***2.9***	2.9-3.0
B	Mill effluent	10/22/2010	NA	1.1	2.2	5.6	5.5-5.8
	Reactor control	11/1/2010	0.000	4.4	8.75	8.6	8.4-8.8
	WBL spike 1	11/1/2010	0.092	2.2	4.4	***6.0***	5.9-6.1
	WBL spike 2	11/1/2010	0.185	2.2	4.4	***3.8***	3.6-3.9
	WBL spike 3	11/1/2010	0.277	1.1	2.2	***2.9***	2.8-2.9
	WBL spike 4	11/1/2010	0.370	1.1	2.2	***2.3***	2.3-2.4
	WBL spike 5	11/1/2010	0.462	1.1	2.2	***1.8***	1.7-1.8
C	Mill effluent	11/4/2010	NA	4.4	8.8	7.5	7.3-7.7
	Reactor control	11/17/2010	0.000	4.4	8.8	11.3	11.0-11.5
	WBL spike 1	11/17/2010	0.083	1.1	2.2	***7.2***	6.8-7.4
	WBL spike 2	11/17/2010	0.167	2.2	4.4	***5.7***	5.5-6.0
	WBL spike 3	11/17/2010	0.250	2.2	4.4	***4.5***	4.4-4.7
	WBL spike 4	11/17/2010	0.334	1.1	2.2	***2.9***	2.8-3.0
	WBL spike 5	11/17/2010	0.417	1.1	2.2	***2.7***	2.5-2.8
D	Mill effluent	2/9/2011	NA	2.2	4.4	5.5	5.4-5.6
	Reactor control	2/21/2011	0.000	4.4	8.8	11.6	11.4-11.9
	WBL spike 1	2/21/2011	0.103	1.1	2.2	***5.6***	5.5-5.8
	WBL spike 2	2/21/2011	0.206	1.1	2.2	***4.9***	4.7-5.1999
	WBL spike 3	2/21/2011	0.309	1.1	2.2	***3.8***	3.6-4.0
	WBL spike 5	2/21/2011	0.515	1.1	2.2	***2.1***	2.0-2.2

NA: Not Available

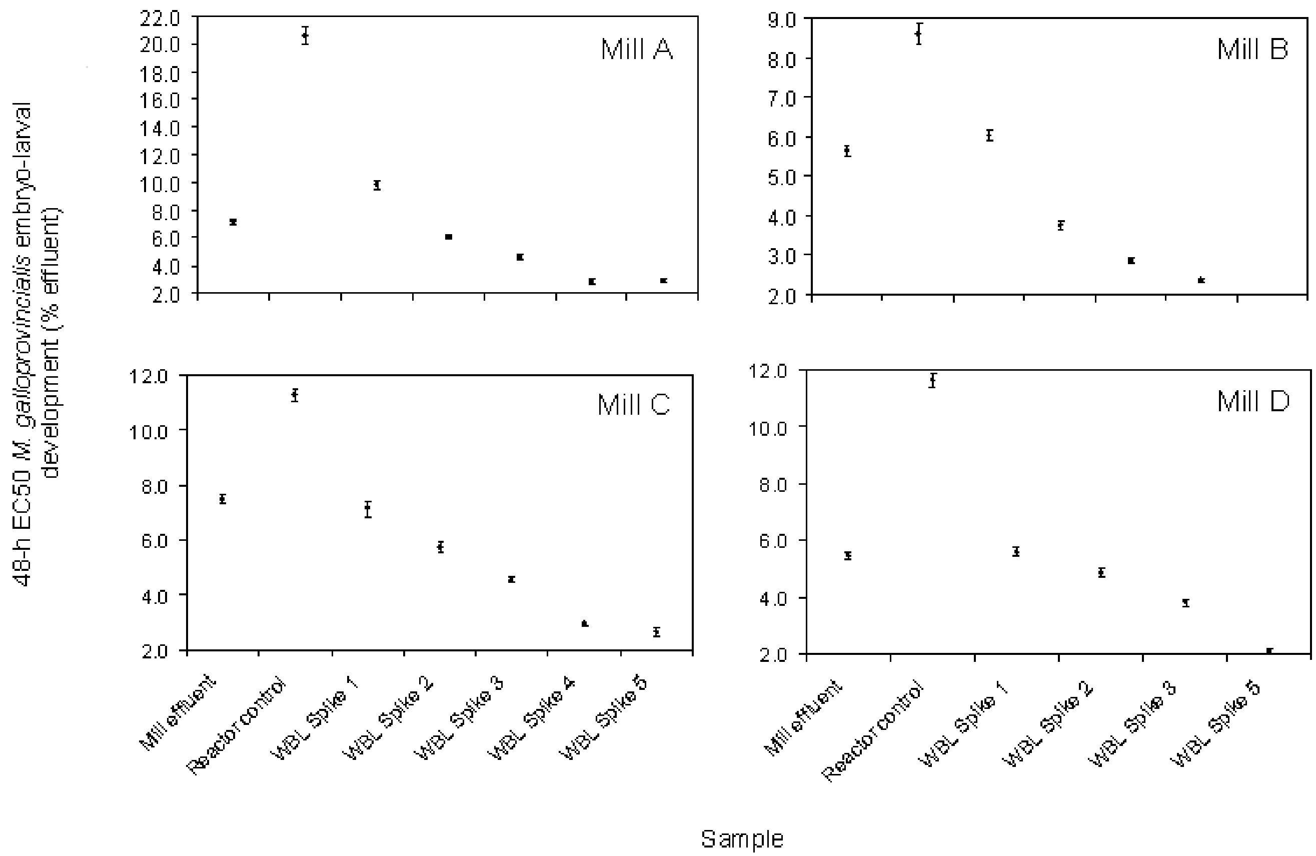


Figure 19. Results of 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests for mill-treated and simulated effluent samples from four bleached kraft mills. Error bars indicate 95% confidence intervals. WBL: weak black liquor.

Reference Toxicant Tests

Results of 48-h *M. galloprovincialis* embryo-larval development toxicity tests with a reference toxicant (copper chloride) are shown in Table 24 and in Figure 20. Concentration response curves are included in Appendix J. The control chart showing performance of mussel stock can be found in Appendix K.

Table 24. Results of 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests with copper chloride (reference toxicant). CI: confidence interval

Mill	Effluent samples tested concurrently	Test date	NOEC	LOEC	EC50	95% CI EC50
A	Mill effluent	9/1/2010	6.6	9.6	11.0	10.9-11.2
	Simulated effluents	9/9/2010	4.5	6.6	10.2	9.9-10.4
B	Mill effluent	10/22/2010	3	4.5	9.0	6.5-11.2
	Simulated effluents	11/1/2010	3	4.5	8.0	7.9-8.0
C	Mill effluent	11/4/2010	3	4.5	8.6	8.5-8.8
	Simulated effluents	11/17/2010	9.6	13.8	8.7	8.5-8.8
D	Mill effluent	2/9/2011	4.5	6.6	8.6	8.5-8.8
	Simulated effluents	2/21/2011	4.5	6.6	8.8	7.8-9.5

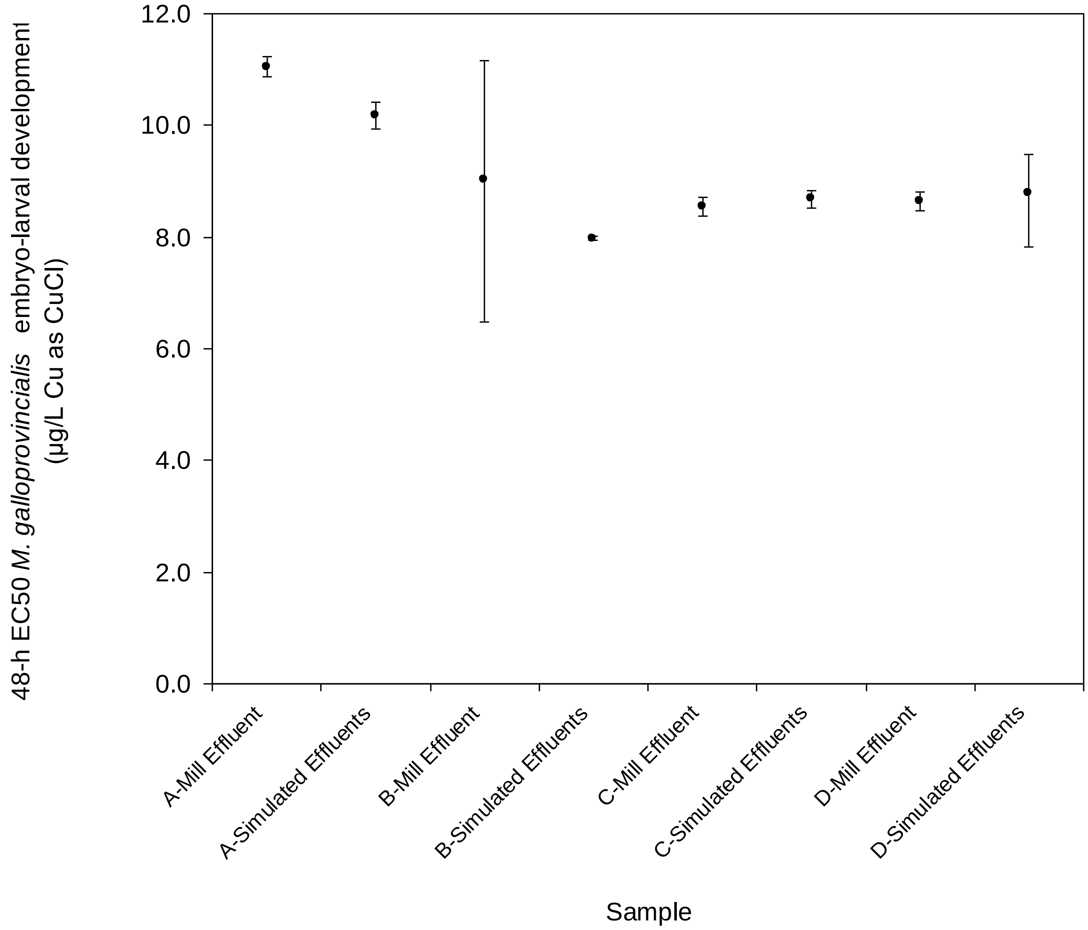


Figure 20. Results of 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests with copper chloride (reference toxicant). Error bars indicate 95% confidence interval. Labels on x-axis indicates which mill (A-D) and effluent type (simulated or mill) the reference toxicant coincides with.

Control Performance

Performance of dilution water controls for 48-h *M. galloprovincialis* embryo-larval development toxicity tests is shown in Table 25. Mean percent survival and mean percent normal in the controls were above the test criteria of >70% for all toxicity tests (Table 25).

Table 25. Control performance (percent normality and percent survival) for 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests. WBL: weak black liquor.

Mill	Sample	Test date	Mean percent survival	Mean percent normal
A	Mill effluent	9/1/2010	97.7	95.8
	Reference toxicant (CuCl)	9/1/2010	96.5	96.0
	Reactor control	9/9/2010	87.1	92.7
	WBL spike 1	9/9/2010	94.5	91.3
	WBL spike 2	9/9/2010	99.0	94.7
	WBL spike 3	9/9/2010	82.9	92.8
	WBL spike 4	9/9/2010	94.8	93.3
	WBL spike 5	9/9/2010	91.5	93.6
	Reference toxicant (CuCl)	9/9/2010	97.0	93.4
B	Mill effluent	10/22/2010	98.4	98.0
	Reference toxicant (CuCl)	10/22/2010	98.6	97.2
	Reactor control	11/1/2010	92.4	92.4
	WBL spike 1	11/1/2010	95.8	93.7
	WBL spike 2	11/1/2010	95.9	94.1
	WBL spike 3	11/1/2010	93.2	95.1
	WBL spike 4	11/1/2010	86.3	93.9
	WBL spike 5	11/1/2010	94.3	93.1
	WBL spike 5 (unsettled)	11/1/2010	96.5	92.1
	Reference toxicant (CuCl)	11/1/2010	89.4	93.2
C	Mill effluent	11/4/2010	93.7	91.8
	Reference toxicant (CuCl)	11/4/2010	96.4	90.3
	Reactor control	11/17/2010	91.7	95.3
	WBL spike 1	11/17/2010	89.7	94.8
	WBL spike 2	11/17/2010	92.1	94.3
	WBL spike 3	11/17/2010	93.2	94.4
	WBL spike 4	11/17/2010	97.5	93.7
	WBL spike 5	11/17/2010	94.9	94.6
	WBL spike 5 (unsettled)	11/17/2010	99.7	93.9
	Reference toxicant (CuCl)	11/17/2010	97.3	94.1
D	Mill effluent	2/9/2011	92.1	95.9
	Reference toxicant (CuCl)	2/9/2011	98.3	93.6
	Reactor control	2/21/2011	80.2	89.8
	WBL spike 1	2/21/2011	82.7	92.8
	WBL spike 2	2/21/2011	72.2	89.3
	WBL spike 3	2/21/2011	79.7	86.6
	WBL spike 5	2/21/2011	88.4	88.0
	Reference toxicant (CuCl)	2/21/2011	88.8	90.9

Settled and Unsettled Effluent Samples

A comparison of results from 48-h *M. galloprovincialis* embryo-larval development toxicity tests with unsettled and settled effluent samples of Mill B WBL spike 5 and Mill C WBL spike 5 simulated effluents are shown in Table 26; summary sheets from ToxCalc can be found in Appendix I. No significant differences in toxicity were observed for Mill B and Mill C WBL spike 5 settled and unsettled simulated effluents. Because there was no significant difference, toxicity results from Mill A (settled) were compared to Mills B, C, and D (unsettled).

Table 26. Comparison of 48-h *Mytilus galloprovincialis* embryo-larval development toxicity tests with settled and unsettled Mill B and Mill C weak black liquor spike 5 simulated effluents. Units for NOEC, LOEC and EC50 are % Effluent. CI: confidence intervals; WBL: weak black liquor.

Mill	Sample	Test date	WBL Solids (g/L)	NOEC	LOEC	EC50	95% CI EC50
B	WBL spike 5 (settled)	11/1/2010	0.462	1.1	2.2	1.8	1.7-1.8
	WBL spike 5 (unsettled)	11/1/2010	0.462	1.1	2.2	1.8	1.7-1.8
C	WBL spike 5 (settled)	11/17/2010	0.417	1.1	2.2	2.7	2.5-2.8
	WBL spike 5 (unsettled)	11/17/2010	0.417	1.1	2.2	2.8	2.5-3.1

3.3.2 7-d *Ceriodaphnia dubia* Survival and Reproduction

Results of the 7-d *C. dubia* survival and reproduction toxicity tests conducted by NCASI researchers at the SABF are shown in Table 27. Summary sheets provided by the NCASI SABF laboratory can be found in Appendix L. The IC25 for *C. dubia* survival was >100% effluent in all but three effluent samples (Mill A WBL spike 5, Mill B WBL spike 2, and Mill B WBL spike 5).

The IC25 for *C. dubia* reproduction was >100% effluent for three of the samples (Mill A mill effluent, Mill A reactor control, and Mill B mill effluent; Table 27). The highest toxicity (i.e. the lowest IC25) for *C. dubia* reproduction was observed in the Mill A WBL spike 5 effluent sample (Table 27). For Mill A, a significant difference in IC25 for reproduction was observed between WBL spike 2 and WBL spike 5 (Table 27). For Mill B, significant differences in IC25 for reproduction were observed between the reactor control and WBL spike 5, and between WBL spike 2 and WBL spike 5 (Table 27). For Mill C, a significant difference in IC25 for reproduction was observed between the mill effluent and the reactor control (Table 27). There were significant differences between all of the Mill C simulated effluent samples, with WBL spike 5 having the highest variability (Table 27). For Mill D, significant differences in IC25 for reproduction were observed between the reactor control and WBL spike 5, and between WBL spike 2 and WBL spike 5 (Table 27). For all simulated effluent samples, as WBL solids increased, the IC25 for *C. dubia* reproduction decreased (i.e. toxicity increased; Table 27).

Table 27. Results of 7-d *Ceriodaphnia dubia* survival and reproduction toxicity tests with mill-treated and simulated effluent samples from four bleached kraft mills. Units for NOEC, LOEC and IC25 are % Effluent. Bold and italicized IC25s indicate spiked effluent samples tests that were significantly different from the reactor control. CI: confidence intervals; WBL: weak black liquor; NC: not calculated; NA: not available.

Mill	Sample	Test date	WBL Solids (g/L)	Survival				Reproduction			
				NOEC	LOEC	IC25	95% CI IC25	NOEC	LOEC	IC25	95% CI IC25
A	Mill effluent	9/8/2010	NA	100	>100	>100	NC	100	>100	>100	NC
	Reactor control	9/14/2010	0.000	100	>100	>100	NC	100	>100	>100	NC
	WBL spike 2	9/14/2010	0.193	100	>100	>100	NC	50	100	***61.6***	57.6-64.2
	WBL spike 5	9/9/2010	0.483	100	>100	***35.4***	31.9-56.3	100	100	***16.7***	14.6-17.5
B	Mill effluent	10/25/2010	NA	100	>100	>100	NC	100	>100	>100	NC
	Reactor control	11/2/2010	0.000	100	>100	>100	NC	50	100	52.2	44.6-57.7
	WBL spike 2	11/2/2010	0.185	100	>100	81.2	NC	50	100	46.5	35.0-58.1
	WBL spike 5	11/2/2010	0.462	100	>100	45.8	NC	25	50	***24.6***	20.9-28.4
C	Mill effluent	11/11/2010	NA	100	>100	>100	NC	50	100	51.6	35.8-55.5
	Reactor control	11/17/2010	0.000	100	>100	>100	NC	50	100	65.4	61.6-66.8
	WBL spike 2	11/17/2010	0.167	100	>100	>100	NC	50	100	***62.2***	59.8-62.7
	WBL spike 5	11/17/2010	0.417	100	>100	>100	NC	50	100	***60.1***	24.4-62.7
D	Mill effluent	2/11/2011	NA	100	>100	>100	NC	50	100	60.5	50.0-66.5
	Reactor control	2/21/2011	0.000	100	>100	>100	NC	25	50	48.1	37.0-57.2
	WBL spike 2	2/21/2011	0.206	100	>100	>100	NC	25	50	***35.2***	32.5-41.3
	WBL spike 5	2/21/2011	0.515	100	>100	>100	NC	25	50	***29.8***	27.1-32.0

3.4 Correlation Analysis

The purpose of the correlation analysis was to determine if there were significant correlations between: 1) WBL solids and simulated effluent chemical parameters (as described in Section 1.4.3); 2) WBL solids and chronic toxicity to *M. galloprovincialis* and *C. dubia*; 3) effluent (mill-treated and simulated) chemical parameters and chronic toxicity to *M. galloprovincialis* and *C. dubia*; and 4) between the two chronic toxicity tests. In cases where both variables met the assumptions for parametric testing, Pearson's r was used with p-value set at 0.05. In cases where one or both parameters did not meet all of the assumptions for parametric testing, Spearman's rho was used with p-value set at 0.01. The conservative p-value for the non-parametric tests was used because the rank-based Spearman's rho cannot calculate an exact p-value when there are tied values. All data were pooled together (rather than examining each mill individually for correlation analysis between: 1) WBL solids and effluent chemical parameters; 2) WBL solids and chronic toxicity to *M. galloprovincialis* and *C. dubia* and 3) between the two chronic toxicity tests. For the correlation analysis between effluent (mill-treated and simulated) chemical parameters and chronic toxicity to *M. galloprovincialis* and *C. dubia*;, data were not pooled, and samples from each mill were examined separately.

Results of Shapriro Wilk's tests were that the following variables did not fit a normal distribution: 48-h EC50 *M. galloprovincialis* embryo-larval development, 7-d IC25 *C. dubia* survival, conductivity, turbidity, hardness, salinity, campesterol, stigmastanol, beta-sitosterol, stigmasterol, pimaric acid, sandracopimaric acid, isopimaric acid, palustric acid, dehydroabietic acid, abietic acid, and neoabietic acid (Appendix M).

There were some cases where variables were normally distributed but contained censored data (i.e. values that were below detection limit or toxicity tests where the IC25 was >100%). The 7-d IC25 for *C. dubia* reproduction met the normality assumption for parametric testing, but contained IC25 values that were >100%. IC25 values >100% were entered as 100%, and the non-parametric Spearman's rho was used instead of Pearson's r. For some of the chemical parameters (primarily the RAs), results were below detection limits. When this was the case, the detection limit was entered and used for the analysis, along with non-parametric Spearman's rho correlation analysis.

3.4.1 Weak Black Liquor Solids and Chemical Parameters

Statistically significant positive correlations were observed between WBL solids and: color (Pearson's $r = 0.54$; $p = 0.007$), DCOD (Pearson's $r = 0.50$; $p = 0.014$), and polyphenols (Pearson's $r = 0.89$; $p >0.001$; Figure 21 - Figure 23; Table 28).

Table 28. Correlation analysis between weak black liquor solids and chemical parameters with simulated effluent samples. *: Statistically significant (Spearman's rho $p \leq 0.01$ and Pearson's r $p \leq 0.05$); NS: not significant; NC: not calculated.

Tested Parameter	Pearson's r	Spearman's rho	p-value
pH	-0.18		NS
Color	0.54		*
Conductivity		0.25	NS
Turbidity		0.33	NS
TSS	0.27		NS
Polyphenols	0.89		*
Hardness		-0.08	NS
Alkalinity	0.20		NS
Salinity		0.18	NS
BOD	0.21		NS
DCOD	0.50		*
DOC	0.07		NS
Campesterol		0.13	NS
Stigmastanol		0.08	NS
Beta-sitosterol		0.34	NS
Stigmasterol		0.19	NS
Pimaric acid		0.16	NS
Sandracopimaric acid		NA	NC
Isopimaric acid		0.17	NS
Palustric acid		0.30	NS
Dehydroabietic acid		0.31	NS
Abietic acid		0.36	NS
Neoabietic acid		0.39	NS

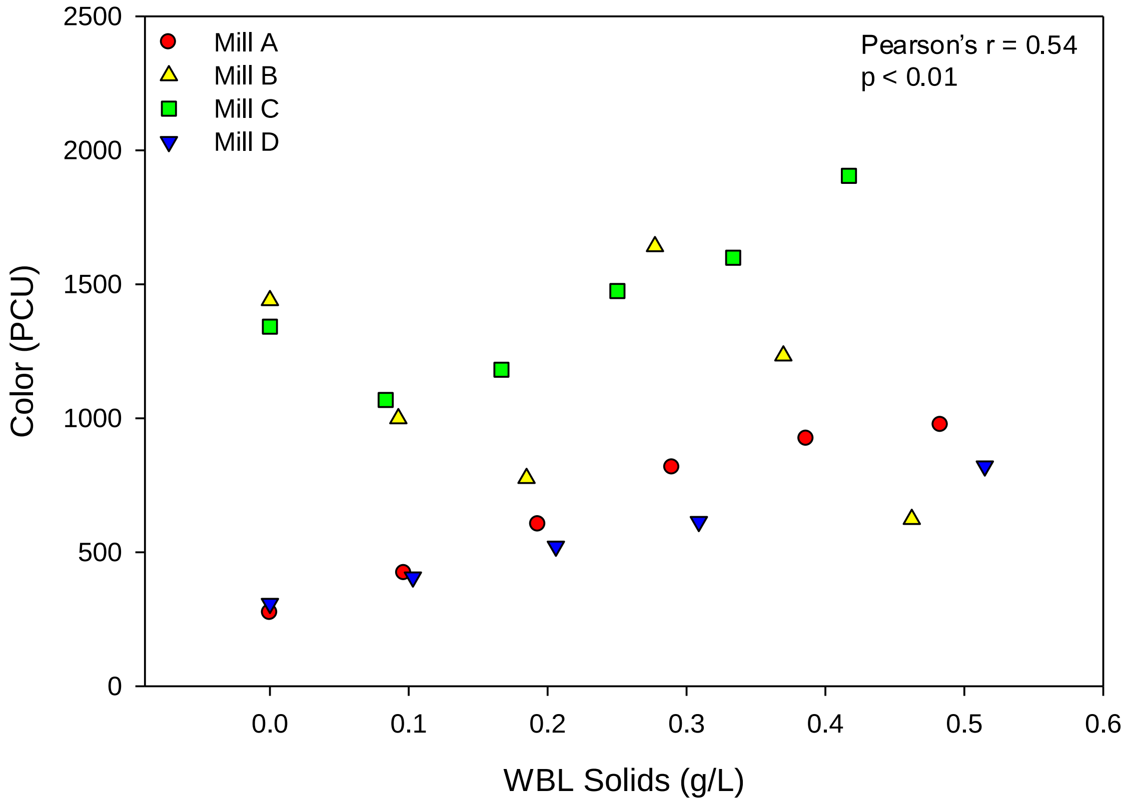


Figure 21. Scatterplot of weak black liquor solids vs. color showing significant positive correlation for pooled data (data coded to denote mill). Correlation significant based on Pearson's r.

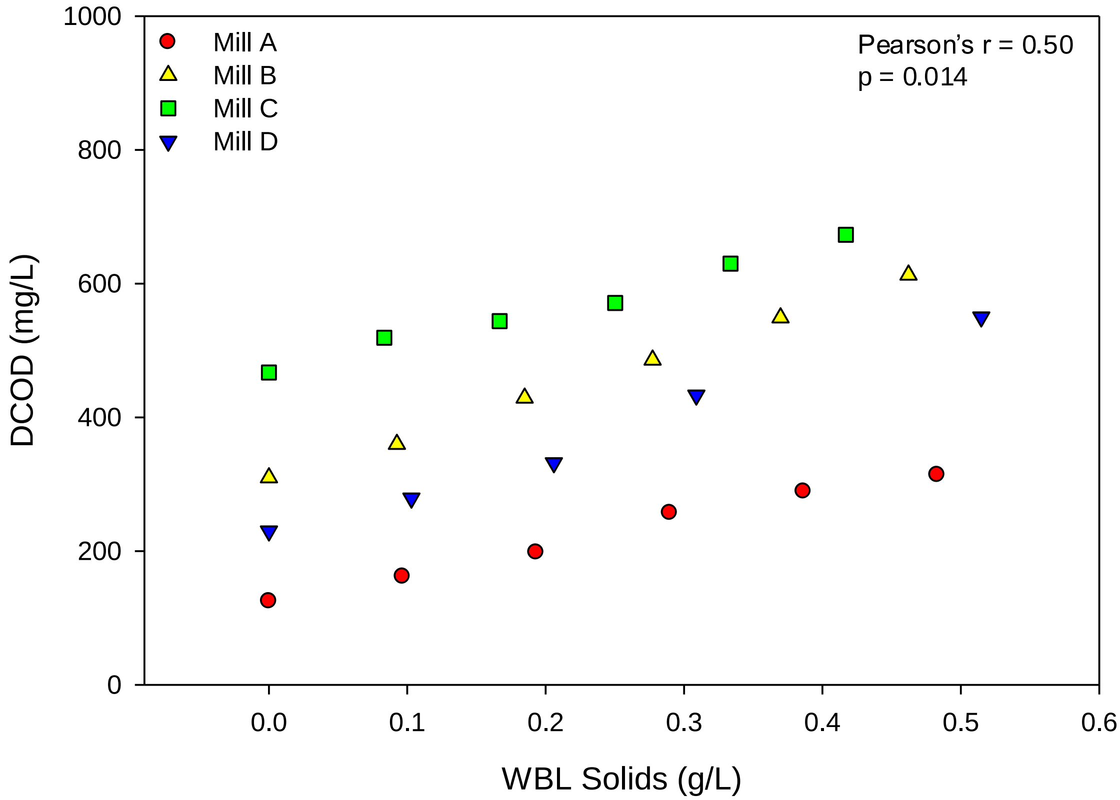


Figure 22. Scatterplot of weak black liquor solids vs. dissolved chemical oxygen demand showing significant positive correlation for pooled data (data coded to denote mill). Correlation significant based on Pearson's r.

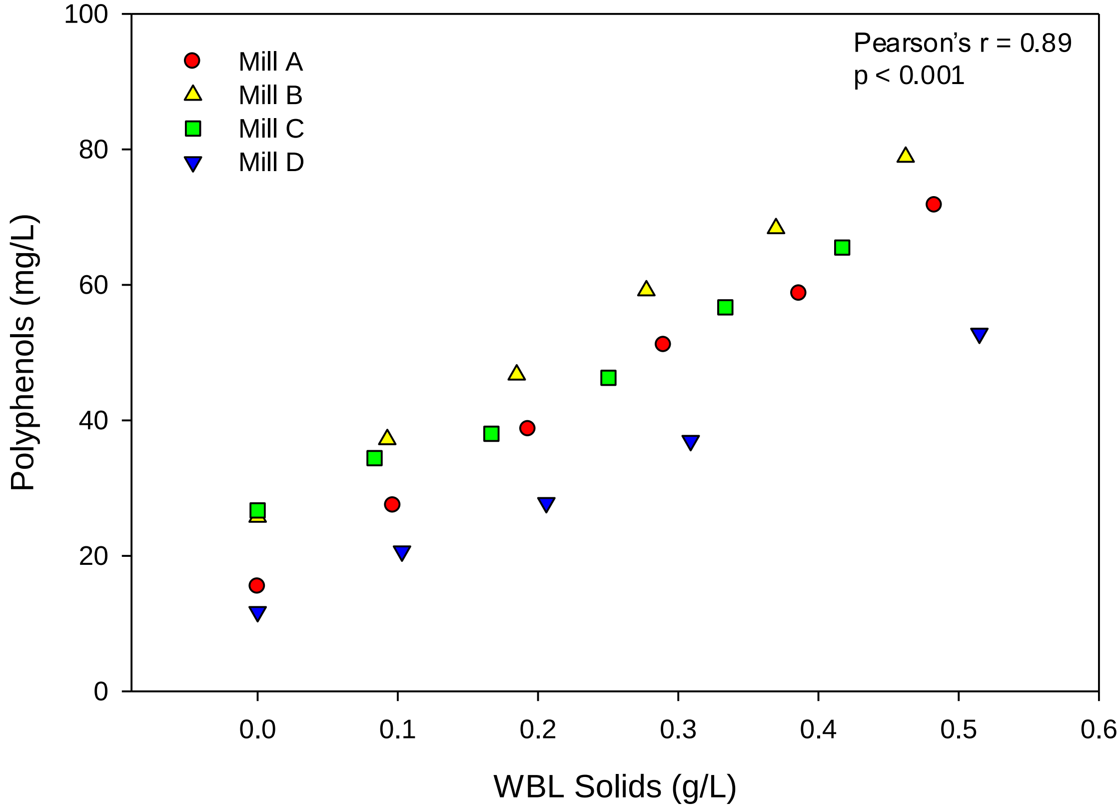


Figure 23. Scatterplot of weak black liquor solids vs. polyphenols showing significant positive correlation for pooled data (data coded to denote mill). Correlation significant based on Pearson's r.

3.4.2 Weak Black Liquor Solids and Toxicity

Statistically significant negative correlations were observed between WBL solids and the 48-h EC50 *M. galloprovincialis* embryo-larval development (Spearman's rho = -0.93; p > 0.001), and between WBL solids and the 7-d IC25 *C. dubia* reproduction (Spearman's rho = -0.73; p > 0.01; Figure 24 and Figure 25, respectively). There was no significant relationship between WBL solids and the 7-d IC25 for *C. dubia* survival.

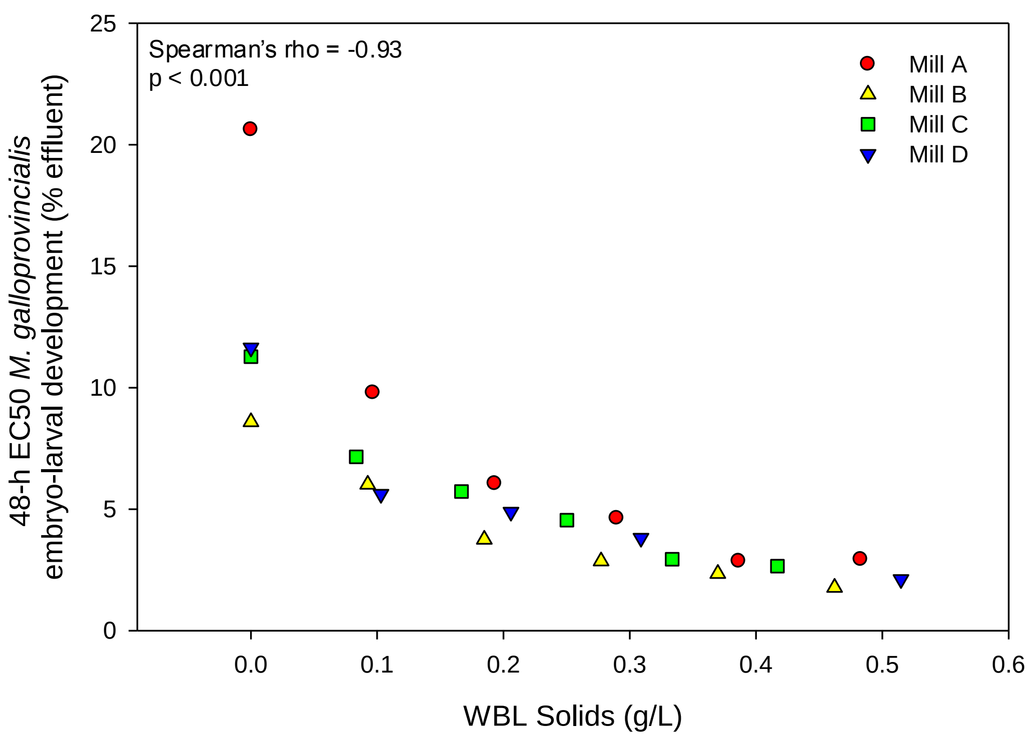


Figure 24. Scatterplot of 48-h EC50 (% effluent) *Mytilus galloprovincialis* embryo-larval development versus weak black liquor solids showing significant negative correlation for pooled data (data coded to denote mill). Correlation significant based on Spearman's rho.

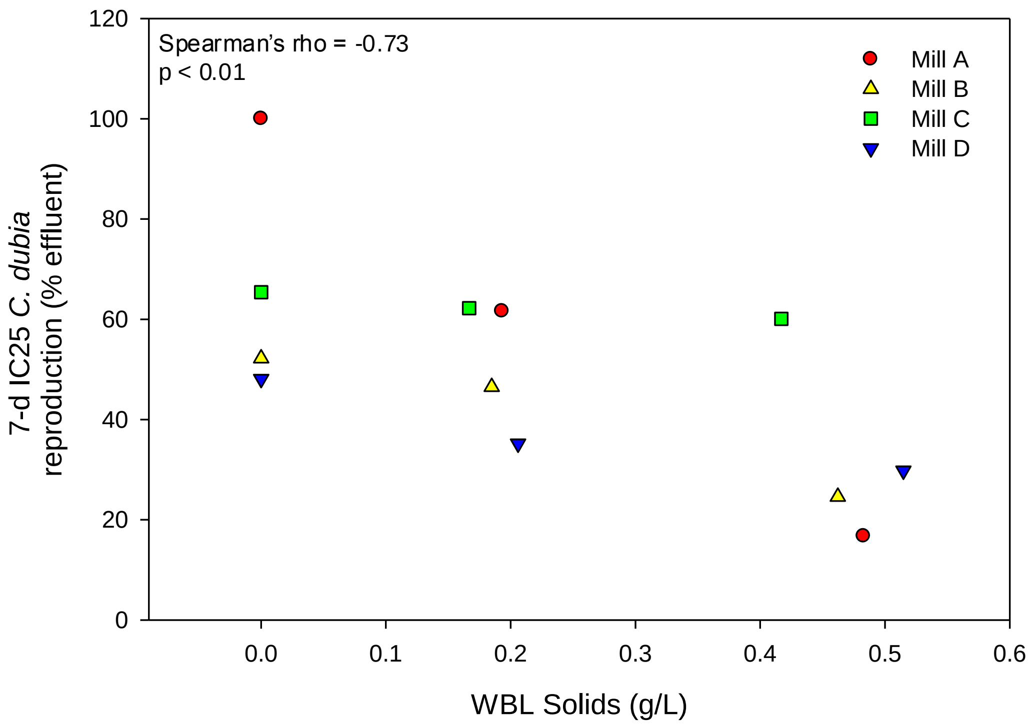


Figure 25. Scatterplot of 7-d IC25 *Ceriodaphnia dubia* reproduction versus weak black liquor solids showing significant negative correlation for pooled data (data coded to denote mill). Correlation significant based on Spearman's rho.

3.4.3 Chemistry and Toxicity

For Mill A, statistically significant negative correlations ($p > 0.01$) were found between the 48-h EC50 *M. galloprovincialis* embryo-larval development and: abietic acid (Spearman's rho = -0.96; $p < 0.01$), color (Spearman's rho = -0.93; $p < 0.01$), and polyphenols (Spearman's rho = -0.93; $p < 0.01$; Figure 26 - Figure 28; Table 29). For Mill B, statistically significant negative correlations ($p > 0.01$) were found between the 48-h EC50 *M. galloprovincialis* embryo-larval development and: color (Spearman's rho = -0.96; $p < 0.01$; Figure 27; Table 29), conductivity (Spearman's rho = -0.96; $p < 0.01$; Figure 29), DCOD (Spearman's rho = -0.96; $p < 0.01$; Figure 30), and polyphenols (Spearman's rho = -0.93; $p < 0.01$; Figure 28; Table 29). For Mill C, statistically significant negative correlations ($p > 0.01$) were found between the 48-h EC50 *M. galloprovincialis* embryo-larval development and: color (Spearman's rho = -1.00; $p < 0.001$; Figure 27), DCOD (Spearman's rho = -1.00; $p < 0.001$; Figure 30), DOC (Spearman's rho = -0.96; $p < 0.01$; Figure 31), and polyphenols (Spearman's rho = -0.96; $p < 0.01$; Figure 28; Table 29). No significant correlations were found between the 48-h EC50 *M. galloprovincialis* embryo-larval development and effluent chemical parameters measured for Mill D (Table 29). No statistically significant correlations were found between any of the effluent chemical parameters and 7-d IC25 for *C. dubia* survival or reproduction (Table 30 and Table 31).

Table 29. Correlation analysis using Spearman's rho between the 48-h EC50 *Mytilus galloprovincialis* embryo-larval development and chemical parameters with mill-treated and simulated effluent samples. *: statistically significant (Spearman's rho $p \leq 0.01$); NS: not significant; TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

	Mill A		Mill B		Mill C		Mill D	
Tested parameter	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**
pH	0.54	NS	0.21	NS	-0.77	NS	-0.26	NS
Color	-0.93	*	-0.96	*	-1.00	*	-0.94	NS
Conductivity	-0.61	NS	-0.96	*	-0.89	NS	-0.66	NS
Turbidity	-0.86	NS	0.18	NS	-0.64	NS	-0.83	NS
TSS	-0.82	NS	0.36	NS	-0.54	NS	-0.89	NS
Polyphenols	-0.93	*	-0.96	*	-0.96	*	-0.94	NS
Hardness	-0.29	NS	0.31	NS	0.34	NS	-0.03	NS
Alkalinity	-0.61	NS	-0.89	NS	-0.46	NS	-0.31	NS
Salinity	0.20	NS	0.61	NS	-0.37	NS	-0.68	NS
BOD	-0.83	NS	0.21	NS	0.39	NS	-0.26	NS
DCOD	-0.61	NS	-0.96	*	-1.00	*	-0.94	NS
DOC	0.54	NS	0.29	NS	-0.96	*	-0.75	NS
Campesterol	-0.79	NS	0.04	NS	-0.32	NS	-0.20	NS
Stigmastanol	-0.86	NS	-0.71	NS	0.07	NS	0.20	NS
Beta-sitosterol	-0.75	NS	-0.63	NS	-0.79	NS	-0.20	NS
Stigmasterol	-0.46	NS	0.00	NS	-0.25	NS	0.26	NS
Pimaric acid	NA	NA	0.58	NS	-0.39	NS	-0.54	NS
Sandracopimaric acid	NA	NA	NA	NA	0.41	NS	0.13	NS
Isopimaric acid	NA	NA	-0.46	NS	-0.43	NS	-0.37	NS
Palustric acid	NA	NA	-0.79	NS	-0.36	NS	-0.43	NS
Dehydroabietic acid	-0.86	NS	-0.57	NS	-0.39	NS	-0.54	NS
Abietic acid	-0.96	*	-0.32	NS	-0.39	NS	-0.43	NS
Neoabietic acid	NA	NA	-0.57	NS	0.39	NS	-0.29	NS

Table 30. Correlation analysis using Spearman's rho between the 7-d IC25 for *Ceriodaphnia dubia* survival and chemical parameters with mill-treated and simulated effluent samples. NS: not significant (Spearman's rho $p \geq 0.01$); TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

	Mill A		**Mill B**		**Mill C**		**Mill D**	
Tested parameter	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**
pH	0.77	NS	-0.21	NS	NA	NA	NA	NA
Color	-0.77	NS	-0.95	NS	NA	NA	NA	NA
Conductivity	-0.26	NS	-0.95	NS	NA	NA	NA	NA
Turbidity	-0.77	NS	-0.11	NS	NA	NA	NA	NA
TSS	-0.77	NS	-0.11	NS	NA	NA	NA	NA
Polyphenols	-0.77	NS	-0.95	NS	NA	NA	NA	NA
Hardness	-0.26	NS	0.00	NS	NA	NA	NA	NA
Alkalinity	-0.26	NS	-0.95	NS	NA	NA	NA	NA
Salinity	0.33	NS	0.54	NS	NA	NA	NA	NA
BOD	-0.87	NS	0.21	NS	NA	NA	NA	NA
DCOD	-0.26	NS	-0.95	NS	NA	NA	NA	NA
DOC	0.26	NS	0.21	NS	NA	NA	NA	NA
Campesterol	-0.77	NS	0.63	NS	NA	NA	NA	NA
Stigmastanol	-0.77	NS	-0.95	NS	NA	NA	NA	NA
Beta-sitosterol	-0.77	NS	-0.74	NS	NA	NA	NA	NA
Stigmasterol	0.26	NS	0.32	NS	NA	NA	NA	NA
Pimaric acid	NA	NA	0.89	NS	NA	NA	NA	NA
Sandracopimaric acid	NA	NA	NA	NA	NA	NA	NA	NA
Isopimaric acid	NA	NA	-0.63	NA	NA	NA	NA	NA
Palustric acid	NA	NA	-0.95	NS	NA	NA	NA	NA
Dehydroabietic acid	-0.77	NS	-0.32	NS	NA	NA	NA	NA
Abietic acid	-0.77	NS	-0.63	NS	NA	NA	NA	NA
Neoabietic acid	NA	NA	-0.95	NS	NA	NA	NA	NA

Table 31. Correlation analysis using Spearman's rho between the 7-d IC25 for *Ceriodaphnia dubia* reproduction and chemical parameters with mill-treated and simulated effluent samples. NS: not significant (Spearman's rho $p \geq 0.01$); TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

	Mill A		Mill B		Mill C		Mill D	
Tested parameter	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**
pH	0.63	NS	-0.40	NS	0.20	NS	-1.00	NS
Color	-0.63	NS	-0.80	NS	0.40	NS	-0.40	NS
Conductivity	-0.11	NS	-0.80	NS	0.20	NS	0.20	NS
Turbidity	-0.95	NS	-0.40	NS	-0.60	NS	-0.40	NS
TSS	-0.95	NS	-0.40	NS	0.00	NS	-0.40	NS
Polyphenols	-0.95	NS	-0.80	NS	-0.40	NS	0.40	NS
Hardness	-0.11	NS	-0.32	NS	-0.40	NS	0.32	NS
Alkalinity	-0.11	NS	-1.00	NS	-1.00	NS	0.20	NS
Salinity	0.54	NS	0.26	NS	-1.00	NS	-0.95	NS
BOD	-1.00	NS	-0.40	NS	-0.40	NS	-0.40	NS
DCOD	-0.11	NS	-0.80	NS	-0.40	NS	-0.40	NS
DOC	0.74	NS	0.00	NS	0.20	NS	-0.11	NS
Campesterol	-0.95	NS	-0.80	NS	0.40	NS	0.40	NS
Stigmastanol	-0.95	NS	-1.00	NS	0.80	NS	0.80	NS
Beta-sitosterol	-0.95	NS	-0.60	NS	-0.80	NS	0.40	NS
Stigmasterol	-0.21	NS	-0.40	NS	-0.80	NS	0.80	NS
Pimaric acid	NA	NA	0.95	NS	-1.00	NS	0.20	NS
Sandracopimaric acid	NA	NA	NA	NA	-0.77	NS	0.77	NS
Isopimaric acid	NA	NA	-0.80	NS	-1.00	NS	0.20	NS
Palustric acid	NA	NA	-1.00	NS	-1.00	NS	0.20	NS
Dehydroabietic acid	-0.95	NS	-0.40	NS	-1.00	NS	0.20	NS
Abietic acid	-0.95	NS	-0.80	NS	-1.00	NS	0.20	NS
Neoabietic acid	NA	NA	-1.00	NS	-1.00	NS	0.32	NS

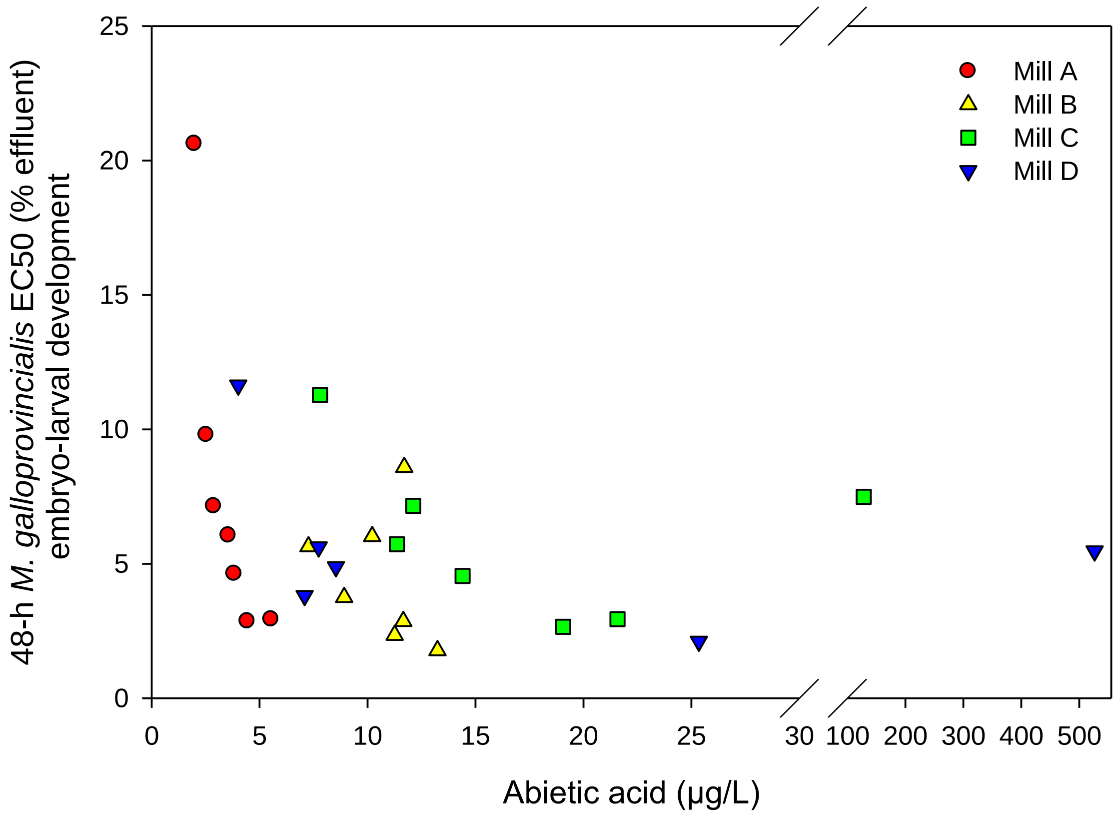


Figure 26. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus abietic acid. Significant negative correlation based on Spearman's rho for Mill A.

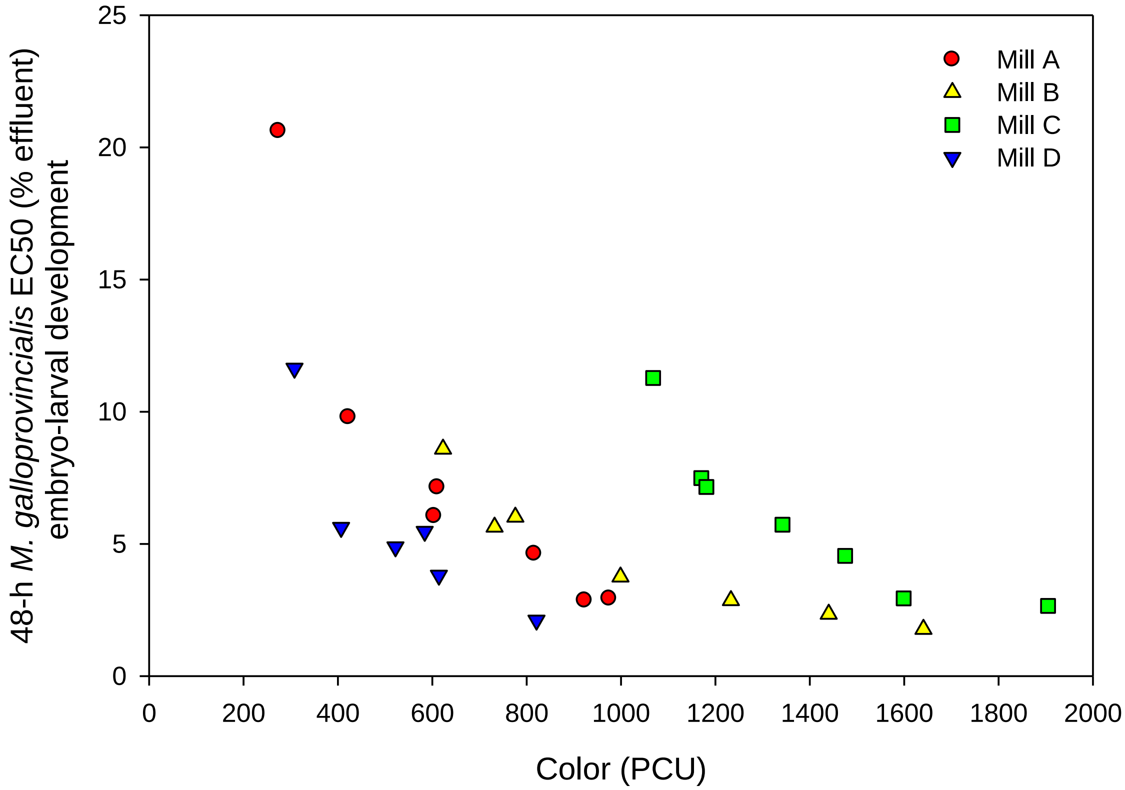


Figure 27. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus color. Significant negative correlations based on Spearman's rho for Mill A, Mill B, and Mill C.

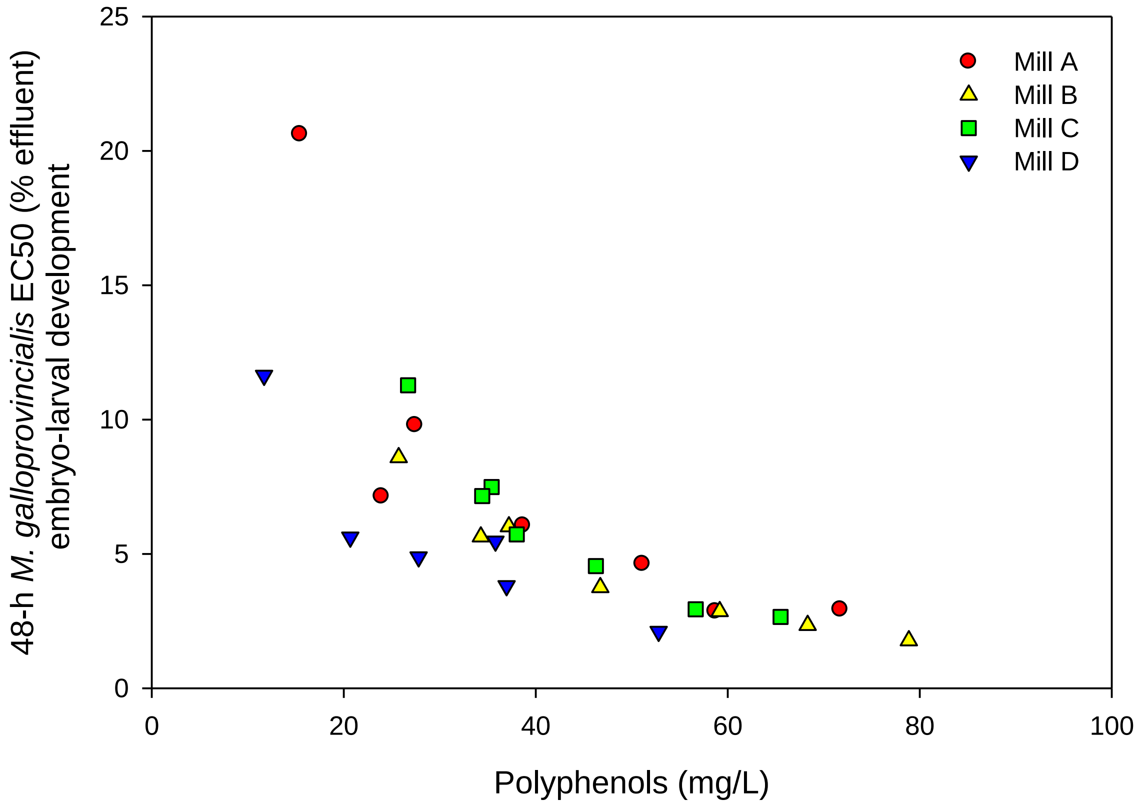


Figure 28. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus polyphenols. Significant negative correlations based on Spearman's rho for Mill A, Mill B, and Mill C.

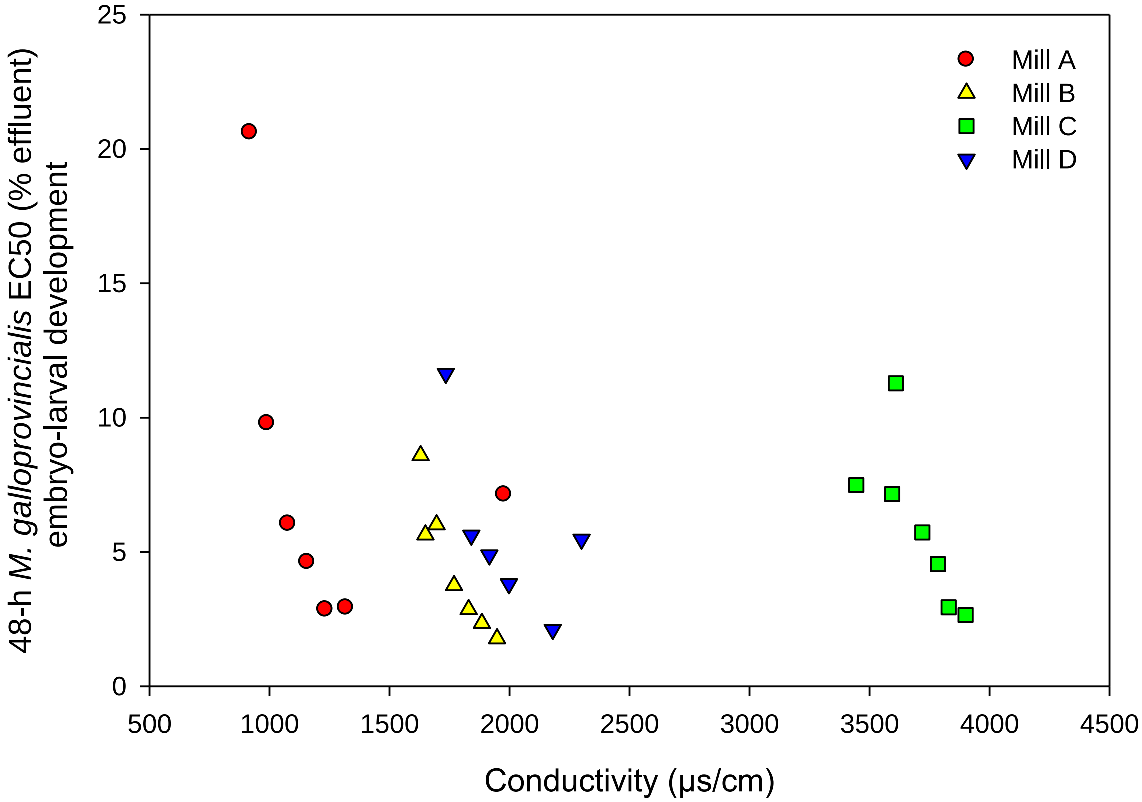


Figure 29. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus conductivity. Significant negative correlation based on Spearman's rho for Mill B.

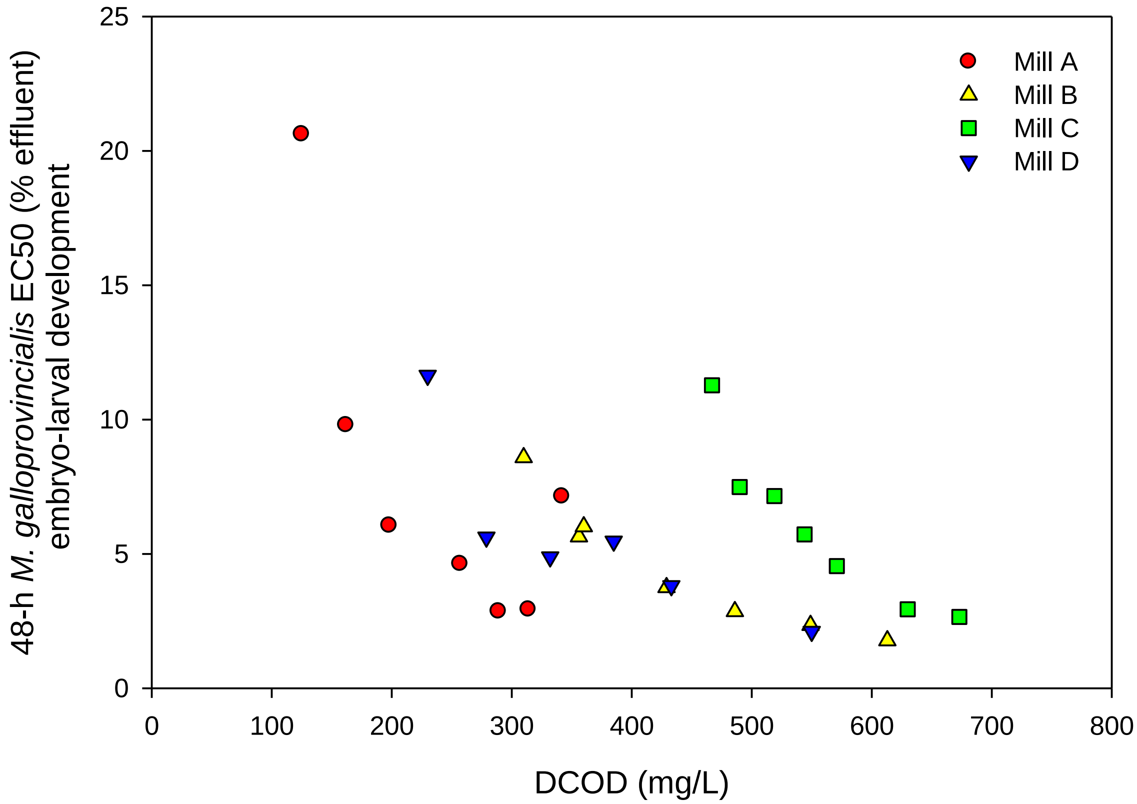


Figure 30. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus dissolved chemical oxygen demand (DCOD). Significant negative correlations based on Spearman's rho for Mill B and Mill C.

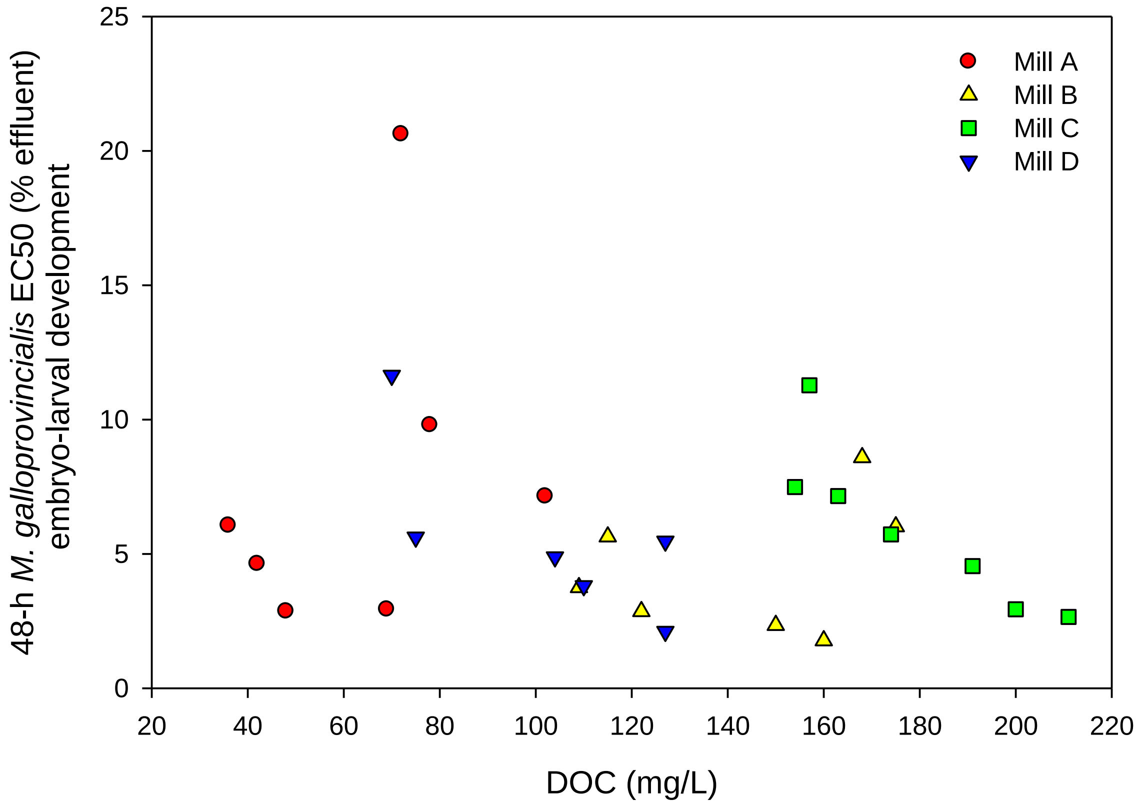


Figure 31. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus dissolved organic carbon (DOC). Significant negative correlation based on Spearman's rho for Mill C.

3.4.4 Toxicity Tests – Organism and Effects Endpoint Comparison

Based on the conservative threshold of $p \leq 0.01$, statistically significant correlations were not observed between the 48-h EC50 *M. galloprovincialis* embryo-larval development and the 7-d IC25 *C. dubia* reproduction (Spearman's rho = 0.60; p = 0.013) or between the 48-h EC50 *M. galloprovincialis* embryo-larval development and the 7-d IC25 *C. dubia* survival (Spearman's rho = 0.54; p = 0.031; Figure 32 and Figure 33, respectively).

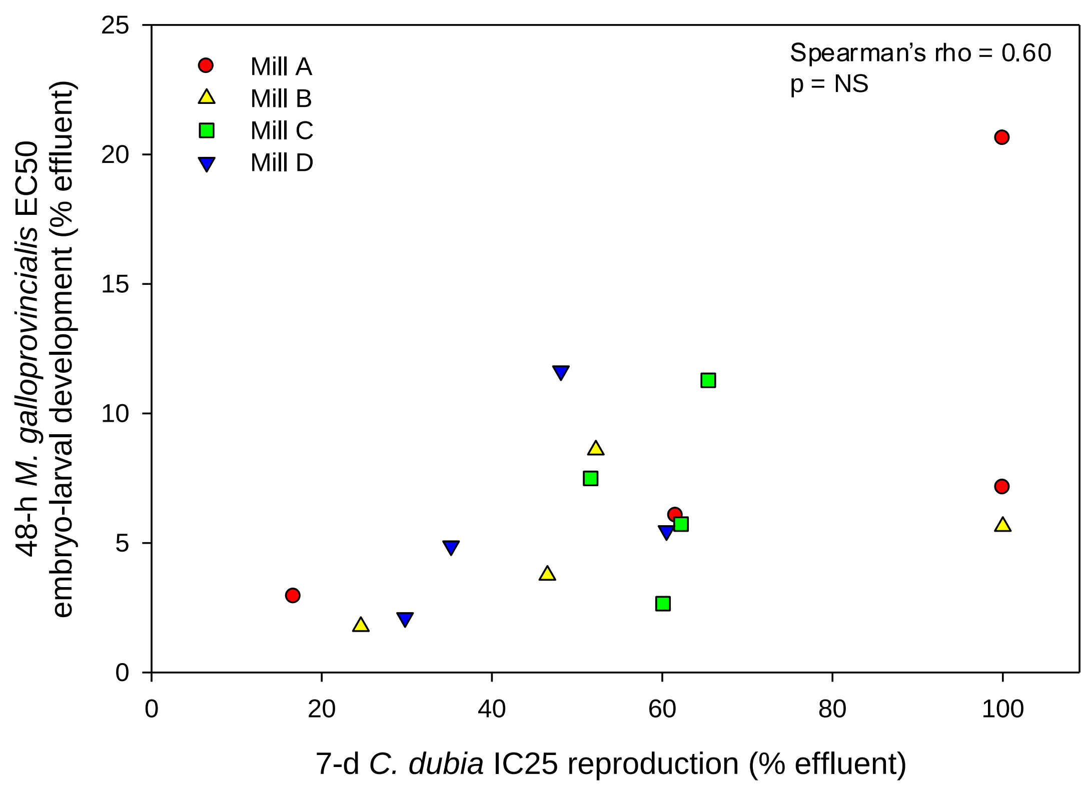


Figure 32. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus 7-d IC25 *Ceriodaphnia dubia* reproduction for pooled data (data coded to denote mill). Correlation not significant based on Spearman's rho. NS: not significant.

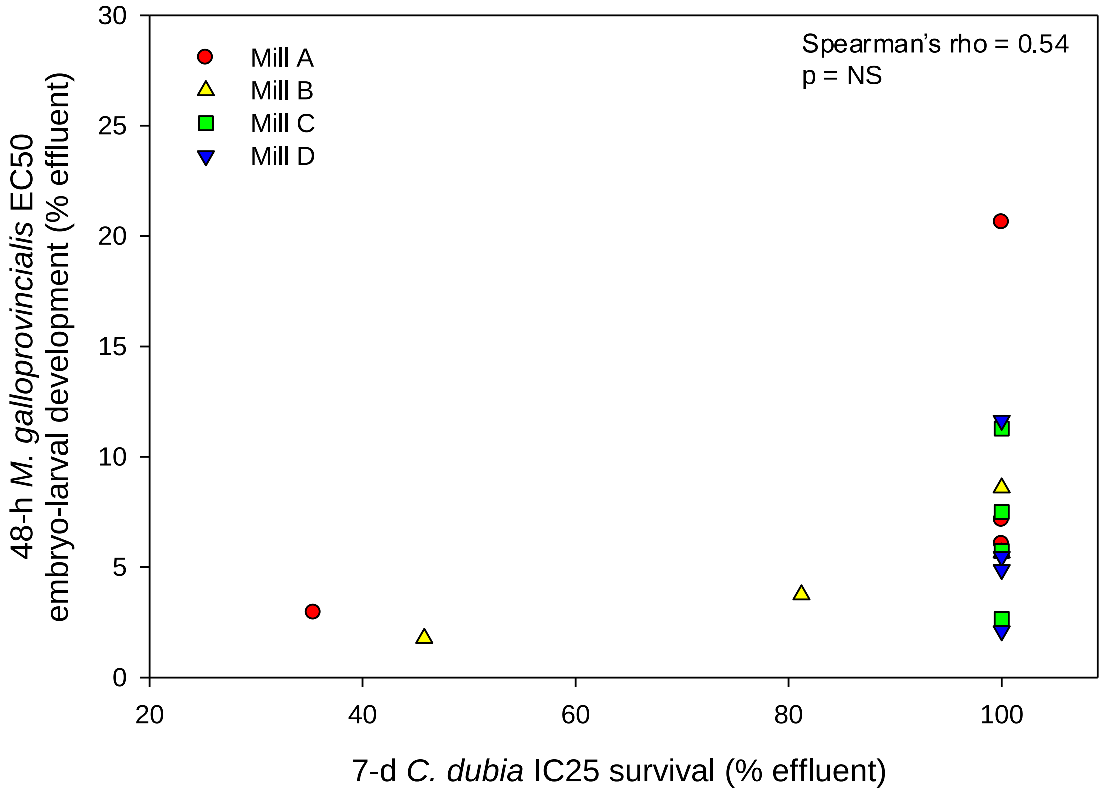


Figure 33. Scatterplot of 48-h EC50 *Mytilus galloprovincialis* embryo-larval development versus 7-d IC25 *Ceriodaphnia dubia* reproduction for pooled data (data coded to denote mill). Correlation not significant based on Spearman's rho. NS: not significant.

3.5 Multivariate Analysis

Using the correlation.r source file developed by Dr. Geoffrey Matthews (Computer Science Department, WWU) correlation coefficients and p-values were calculated between all 23 of the effluent chemistry parameter-pairs to examine relationships and identify and eliminate redundant variables from the multivariate analysis. Non-parametric correlation analysis (Spearman's rho) found 96 significant correlations between the variables (Appendix N). A significant positive correlation (Speraman's rho = 0.86, $p > 0.001$) was found between hardness and alkalinity. Hardness and alkalinity were both positively correlated with DCOD, DOC, and six of seven RAs. Hardness and alkalinity were also negatively correlated with campesterol. Because both hardness and alkalinity are measured in mg of $CaCO_3$ and show a positive correlation these were considered redundant and alkalinity was excluded from multivariate analysis. The results of multivariate analysis can be found in sections 3.5.1 through 3.5.4.

3.5.1 Hierarchical Clustering

Hierarchical clustering was used to explore how simulated effluent samples were grouping together based on the chemical analysis. The goal of the hierarchical clustering was to determine if samples with similar toxicity or similar additions of WBL solids grouped together. Because correlation analysis showed a non-random mill effluent effect (i.e. it appears that samples from the sample mill are grouping together), the first step of this exploratory analysis was to use clustering to determine if samples from the same mill group together using all of the chemistry data, Because some of the chemical parameters included probable outliers, hierarchical distance and clustering methods were selected that were robust to outliers. Three combinations of distance/clustering methods were used, including: 1) Euclidean distances with Average clustering; 2) Euclidean distances with Centroid clustering: and 3) Canberra distances with Average clustering. More than one hierarchical distance/clustering combination was used to confirm that patterns observed in the data were consistent across different distance/clustering combinations. Euclidean and Canberra distances are more robust with respect to heteroscedastic variance, while the Average (Bayesian) and Centroid clustering methods are less sensitive to outliers.

As a first step, four clusters were requested to see if samples were clustering according to mill (based on the chemical analysis of simulated effluent samples). Requesting four clusters, hierarchical clustering with Canberra distances and the average clustering method put samples from each of the four mills into their own group (Figure 34). Euclidean distances with Average and Centroid clustering placed samples from Mill A and Mill C into their own group (Figure 35). Euclidean distances with Average and Centroid clustering also put all samples from Mill D into the one cluster; however samples from Mill B were split between two clusters (Figure 35). Results of association analysis with four clusters and the three different methods showed that clustering was not random (Table 32), and that samples were not clustering based on toxicity or WBL solids (as clusters could be classified by mill regardless of EC50 or amount of WBL solids added).

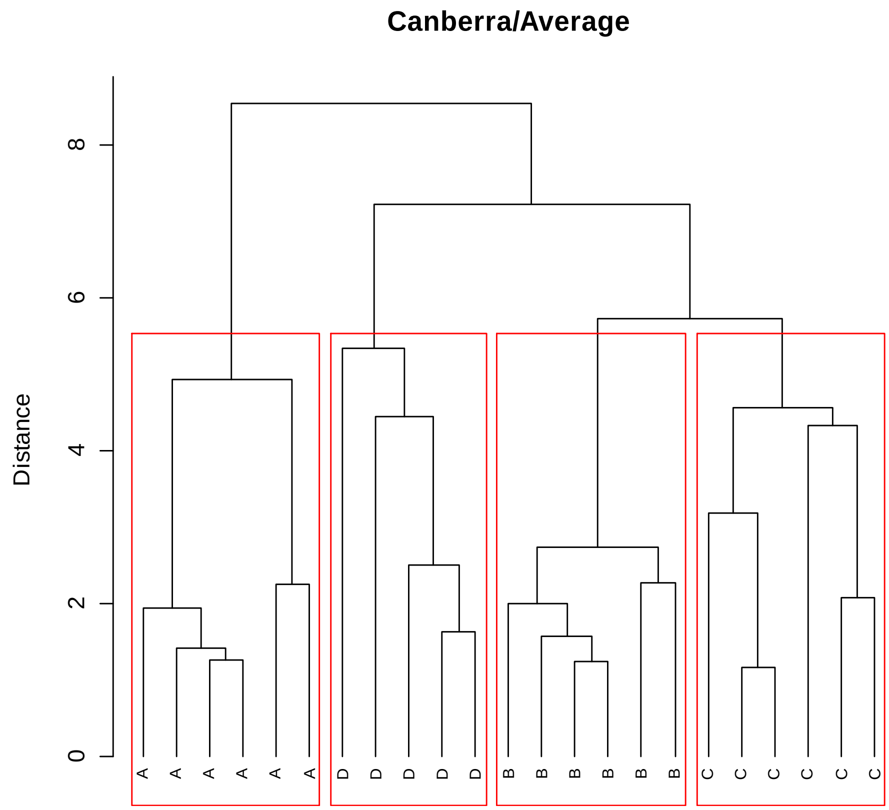


Figure 34. Hierarchical clustering of simulated effluent samples using chemical parameters and Canberra distances with the Average cluster method and four clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill.

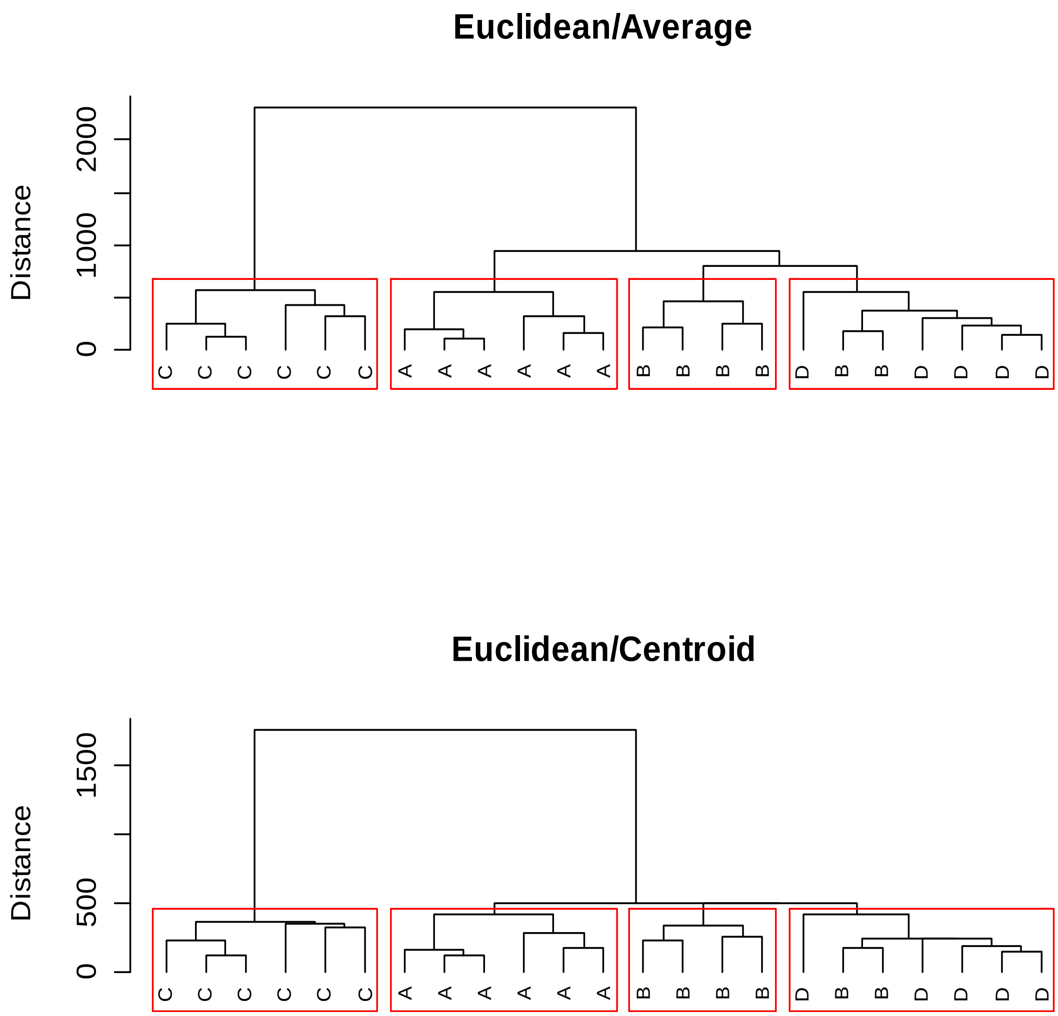


Figure 35. Hierarchical clustering of simulated effluent samples using chemical parameters and Euclidean distances with the Average and Centroid cluster methods and four clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill

Table 32. Hierarchical clustering association analysis table for simulated effluent samples based on chemical parameters with four clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster. df: degrees of freedom.

Distance/Cluster	**Mill**	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**	χ^2	**df**	**p-value**
Euclidean/Average	A	6	0	0	0	56.9524	9	5.16E-09
	B	0	2	4	0			
	C	0	0	0	6			
	D	0	5	0	0			
Euclidean/Centroid	A	6	0	0	0	56.9524	9	5.16E-09
	B	0	2	4	0			
	C	0	0	0	6			
	D	0	5	0	0			
Canberra/Average	A	6	0	0	0	69	9	2.39E-11
	B	0	6	0	0			
	C	0	0	6	0			
	D	0	0	0	5			

Requesting three clusters, hierarchical clustering with Canberra distances and the average clustering method put samples from Mill A and Mill D each into their own group, with all samples from Mill B and Mill C in the same cluster (Figure 36). Euclidean distances with Average and Centroid clustering placed samples from Mill A and Mill C into their own group with all samples from Mill B and Mill D in the same cluster (Figure 37). Results of association analysis with three clusters and the three different methods showed that clustering was not random (Table 33), and that samples were not clustering based on toxicity or WBL solids.

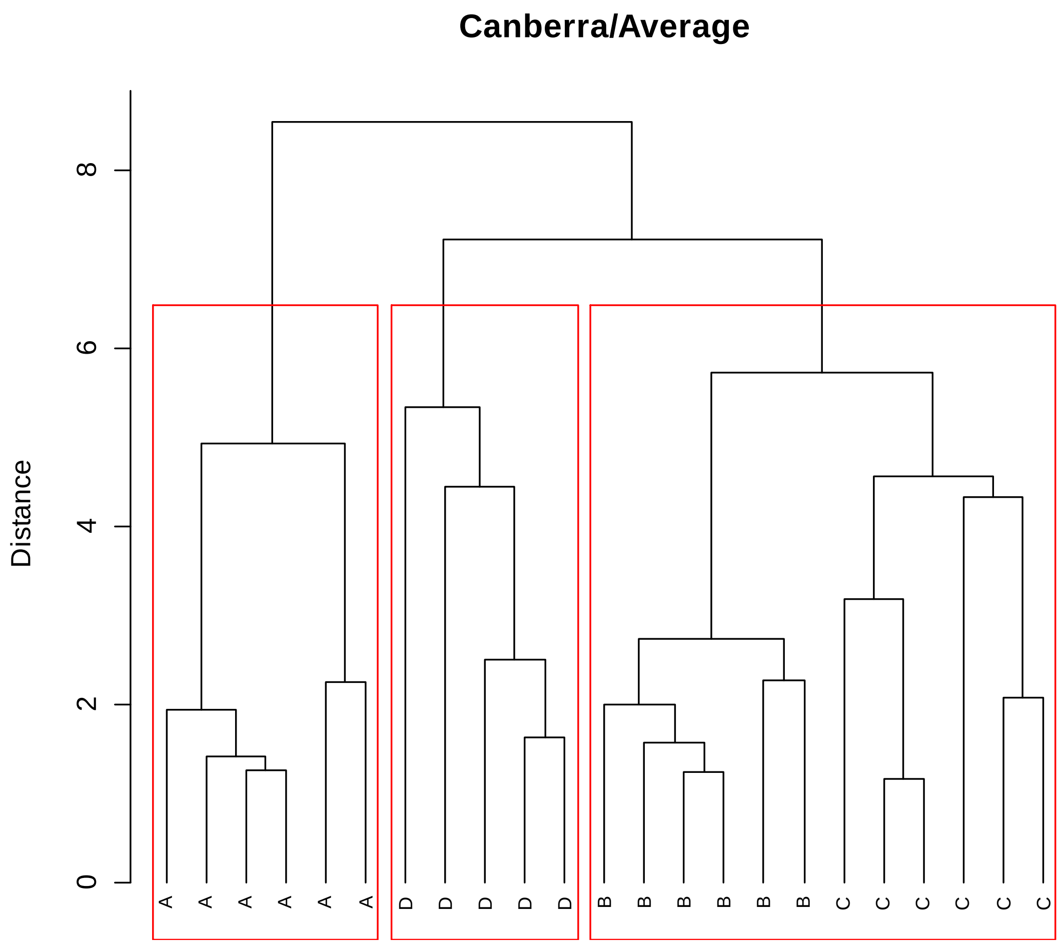
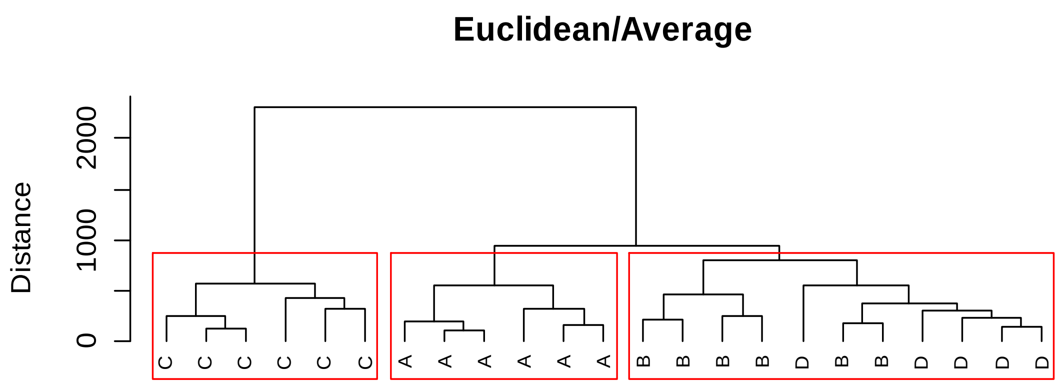


Figure 36. Hierarchical clustering of simulated effluent samples using chemical parameters and Canberra distances with the Average cluster method and three clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill.



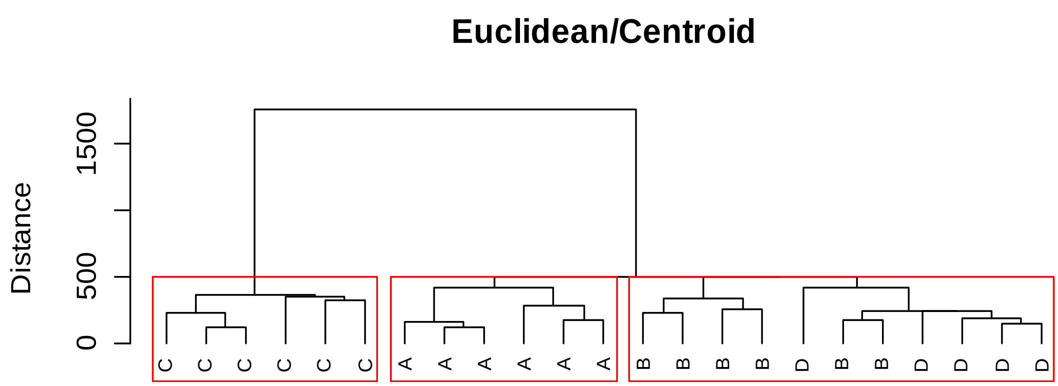


Figure 37. Hierarchical clustering of simulated effluent samples using chemical parameters and Euclidean distances with the Average and Centroid cluster methods and three clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill.

Table 33. Hierarchical clustering association analysis table for simulated effluent samples based on chemical parameters with three clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Distance/Cluster	Mill	Cluster 1	Cluster 2	Cluster 3	χ^2	df	p-value
Euclidean/Average	A	6	0	0	46	6	2.96E-08
	B	0	6	0			
	C	0	0	6			
	D	0	5	0			
Euclidean/Centroid	A	6	0	0	46	6	2.96E-08
	B	0	6	0			
	C	0	0	6			
	D	0	5	0			
Canberra/Average	A	6	0	0	46	6	2.96E-08
	B	0	6	0			
	C	0	6	0			
	D	0	0	5			

Finally, requesting two clusters, hierarchical clustering with Canberra distances and the average clustering method put samples from Mill A into their own group, with all remaining samples in the second cluster (Figure 38). Euclidean distances with Average and Centroid clustering placed samples from Mill A and Mill C into their own group with all samples from Mill B and Mill D in the same cluster (Figure 39). Results of association analysis with three clusters and the three different methods showed that clustering was not random (Table 34), and that samples were not clustering based on toxicity or WBL solids.

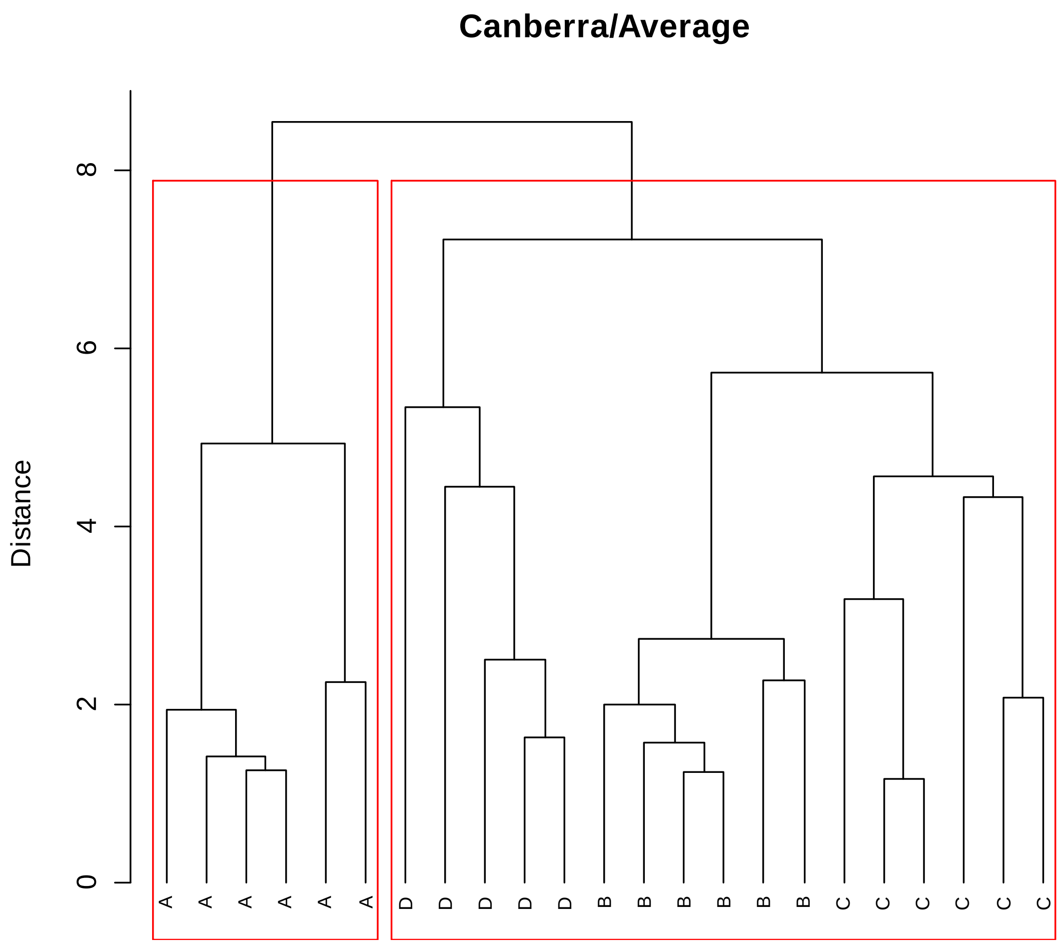


Figure 38. Hierarchical clustering of simulated effluent samples using chemical parameters and Canberra distances with the Average cluster method and two clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill.

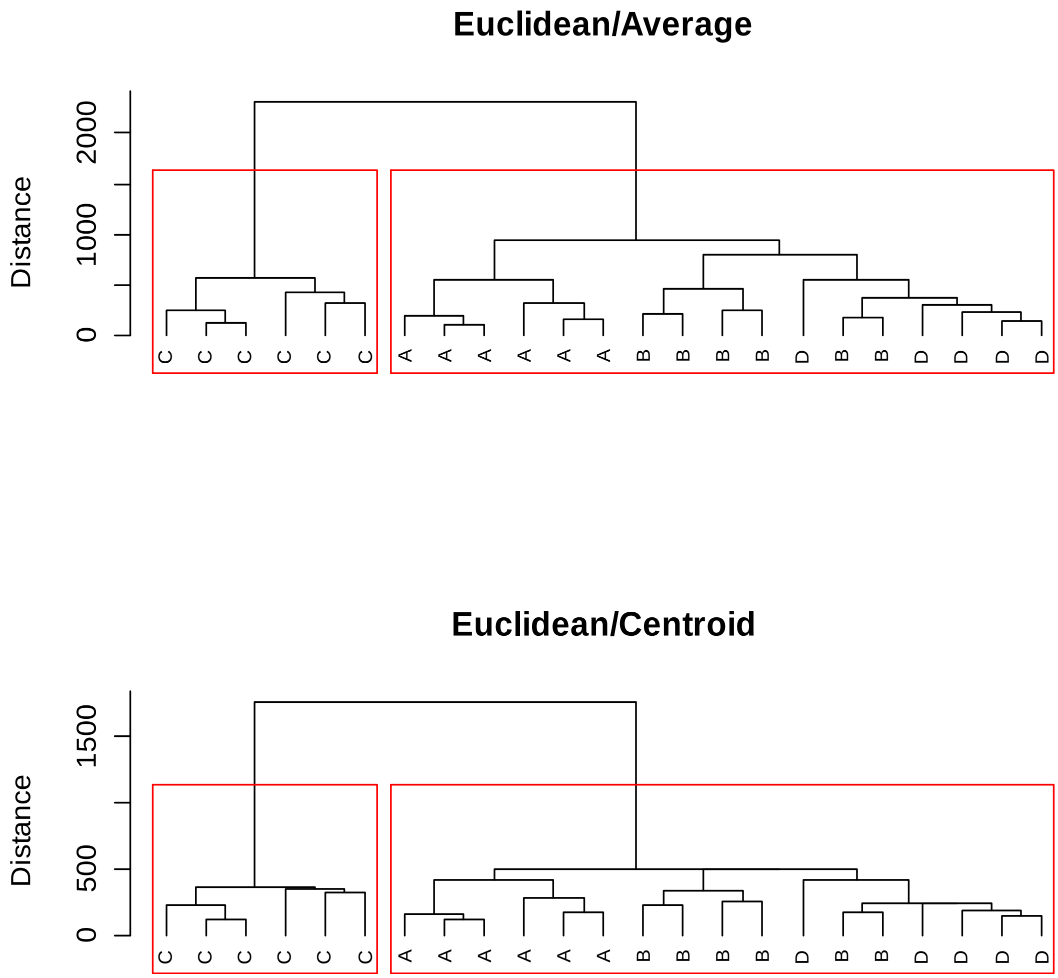


Figure 39. Hierarchical clustering of simulated effluent samples using chemical parameters and Euclidean distances with the Average and Centroid cluster methods and three clusters. Letters (A, B, C, and D) on the x-axis correspond to the mill.

Table 34. Hierarchical clustering association analysis table for simulated effluent samples based on chemical parameters with two clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Distance/Cluster	Mill	Cluster 1	Cluster 2	χ^2	df	p-value
Euclidean/Average	A	6	0	23	3	4.04E-05
	B	6	0			
	C	0	6			
	D	5	0			
Euclidean/Centroid	A	6	0	23	3	4.04E-05
	B	6	0			
	C	0	6			
	D	5	0			
Canberra/Average	A	6	0	23	3	4.04E-05
	B	0	6			
	C	0	6			
	D	0	5			

3.5.2 Kmeans Clustering

Kmeans clustering was also used to explore how the simulated effluent chemistry data grouped together based on the chemical analysis. As with hierarchical clustering, this clustering technique was used to see if simulated effluent samples with similar toxicity or WBL solids grouped together. Kmeans clustering shows how strongly each variable contributes to the separation between the different clusters. Therefore if samples with similar toxicity or WBL solids are clustered together, it may be possible to identify the specific chemical parameters that are driving the trend. Because results of kmeans clustering is based on iterations, results can change each time the analysis is conducted; as such, repeated kmeans clustering runs were used to verify that results were stable. Association analysis was used to determine if kmeans clustering results were random when samples were classified by mill or WBL treatment.

First, kmeans clustering was used to determine if samples would cluster into four groups (i.e. if the samples from the four mills would each cluster into their own group). Repeated kmeans runs found that 80% of the time (4 out of 5 runs), samples from Mill A and samples from Mill C each had their own cluster, samples from Mill D were all in the same cluster, and samples from Mill B were split between two clusters (Table 35). Association analysis showed that clustering with samples classified by mill was non-random (Table 35). Two variables with good separation between cluster centers (Appendix O) were chosen to plot the results of the multivariate kmeans clustering with four groups (Figure 40). Association analysis showed that kmeans clustering using the simulated effluent chemistry data was not able to separate the samples into four groups with samples classified by WBL treatment, and that clustering was random (Table 36).

Table 35. Kmeans clustering association analysis table for simulated effluent samples with four clusters and samples classified by mill. Values in the “Cluster” columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	Mill	Cluster 1	Cluster 2	Cluster 3	Cluster 4	χ^2	df	p-value
Run 1	A	0	0	6	0	56.9524	9	5.16E-09
	B	0	4	0	2			
	C	6	0	0	0			
	D	0	0	0	5			
Run 2	A	0	6	0	0	56.9524	9	5.16E-09
	B	0	0	2	4			
	C	6	0	0	0			
	D	0	0	5	0			
Run 3	A	0	6	0	0	56.9524	9	5.16E-09
	B	2	0	4	0			
	C	0	0	0	6			
	D	5	0	0	0			
Run 4	A	0	0	6	0	56.9524	9	5.16E-09
	B	2	4	0	0			
	C	0	0	0	6			
	D	5	0	0	0			
Run 5	A	6	0	0	0	30.9919	9	2.97E-04
	B	2	4	0	0			
	C	0	0	3	3			
	D	3	2	0	0			

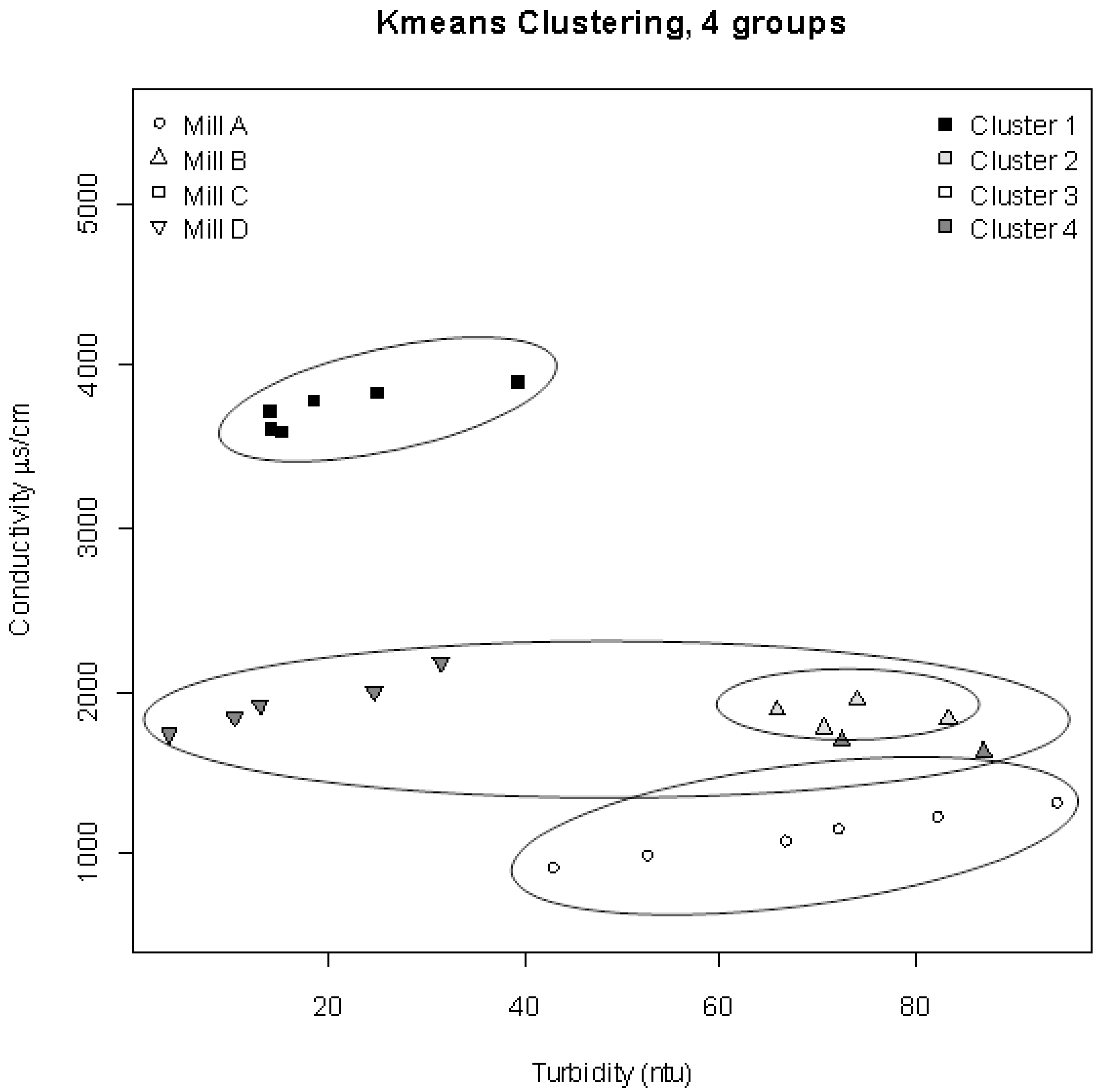


Figure 40. Kmeans clustering bivariate plot using turbidity and conductivity with simulated effluent samples from four bleached kraft mills and four clusters.

Table 36. Kmeans clustering association analysis table for simulated effluent samples with four clusters and samples classified by weak black liquor treatment. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	WBL Solids	Cluster 1	Cluster 2	Cluster 3	Cluster 4	χ^2	df	p-value
Run 1	Reactor control	1	2	1	0	4.4038	15	0.996
	WBL spike 1	1	2	1	0			
	WBL spike 2	1	1	1	1			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	0	1	1			
	WBL spike 5	1	1	1	1			
Run 2	Reactor control	1	0	1	2	4.4038	15	0.996
	WBL spike 1	1	0	1	2			
	WBL spike 2	1	1	1	1			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	1	1	1			
Run 3	Reactor control	1	0	2	1	4.4038	15	0.996
	WBL spike 1	1	0	2	1			
	WBL spike 2	1	1	1	1			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	0	1			
	WBL spike 5	1	1	1	1			
Run 4	Reactor control	2	0	1	1	6.3598	15	0.973
	WBL spike 1	2	0	1	1			
	WBL spike 2	2	0	1	1			
	WBL spike 3	2	1	0	1			
	WBL spike 4	1	1	0	1			
	WBL spike 5	2	1	0	1			
Run 5	Reactor control	1	0	2	1	6.3598	15	0.973
	WBL spike 1	1	0	2	1			
	WBL spike 2	1	0	2	1			
	WBL spike 3	1	1	2	0			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	1	2	0			

Next, kmeans was also used to determine if samples would cluster into three groups. Consistent across most repeated kmeans clustering runs was Mill C simulated effluent samples grouping separate and simulated effluent samples from Mills B and D grouping together (Table 37). Two variables with good separation between cluster centers (Appendix O) were chosen to plot the results of the multivariate kmeans clustering with three groups (Figure 41). Results of association analysis showed that kmeans clustering was not able to separate the samples into three groups with samples classified by WBL treatment, and that clustering was random (Table 38).

Table 37. Kmeans clustering association analysis table for simulated effluent samples with three clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	Mill	Cluster 1	Cluster 2	Cluster 3	χ^2	df	p-value
Run 1	A	0	6	0	46	6	2.96E-08
	B	6	0	0			
	C	0	0	6			
	D	5	0	0			
Run 2	A	6	0	0	46	6	2.96E-08
	B	0	0	6			
	C	0	6	0			
	D	0	0	5			
Run 3	A	6	0	0	30.9919	6	2.54E-05
	B	2	4	0			
	C	0	0	6			
	D	3	2	0			
Run 4	A	0	6	0	30.9919	6	2.54E-05
	B	0	2	4			
	C	6	0	0			
	D	0	3	2			
Run 5	A	0	0	6	23	6	7.97E-04
	B	0	0	6			
	C	3	3	0			
	D	0	0	5			

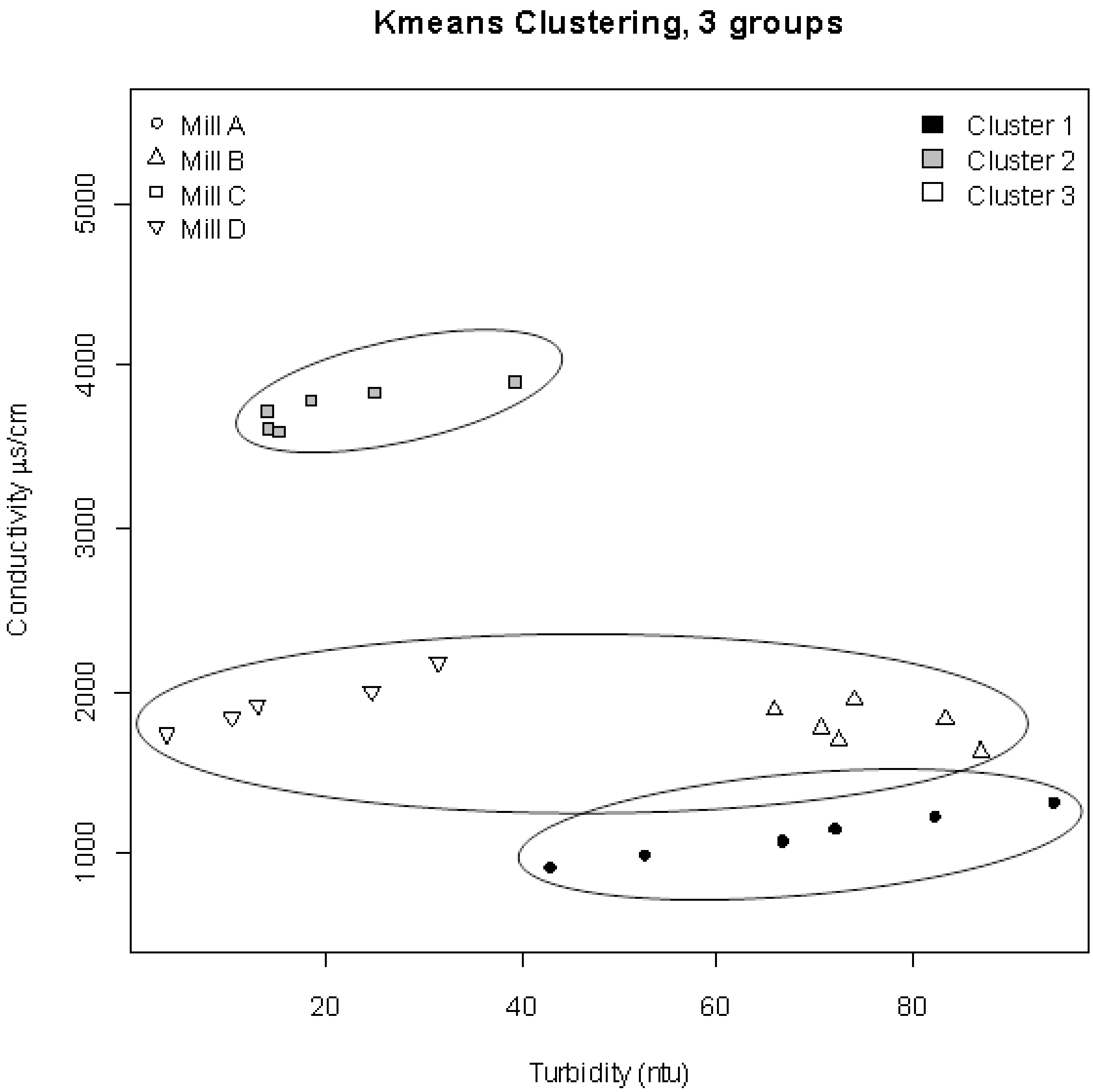


Figure 41. Kmeans clustering bivariate plot using turbidity and conductivity with simulated effluent samples from four bleached kraft mills and three clusters.

Table 38. Kmeans clustering association analysis table for simulated effluent samples with three clusters and samples classified by weak black liquor treatment. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	WBL Solids	Cluster 1	Cluster 2	Cluster 3	χ^2	df	p-value
Run 1	Reactor control	1	2	1	0.2904	10	1.000
	WBL spike 1	1	2	1			
	WBL spike 2	1	2	1			
	WBL spike 3	1	2	1			
	WBL spike 4	1	1	1			
	WBL spike 5	1	2	1			
Run 2	Reactor control	3	0	1	6.2146	10	0.797
	WBL spike 1	3	0	1			
	WBL spike 2	2	1	1			
	WBL spike 3	1	2	1			
	WBL spike 4	1	1	1			
	WBL spike 5	1	2	1			
Run 3	Reactor control	1	1	2	0.2904	10	1.000
	WBL spike 1	1	1	2			
	WBL spike 2	1	1	2			
	WBL spike 3	1	1	2			
	WBL spike 4	1	1	1			
	WBL spike 5	1	1	2			
Run 4	Reactor control	2	1	1	0.2904	10	1.000
	WBL spike 1	2	1	1			
	WBL spike 2	2	1	1			
	WBL spike 3	2	1	1			
	WBL spike 4	1	1	1			
	WBL spike 5	2	1	1			
Run 5	Reactor control	1	1	2	0.2904	10	1.000
	WBL spike 1	1	1	2			
	WBL spike 2	1	1	2			
	WBL spike 3	1	1	2			
	WBL spike 4	1	1	1			
	WBL spike 5	1	1	2			

Finally, kmeans was used to determine if samples would cluster into two groups. Repeated kmeans runs found that results were stable, with Mill C simulated effluent samples grouping separate from simulated effluent samples from the other three mills (Table 39). Association analysis showed that kmeans clustering with samples classified by mill was non-random (Table 39). Two variables with good separation between cluster centers (Appendix O) were chosen (conductivity and turbidity) to plot the results of the multivariate kmeans clustering with two groups (Figure 42). Association analysis showed that kmeans clustering was not able to separate the samples into two groups with samples classified by WBL treatment, and that clustering was random (Table 40).

Table 39. Kmeans clustering association analysis table for simulated effluent samples with two clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	Mill	Cluster 1	Cluster 2	χ^2	df	p-value
Run 1	A	6	0	23	3	4.04E-05
	B	6	0			
	C	0	6			
	D	5	0			
Run 2	A	6	0	23	3	4.04E-05
	B	6	0			
	C	0	6			
	D	5	0			
Run 3	A	6	0	23	3	4.04E-05
	B	6	0			
	C	0	6			
	D	5	0			
Run 4	A	0	6	23	3	4.04E-05
	B	0	6			
	C	6	0			
	D	0	5			
Run 5	A	0	6	23	3	4.04E-05
	B	0	6			
	C	6	0			
	D	0	5			

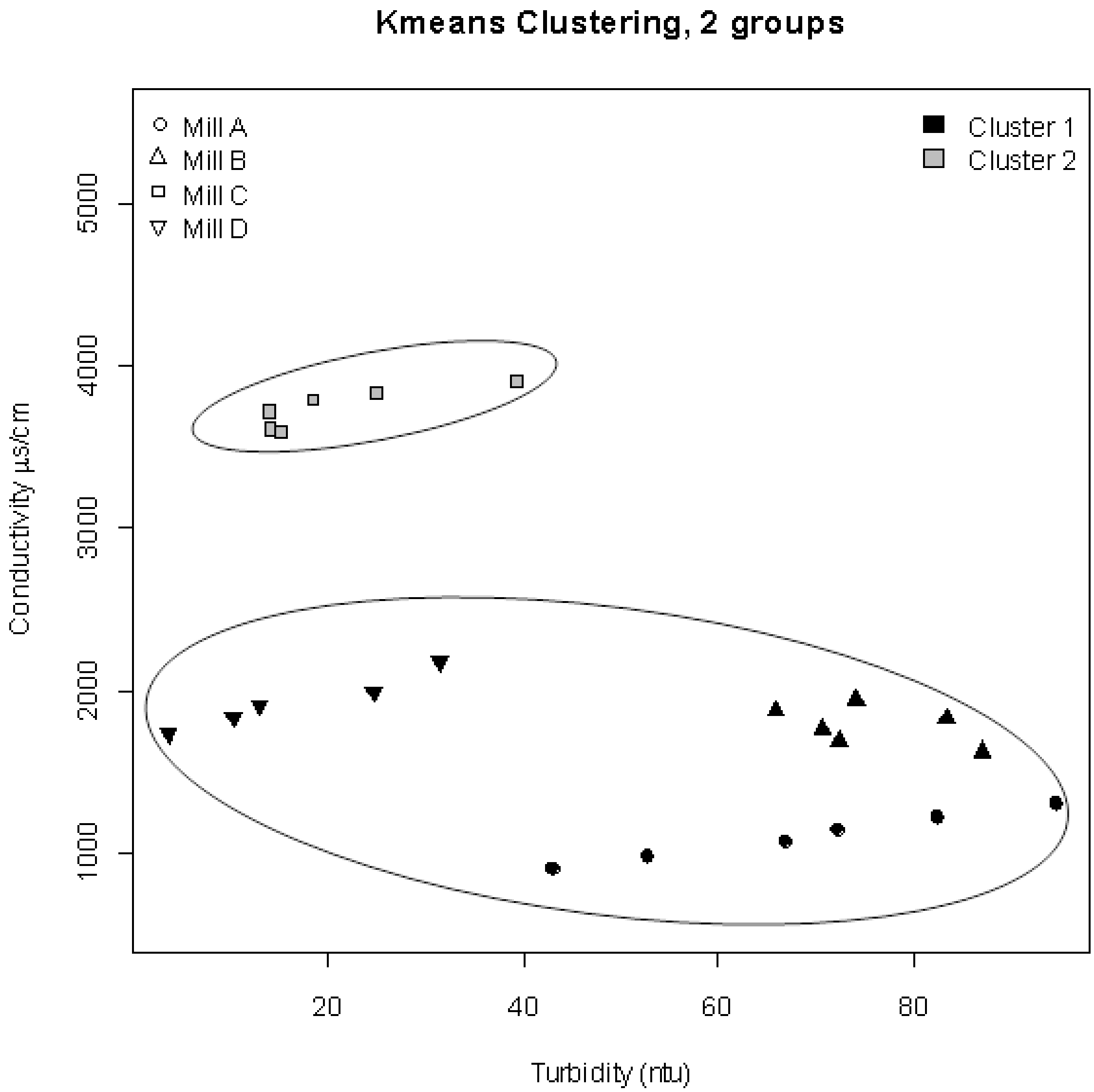


Figure 42. Kmeans clustering bivariate plot using turbidity and conductivity with simulated effluent samples from four bleached kraft mills and two clusters.

Table 40. Kmeans clustering association analysis table for simulated effluent samples with two clusters and samples classified by weak black liquor treatment. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	WBL Solids	Cluster 1	Cluster 2	χ^2	df	p-value
Run 1	Reactor control	1	3	0.094	5	1.000
	WBL spike 1	1	3			
	WBL spike 2	1	3			
	WBL spike 3	1	3			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 2	Reactor control	1	3	0.094	5	1.000
	WBL spike 1	1	3			
	WBL spike 2	1	3			
	WBL spike 3	1	3			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 3	Reactor control	1	3	0.094	5	1.000
	WBL spike 1	1	3			
	WBL spike 2	1	3			
	WBL spike 3	1	3			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 4	Reactor control	3	1	0.094	5	1.000
	WBL spike 1	3	1			
	WBL spike 2	3	1			
	WBL spike 3	3	1			
	WBL spike 4	2	1			
	WBL spike 5	3	1			
Run 5	Reactor control	1	3	0.094	5	1.000
	WBL spike 1	1	3			
	WBL spike 2	1	3			
	WBL spike 3	1	3			
	WBL spike 4	1	2			
	WBL spike 5	1	3			

3.5.3 Riffle Clustering

Using the simulated effluent chemistry data, Riffle clustering was used to see if simulated effluent samples grouped by WBL treatment or by mill. Riffle clustering is a non-metric and non-parametric clustering method. Riffle does not use distance metrics (like hierarchical and kmeans clustering), rather each variable is examined independently (Matthews 2011). Another feature of Riffle clustering is the ability to identify variables that don't contribute to clustering, and exclude them (Matthews 2011). For this reason, Riffle is a good option when variance is heteroscedastic. Similar to kmeans clustering, this technique shows which variables are contributing to separation between clusters using proportional reduction in error (PRE) scores. Variables with high PRE scores are those that contribute most to the separation seen between clusters. As is the case for kmeans clustering, repeated analysis (Riffle runs) can produce different results. For this reason, multiple Riffle runs were conducted to determine if results remained stable.

Requesting four clusters, Riffle runs separated the samples into four groups. Association analysis showed that Riffle was unable to cluster the data based on mill, although clustering was non-random (Table 41). Consistent across all Riffle runs, simulated effluent samples from Mill A clustered separate from other samples (Table 41). Two variables with high PRE scores (Appendix P) were chosen to plot the results of the multivariate Riffle clustering with four groups (Figure 43). Association analysis also found that Riffle clustering was not able to separate the data into four groups based on WBL treatment (Table 42).

Table 41. Riffle clustering association analysis table for simulated effluent samples with four clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Kmeans Run	Mill	Cluster 1	Cluster 2	Cluster 3	Cluster 4	χ^2	df	p-value
Run 1	A	6	0	0	0	44.9048	9	9.61E-07
	B	0	6	0	0			
	C	0	0	6	0			
	D	0	3	1	1			
Run 2	A	6	0	0	0	49.45	9	1.37E-07
	B	0	5	0	1			
	C	0	0	5	1			
	D	0	0	1	4			
Run 3	A	6	0	0	0	49.45	9	1.37E-07
	B	0	5	0	1			
	C	0	0	5	1			
	D	0	0	1	4			
Run 4	A	6	0	0	0	43.0307	9	2.13E-06
	B	0	1	5	0			
	C	0	0	0	6			
	D	0	0	4	1			
Run 5	A	6	0	0	0	55.2256	9	1.10E-08
	B	0	5	0	1			
	C	0	0	6	0			
	D	0	1	0	4			

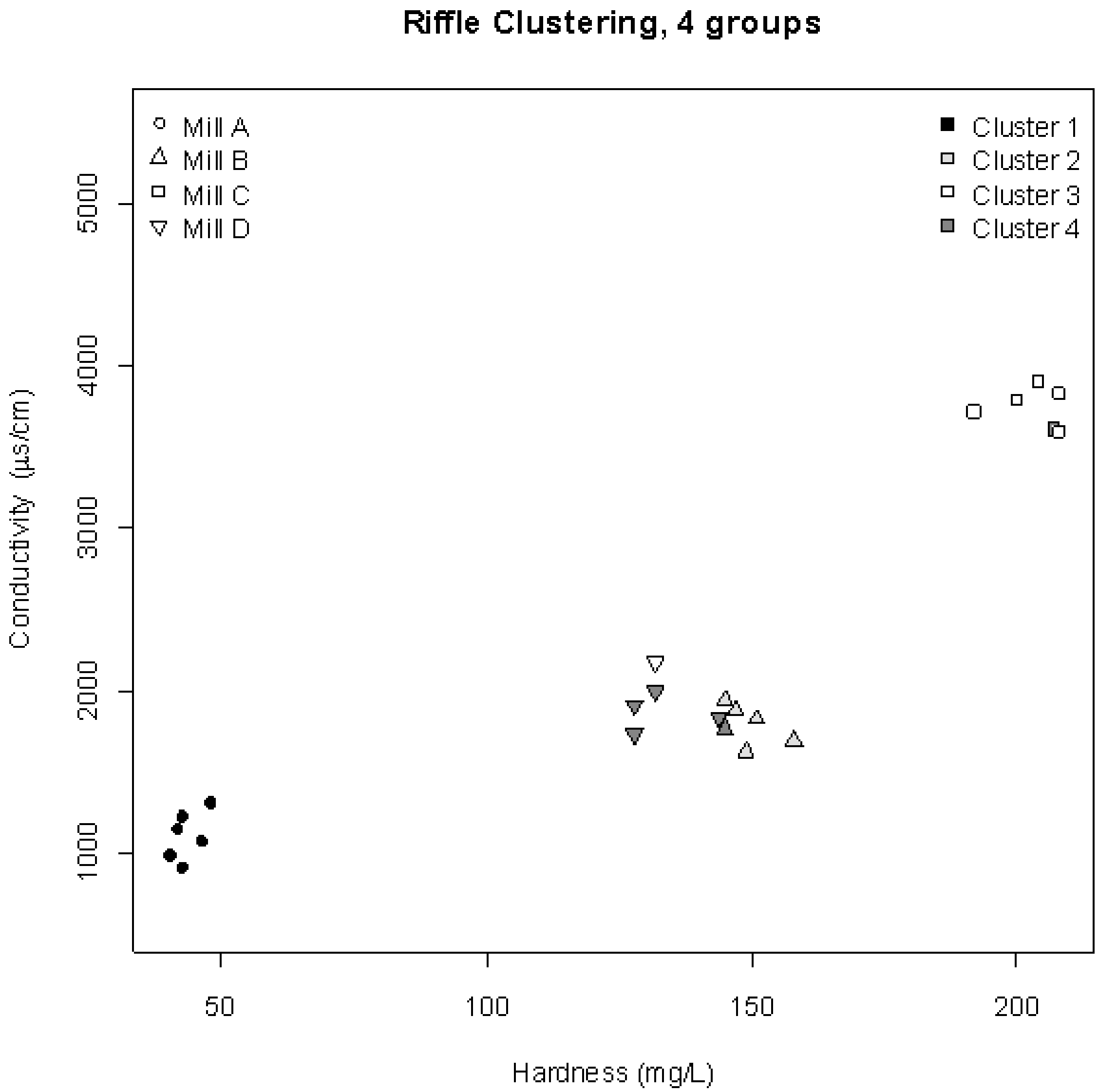


Figure 43. Riffle clustering bivariate plot using hardness and conductivity with simulated effluent samples from four bleached kraft mills and four clusters.

Table 42. Riffle clustering association analysis table for simulated effluent samples with four clusters and samples classified by weak black liquor treatment. Values in the “Cluster” columns indicate the number of effluent samples placed into each cluster.

Riffle Run	WBL Solids	Cluster 1	Cluster 2	Cluster 3	Cluster 4	χ^2	df	p-value
Run 1	Reactor control	1	2	1	0	6.2824	15	0.975
	WBL spike 1	1	2	1	0			
	WBL spike 2	1	2	1	0			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	1	2	0			
Run 2	Reactor control	1	1	0	2	6.7722	15	0.964
	WBL spike 1	1	1	1	1			
	WBL spike 2	1	0	1	2			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	1	2	0			
Run 3	Reactor control	1	1	1	1	0.9583	15	1.000
	WBL spike 1	1	1	1	1			
	WBL spike 2	1	1	1	1			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	1	1	1			
Run 4	Reactor control	2	1	0	1	10.6694	15	0.776
	WBL spike 1	1	1	1	1			
	WBL spike 2	1	1	0	2			
	WBL spike 3	1	1	1	1			
	WBL spike 4	1	1	1	0			
	WBL spike 5	1	0	3	0			
Run 5	Reactor control	3	1	0	0	15.5729	15	0.411
	WBL spike 1	1	2	0	1			
	WBL spike 2	1	1	1	1			
	WBL spike 3	1	0	2	1			
	WBL spike 4	1	0	2	0			
	WBL spike 5	1	0	3	0			

Next, Riffle clustering was used to determine if samples could be grouped into three clusters. Association analysis showed that Riffle clustering with samples classified by mill was non-random, although there were several misclassifications (Table 43). Two variables with high PRE scores (Appendix P) were chosen to plot the results of the multivariate Riffle clustering with three groups (Figure 44). Riffle clustering was not able to separate the data into three groups based on WBL treatment; association analysis showed that clustering with samples classified by WBL treatment was random (Table 44).

Table 43. Riffle clustering association analysis table for simulated effluent samples with three clusters and samples classified by mill. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Riffle Run	Mill	Cluster 1	Cluster 2	Cluster 3	χ^2	df	p-value
Run 1	A	6	0	0	28.0868	6	9.05E-05
	B	0	4	2			
	C	0	5	1			
	D	1	0	4			
Run 2	A	6	0	0	22.9589	6	8.10E-04
	B	0	4	2			
	C	0	1	5			
	D	2	2	1			
Run 3	A	5	1	0	24.0268	6	5.16E-04
	B	2	4	0			
	C	0	3	3			
	D	0	0	5			
Run 4	A	6	0	0	31.1732	6	2.35E-05
	B	0	6	0			
	C	0	1	5			
	D	2	1	2			
Run 5	A	6	0	0	22.9589	6	8.10E-04
	B	0	4	2			
	C	0	1	5			
	D	2	2	1			

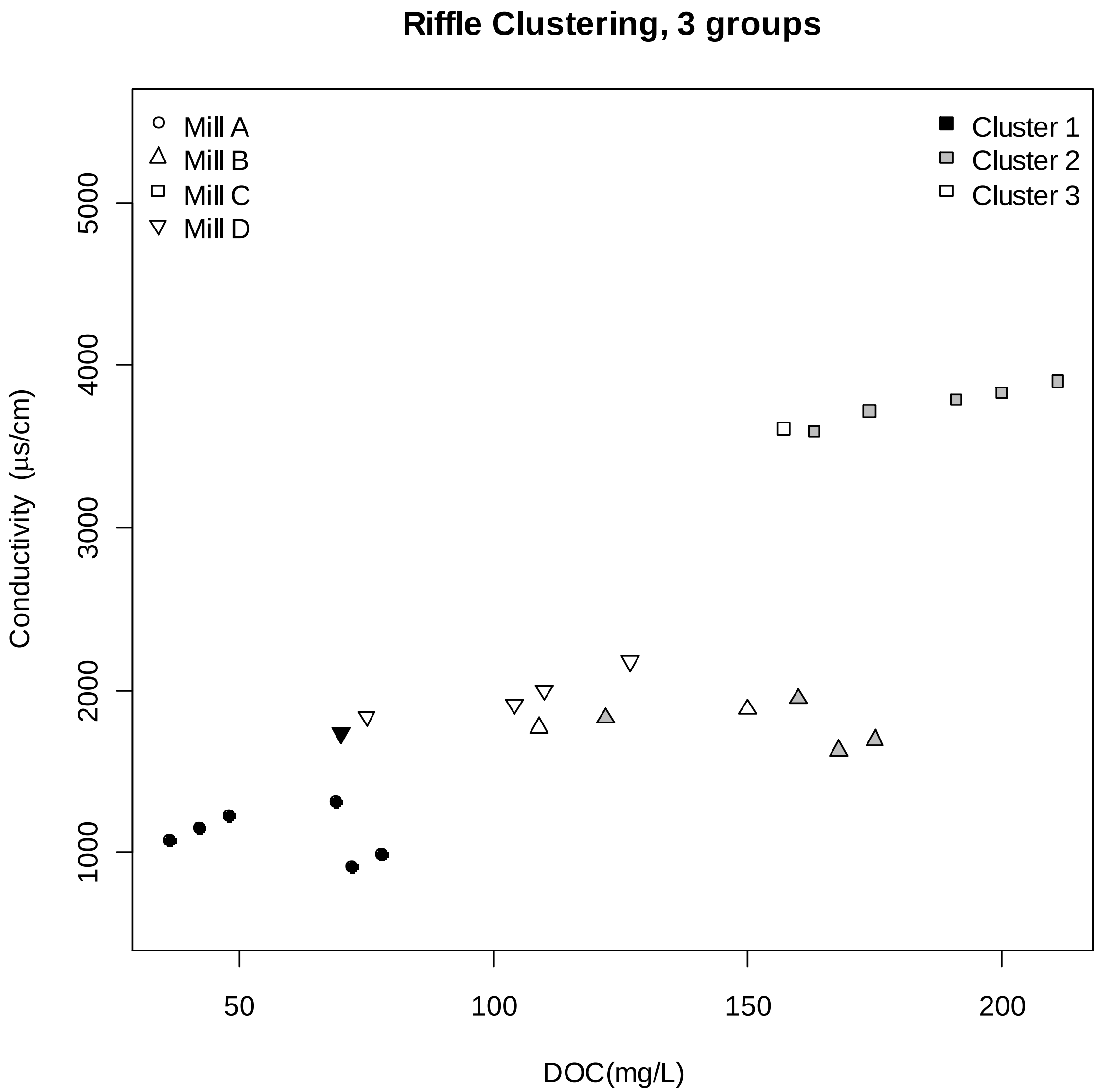


Figure 44. Riffle clustering bivariate plot using dissolved organic carbon and conductivity with simulated effluent samples from four bleached kraft mills and three clusters.

Table 44. Riffle clustering association analysis table for simulated effluent samples with three clusters and samples classified by weak black liquor treatment. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster.

Riffle Run	WBL Solids	Cluster 1	Cluster 2	Cluster 3	χ^2	df	p-value
Run 1	Reactor control	3	1	0	5.9873	10	0.816
	WBL spike 1	1	2	1			
	WBL spike 2	2	1	1			
	WBL spike 3	2	0	2			
	WBL spike 4	1	1	1			
	WBL spike 5	1	1	2			
Run 2	Reactor control	3	0	1	8.7961	10	0.552
	WBL spike 1	2	1	1			
	WBL spike 2	1	1	2			
	WBL spike 3	1	2	1			
	WBL spike 4	1	2	0			
	WBL spike 5	0	2	2			
Run 3	Reactor control	1	1	2	4.4836	10	0.923
	WBL spike 1	1	1	2			
	WBL spike 2	2	1	1			
	WBL spike 3	2	1	1			
	WBL spike 4	1	2	0			
	WBL spike 5	1	2	1			
Run 4	Reactor control	0	1	3	8.3854	10	0.591
	WBL spike 1	1	1	2			
	WBL spike 2	2	1	1			
	WBL spike 3	2	1	1			
	WBL spike 4	1	2	0			
	WBL spike 5	2	0	2			
Run 5	Reactor control	3	0	1	5.4762	10	0.857
	WBL spike 1	1	1	2			
	WBL spike 2	1	2	1			
	WBL spike 3	1	2	1			
	WBL spike 4	1	1	1			
	WBL spike 5	1	1	2			

Finally, requesting two clusters, Riffle separated the samples into two groups. Association analysis showed that Riffle clustering with samples classified by mill was non-random, although for most Riffle runs samples from the same mill were split between two different clusters (Table 45). No patterns in groupings were apparent across repeated Riffle runs. Two variables with consistently high PRE scores (Appendix P) were chosen to plot the results of the multivariate Riffle clustering with three groups (Figure 45). Riffle clustering was not able to separate the data into two groups with samples classified by WBL treatment; association analysis showed that clustering was random (Table 46).

Table 45. Riffle clustering association analysis table for simulated effluent samples with two clusters and samples classified by mill. Values in the “Cluster” columns indicate the number of effluent samples placed into each cluster.

Riffle Run	Mill	Cluster 1	Cluster 2	χ^2	df	p-value
Run 1	A	6	0	12.8475	3	4.98E-03
	B	4	2			
	C	0	6			
	D	2	3			
Run 2	A	6	0	16.4543	3	9.15E-04
	B	0	6			
	C	1	5			
	D	4	1			
Run 3	A	6	0	12.8475	3	4.98E-03
	B	4	2			
	C	0	6			
	D	2	3			
Run 4	A	6	0	16.4543	3	9.15E-04
	B	1	5			
	C	0	6			
	D	4	1			
Run 5	A	5	1	19.6604	3	2.00E-04
	B	6	0			
	C	0	6			
	D	0	5			

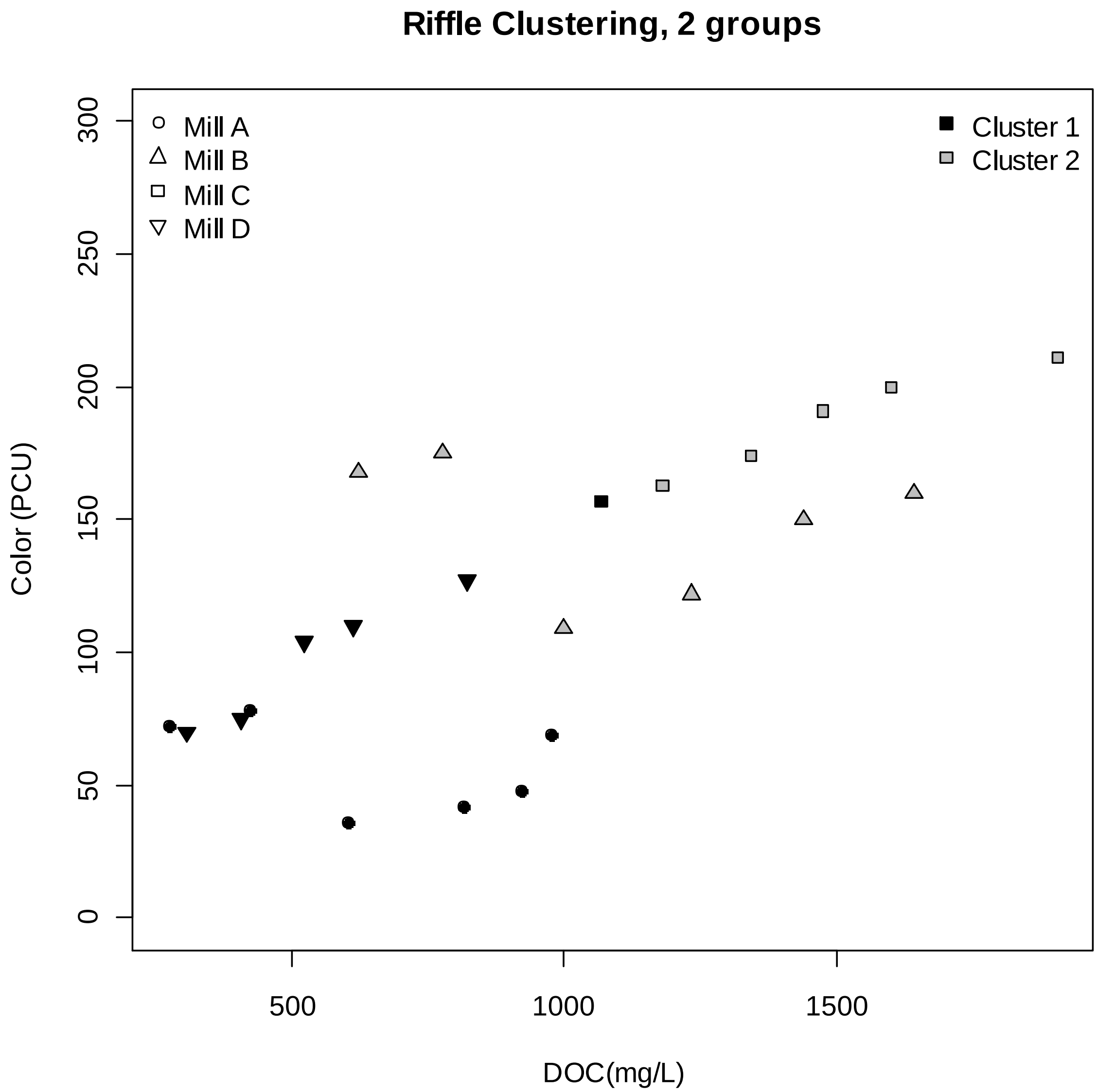


Figure 45. Riffle clustering bivariate plot using color and dissolved organic carbon with simulated effluent samples from four bleached kraft mills and two clusters.

Table 46. Riffle clustering association analysis table for simulated effluent samples with two clusters and samples classified by weak black liquor treatment. Values in the "Cluster" columns indicate the number of effluent samples placed into each cluster

Riffle Run	WBL Solids	Cluster 1	Cluster 2	χ^2	df	p-value
Run 1	Reactor control	3	1	3.2961	5	0.654
	WBL spike 1	3	1			
	WBL spike 2	2	2			
	WBL spike 3	2	2			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 2	Reactor control	3	1	2.2942	5	0.807
	WBL spike 1	2	2			
	WBL spike 2	2	2			
	WBL spike 3	2	2			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 3	Reactor control	3	1	3.2961	5	0.654
	WBL spike 1	3	1			
	WBL spike 2	2	2			
	WBL spike 3	2	2			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 4	Reactor control	2	2	2.2942	5	0.807
	WBL spike 1	2	2			
	WBL spike 2	3	1			
	WBL spike 3	2	2			
	WBL spike 4	1	2			
	WBL spike 5	1	3			
Run 5	Reactor control	1	3	1.2923	5	0.936
	WBL spike 1	2	2			
	WBL spike 2	2	2			
	WBL spike 3	2	2			
	WBL spike 4	2	1			
	WBL spike 5	2	2			

3.5.4 Principal Component Analysis

Principal component analysis found that the first three principal components (PC) explained 82.7% of cumulative variance between samples (Table 47). An exponential drop-off of proportion of variance was also observed, which indicates sample ordination (Appendix Q). Principal component 1 included turbidity, TSS, beta-sitosterol, stigmasterol, and campesterol; PC 2 included polyphenols, color, turbidity, stigmasterol TSS, stigmastanol, beta-sitosterol, isopimaric acid, campesterol, palustric acid, DCOD, and neoabietic acid (Appendix Q). Principal component analysis variable loadings for the first two principal components are shown in Figure 46. Results of PCA found that simulated effluent samples appeared to ordinate by mill (Figure 47) rather than by WBL treatment (Figure 48).

Randomization testing was also used to determine the strength of the PCA analysis. Three data files were created where the association between known groups (mill and WBL treatment) and the results of the chemical analysis were randomized. Principal component analysis using the three random data files found that data did not ordinate by mill (Appendix Q). Variance plots for the three random files showed a gentle decline in proportion of variance in contrast to the exponential drop-off observed in the non-random data file (Appendix Q). For the random data files, only 39.2, 39.4, and 40.3% of the variance was explained by the first three principal components as compared to 82.7% for the non-random data (Appendix Q). A visual evaluation of PCA ordination plots found that the three randomized data files do not show ordination by mill (Appendix Q).

Table 47. Proportional and cumulative variance for first 10 principal components using simulated effluent chemistry data.

	Comp.1	**Comp.2**	**Comp.3**	**Comp.4**	**Comp.5**	**Comp.6**	**Comp.7**	**Comp.8**	**Comp.9**	**Comp.10**
Standard deviation	3.186489	2.191155	1.484539	1.238598	0.815213	0.704011	0.540579	0.539932	0.405287	0.375475
Proportion of Variance	0.48351	0.228627	0.104946	0.073054	0.031646	0.023602	0.013915	0.013882	0.007822	0.006713
Cumulative proportion	0.48351	0.712137	0.817082	0.890136	0.921782	0.945384	0.959299	0.973181	0.981003	0.987716

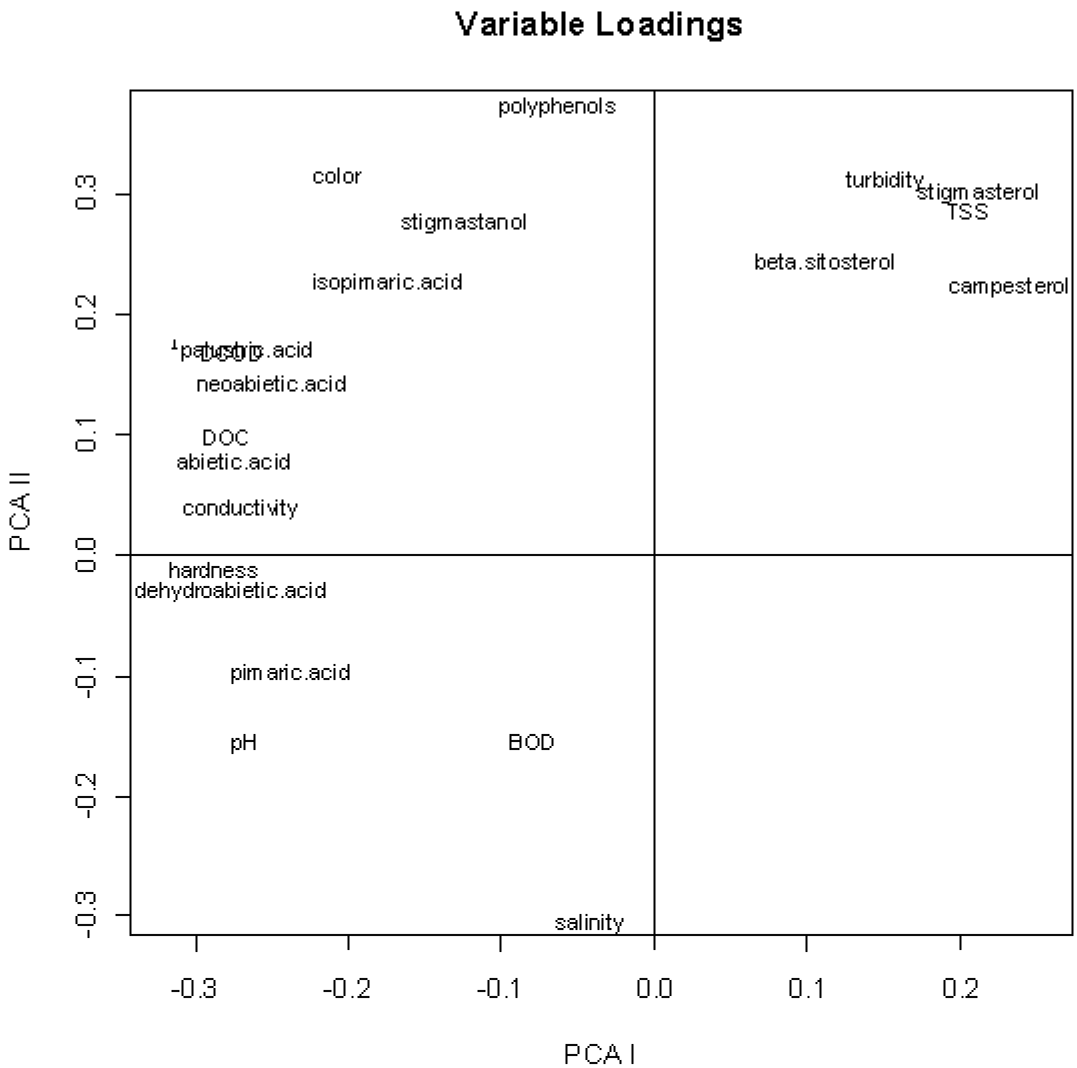


Figure 46. Principal component analysis variable loading of simulated effluent samples for first two principal components. [1]Palustric acid and dissolved chemical oxygen demand (DCOD).

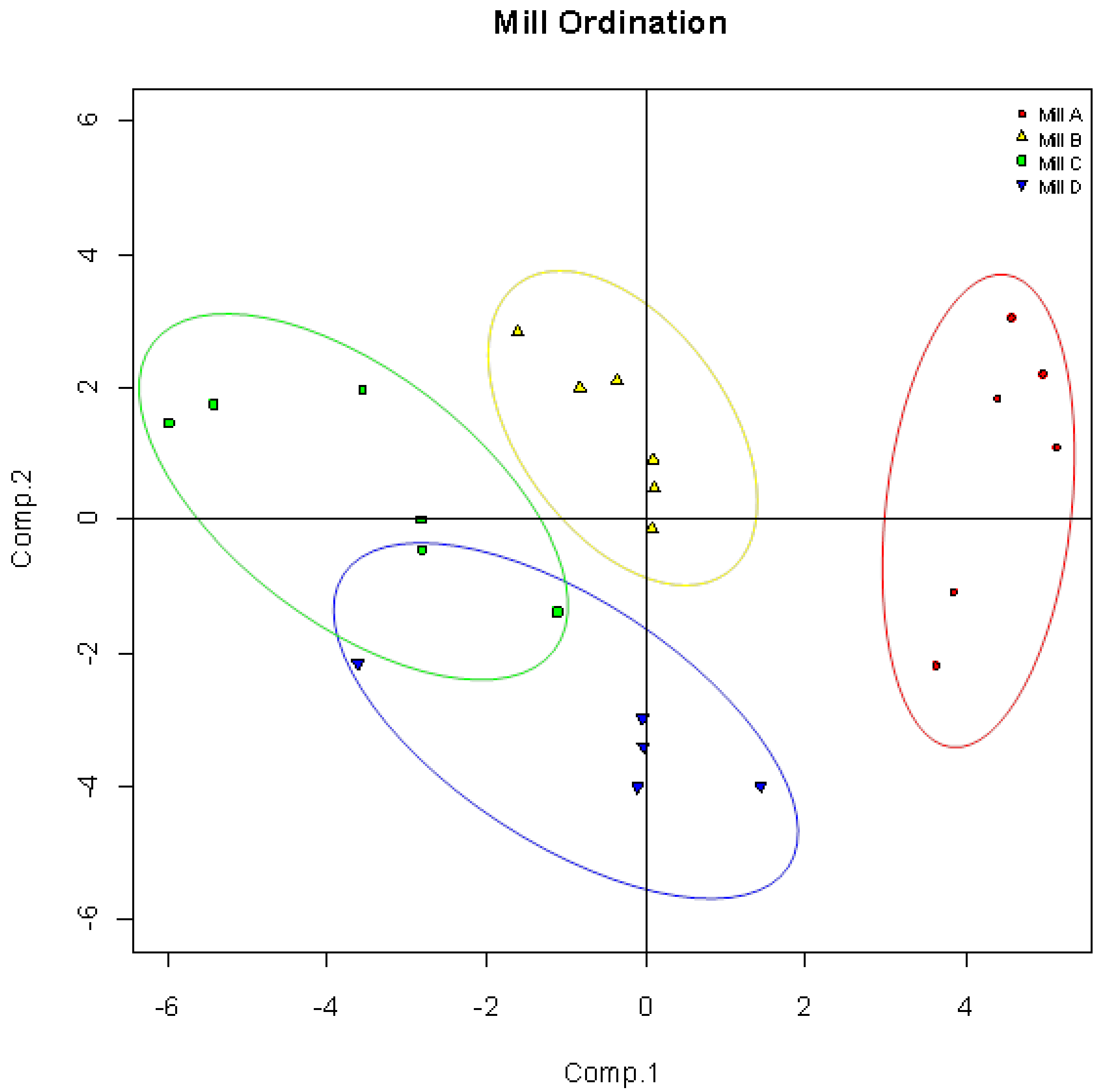


Figure 47. Principal component analysis ordination of simulated effluent samples. Samples coded to denote mill.

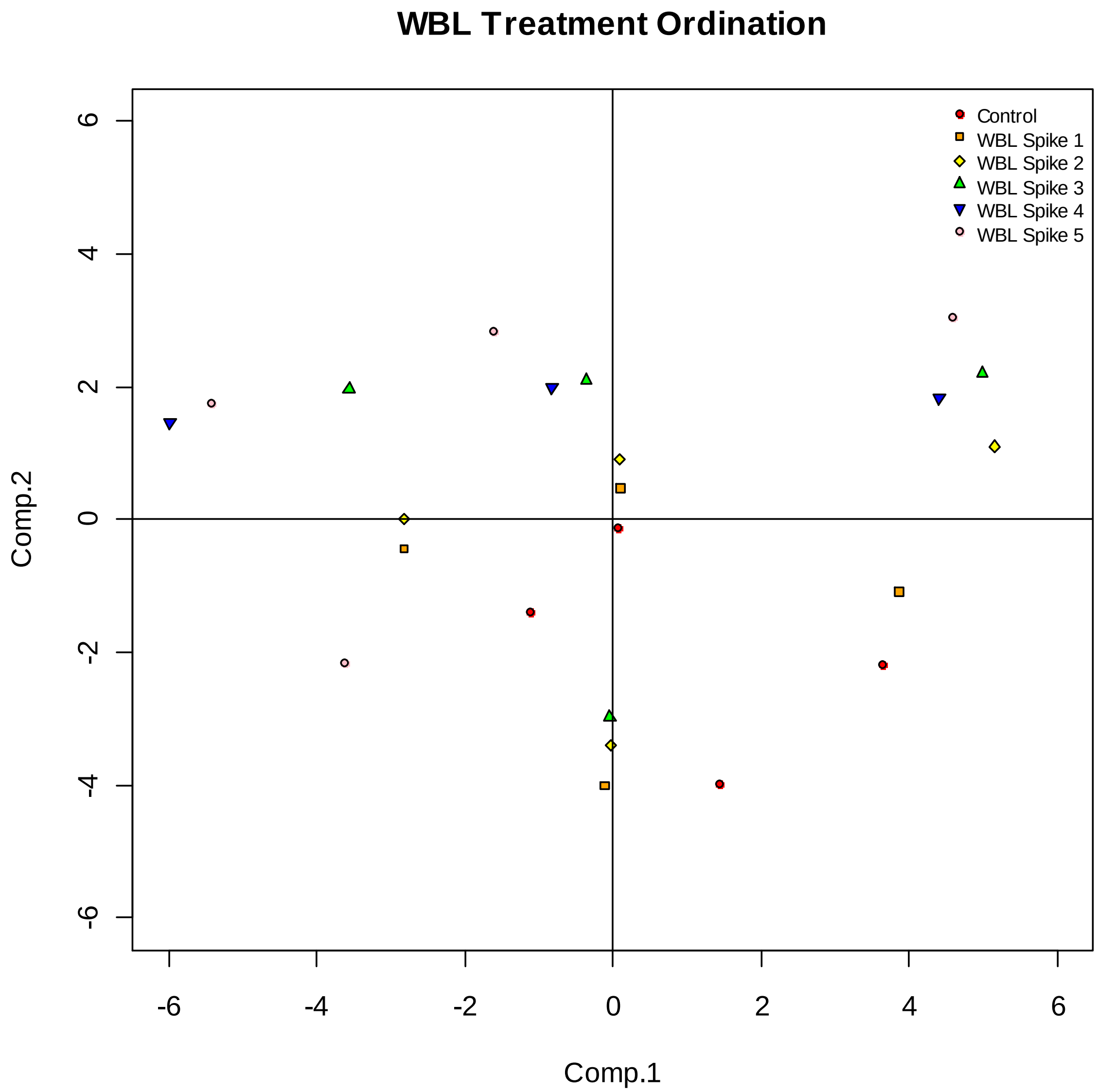


Figure 48. Principal component analysis ordination of simulated effluent samples. Samples coded to denote weak black liquor treatment.

4.0 DISCUSSION

The 48-h EC50 for *M. galloprovincialis* embryo-larval development appears to be a more sensitive endpoint than the 7-d *C. dubia* reproduction with respect to both mill-treated and simulated effluent samples. Some effluent samples that showed chronic toxicity to *M. galloprovincialis* did not show chronic toxicity to *C. dubia*. Due to the sensitivity of *M. galloprovincialis*, significant differences in the 48-h EC50 for embryo-larval development were observed between simulated effluent samples. For all four mills, as the concentration of WBL solids in the simulated effluent samples increased, toxicity to *M. galloprovincialis* increased (i.e. the EC50 decreased).

Correlation analysis was able to find significant relationships between WBL solids and three effluent chemical parameters. Significant positive correlations were found between WBL solids and color, DCOD, and polyphenols. Correlation analysis only indicates a significant relationship (not causation), so it may be that some other chemical parameter not measured during this study is more indicative of WBL losses and co-varies with some (or all) of these three chemical parameters. Currently, mill personnel monitor various chemical parameters such as COD, conductivity, and color for the purpose of monitoring WBL losses; these parameters, however, are not specific to WBL and other sources such as the bleach plant contribute to the base load (Steve Stratton, Regional Manager, NCASI, personal communication). Results from this study confirmed the relationship between WBL solids and color, and between WBL solids and COD (measured in this study as DCOD).

Results of correlation analysis using the mill-treated and simulated effluent samples also found significant relationships between chemical parameters and toxicity to

M. galloprovincialis. For three out of the four mills (Mill A, Mill B, and Mill C), color and polyphenols were negatively correlated with the 48-h EC50 for *M. galloprovincialis* embryo-larval development (i.e. as the EC50 decreased (toxicity increased) these chemical parameters increased). For two out of four mills (Mill B and Mill C), DCOD was negatively correlated with the 48-h EC50 for *M. galloprovincialis* embryo development. Significant negative correlations were observed between the 48-h EC50 for *M. galloprovincialis* embryo-larval development and abietic acid (Mill A), conductivity (Mill B), and DOC (Mill C). No significant correlations were found between the 48-h EC50 for *M. galloprovincialis* embryo-larval development and Mill D effluent chemical parameters. Again, correlation analysis between these variables only indicates a significant relationship (not causation); further studies could determine whether or not increased abietic acid, color, conductivity, DOC, DCOD, and polyphenols contribute directly to increased toxicity, or if instead they co-vary with another chemical substance that was not measured during this study. Because color, DCOD, and polyphenols correlate with both WBL solids and increased toxicity to aquatic organisms, examination of these endpoints could be useful to mill personnel in monitoring the potential significance of WBL losses.

Correlation analysis found significant negative correlations between WBL solids and the 48-h EC50 for *M. galloprovincialis* embryo-larval development, and between WBL solids and the 7-d IC25 *C. dubia* reproduction (i.e. as WBL solids increased the toxicity increased (as indicated by a decrease in EC50/IC25)). Results from this study confirm findings reported by Carey et al. (2002) who observed a general trend of increased sub-lethal toxicity (reproduction) to *C. dubia* as estimated WBL losses increased. A significant correlation was not observed between *C. dubia* survival and WBL solids. Whether or not increased WBL is the cause of increased toxicity could be confirmed with further studies that use replication of WBL treatments within a mill instead of a single replicate per WBL treatment as was done here (i.e. simulated effluents from the same mill having the same additions of WBL solids across multiple replicates) and linear regression to predict toxicity based on addition of WBL solids. For this to be logistically feasible (due to laboratory space and time constraints), it is likely that fewer WBL treatment concentrations (i.e. spike levels) would be tested at the same time so that each WBL treatment concentration being examined could be tested in replicate concurrently.

Multivariate methods including cluster analysis (hierarchical, kmeans, and non-metric Riffle) and PCA were used to explore the data for patterns, with the goal of identifying effluent chemical parameters that might relate to WBL solids or effluent toxicity. Consistent across all multivariate methods, simulated effluent samples appeared to be grouping together based on mill rather than on the amount of WBL added (i.e. WBL treatment). Because simulated effluent samples from the same mill were grouping together regardless of WBL solids, it was not possible to identify effluent chemical parameters that were related to increased WBL solids with multivariate methods.

Multivariate methods were also used to identify effluent chemical parameters that might correspond to increased effluent toxicity (i.e. decreased EC50/IC25). If effluent samples with similar toxicity (i.e. similar IC25/EC50s) grouped together, then these methods may have identified the specific chemical parameters driving this trend. Because samples grouped together based on mill (A, B, C, or D) rather than on toxicity (i.e. all effluent samples from Mill A were grouping together and represented samples with a range of IC25s/EC50s), it was not possible to determine specific effluent chemical parameters that consistently corresponded to increased toxicity using multivariate methods.

It was unclear using data from the 48-h embryo-larval development *M. galloprovincialis* toxicity tests whether furnish type (i.e. the type of wood being pulped) was an important factor in the toxicity of biologically treated WBL in kraft mill effluents. The highest toxicity (i.e. lowest EC50s) of WBL-spiked simulated effluent samples were observed for Mill B samples, which uses primarily softwood, while the lowest toxicity (i.e. the highest EC50s) was observed in samples from a mill that also uses softwood (Mill A).

Results from the 7-d *C. dubia* survival and reproduction tests also did not clearly show that furnish type was an important factor in toxicity of biologically treated WBL. For WBL spiked simulated effluent samples, Mill C had the lowest toxicity (i.e. the highest IC25s); across the highest WBL spiked simulated effluent samples (WBL spike 5), Mill A had the highest toxicity (i.e. the lowest IC25).

As with most laboratory studies, the application of results to field situations (or in this case - mill operations) can be challenging. Although the laboratory biotreatment was designed to replicate secondary (biological) treatment at a bleached kraft mill, it is not possible to fully reproduce this process in the laboratory. Because biotreatment ranged from 5-10 d depending on the mill, it may be that some of the simulated effluent samples were more fully treated than others. It is possible that the five day biotreatment of Mill B simulated effluent samples was not sufficient to reduce toxicity, and accounted for simulated effluent samples from this mill having the highest toxicity. Overall DCOD reduction in the

Mill B reactors during biotreatment was 31-37% compared to 41-53% for Mill A, 44-47% for Mill C, and 37-65% for Mill D. Analysis of RAs measured before and after biotreatment to estimate treatment efficiencies for the highest spiked reactor (WBL spike 5) and the reactor control for Mill B and Mill C, however, found that estimated treatment efficiencies ranged from 96.1 to 100% for the highest spiked reactor (WBL spike 5) and from 67.2 to 100% for the reactor control. Due to heavy foaming and high solids content that clogged the aquarium pumps, a longer biotreatment (10 days) was required for Mill C simulated effluent samples. This additional time was required to allow the DCOD to stabilize. Based on the estimated treatment efficiencies using the DCOD data, however, biotreatment of Mill C simulated effluent samples appear similar to those for Mills A and D.

During this study, biotreatment efficiency was measured in terms of overall DCOD reduction. Because reactors from all four mills showed similar reductions in DCOD, all data were pooled together to examine the relationship between WBL solids and effluent chemical parameters. It could be, however, that not all effluent chemical parameters were reduced by the same degree across all four mills during the laboratory biotreatment. For this reason, future studies could examine the relationship between WBL solids and effluent chemical parameters for un-pooled data (i.e. each mill examined individually). Future work might also measure effluent chemical parameters before and after laboratory biotreatment to examine if individual effluent chemical parameters correlate with increased WBL solids before and after biotreatment (for the un-pooled data), and determine if trends are consistent across different mills

Three months prior to the sampling of Mill D for this study, aeration was decreased in the ASB treatment pond which lead to lowered treatment pond DO levels and high effluent BOD. In an effort to increase DO in the final effluent, the mill used pumps to recycle effluent back through the treatment system. During this same time period, the mill experienced a fiber line liquor (dilute WBL) spill. Once BOD levels had decreased, the amount of effluent being recycled through the treatment system was reduced. When Mill D was sampled for this study, approximately 20% of the effluent was still being recycled back into the mill treatment system. According to mill personnel, the treatment system was almost back at normal levels and the treatment system process was fairly representative (Terry Bousquet, Project Leader, NCASI, personal communication). Results of the RA analysis of the Mill D mill-treated effluent sample, however, were over 1 ppm, which is usually indicative of seeing a black liquor spill at a mill (Diana Cook, Principal Research Scientist, NCASI, personal communication). Estimated treatment efficiencies (based on DCOD reductions) for the Mill D simulated effluent samples were also highly variable. Compared to the three other mill-treated effluent samples, Mill D showed the highest toxicity to *M. galloprovincialis*. For the *C. dubia* tests, however, the Mill C mill-treated effluent sample was more toxic compared to the Mill D mill-treated effluent sample.

Although *M. galloprovincialis* was the primary test organism for this study, the majority of pulp mills in the U.S. have NPDES permits that require the use of *C. dubia* for chronic WET testing (U.S. EPA 2011b). Due to logistical constraints, it was not feasible to conduct toxicity tests with *C. dubia* on all of the simulated effluent samples, so a subset of samples was also tested with *C. dubia*. Results from this study were that *M. galloprovincialis* are a more sensitive test organism compared to *C. dubia* chronic endpoints with respect to WBL. Although a significant correlation was not found between the toxicity results of the two organisms for this study, analysis of unpublished data generated by NCASI using 124 effluent samples found a significant positive correlation between the IC25 for *C. dubia* reproduction and the 48-h EC50 for *M. galloprovincialis* embryo-larval development (Spearman's rho = 0.57, $p < 0.001$). The lack of a significant correlation between those measures in this study could be due to not enough tests being conducted concurrently.

While there was a significant correlation between WBL solids and toxicity to both test species, it may be that different chemical parameters present in WBL are responsible for the increased toxicity. In this study, none of the effluent chemical parameters examined correlated with increased toxicity to *C. dubia* chronic endpoints. The lack of a significant correlation between effluent chemical parameters and toxicity to *C. dubia* chronic endpoints in this study could be due to not enough tests being conducted. Future work should investigate the relationship between these two chronic tests to determine if increased toxicity is due to the same effluent chemical parameter(s). One approach would be to examine individual chemical parameters that correlated with increased toxicity to *M. galloprovincialis* (i.e. abietic acid, color, conductivity, DOC, DCOD, and polyphenols) to see if there is a relationship between these parameters and increased toxicity to *C. dubia* chronic endpoints.

Analysis by Carey et al. (2002) using Cycle 2 EEM data from six bleached kraft mills in British Columbia found a general trend of increased sub-lethal (reproductive) toxicity to fish with increased.estimated WBL losses. Because the 7-d *P. promelas* survival and growth toxicity tests is the second most commonly used freshwater WET test for evaluating the chronic toxicity of mill effluents to aquatic organisms under the NPDES permitting system (U.S. EPA 2011b), further studies might also be conducted to confirm findings by Carey et al. (2002). Future work might focus on other marine and estuarine WET test species; those currently used for evaluating chronic WET of pulp mill effluents include *Menidia spp* and *A, bahia*; (U.S. EPA 2011b).

5.0 CONCLUSIONS

This study provides the first results about the chronic and sub-lethal toxicity of treated WBL to aquatic organisms. Similar to studies previously conducted using untreated WBL, an increase in chronic toxicity was observed as the amount of treated WBL increased. Although increased WBL solids were related to increased chronic toxicity to both test organisms, *M. galloprovincialis* appear to be a more sensitive test organism with respect to WBL. Color, DCOD, and polyphenols were positively correlated with increased WBL, and of those three parameters, color and polyphenols were negatively correlated with the 48-h EC50 for *M. galloprovincialis* embryo-larval development.

Future research could focus on individual effluent chemical parameters that correlated with either toxicity or WBL solids (i.e. abietic acid, color, conductivity, DOC, DCOD or polyphenols) to further determine effluent components in WBL that might contribute to final mill effluent toxicity. One approach could be to determine the chemistry and toxicity of biotreated mill effluent samples with variable levels (or extreme high or extreme low levels) of these individual chemical parameters. This would provide information about whether or not the relationship between the chemical parameter and WBL solids, or between the chemical parameter and toxicity is consistent across a wide range of possible values. To confirm if an individual chemical parameter correlates with WBL solids and effluent toxicity, a TIE correlation or species sensitivity approach could be used. The TIE correlation approach uses toxic units (TUs) to examine the relationship between effluent toxicity and the toxicity of an individual chemical parameter to determine if there is a consistent relationship between the concentration of a specific toxicant (i.e. chemical parameter) and effluent toxicity (Mount and Norberg-King 1993). This would require additional testing of mill effluents having a wide range of toxicities (in addition do a wide range of chemical measurements). The TIE species sensitivity approach uses toxicity tests with two different organisms of varying sensitivities to determine the ratio of toxicity of a suspect toxicant (i.e. individual chemical parameter) to the toxicity of the effluent sample. If the individual chemical parameter is responsible for toxicity, the ratio between toxicity of the individual parameter and toxicity of mill effluent will be the same for the two test organisms (Mount and Norberg-King 1993). It is possible, however, that some unmeasured effluent chemical parameter(s) may be related to increased toxicity and WBL losses, so future work might also use different tools to identify the unknown parameters(s). One such approach might be to use a combination of GC-MS and PCA to look for trends in the more comprehensive chemical data. Another factor to consider is particle size analysis of the WBL solids; it may be that particle size of WBL components is an important factor in bioavailability and toxicity to aquatic organisms.

Results of the multivariate analysis were that simulated effluent samples were most similar based on mill origin, rather than factors such as chronic toxicity or WBL solids. Because effluent composition between mills was so variable, it is difficult to apply findings from this study to other bleached kraft mills in the U.S. To overcome this challenge, one approach might be to use composite samples from several mills to evaluate the chronic toxicity and composition of WBL. The composite sample approach would however need to take into account several factors, including whether the resulting composite sample would have an additive, antagonistic, or synergistic, effect on chronic toxicity. The overall goal of this approach would be to determine more broadly which WBL chemical parameters generally contribute to increased toxicity.

REFERENCES

Adams TN, Frederick WJ, Grace TM, Hupa M, Lisa K, Jones AK, Tran H. 1997. *Kraft Recovery Boilers*. TAPPI Press, Atlanta, GA, USA.

American Public Health Association (APHA). 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Washington, DC, USA.

Balcan M, Gupta P. 2010. Robust Hierarchical Clustering. In Kalai AT, Mohri M, eds, *Proceedings of the 23rd Conference on Learning Theory (COLT)*. OmniPress for the Association for Computational Learning, Madison, WI, USA, pp 282-294.

Barkley WA, Hardan KJ, Anderson DH, Folster HG. 1986. Characterization of weak black liquor and its effect on aerated lagoon operation. In Water Pollution Control Federation, ed, *Proceedings of the Industrial Wastes Symposia*. Water Pollution Control Federation, Alexandria, VA, USA, pp 1-16.

Biermann CJ. 1996. *Handbook of Pulping and Papermaking*. Academic Press, San Diego, CA, USA.

Burnison BK, Hodson PV, Nuttley DJ, Efler S. 1996. A bleached-kraft mill effluent fraction causing induction of a fish mixed-function oxygenase enzyme. *Environ Toxicol Chem* 15:1524-1531.

Carey JC, Hall E, McCubbin N. 2002. Review of scientific basis for AOX effluent standard in British Columbia. Report prepared for Minister of Water, Land and Air Protection of British Columbia. Ministry of Water, Land and Air Protection in British Columbia, Vancouver, BC, Canada.

Chandrasekaran K, Reis R, Tanner G, Rogers H. 1978. Removing toxicity in an aerated stabilization basin. *Pulp and Paper Canada* 79:65-75.

Crawley MJ. 2007. *The R Book*. John Wiley & Sons Ltd., Chichester, UK.

den Besten PJ. 2005. *Ecological Testing of Freshwater and Marine Systems*. Taylor & Francis Group, LLC, Boca Raton, FL, USA.

Environment Canada. 2005 (with June 2007 amendments). Guidance document on statistical methods for environmental toxicity tests. EPS 1/RM/46. Environment Canada, Method Development and Applications Section, Environmental Technology Centre, Ottawa, ON, Canada.

Hach. 2003. *Water Analysis Handbook*. Hach Company, Loveland, CO, USA.

Hanmer R. 1988. Environmental protection in the United States pulp, paper, and paperboard industry; an overview of regulation of wastewater under the U.S. Clean Water Act. *Water Sci Technol* 20:1-7.

Hewitt LM, Kovacs TG, Dubé MG, MacLatchy DL, Martel PH, McMaster ME, Paice MG, Parrott JL, van den Heuvel MR, Van Der Kraak GJ. 2008. Altered reproduction in fish exposed to pulp and paper mill effluents; roles of individual compounds and mill operating conditions. *Environ Toxicol Chem* 27:682-697.

Hodson PV, Maj MK, Efler S, Burnison BK, van Heiningen ARP, Girard R, Carey JH. 1997. MFO induction in fish by spent cooking liquors from kraft pulp mills. *Environ Toxicol Chem* 16:908-916.

Johnson D. 1988. Development of *Mytilus edulis* embryos: a bioassay for polluted waters. *Mar Ecol Prog Ser* 45:135-138.

Kelly CR, Hargreaves TL, Golden R, Holm SE, Deardorff TL, Festa JL. 2004. Toxicity investigations associated with *Daphnia magna* and *Pimephales promelas* exposed to spent pulping liquor from an elemental chlorine free kraft mill. In Borton DL Hall TJ, Fisher RL, Thomas JF. eds, *Pulp and Paper Mill Effluent Environmental Fate and Effects*. DEStech Publications, Inc., Lancaster, PA, USA, pp 304-309.

Lowell RB, Ring B, Pastershank G, Walker S, Trudel L, Hedley K. 2005. National assessment of pulp and paper environmental effects monitoring data: Findings from cycles 1 through 3. NWRI Scientific Assessment Report Series 5. National Water Research Institute, Burlington, ON, Canada.

Meriläinen P, Oikari A. 2008. Exposure assessment of fishes to a modern pulp and paper mill effluents after a black liquor spill. *Environ Monit Assess* 144:419-435.

Matthews, R.A. 2011. Multivariate analysis – nonmetric clustering. Western Washington University, Bellingham, WA, USA. Retrieved August 30th, 2011 from http://ceratium.ietc.wwu.edu/IWS2/people/classes/classes/ESCI503/cluster2BMR.pdf

Matthews G, Hearne J. 1991. Clustering without a metric. *IEEE Trans Pattern Anal Mach Intell* 13:175-184.

Matthews GB, Matthews RA, Landis WG. 1995. Nonmetrics clutering and association analysis: implications for the evaluation of multispecies toxicity test and field monitoring. In Hughes JS, Biddinger GR, Mones E, eds, *Environmental Toxicology and Risk Assessment,* 3rd Vol. American Society for Testing and Materials, Philadelphia, PA, USA, pp 79-93.

Mount DI, Norberg-King TJ. 1993. Methods for aquatic toxicity identification evaluations: phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA-600-R-92-081. U.S. Environmental Protection Agency, Office of Research and Development, Deluth, MN, USA.

National Council for Air and Stream Improvement, Inc. (NCASI). 1997. Methods manual. National Council for Air and Stream Improvement, Inc., Research Triangle Park, NC, USA.

______. 2000. An update of procedures for the measurement of color in pulp mill wastewaters. Technical Bulletin No. 803. National Council for Air and Stream Improvement, Inc., Research Triangle Park, NC, USA.

______.2011. NCASI study of GHG and energy benefits of using black liquor presented to EPA. NCASI Bulletin Board Vol 37(2): 1.

Newman MC, Unger MA. 2003. *Fundamentals of Ecotoxicology*. CRC Press, Boca Raton, FL, USA.

Rand GM. 1995. *Fundamentals of Aquatic Toxicology*, 2nd ed. Taylor & Francis, Washington, DC, USA.

Rod'ko Y, Scott BF, Carey JH. 1996. Analysis of pulp mill black liquor for organosulfur compounds using GC/atomic emission detection. In Servos MS, Munkittrick KR, Carey JH, Van der Kraak GV, eds, *Environmental Fate and Effects of Pulp and Paper Mill Effluents*, St. Lucie Press, Delray Beach, FL, USA, pp 195-202.

Servizi JA, Gordon RW. 1973. Detotoxification of kraft pulp-mill effluent by an aerated lagoon. *Pulp and Paper Magazine of Canada* 74:103-110.

Schnell A, Hodson PV, Steel P, Melcer H, Carey JH. 1993. Optimized biological treatment of bleached kraft mill effluents for the enhanced removal of toxic compounds and MFO induction response in fish. In Canadian Pulp and Paper Association, ed, *Proceedings of the 1993 Environment Conference Technical Section*, Montréal, Québec, Canada, pp 97-111.

Smook GA. 1990. *Handbook of Pulp and Paper Terminology*. Angus Wilde Publications, Vancouver, BC, Canada.

Sturm A, Hodson PV, Carey JH, Hansen P-D. 1999. Hepatic UDP-glucuronosyltransferase in Rainbow trout (*Oncorhynchus mykiss*) and preliminary assessment of respond to pulp mill cooking liquor. *Bull Environ Contam Toxicol* 62:608-615.

Technical Association of the Pulp and Paper Industry (TAPPI). 1996. *Dictionary of Paper*, 5th ed. TAPPI Press, Atlanta, GA, USA.

_____. 2009. T650 om-09 solids content of black liquor. TAPPI Press, Atlanta, GA, USA.

Timbrell J. 2000. *Principles of Biochemical Toxicology*, 3rd ed, Taylor & Francis, Philadelphia, PA, USA.

Torres GJ, Basnet RB, Sung AH, Mukkamala S, Ribeiro BM. 2009. A similarity measure for clustering and its applications. *Int J Electr Comput Syst Eng* 3:164-170.

U. S. Environmental Protection Agency (U. S. EPA). 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms, 1st ed. EPA-600-R-95-136. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH, USA.

______. 2002a. Profile of the pulp and paper industry. EPA-310-R-02-002. U.S. Environmental Protection Agency, Office of Compliance Sector Notebook Project, Washington, DC, USA.

______.2002b. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th ed. EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

______.2002c. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 4th ed. EPA-821-R-02-013. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

______. 2002d. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms, 3rd ed. EPA-821-R-02-014. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA.

______.2009. *Ceriodaphnia dubia* chronic toxicity test report form. U.S. Environmental Protection Agency, Region 8, Denver, CO, USA. Retrieved November 1st, 2011 from http://www.epa.gov/region8/water/wet/pdf/Ceriodaphnia%20dubia%20CHRONIC%20TOXICITY%20TEST%20REPORT%20FORMAT.pdf.

______.2011a. Effluent guidelines – pulp and paper rulemaking actions- final pulp and paper cluster rule. U.S. Environmental Protection Agency, Office of Water, Washington, DC, USA. Retrieved July 1st, 2011 from http://water.epa.gov/scitech/wastetech/guide/pulppaper/cluster.cfm.

______. 2011b. Water data systems – permit compliance system. U.S. Environmental Protection Agency, Office of Compliance, Washington, DC, USA. Retrieved July 13th, 2011 from http://www.epa.gov/oecaerth/data/systems/water/index.html.

Vadodaria SJ. 1999. Effect of black liquor on the activated sludge process: An experimental investigation of black liquor on the activated sludge treatment of bleached kraft mill effluents. M.S. Thesis. McGill University, Montreal, QC,Canada.

Washington State Department of Ecology (WDOE). 2008. Laboratory guidance and whole effluent toxicity test review criteria. WQ-R-95-80. Washington State Department of Ecology, Water Quality Program, Olympia, WA, USA.

Zacharewski TR, Berhane K, Gillesby BE. 1995. Detection of estrogen- and dioxin-like activity in pulp and paper mill black liquor and effluent using *in vitro* recombinant receptor/reporter gene assays. *Environ Sci Technol* 29:2140-2146.

Xiao, C. 2005. Black liquor from crop straw pulping as a potassium source and soil amendment. PhD thesis. Washington State University, Pullman, WA, USA.

APPENDIX A

West Coat Regional Center Laboratory Biotreatment Standard Operating Procedure

1.0 Sampling and background analysis

Sample bottles and blue ice need to be shipped to the mill and arrangements made for sample collection at least two weeks prior to sampling to avoid overnight or short term shipping costs. Ideally, the mill should collect the materials on Monday and ship Tuesday, but with this large a volume we found that some of the mills had problems with this, so it is very difficult to anticipate exactly when the reactor set-up begins.

Sample Type	**Volume Collected**
FE - Final Effluent	7 x 1L
INF - 1° Clarifier Effluent (Influent to Treatment)	20 gallons
TPWW-Treatment Pond Wastewater	8 gallons
WBL – Weak Black Liquor	20 x 30 ml

Note: We asked the mill to collect and ship the WBL prior to sampling so we could conduct solids and other analytical measurements in advance of the reactor set-up. Shipping of WBL requires special handling in order to be shipped under a small quantity exception for dangerous goods.

Treated effluent:

- WCRC –3 Liters split and preserved as follows:
  - 1 L pH 2 preserved (as per phytosterols)
  - 500 ml pH 10 preserved (as per RAs)
  - 500 ml unpreserved for GC/MS screening
  - 100 ml to be filtered and preserved to pH 2 for DOC and DCOD
- NABF:
  - 2 liters amber glass, unpreserved for NABF bioassays and chemistry
- SABF
  - 2 liter amber glass, unpreserved for bioassays

Weak Black Liquor: Composite all 20 containers of WBL

- WBL Neat: Conduct a solids determination from the composite sample.
- WBL reactor spiking solution: Dilute a portion 1:1 and pH adjust to between 9 and 10 in order to facilitate transfer of WBL aliquots to reactors and minimize the need to adjust reactor pH after spiking.
- WBL Analytical Dilution: Prepare 1:200 dilution of WBL for COD, DCOD, BOD, and TOC background analyses.

Influent and treatment pond wastewaters:

- Aliquots of influent and treatment pond wastewaters should be collected and preserved as needed for background analyses of solids, COD, DCOD, BOD and DOC.
- Influent and treatment pond wastewaters should be partitioned into BLSS reactors on the day of arrival.

2.0 Equipment and operations

2.1 Monitoring with YSI probes:

A YSI 5200 Recirculation System Monitor and a YSI 5562 probe module will be used for measuring dissolved oxygen (DO), temperature, pH, oxidation reduction potential (ORP), and conductivity.

Acquisition of the YSI 5200 monitors data output is made via a RJ-45 Cat 5 network cable connected to a serial port on the computer and the data is viewed and managed by AquaManager-Windows software.

The probes sensors will be checked for proper performance before runs each day of testing by using appropriate standards for calibration or calibration verification.

2.2 Reactor chamber and gas flow regulation:

A glass three door refrigerator is used for maintaining temperature equilibrium in the reactors. Temperature in the carboy will be maintained at 30 ± 2°C by a separate carboy filled with water which is wrapped with a 12 **X** 24 inch rubber silicone heating pad controlled by a cyclic on/off controller and a rheostat power controller. Temperature control is verified using the YSI probe.

Compressed air and nitrogen with two stage regulators are connected to a stainless steel three-way valve, an on/off toggle valve and a flow meter for flow management.

Flow rate is monitored by a 0-2 L/min flow meter calibrated at test flow rates with a 1000 ml glass bubble meter.

The probe module, gas inlet and a ¼ inch OD Teflon® exit line are positioned in holes cut into a # 12 silicone rubber stopper.

2.3 Miscellaneous equipment

Syringe filters, Acrodisc glass, 25 mm dia. (1.0 μm) VWR, Cat. # 28143-986

Syringe filters 0.45 μm

Ammonia dipsticks

3.0 Reactor set-up

3.1 Reactor preparation

To each of six reactors add 8.5 L of ASB influent into a glass carboy. In order to ensure each reactor make-up is the same, mix the contents of 3 gallon containers into a large carboy and transfer 2 liters of the combined mix to each carboy, continue to combine and distribute ASB influent until each reactor contains 8.5 L. Attach a submersible aquarium pump, via the pumps suction cup feet, to the bottom on one side of the carboy. Insert a 1 inch spherical air stone connected with Tygon® tubing to an aquarium pump manifold on the opposite side and purge with room air at approximately 1.5L/min for one hour then add 1.5 L of ASB-TPWW to each reactor and continue to purge with air overnight. Test the ammonia level in the control reactor using a dipstick and add nutrients to all reactors, if necessary. Collect an aliquot of sample from one of the reactors to conduct background COD and DCOD measurements. It is necessary to run the reactors overnight to promote biological activity and achieve the desired reactor temperatures.

Place YSI 5200 probes in the control and (RWBL-5). Monitor for DO, temperature, pH, ORP and conductivity. Nutrients are likely to be consumed and DO levels will likely drop overnight. Therefore, the nutrient levels should be checked again prior to spiking with WBL. After purging overnight, spike each reactor with WBL. The WBL volumes are determined based on the WBL solids content in an attempt to ensure that nominally equivalent amounts of WBL were added in each study.

3.2 Reactor monitoring and analysis

Day 0: Take an aliquot from each reactor for COD/DCOD measurements. Take an 800 ml aliquot from the control and RWBL-5, allow solids to settle, decant off ~ 600 ml, filter a 30 ml portion for ortho-phosphate and ammonia analysis and pH 10 preserve the remainder for RA/FA analyses. If necessary, add nutrients. Ensure the aeration systems are not plugged.

Daily: Take an aliquot from each reactor for DCOD measurements, check DO levels and ammonia levels. Add nutrients, if necessary. Run DCOD analyses daily or every other day to evaluate reactor treatment processes. Once the DCOD measurements have stabilized, discontinue the reactor (Day n) study.

Day n: Take an aliquot from each reactor for COD/DCOD measurements. Discontinue aeration and allow solids to settle for at least one hour. Siphon the effluent from each reactor avoiding the settled solids into their respective sample containers as noted below

Lab	Sample volumes/preservation
NABF	2 L unpreserved
SABF	2 L unpreserved
WCRC	1 L unpreserved
WCRC	1 L pH 2 preserved
WCRC	1 L pH 10 preserved
WCRC	0.03 L (Ctrl nutrients)

Ship aliquots to the respective laboratories on the date the reactors are discontinued.

Clean-up reactors, fill with water and ~ 100 ml RBS soap and let soak overnight. Rinse and set aside for next set-up.

APPENDIX B

Test Chamber Chemistry for *Mytilus galloprovincialis* Toxicity Tests

Table 48. Test chamber chemistry for 48-h *Mytilus galloprovincialis* embryo-larval development tests measured at test initiation and test termination for Mill A mill-treated and simulated effluent samples. NM: not measured; DO: dissolved oxygen; WBL: weak black liquor.

		Test Initiation			Test Termination		
Effluent Sample	**Test treatment**	**pH**	**Salinity (‰)**	**Temperature (°C)**	**pH**	**DO (mg/L)**	**Salinity (‰)**
Mill-treated effluent	Control	NM	31	NM	7.8	6.9	30
	Brine Control	NM	30.5	NM	7.9	6.7	30
	1.1% effluent	NM	30	NM	8.0	6.9	31
	2.2% effluent	NM	30	NM	8.0	6.9	30
	4.4% effluent	NM	30	NM	8.0	7.0	31
	8.75% effluent	NM	30	NM	8.1	7.0	31
	17.5% effluent	NM	31	NM	8.2	7.0	31
	35% effluent	NM	31	NM	8.3	7.0	31
	70% effluent	NM	33	NM	8.4	6.9	33
Reactor Control	Control	7.78	32	19.0	7.89	6.85	30
	Brine Control	7.90	30	21.0	7.78	6.96	32
	1.1% effluent	7.90	32	20.5	7.88	6.91	32
	2.2% effluent	7.95	32	20.5	NM	NM	NM
	4.4% effluent	7.94	32	20.0	NM	NM	NM
	8.75% effluent	7.96	33	20.5	NM	NM	NM
	17.5% effluent	7.98	31	20.5	7.92	6.77	32
	35% effluent	8.04	32	20.5	NM	NM	NM
	70% effluent	8.10	32	21.0	NM	NM	NM
WBL Spike 1	Control	7.78	32	19.0	7.88	7.08	32
	Brine Control	7.90	30	21.0	7.79	7.13	32
	1.1% effluent	7.96	NM	21.0	7.83	7.25	32
	2.2% effluent	7.99	32	21.0	NM	NM	NM
	4.4% effluent	7.98	32	21.0	NM	NM	NM
	8.75% effluent	8.00	32	21.0	NM	NM	NM
	17.5% effluent	7.99	32	21.0	NM	NM	NM
	35% effluent	8.02	32	20.5	NM	NM	NM
	70% effluent	8.06	32	20.5	8.07	7.13	33

Effluent Sample	Test treatment	Test Initiation pH	Salinity (‰)	Temperature (°C)	Test Termination pH	DO (mg/L)	Salinity (‰)
WBL spike 2	Control	7.78	32	19.0	7.90	7.25	32
	Brine Control	7.90	30	21.0	7.76	7.23	32
	1.1% effluent	7.98	32	21.0	NM	NM	33
	2.2% effluent	8.00	32	20.5	7.89	7.32	32
	4.4% effluent	NM	NM	20.5	NM	NM	NM
	8.75% effluent	8.01	31	20.0	NM	NM	NM
	17.5% effluent	8.06	32	21.0	NM	NM	NM
	35% effluent	8.09	30	20.0	7.98	7.23	32
	70% effluent	8.13	32	20.0	NM	NM	32
WBL spike 3	Control	7.78	32	19.0	7.86	7.23	32
	Brine Control	7.90	30	21.0	7.75	7.19	31
	1.1% effluent	8.03	32	21.0	NM	NM	33
	2.2% effluent	NM	32	21.0	7.87	7.23	32
	4.4% effluent	NM	32	20.5	NM	NM	NM
	8.75% effluent	NM	32	20.0	NM	NM	NM
	17.5% effluent	NM	32	20.0	NM	NM	NM
	35% effluent	NM	32	20.5	NM	NM	NM
	70% effluent	8.15	32	20.0	8.08	7.14	33
WBL spike 4	Control	7.78	32	19.0	7.90	7.27	32
	Brine Control	7.90	30	21.0	7.78	7.29	31
	1.1% effluent	7.91	32	20.0	7.86	7.20	32
	2.2% effluent	NM	32	20.0	NM	NM	NM
	4.4% effluent	NM	32	20.0	NM	NM	NM
	8.75% effluent	NM	32	19.0	NM	NM	NM
	17.5% effluent	NM	32	18.5	NM	NM	NM
	35% effluent	NM	32	18.5	NM	NM	NM
	70% effluent	8.19	32	20.5	8.13	7.15	33

(Continued on next page)

Effluent Sample	Test treatment	Test Initiation pH	Salinity (‰)	Temperature (°C)	Test Termination pH	DO (mg/L)	Salinity (‰)
WBL spike 5	Control	7.78	32	19.0	7.82	NM	NM
	Brine Control	7.90	30	21.0	7.85	7.07	32
	1.1% effluent	7.97	32	21.0	7.90	6.99	32
	2.2% effluent	NM	32	21.0	NM	NM	NM
	4.4% effluent	NM	32	20.5	NM	NM	NM
	8.75% effluent	NM	32	20.0	NM	NM	NM
	17.5% effluent	NM	32	21.0	NM	NM	NM
	35% effluent	NM	32	21.0	NM	NM	NM
	70% effluent	8.19	32	21.0	8.13	6.87	32

Table 49. Test chamber chemistry for 48-h *Mytilus. galloprovincialis* embryo-larval development tests measured at test initiation and test termination for reference toxicant tests run with Mill A samples. NM: not measured; DO: dissolved oxygen.

Effluent samples tested concurrently	Test treatment	Test Initiation			Test Termination		
		pH	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Salinity (‰)
Mill effluent	Control	NM	31	NM	8.1	7.0	30
	3.0 µg/L Cu	NM	31	NM	8.0	6.9	31
	4.4 µg/L Cu	NM	30	NM	8.0	7.0	30
	6.5 µg/L Cu	NM	30	NM	8.0	7.0	30
	9.5 µg/L Cu	NM	30	NM	8.0	6.9	30
	13.9 µg/L Cu	NM	31	NM	8.0	6.9	31
	20.4 µg/L Cu	NM	31	NM	8.0	6.9	31
Simulated effluents	Control	7.78	32	19.0	7.93	7.01	31
	3.0 µg/L Cu	7.73	31	19.5	NM	6.90	32
	4.4 µg/L Cu	7.72	32	19.0	NM	NM	NM
	6.5 µg/L Cu	7.75	32	19.5	NM	NM	NM
	9.5 µg/L Cu	7.60	32	19.5	NM	NM	NM
	13.9 µg/L Cu	7.74	32	19.0	NM	NM	NM
	20.4 µg/L Cu	7.77	32	19.0	7.89	6.98	32

Table 50. Test chamber chemistry for 48-h *Mytilus galloprovincialis* embryo-larval development tests measured at test initiation and test termination for Mill B mill-treated and simulated effluent samples. NM: not measured; DO: dissolved oxygen; WBL: weak black liquor.

		Test Initiation			Test Termination		
Effluent Sample	**Test treatment**	**pH**	**Salinity (‰)**	**Temperature (°C)**	**pH**	**DO (mg/L)**	**Salinity (‰)**
Mill-treated effluent	Control	7.74	32	18.60	8.06	7.56	32
	Brine Control	8.15	32	18.60	8.08	7.77	31
	1.1% effluent	7.70	31	17.90	8.13	7.44	32
	2.2% effluent	NM	32	NM	NM	NM	32
	4.4% effluent	NM	32	NM	NM	NM	31
	8.75% effluent	NM	32	NM	NM	NM	32
	17.5% effluent	NM	28	NM	NM	NM	28
	35% effluent	NM	32	NM	NM	NM	32
	70% effluent	7.67	32	18.70	8.41	7.38	32
Reactor control	Control	7.80	32	17.9	7.99	7.03	32
	Brine Control	8.14	32	18.1	8.01	7.20	32
	1.1% effluent	7.85	32	18.1	8.04	7.01	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.16	32	17.8	8.35	6.94	32
WBL spike 1	Control	NM	NM	NM	8.10	7.22	32
	Brine Control	NM	NM	NM	8.05	7.32	31
	1.1% effluent	7.84	32	18.0	8.05	7.03	33
	2.2% effluent	32.00	32	NM	NM	NM	NM
	4.4% effluent	32.00	32	NM	NM	NM	NM
	8.75% effluent	32.00	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.11	32	18.1	8.40	7.05	32

(Continued on next page)

Effluent Sample	Test treatment	Test Initiation			Test Termination		
		pH	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Salinity (‰)
WBL spike 2	Control	NM	NM	NM	NM	NM	NM
	Brine Control	NM	NM	NM	NM	NM	NM
	1.1% effluent	7.86	32	18.3	8.07	7.07	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.20	32	18.3	8.41	6.94	33
WBL spike 3	Control	NM	NM	NM	8.09	7.21	32
	Brine Control	NM	NM	NM	8.02	7.18	31
	1.1% effluent	7.86	32	NM	8.04	7.27	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.20	34	18.3	8.42	7.11	33
WBL spike 4	Control	NM	NM	NM	NM	NM	NM
	Brine Control	NM	NM	NM	NM	NM	NM
	1.1% effluent	7.86	33	18.0	8.12	7.15	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	33	NM	NM	NM	NM
	8.75% effluent	NM	33	NM	NM	NM	NM
	17.5% effluent	NM	34	NM	NM	NM	NM
	35% effluent	NM	34	NM	NM	NM	NM
	70% effluent	8.15	34	18.3	8.46	7.13	34

(Continued on next page)

Effluent Sample	Test treatment	Test Initiation pH	Test Initiation Salinity (‰)	Test Initiation Temperature (°C)	Test Termination pH	Test Termination DO (mg/L)	Test Termination Salinity (‰)
WBL spike 5	Control	NM	NM	NM	NM	NM	NM
	Brine Control	NM	NM	NM	NM	NM	NM
	1.1% effluent	7.85	33	18.1	8.11	7.16	33
	2.2% effluent	NM	33	NM	NM	NM	NM
	4.4% effluent	NM	34	NM	NM	NM	NM
	8.75% effluent	NM	34	NM	NM	NM	NM
	17.5% effluent	NM	34	NM	NM	NM	NM
	35% effluent	NM	34	NM	NM	NM	NM
	70% effluent	8.13	34	18.1	8.47	7.11	34
WBL spike 5	Control	7.80	32	17.9	8.06	7.20	32
(unsettled sample)	Brine Control	8.14	32	18.1	8.03	7.12	32
	1.1% effluent	7.76	32	18.0	8.07	7.09	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	3.3% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	34	NM	NM	NM	NM
	35% effluent	8.19	34	18.0	8.45	7.21	32

Table 51. Test chamber chemistry for 48-h *Mytilus galloprovincialis* embryo-larval development tests measured at test initiation and test termination for reference toxicant tests run with Mill B samples. NM: not measured; DO: dissolved oxygen.

Effluent samples tested concurrently	Test treatment	Test Initiation			Test Termination		
		pH	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Salinity (‰)
Mill effluent	Control	7.74	32	18.60	8.13	7.55	30
	3.0 µg/L Cu	8.15	32	18.00	8.13	7.55	30
	4.4 µg/L Cu	7.88	32	NM	NM	NM	NM
	6.5 µg/L Cu	NM	32	NM	NM	NM	NM
	9.5 µg/L Cu	NM	32	NM	NM	NM	NM
	13.9 µg/L Cu	NM	32	NM	NM	NM	NM
	20.4 µg/L Cu	7.89	32	18.40	8.12	7.51	31
Simulated effluents	Control	7.80	32	17.9	8.09	7.16	32
	3.0 µg/L Cu	7.89	32	18.1	8.05	7.15	33
	4.4 µg/L Cu	NM	32	NM	NM	NM	NM
	6.5 µg/L Cu	NM	32	NM	NM	NM	NM
	9.5 µg/L Cu	NM	32	NM	NM	NM	NM
	13.9 µg/L Cu	NM	32	NM	NM	NM	NM
	20.4 µg/L Cu	7.88	31	18.0	8.06	6.96	32

Table 52. Test chamber chemistry for 48-h *Mytilus galloprovincialis* embryo-larval development tests measured at test initiation and test termination for Mill C mill-treated and simulated effluent samples. NM: not measured; DO: dissolved oxygen; WBL: weak black liquor

		Test Initiation			Test Termination		
Effluent Sample	**Test treatment**	**pH**	**Salinity (‰)**	**Temperature (°C)**	**pH**	**DO (mg/L)**	**Salinity (‰)**
Mill-treated effluent	Control	7.79	32	18.4	8.07	7.64	32
	Brine Control	8.29	30	18.6	8.09	7.64	31
	1.1% effluent	7.77	32	18.6	8.08	7.57	32
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	33	NM	NM	NM	NM
	70% effluent	7.87	33	19.1	8.52	7.42	33
Reactor control	Control	7.75	32	19.2	7.44	7.33	31
	Brine Control	8.06	30	18.8	7.74	7.27	32
	1.1% effluent	7.78	31	18.6	7.90	7.31	32
	2.2% effluent	NM	31	NM	NM	NM	NM
	4.4% effluent	NM	31	NM	NM	NM	NM
	8.75% effluent	NM	31	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.10	32	18.4	8.26	7.25	33
WBL spike 1	Control	7.75	32	19.2	NM	NM	NM
	Brine Control	8.06	30	18.8	NM	NM	NM
	1.1% effluent	7.83	31	18.6	8.03	7.37	32
	2.2% effluent	NM	31	NM	NM	NM	NM
	4.4% effluent	NM	31	NM	NM	NM	NM
	8.75% effluent	NM	31	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.13	32	18.8	8.30	7.21	34

(Continued on next page)

Effluent Sample	Test treatment	Test Initiation pH	Salinity (‰)	Temperature (°C)	Test Termination pH	DO (mg/L)	Salinity (‰)
WBL spike 2	Control	7.75	32	19.2	7.97	7.44	32
	Brine Control	8.06	30	18.8	7.92	7.35	32
	1.1% effluent	7.83	31	18.9	7.93	7.53	31
	2.2% effluent	NM	31	NM	NM	NM	NM
	4.4% effluent	NM	31	NM	NM	NM	NM
	8.75% effluent	NM	31	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.16	32	18.8	8.32	7.78	33
WBL spike 3	Control	7.75	32	19.2	NM	NM	NM
	Brine Control	8.06	30	18.8	NM	NM	NM
	1.1% effluent	7.83	32	18.6	8.06	7.39	34
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.14	32	18.9	8.31	7.27	34
WBL spike 4	Control	7.75	32	19.2	7.93	7.32	32
	Brine Control	8.06	30	18.8	7.89	7.56	32
	1.1% effluent	7.83	32	18.8	7.93	7.43	32
	2.2% effluent	NM	31	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	8.14	33	18.9	8.31	7.29	34

(Continued on next page)

Effluent Sample	Test treatment	Test Initiation pH	Salinity (‰)	Temperature (°C)	Test Termination pH	DO (mg/L)	Salinity (‰)
WBL spike 5	Control	7.75	32	19.2	NM	NM	NM
	Brine Control	8.06	30	18.8	NM	NM	NM
	1.1% effluent	7.84	31	18.8	7.95	7.28	32
	2.2% effluent	NM	31	NM	NM	NM	NM
	3.3% effluent	NM	31	NM	NM	NM	NM
	4.4% effluent	NM	31	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	8.05	32	19.0	8.20	7.44	32
WBL spike 5	Control	7.75	32	19.2	7.99	7.34	32
(unsettled sample)	Brine Control	8.06	30	18.8	7.91	7.36	32
	1.1% effluent	7.82	31	19.1	7.94	7.42	31
	2.2% effluent	NM	31	NM	NM	NM	NM
	3.3% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	32	NM	NM	NM	NM
	17.5% effluent	NM	32	NM	NM	NM	NM
	35% effluent	8.12	33	18.5	8.20	7.16	32

Table 53. Test chamber chemistry for 48-h *Mytilus. galloprovincialis* embryo-larval development tests measured at test initiation and test termination for reference toxicant tests run with Mill C samples. NM: not measured; DO: dissolved oxygen

Effluent samples tested concurrently	Test treatment	Test Initiation			Test Termination		
		pH	**Salinity (‰)**	**Temperature (°C)**	**pH**	**DO (mg/L)**	**Salinity (‰)**
Mill effluent	Control	7.79	32	18.4	8.16	7.65	32
	3.0 µg/L Cu	7.96	32	18.5	8.14	7.51	32
	4.4 µg/L Cu	NM	32	NM	NM	NM	NM
	6.5 µg/L Cu	NM	32	NM	NM	NM	NM
	9.5 µg/L Cu	NM	32	NM	NM	NM	NM
	13.9 µg/L Cu	NM	32	NM	NM	NM	NM
	20.4 µg/L Cu	7.92	32	18.7	8.13	7.48	32
Simulated effluents	Control	7.75	32	19.2	NM	NM	NM
	3.0 µg/L Cu	7.86	32	19.0	7.95	7.41	32
	4.4 µg/L Cu	NM	32	NM	NM	NM	NM
	6.5 µg/L Cu	NM	32	NM	NM	NM	NM
	9.5 µg/L Cu	NM	32	NM	NM	NM	NM
	13.9 µg/L Cu	NM	32	NM	NM	NM	NM
	20.4 µg/L Cu	7.83	31	19.0	7.93	7.37	32

Table 54. Test chamber chemistry for 48-h *Mytilus. galloprovincialis* embryo-larval development tests measured at test initiation and test termination for Mill D mill-treated and simulated effluent samples. NM: not measured; DO: dissolved oxygen; WBL: weak black liquor.

		Test Initiation			Test Termination		
Effluent Sample	**Test treatment**	**pH**	**Salinity (‰)**	**Temperature (°C)**	**pH**	**DO (mg/L)**	**Salinity (‰)**
Mill-treated effluent	Control	7.79	32	20.2	7.85	7.19	30
	Brine Control	7.97	32	18.8	7.88	7.36	30
	1.1% effluent	7.66	32	19.1	7.95	7.23	30
	2.2% effluent	NM	32	NM	NM	NM	NM
	4.4% effluent	NM	32	NM	NM	NM	NM
	8.75% effluent	NM	31	NM	NM	NM	NM
	17.5% effluent	NM	31	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	7.63	32	20.8	8.30	7.19	33
Reactor control	Control	7.60	30	19.4	NM	NM	NM
	Brine Control	7.83	30	19.4	NM	NM	NM
	1.1% effluent	7.53	30	17.9	NM	NM	NM
	2.2% effluent	NM	30	NM	NM	NM	NM
	4.4% effluent	NM	30	NM	NM	NM	NM
	8.75% effluent	NM	30	NM	NM	NM	NM
	17.5% effluent	NM	30	NM	NM	NM	NM
	35% effluent	NM	31	NM	NM	NM	NM
	70% effluent	7.89	32	19.8	NM	NM	NM
WBL spike 1	Control	7.60	30	19.4	NM	NM	NM
	Brine Control	7.83	30	19.4	NM	NM	NM
	1.1% effluent	7.89	30	19.7	NM	NM	NM
	2.2% effluent	NM	30	NM	NM	NM	NM
	4.4% effluent	NM	30	NM	NM	NM	NM
	8.75% effluent	NM	30	NM	NM	NM	NM
	17.5% effluent	NM	31	NM	NM	NM	NM
	35% effluent	NM	32	NM	NM	NM	NM
	70% effluent	7.94	32	19.4	NM	NM	NM

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Effluent Sample	Test treatment	Test Initiation pH	Salinity (‰)	Temperature (°C)	Test Termination pH	DO (mg/L)	Salinity (‰)
WBL spike 2	Control	7.60	30	19.4	NM	NM	NM
	Brine Control	7.83	30	19.4	NM	NM	NM
	1.1% effluent	7.87	30	19.6	NM	NM	NM
	2.2% effluent	NM	30	NM	NM	NM	NM
	4.4% effluent	NM	30	NM	NM	NM	NM
	8.75% effluent	NM	30	NM	NM	NM	NM
	17.5% effluent	NM	31	NM	NM	NM	NM
	35% effluent	NM	31	NM	NM	NM	NM
	70% effluent	7.94	32	19.5	NM	NM	NM
WBL spike 3	Control	7.60	30	19.4	NM	NM	NM
	Brine Control	7.83	30	194.0	NM	NM	NM
	1.1% effluent	8.01	30	18.9	NM	NM	NM
	2.2% effluent	NM	30	NM	NM	NM	NM
	4.4% effluent	NM	30	NM	NM	NM	NM
	8.75% effluent	NM	30	NM	NM	NM	NM
	17.5% effluent	NM	31	NM	NM	NM	NM
	35% effluent	NM	31	NM	NM	NM	NM
	70% effluent	7.95	32	18.9	NM	NM	NM
WBL spike 5	Control	7.60	30	19.4	NM	NM	NM
	Brine Control	7.83	30	19.4	NM	NM	NM
	1.1% effluent	7.92	30	18.7	NM	NM	NM
	2.2% effluent	NM	30	NM	NM	NM	NM
	3.3% effluent	NM	30	NM	NM	NM	NM
	4.4% effluent	NM	30	NM	NM	NM	NM
	8.75% effluent	NM	31	NM	NM	NM	NM
	17.5% effluent	NM	31	NM	NM	NM	NM
	35% effluent	7.95	32	18.7	NM	NM	NM

Table 55. Test chamber chemistry for 48-h *Mytilus galloprovincialis* embryo-larval development tests measured at test initiation and test termination for reference toxicant tests run with Mill D samples. NM: not measured; DO: dissolved oxygen.

Effluent samples tested concurrently	Test treatment	Test Initiation			Test Termination		
		pH	Salinity (‰)	Temperature (°C)	pH	DO (mg/L)	Salinity (‰)
Mill effluent	Control	7.79	32	20.2	8.00	7.19	31
	3.0 µg/L Cu	7.71	32	20.1	7.88	7.72	32
	4.4 µg/L Cu	NM	32	NM	NM	NM	NM
	6.5 µg/L Cu	NM	32	NM	NM	NM	NM
	9.5 µg/L Cu	NM	32	NM	NM	NM	NM
	13.9 µg/L Cu	NM	32	NM	NM	NM	NM
	20.4 µg/L Cu	7.69	32	20.4	7.92	7.54	32
Simulated effluents	Control	7.60	30	19.4	NM	NM	NM
	3.0 µg/L Cu	7.92	30	17.9	NM	NM	NM
	4.4 µg/L Cu	NM	30	NM	NM	NM	NM
	6.5 µg/L Cu	NM	30	NM	NM	NM	NM
	9.5 µg/L Cu	NM	30	NM	NM	NM	NM
	13.9 µg/L Cu	NM	30	NM	NM	NM	NM
	20.4 µg/L Cu	7.87	30	18.2	NM	NM	NM

APPENDIX C

Bivalve Spawning Data Sheet

Date: ____________________________ Test description: ____

Species: ____________________________ Source: ____________________________ Date Received: ______________

Time spawning attempt began: ______________

Beaker	Time of spawn	Sex	Beaker	Time of spawn	Sex
1	______________________	_______________	7	______________________	_____________
2	______________________	_______________	8	______________________	_____________
3	______________________	_______________	9	______________________	_____________
4	______________________	_______________	10	______________________	_____________
5	______________________	_______________	11	______________________	_____________
6	______________________	_______________	12	______________________	_____________

Number of natural spawning: _______ males _______ females

Number of strip spawned used for fertilization: _______ males _______ females

Number or natural spawned used for fertilization: _______ males _______ females

Time of fertilization: ______________

Trial sperm dilution volume: Beaker 1: ___________ Beaker 2: ___________ Beaker 3: ___________

Sperm suspension selected: ________________

Egg count volume: _________ Fertilized: ___________ Unfertilized: ___________ % Fertilized: ___________

Fertilized: ___________ Unfertilized: ___________ % Fertilized: ___________

Fertilized: ___________ Unfertilized: ___________ % Fertilized: ___________

Mean fertilized: ___________ Mean % fertilized: ___________

Volume of embryo suspension added to test chambers: ______________

Test stocking time: ___________ Test volume: ______________ Embryo density in test chambers: ____________

APPENDIX D

Environmental Chamber Temperature Summary for *Mytilus galloprovincialis* Toxicity Tests

Table 56. Minimum, maximum, and mean temperatures of environmental chamber during 48-h *Mytilus galloprovincialis* embryo-larval development tests for Mill B, Mill C, and Mill D. Temperature measured continuously using Onset Stow Away TidbiT Temp Loggers.

	Test Date		Temperature (°C)		
Mill	**Initiation**	**Termination**	**Min**	**Max**	**Mean**
B	10/22/2010	10/24/2010	15.20	15.99	15.54
	11/1/2010	11/3/2010	17.14	18.26	17.61
C	11/4/2010	11/6/2010	17.14	18.10	17.50
	11/17/2010	11/19/2010	17.95	18.92	18.33
D	2/9/2011	2/9/2011	17.62	18.64	18.15
	2/21/2011	2/23/2011	17.62	18.74	18.20

APPENDIX E

Bivalve Bioassay Data Sheet

Species:		Corvallis code:		NOEC:	
Start date/time:		End date/time:		LOEC:	
Date brine prepared:		Analyst:		EC50:	
Sample Description:				Date sampled:	

Stocking densities:	A	B	C	D	E	F	Mean
Number fertilized:							

	Replicate A				Replicate B				Replicate C				Replicate D			
	Norm	Abnorm	Total	% Norm	Norm	Abnorm	Total	% Norm	Norm	Abnorm	Total	% Norm	Norm	Abnorm	Total	% Norm
Control																
Brine Control																
Treatment 1																
Treatment 2																
Treatment 3																
Treatment 4																
Treatment 5																
Treatment 6																
Treatment 7																

APPENDIX F

Northwest Aquatic Biology Facility Control Chart Criteria and Method Detection Limits

Standards

When a standard value is greater or less than 2 SD from the mean it is said to have exceeded warning limits (WL) and 3 SD from the baseline is the action limit (AL; Table D-1). Exceedance of WL indicates that the quality of the analysis needs to be considered along with other factors (e.g. duplicates and sample concentrations) and a redo of the standard is recommended. Exceedance of AL should be corrected before any samples or duplicates are analyzed. The standard should be repoured, the glassware recleaned, and the stock standard should be remade if necessary.

Table 57. Northwest Aquatic Biology Facility control chart criteria for check standards. SD: standard deviation; BOD: biochemical oxygen demand; TSS: total suspended solids.

Parameter	Check Standard	Mean	SD	Warning Limits	Action Limits
pH	8.00	7.99	0.03	7.931-8.049	7.902-8.075
Color (PCU)	500	499.7	4.1	491.5-507.9	487.3-512.1
Conductivity (µs/cm)	1000	1005.4	5.1	995.1-1015.7	990-1020.8
	1990	2005.8	8.7	1988.4-2023.2	1979.7-2031.9
Turbidity (NTU)	20.0	20.1	0.2	19.68-20.59	19.45-20.82
BOD (mg/L)	198	202	26	150.0-254.0	124.0-279.9
TSS (mg/L)	20	18.1	1.8	14.5-21.7	12.6-23.5
Hardness (mg/L)	100	96.4	2.8	90.7-102.0	87.9-104.8
Alkalinity (mg/L)	100	98	2.9	92.1-103.8	89.2-106.7
Polyphenols (mg/L)	8.0	7.9	0.1	7.6-8.1	7.4-8.3

Duplicates

Duplicate control charting consists of recording the values of two duplicates and comparing the ongoing trend of the difference between them. The difference is not based on the absolute difference between them but on the relative percent difference (RPD) which is computed by the following formula:

$$RPD = [(duplicate\ 1 - duplicate\ 2) \div (mean\ of\ the\ two\ duplicates)] \times 100$$

WL and AL are then based on the SD of the RPD and are thus similar to standard control charts: WL equals 2 SD and AL equals 3 SD. These are not compared to a mean, but to zero (i.e., a baseline of no difference between the two duplicates; Table D-2).

Table 58. Northwest Aquatic Biology Facility control chart criteria for check duplicates. SD: standard deviation; RPD: relative percent difference; BOD: biochemical oxygen demand; TSS: total suspended solids; NA: not available..

Parameter	SD	Warning Limits (% RPD)	Action Limits (% RPD)
pH	1.35	2.7	4.05
Color (PCU)	7.13	14.26	21.4
Conductivity (µs/cm)	8.16	16.32	24.49
Turbidity (NTU)	1.65	3.3	4.96
[a]BOD (mg/L)	NA	NA	NA
TSS (mg/L)	18.13	36.27	54.4
Hardness (mg/L)	4.02	8.03	12.05
Alkalinity (mg/L)	1.74	3.47	5.21
Polyphenols (mg/L)	1.85	3.7	5.55

[a]BOD duplicate criteria based on method requirement of minimum dissolve d oxygen depletion of 2 mg/L.

Method Detection Limits

Method detection limits (MDLs) are determined for water quality parameters at NABF. The MDL is defined as the lowest quantity of a substance that can be measured with 99% confidence that the analyte concentration is greater than zero. MDLs vary lab to lab and depend on a variety of factors such as reagent lot, analyst, instrument, and sample type. For reporting purposes at NABF, any value below the MDL is considered non-detect (ND). An MDL should be determined for each water quality parameter (exceptions are BOD, conductivity, and pH). Current MDLs were either calculated based on control chart standard data or on seven measure of a standard. Any MDL should be recalculated if there are changes in method, instrument, analyst, or when a new calibration curve is prepared.

Procedure

1. Estimate the detection limit for the water quality parameter. For example, Hach provides an EDL (Estimated Detection Limit) for each of its methods, and this value can be a starting concentration for determining the lab MDL.
2. Prepare a standard between 1 and 5 times the estimated MDL.
3. Prepare a minimum of seven aliquots of the standard and process each through the entire analytical method.
4. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. Average the blank measurements and subtract this value from sample measurements.

Calculation in Excel

1. Determine the mean: = *average (range).*
2. Determine the pooled standard deviation: = *stdevp (range).*
3. Determine the % relative standard deviation (RSD): = *(pooled SD/mean)*100.*
4. Determine % recovery: = *(standard value/standard result)*100.*
5. Determine the one-sided t distribution for seven samples. For seven samples (with six degrees of freedom), the t value for a 99% confidence interval is 3.143. If more than seven samples are used to determine the MDL, a t distribution calculator can be easily found on-line.
6. Determine the MDL (seven samples): *=stdevp*3.143.*

MDLs determined at NABF for 2010 for each water quality parameter are located in the following summary table (D3).

Table 59. Summary of National Council for Air and Stream Improvement Northwest Aquatic Biology Facility 2010 water quality method detection limits. MDL: method detection limit, RSD: relative standard deviation; BOD: biochemical oxygen demand; TSS: total suspended solids.

Parameter	MDL	Standard Concentration	%RSD	% Recovery
Alkalinity	8 mg/L	100 mg/L	3	98.5
BOD[a]	2 mg/L			
Color[b]	5 PCU	14 PCU =river water sample	12.3	
Hardness	3 mg/L	15 mg/L	8.9	96.2
Polyphenols	0.01 mg/L	0.2 mg/L	2.2	101
TSS	4 mg/L	20 mg/L	8.2	92.1
Turbidity	0.6 NTU	20 NTU	1.3	100

[a] BOD MDL based on method requirement of minimum dissolved oxygen depletion of 2 mg/L

[b] River water sample used for color MDL determinations

APPENDIX G

Northwest Aquatic Biology Facility Polyphenols Calibration Curve

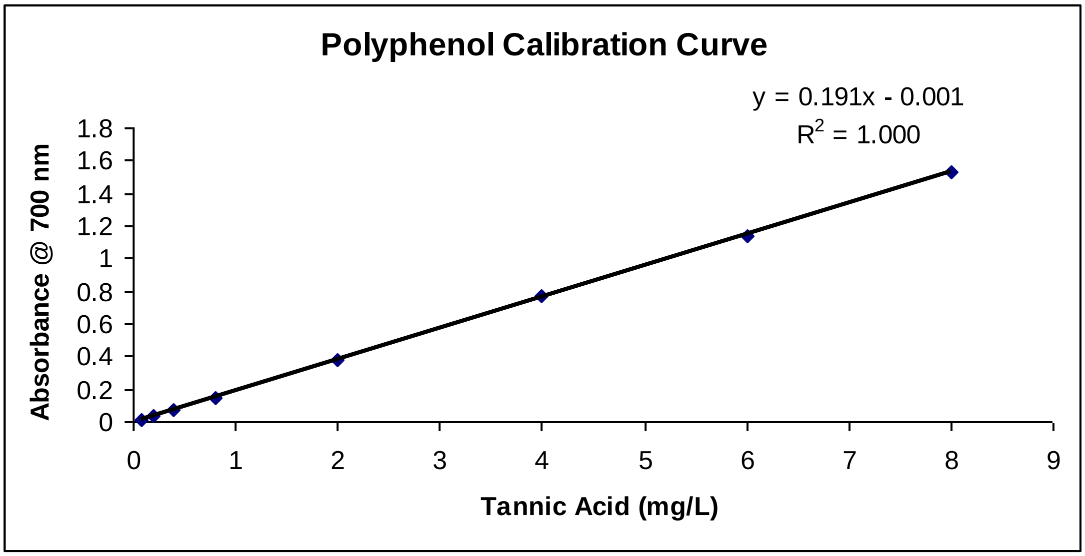

Figure 49. National Council for Air and Stream Improvement Northwest Aquatic Biology Facility polyphenols calibration curve.

APPENDIX H

West Coast Regional Center QA/QC and Lower Calibration Limits for Phytosterols and Resin Acids

Table 60. National Council for Air and Stream Improvement West Coast Regional Center QA/QC criteria for phytosterols. RPD: relative percent difference.

Method: NCASI STER 97 (NCASI 1997)					
Parameter	Daily Calibration Verification % deviation from average response factor	Precision RPD	Matrix Spike Recovery (%)	Surrogate Recovery (%)	Method Blanks (µg/L)
Campesterol	±15	<15	40 - 120		<0.50
Stigmasterol	±15	<15	40 - 120		<0.50
β-Sitosterol	±15	<15	40 - 120		<0.50
Stigmastanol	±15	<15	40 - 120		<0.50
Cholesterol (Surrogate)	±15	<15	*NA	40 -125	<0.50

*NA=not applicable

Note: Instrument DFTPP tune criteria must meet or exceed method specifications

Table 61. National Council for Air and Stream Improvement West Coast Regional Center lower calibration limits (LCLs) for phytosterols.

Parameter	**LCL**
Campesterol (µg/L)	1.35
Stigmasterol (µg/L)	1.15
Beta-sitosterol (µg/L)	2.44
Stigmastanol (µg/L)	1.00

Table 62. National Council for Air and Stream Improvement West Coast Regional Center QA/QC criteria for resin acids (RAs). RPD: relative percent difference.

Method: NCASI RA/FA 85.02 (NCASI 1997)					
Parameter	Daily Calibration Verification % deviation from average response factor	Precision RPD	Matrix Spike % Recovery	Surrogate % Recovery	Method Blanks (µg/L)
Pimaric acid	±15	<15	60 - 120		<0.50
Sandracopimaric acid	±15	<15	65 - 120		<0.50
Isopimaric acid	±15	<15	65 - 120		<0.50
Palustric acid	±15	<15	40 - 120		<0.50
Dehydroabietic acid	±15	<15	70 - 120		<0.50
Abietic acid	±15	<15	70 - 120		<0.50
Neoabietic acid	±15	<15	50 - 120		<0.50
Heptadecanoic acid (Surrogate)	±15	<15	NA	70 - 120	<0.50
O-Methyl podocarpic acid (Surrogate)	±15	<15	NA	70 - 120	<0.50

Note: Instrument DFTPP tune criteria must meet or exceed method specifications

Table 63. National Council for Air and Stream Improvement West Coast Regional Center lower calibration limits (LCLs) for resin acids (RAs).

Parameter	LCL
Pimaric acid (µg/L)	0.92
Sandracopimaric acid (µg/L)	0.94
Isopimaric acid (µg/L)	1.12
Palustric acid (µg/L)	0.99
Dehydroabietic acid (µg/L)	1.10
Abietic acid (µg/L)	1.00
Neoabietic acid (µg/L)	0.99

APPENDIX I
ToxCalc Summary Sheets for *Mytilus galloprovincialis* Effluent Tests

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/1/2010	Test ID:	24667		Sample ID:	Mill A Mill Effluent	
End Date:	9/3/2010	Lab ID:	NABF		Sample Type:	Bleached Kraft	
Sample Dat	8/31/2010	Protocol:	EPAW 95-EPA West Coast		Test Species:	MG-Mytilus galloprovincialis	
Comments:	Mill A mill-treated effluent						

Conc-%	1	2	3	4
B-Control	0.9742	0.9397	0.9470	0.9430
D-Control	0.9669	0.9638	0.9459	0.9573
1.09	0.9338	0.9517	0.9786	0.9632
2.19	0.9329	0.9281	0.9820	0.9576
4.38	0.9470	0.9104	0.9177	0.9136
8.75	0.3394	0.2039	0.2439	0.1304
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9510	0.9922	1.3501	1.3226	1.4095	2.971	4				
D-Control	0.9585	1.0000	1.3665	1.3362	1.3878	1.665	4			25	601
1.09	0.9568	0.9983	1.3654	1.3106	1.4239	3.495	4	17.00	10.00		
2.19	0.9502	0.9913	1.3520	1.2994	1.4364	4.653	4	16.00	10.00		
4.38	0.9222	0.9621	1.2894	1.2669	1.3385	2.573	4	11.00	10.00	47	605
*8.75	0.2294	0.2394	0.4941	0.3695	0.6219	21.229	4	10.00	10.00	454	596
*17.5	0.0000	0.0000	0.0468	0.0418	0.0574	15.555	4	10.00	10.00	478	478
*35	0.0000	0.0000	0.0452	0.0435	0.0471	3.206	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9248849	0.896	0.2656339	2.9034188
Bartlett's Test indicates unequal variances (p = 1.63E-05)	32.009022	16.811893		
The control means are not significantly different (p = 0.50)	0.711848	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	7.1360	6.9438	7.3336
10.0%	7.0316	6.8317	7.2372
20.0%	6.8819	6.6839	7.0856
Auto-3.8%	7.1640	6.9742	7.3589

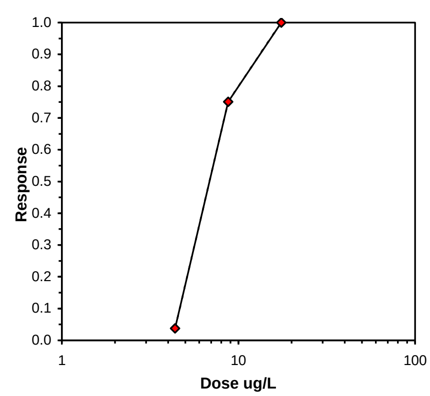

Figure 50. ToxCalc summary sheet including dose-response curve for Mill A mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/9/2010	Test ID:	24668	Sample ID:	Mill A Reactor Control
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis

Comments: Mill A reactor control

Conc-%	1	2	3	4
B-Control	0.9172	0.9272	0.9178	0.9106
D-Control	0.9744	0.8810	0.9073	0.9462
1.09	0.9054	0.9600	0.9220	0.9359
2.19	0.9357	0.9286	0.9259	0.8944
4.38	0.9048	0.9343	0.9143	0.9291
8.75	0.8958	0.9128	0.9396	0.8828
17.5	0.6807	0.6667	0.6241	0.7192
35	0.0000	0.0000	0.0000	
70	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9182	0.9903	1.2809	1.2671	1.2975	0.978	4					
D-Control	0.9272	1.0000	1.3066	1.2185	1.4100	6.462	4				40	563
1.09	0.9308	1.0039	1.3075	1.2582	1.3694	3.618	4	-0.029	2.651	0.0822		
2.19	0.9211	0.9935	1.2874	1.2398	1.3145	2.547	4	0.619	2.651	0.0822		
4.38	0.9206	0.9929	1.2859	1.2571	1.3116	1.945	4	0.668	2.651	0.0822		
8.75	0.9077	0.9790	1.2642	1.2213	1.3225	3.468	4	1.366	2.651	0.0822	54	587
*17.5	0.6727	0.7255	0.9622	0.9108	1.0123	4.352	4	11.102	2.651	0.0822	182	556
*35	0.0000	0.0000	0.0496	0.0484	0.0508	2.462	3	37.507	2.651	0.0888	305	305
*70	0.0000	0.0000	0.0451	0.0400	0.0498	9.013	4	40.659	2.651	0.0822		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9689444	0.902	0.2964832	1.0749424
Bartlett's Test indicates unequal variances (p = 4.72E-04)	26.155411	18.475307		
The control means are not significantly different (p = 0.57)	0.6030847	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	8.75	17.5	12.374369		0.0471021	0.0505484	1.1458036	0.0019254	1.9E-24	7, 23
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	20.621	20.021	21.239
10.0%	20.954	20.250	21.682
20.0%	21.503	20.396	22.670
Auto-2.3%	20.429	19.868	21.006

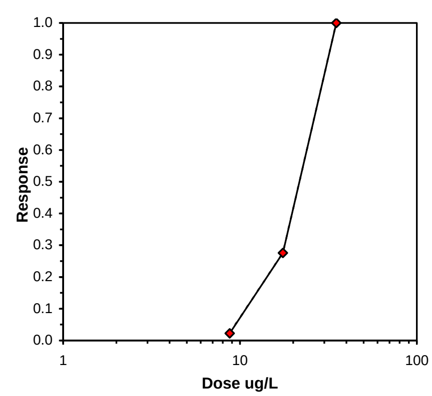

Figure 51. ToxCalc summary sheet including dose-response curve for Mill A reactor control simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/9/2010	Test ID:	24669	Sample ID:	Mill A WBL Spike 1
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill A WBL spike 1				

Conc-%	1	2	3	4
B-Control	0.9091	0.8832	0.9200	0.9343
D-Control	0.9067	0.9301	0.9128	0.9014
1.09	0.9351	0.9362	0.9058	0.9313
2.19	0.8533	0.9085	0.9016	0.8766
4.38	0.9235	0.8855	0.9206	0.9449
8.75	0.8456	0.8162	0.7094	0.1543
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9117	0.9988	1.2705	1.2220	1.3116	2.965	4				
D-Control	0.9127	1.0000	1.2715	1.2514	1.3032	1.777	4			51	584
1.09	0.9271	1.0157	1.2982	1.2588	1.3154	2.049	4	23.00	10.00		
2.19	0.8850	0.9697	1.2262	1.1778	1.2635	3.191	4	13.00	10.00		
4.38	0.9186	1.0065	1.2838	1.2256	1.3338	3.465	4	20.00	10.00	46	567
*8.75	0.6314	0.6917	0.9250	0.4037	1.1670	38.336	4	10.00	10.00	217	551
*17.5	0.0000	0.0000	0.0461	0.0430	0.0505	8.003	4	10.00	10.00	478	478
*35	0.0000	0.0000	0.0526	0.0424	0.0715	25.360	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.6134204	0.896	-2.709173	13.978432
Bartlett's Test indicates unequal variances (p = 1.47E-10)	57.461887	16.811893		
The control means are not significantly different (p = 0.97)	0.041686	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	9.791	9.522	10.068
5.0%	9.900	9.595	10.214
10.0%	10.006	9.652	10.373
20.0%	10.204	9.681	10.754
Auto-0.0%	9.791	9.522	10.068

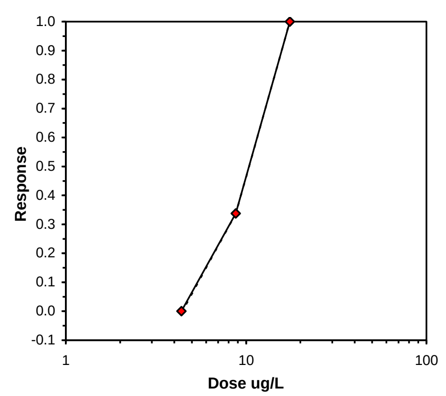

Figure 52. ToxCalc summary sheet including dose-response curve for Mill A weak black liquor spike 1 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/9/2010	Test ID:	24670	Sample ID:	Mill A WBL Spike 2
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill A WBL spike 2				

Conc-%	1	2	3	4
B-Control	0.9343	0.9214	0.9141	0.9241
D-Control	0.9427	0.9548	0.9359	0.9545
1.09	0.9321	0.9220	0.9143	
2.19	0.9324	0.9225	0.8867	0.9291
4.38	0.8113	0.8944	0.8583	0.8960
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9235	0.9752	1.2908	1.2733	1.3116	1.231	4				42	550
D-Control	0.9470	1.0000	1.3391	1.3148	1.3567	1.544	4					
1.09	0.9228	0.9744	1.2895	1.2737	1.3072	1.305	3	0.057	2.567	0.0586		
2.19	0.9177	0.9690	1.2813	1.2274	1.3078	2.871	4	0.450	2.567	0.0542	46	554
*4.38	0.8650	0.9134	1.1971	1.1215	1.2424	4.762	4	4.435	2.567	0.0542	66	500
*8.75	0.0000	0.0000	0.0424	0.0410	0.0442	3.235	4	59.088	2.567	0.0542	559	559
*17.5	0.0000	0.0000	0.0444	0.0420	0.0498	8.177	4	58.992	2.567	0.0542		

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.8787702		0.881		-1.067292	2.799029
Bartlett's Test indicates unequal variances (p = 3.40E-05)					28.150763		15.086272			
The control means are significantly different (p = 0.01)					3.7029811		2.4469118			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Bonferroni t Test	2.19	4.38	3.0971277		0.0312405	0.0338231	1.5570618	0.0008929	2.1E-22	5, 17
Treatments vs B-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	6.0506	5.9208	6.1833
10.0%	6.0549	6.0047	6.1056
20.0%	6.0549	6.0047	6.1056
Auto-0.7%	5.9493	5.8598	6.0403

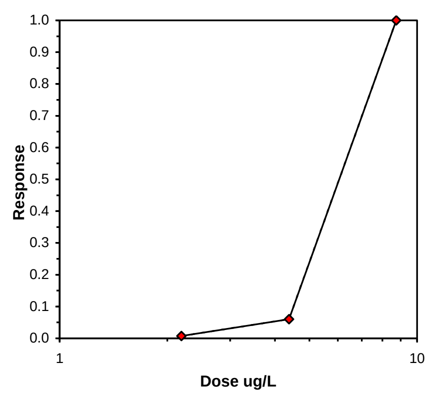

Figure 53. ToxCalc summary sheet including dose-response curve for Mill A weak black liquor spike 2 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal						
Start Date:	9/9/2010	Test ID:	24671	Sample ID:	Mill A WBL Spike 3	
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent	
Sample Date:	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments:	Mill A WBL spike 3					

Conc-%	1	2	3	4
B-Control	0.8966	0.9041	0.9325	0.9412
D-Control	0.9203	0.9535	0.9097	0.9302
1.09	0.9026	0.8684	0.9120	0.9252
2.19	0.8851	0.9385	0.9040	0.9531
4.38	0.4658	0.7613	0.4141	0.3936
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9186	0.9894	1.2833	1.2433	1.3258	3.105	4				
D-Control	0.9284	1.0000	1.3018	1.2656	1.3534	2.899	4			39	540
1.09	0.9020	0.9716	1.2541	1.1996	1.2937	3.183	4	13.00	10.00		
2.19	0.9202	0.9911	1.2883	1.2249	1.3526	4.530	4	16.00	10.00	46	557
*4.38	0.5087	0.5479	0.7972	0.6782	1.0603	22.339	4	10.00	10.00	230	494
*8.75	0.0000	0.0000	0.0469	0.0432	0.0524	8.626	4	10.00	10.00	462	462
*17.5	0.0000	0.0000	0.0452	0.0435	0.0484	4.944	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7904604	0.884	2.092398	8.2972673
Bartlett's Test indicates unequal variances (p = 1.59E-07)	39.859138	15.086272		
The control means are not significantly different (p = 0.53)	0.6740269	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2.19	4.38	3.0971277	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	4.6240	4.4678	4.7856
10.0%	4.6508	4.4734	4.8352
20.0%	4.7039	4.4583	4.9631
Auto-1.1%	4.6031	4.4597	4.7512

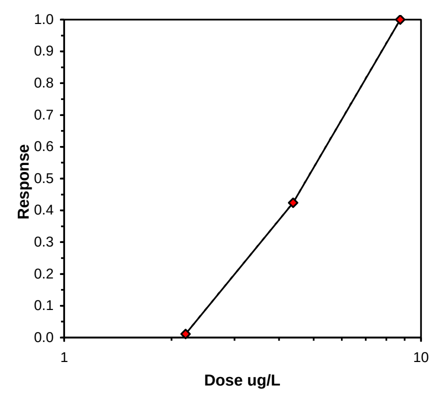

Figure 54. ToxCalc summary sheet including dose-response curve for Mill A weak black liquor spike 3 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/9/2010	Test ID:	24672	Sample ID:	Mill A WBL Spike 4
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill A WBL spike 4				

Conc-%	1	2	3	4
B-Control	0.9249	0.8623	0.9304	0.9103
D-Control	0.9275	0.9281	0.9103	0.9667
1.09	0.8983	0.9660	0.9167	0.9363
2.19	0.8828	0.8915	0.8792	0.8723
4.38	0.0000	0.0206	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9070	0.9719	1.2635	1.1907	1.3038	4.040	4				
D-Control	0.9331	1.0000	1.3128	1.2666	1.3872	3.950	4			39	583
1.09	0.9293	0.9959	1.3063	1.2462	1.3853	4.581	4	17.00	10.00	39	566
*2.19	0.8815	0.9446	1.2194	1.2054	1.2351	1.014	4	10.00	10.00	65	547
*4.38	0.0052	0.0055	0.0747	0.0397	0.1441	63.754	4	10.00	10.00	417	419
*8.75	0.0000	0.0000	0.0437	0.0413	0.0457	4.979	4	10.00	10.00	526	526
*17.5	0.0000	0.0000	0.0423	0.0404	0.0442	5.041	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8566586	0.884	1.0384292	1.5329965
Bartlett's Test indicates unequal variances (p = 8.34E-06)	31.256424	15.086272		
The control means are not significantly different (p = 0.22)	1.3546336	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	13.848651	0.9515066	11.983698	15.713604
Intercept	-1.315993	0.4109022	-2.121361	-0.510625
TSCR	0.0678851	0.007421	0.05334	0.0824303

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.0668954	0.0092966	5.9914646	1	0.4560728	0.0722092	5

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.9412839	1.8179533	2.0493822
EC05	3.355	2.1741986	2.0604613	2.2776099
EC10	3.718	2.3095797	2.1998299	2.4126331
EC15	3.964	2.4056517	2.2975738	2.5099457
EC20	4.158	2.4848489	2.3772625	2.5912587
EC25	4.326	2.5548677	2.4469743	2.6640426
EC40	4.747	2.740178	2.6278786	2.8609442
EC50	5.000	2.8580694	2.7402533	2.9894255
EC60	5.253	2.9810329	2.8553774	3.125926
EC75	5.674	3.197254	3.0533549	3.3713903
EC80	5.842	3.2873471	3.134427	3.4754483
EC85	6.036	3.3955709	3.230885	3.6016508
EC90	6.282	3.5368168	3.3554443	3.7681349
EC95	6.645	3.7570444	3.5471343	4.0312102
EC99	7.326	4.2078138	3.9321112	4.5806328

Significant heterogeneity detected (p < 0.01)

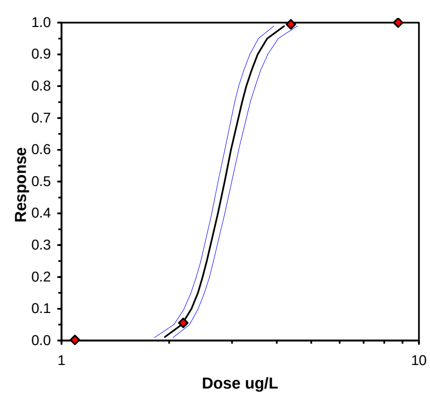

Figure 55. ToxCalc summary sheet including dose-response curve for Mill A weak black liquor spike 4 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/9/2010	Test ID:	24673	Sample ID:	Mill A WBL Spike 5
End Date:	9/11/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	9/8/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill A WBL spike 5				

Conc-%	1	2	3	4
B-Control	0.9638	0.9217	0.8667	0.9236
D-Control	0.9322	0.9658	0.9149	0.9308
1.09	0.9154	0.9051	0.9051	0.8958
2.19	0.8689	0.8014	0.8333	0.8583
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9189	0.9819	1.2886	1.1970	1.3793	5.776	4				
D-Control	0.9359	1.0000	1.3178	1.2748	1.3847	3.561	4			34	535
1.09	0.9054	0.9674	1.2583	1.2422	1.2756	1.088	4	11.00	10.00	52	548
*2.19	0.8405	0.8980	1.1611	1.1089	1.2002	3.493	4	10.00	10.00	82	510
*4.38	0.0000	0.0000	0.0475	0.0440	0.0488	4.889	4	10.00	10.00	446	446
*8.75	0.0000	0.0000	0.0429	0.0406	0.0440	3.652	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8871235	0.868	0.5316549	2.4308351
Bartlett's Test indicates unequal variances (p = 1.28E-05)	27.947399	13.276704		
The control means are not significantly different (p = 0.53)	0.6656354	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	2.9318	2.8688	2.9961
10.0%	2.9750	2.8759	3.0775
20.0%	2.9752	2.9407	3.0101
Auto-3.3%	2.9039	2.8438	2.9653

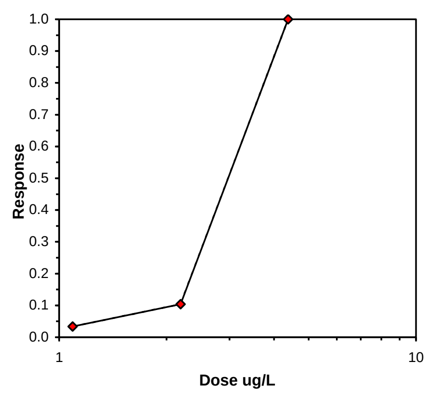

Figure 56. ToxCalc summary sheet including dose-response curve for Mill A weak black liquor spike 5 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	10/22/2010	Test ID:	24776	Sample ID:	Mill B Mill Effluent
End Date:	10/24/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Dat	10/19/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis

Comments: Mill B mill-treated effluent

Conc-%	1	2	3	4
B-Control	0.9813	0.9871	0.9806	0.9821
D-Control	0.9819	0.9874	0.9787	0.9737
1.09	0.9749	0.9679	0.9708	0.9739
2.19	0.9394	0.9709	0.9733	0.9618
4.38	0.7169	0.7964	0.8690	0.9247
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9828	1.0024	1.4395	1.4310	1.4570	0.823	4				
D-Control	0.9804	1.0000	1.4317	1.4079	1.4584	1.483	4			12	618
1.09	0.9719	0.9912	1.4024	1.3907	1.4117	0.677	4	12.00	10.00		
*2.19	0.9614	0.9805	1.3756	1.3221	1.4065	2.783	4	10.00	10.00	23	622
*4.38	0.8267	0.8432	1.1514	1.0097	1.2927	10.618	4	10.00	10.00	114	647
*8.75	0.0000	0.0000	0.0437	0.0377	0.0493	11.645	4	10.00	10.00	540	540
*17.5	0.0000	0.0000	0.0434	0.0402	0.0511	11.779	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8071967	0.884	-0.056365	6.0206501
Bartlett's Test indicates unequal variances (p = 8.35E-07)	36.279243	15.086272		
The control means are not significantly different (p = 0.54)	0.6481779	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	5.6405	5.5132	5.7708
10.0%	5.7437	5.5798	5.9125
20.0%	5.7962	5.7149	5.8787
Auto-1.8%	5.5554	5.4398	5.6736

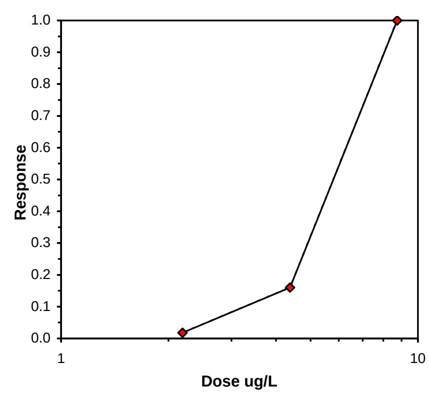

Figure 57. ToxCalc summary sheet including dose-response curve for Mill B mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal							
Start Date:	11/1/2010	Test ID:	24917	Sample ID:	Mill B Reactor Control		
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Simulated effluent		
Sample Dat	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis		
Comments:	Mill B Reactor Control						

Conc-%	1	2	3	4
B-Control	0.8868	0.9123	0.9091	0.9103
D-Control	0.9308	0.9222	0.9188	0.9250
1.09	0.9419	0.8704	0.9067	0.9532
2.19	0.9733	0.9394	0.9474	0.9554
4.38	0.9392	0.9067	0.9398	0.9645
8.75	0.4558	0.4810	0.4029	0.4013
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9046	0.9788	1.2572	1.2276	1.2701	1.579	4			63	662
D-Control	0.9242	1.0000	1.2920	1.2817	1.3046	0.750	4				
1.09	0.9180	0.9933	1.2857	1.2025	1.3528	5.274	4	19.00	10.00		
2.19	0.9539	1.0321	1.3565	1.3221	1.4068	2.695	4	26.00	10.00		
4.38	0.9375	1.0144	1.3215	1.2603	1.3814	3.739	4	23.00	10.00	38	605
*8.75	0.4352	0.4709	0.7203	0.6860	0.7664	5.553	4	10.00	10.00	339	601
*17.5	0.0000	0.0000	0.0423	0.0406	0.0435	2.966	4	10.00	10.00	560	560
*35	0.0000	0.0000	0.0404	0.0386	0.0429	4.839	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9443072	0.896	-0.147528	0.6148287
Bartlett's Test indicates unequal variances (p = 8.99E-06)	33.346691	16.811893		
The control means are significantly different (p = 0.02)	3.149533	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs B-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	8.5923	8.3533	8.8381
5.0%	8.5762	8.3118	8.8490
10.0%	8.5601	8.2648	8.8660
20.0%	8.5282	8.1431	8.9315
Auto-0.0%	8.5923	8.3533	8.8381

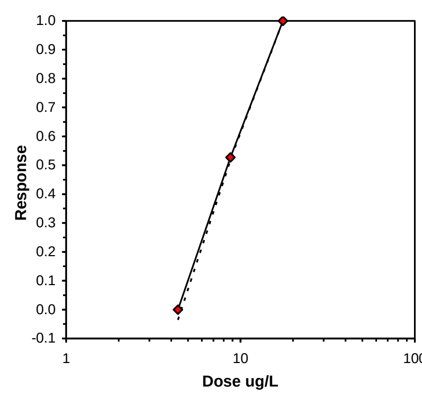

Figure 58. ToxCalc summary sheet including dose-response curve for Mill B reactor control simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal						
Start Date:	11/1/2010	Test ID:	24918	Sample ID:	Mill B WBL spike 1	
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Simulated effluent	
Sample Dat	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments:	Mill B WBL spike 1					

Conc-%	1	2	3	4
B-Control	0.9028	0.9282	0.9392	0.9337
D-Control	0.9589	0.9153	0.9382	0.9358
1.09	0.9714	0.9618	0.9568	0.9045
2.19	0.9647	0.9325	0.9148	0.9299
4.38	0.8713	0.8563	0.8916	0.8590
8.75	0.0000	0.0000	0.0000	
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9260	0.9882	1.2963	1.2537	1.3217	2.301	4					
D-Control	0.9370	1.0000	1.3191	1.2754	1.3667	2.833	4				44	688
1.09	0.9486	1.0124	1.3483	1.2566	1.4010	4.696	4	-1.092	2.567	0.0686		
2.19	0.9355	0.9983	1.3168	1.2745	1.3818	3.475	4	0.085	2.567	0.0686	43	666
*4.38	0.8696	0.9280	1.2018	1.1820	1.2353	2.025	4	4.389	2.567	0.0686	90	697
*8.75	0.0000	0.0000	0.0410	0.0397	0.0426	3.572	3	44.266	2.567	0.0741	447	447
*17.5	0.0000	0.0000	0.0423	0.0397	0.0447	4.958	4	47.766	2.567	0.0686		

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9209279		0.881		-0.487987	1.9439788
Bartlett's Test indicates unequal variances (p = 1.23E-04)					25.272398		15.086272			
The control means are not significantly different (p = 0.38)					0.9513267		2.4469118			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Bonferroni t Test	2.19	4.38	3.0971277		0.0371162	0.0395709	1.5433696	0.0014291	1.2E-20	5, 17
Treatments vs D-Control										

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	6.0205	5.9094	6.1336
10.0%	6.0325	5.9862	6.0792
20.0%	6.0325	5.9862	6.0792
Auto-0.1%	5.9003	5.8218	5.9798

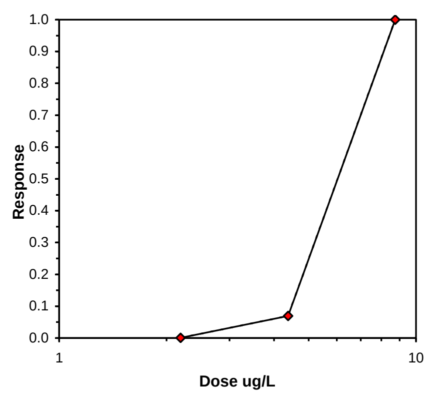

Figure 59. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 1 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/1/2010	Test ID:	24919	Sample ID:	Mill B WBL Spike 2
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Dat	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill B WBL spike 2				

Conc-%	1	2	3	4
B-Control	0.9005	0.8994	0.9557	0.9527
D-Control	0.9441	0.9245	0.9553	0.9416
1.09	0.9632	0.9577	0.9500	0.9240
2.19	0.9116	0.9359	0.9157	0.9265
4.38	0.2980	0.1898	0.3172	0.3419
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		Resp	Number
B-Control	0.9271	0.9848	1.3020	1.2481	1.3587	4.715	4					
D-Control	0.9414	1.0000	1.3273	1.2925	1.3578	2.024	4				38	653
1.09	0.9487	1.0078	1.3446	1.2915	1.3777	2.813	4	21.00	10.00			
2.19	0.9224	0.9799	1.2890	1.2688	1.3148	1.607	4	12.00	10.00	0.0683878	48	617
*4.38	0.2867	0.3046	0.5628	0.4507	0.6246	13.706	4	10.00	10.00	0.0683878	418	588
*8.75	0.0000	0.0000	0.0431	0.0367	0.0481	13.766	4	10.00	10.00	0.0683878	563	563
*17.5	0.0000	0.0000	0.0413	0.0394	0.0435	4.357	4	10.00	10.00			

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8606325	0.884	-1.566949	4.9737749
Bartlett's Test indicates unequal variances (p = 5.18E-05)	27.215322	15.086272		
The control means are not significantly different (p = 0.48)	0.7523713	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2.19	4.38	3.0971277	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	3.7542	3.6462	3.8655
10.0%	3.7090	3.5934	3.8283
20.0%	3.6316	3.5020	3.7660
Auto-2.1%	3.7821	3.6786	3.8885

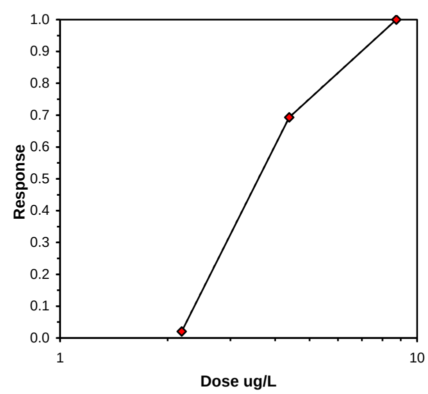

Figure 60. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 2 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal							
Start Date:	11/1/2010	Test ID:	24920	Sample ID:	Mill B WBL Spike 3		
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft		
Sample Dat	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis		
Comments:	Mill B WBL spike 3						

Conc-%	1	2	3	4
B-Control	0.9351	0.9533	0.9222	0.9474
D-Control	0.9273	0.9557	0.9695	0.9504
1.09	0.9253	0.9360	0.9074	0.9244
2.19	0.8344	0.8320	0.8170	0.7857
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9395	0.9882	1.3234	1.2880	1.3531	2.176	4				
D-Control	0.9507	1.0000	1.3495	1.2977	1.3953	2.987	4			31	628
1.09	0.9233	0.9712	1.2907	1.2616	1.3151	1.707	4	11.00	10.00	52	680
*2.19	0.8173	0.8597	1.1296	1.0895	1.1517	2.531	4	10.00	10.00	133	734
*4.38	0.0000	0.0000	0.0401	0.0388	0.0418	3.508	4	10.00	10.00	625	625
*8.75	0.0000	0.0000	0.0403	0.0394	0.0407	1.545	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.870204	0.868	-0.5793	1.7275793
Bartlett's Test indicates unequal variances (p = 2.59E-06)	31.35791	13.276704		
The control means are not significantly different (p = 0.33)	1.0528099	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	2.8591	2.8016	2.9178
10.0%	2.9139	2.8371	2.9929
20.0%	2.9290	2.8943	2.9642
Auto-2.9%	2.8267	2.7724	2.8820

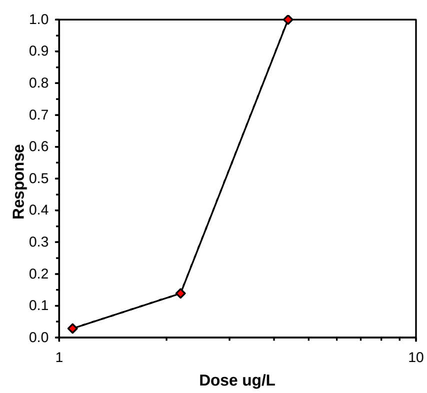

Figure 61. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 3 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal						
Start Date:	11/1/2010	Test ID:	24921	Sample ID:	Mill B WBL Spike 4
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Date:	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill B WBL spike 4				

Conc-%	1	2	3	4
B-Control	0.9627	0.9382	0.9503	0.9408
D-Control	0.9477	0.9655	0.9394	0.9014
1.09	0.9114	0.9306	0.9247	0.9167
2.19	0.5338	0.5466	0.5568	0.6042
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9480	1.0101	1.3418	1.3196	1.3765	1.924	4				
D-Control	0.9385	1.0000	1.3244	1.2514	1.3840	4.167	4			36	601
1.09	0.9208	0.9812	1.2859	1.2685	1.3043	1.229	4	14.00	10.00	49	621
*2.19	0.5603	0.5970	0.8460	0.8192	0.8903	3.670	4	10.00	10.00	300	686
*4.38	0.0000	0.0000	0.0422	0.0413	0.0435	2.352	4	10.00	10.00	563	563
*8.75	0.0000	0.0000	0.0398	0.0392	0.0404	1.319	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8551311	0.868	-0.357895	3.7616026
Bartlett's Test indicates unequal variances (p = 7.93E-08)	38.726707	13.276704		
The control means are not significantly different (p = 0.59)	0.5706262	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	2.3441	2.2767	2.4134
10.0%	2.3628	2.2862	2.4419
20.0%	2.3996	2.2920	2.5121
Auto-2.0%	2.3329	2.2698	2.3978

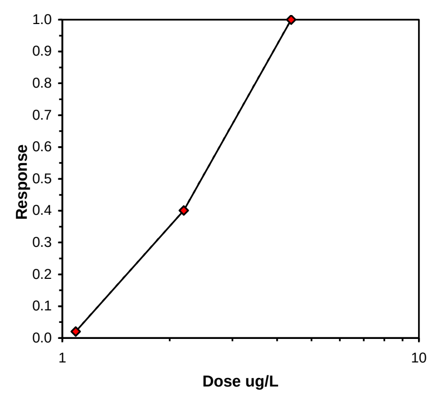

Figure 62. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 4 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/1/2010	Test ID:	24922	Sample ID:	Mill B WBL Spike 5
End Date:	11/3/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Dat	10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill B WBL spike 5				

Conc-%	1	2	3	4
B-Control	0.9276	0.9217	0.9286	0.9494
D-Control	0.9136	0.9477	0.9294	0.9324
1.09	0.9645	0.8993	0.9176	0.8750
2.19	0.2229	0.2680	0.1838	0.1722
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9318	1.0011	1.3074	1.2872	1.3438	1.909	4				
D-Control	0.9308	1.0000	1.3055	1.2724	1.3400	2.123	4			45	652
1.09	0.9141	0.9821	1.2796	1.2094	1.3812	5.754	4	15.00	10.00	53	630
*2.19	0.2117	0.2275	0.4767	0.4279	0.5441	11.021	4	10.00	10.00	477	606
*4.38	0.0000	0.0000	0.0406	0.0392	0.0417	2.775	4	10.00	10.00	609	609
*8.75	0.0000	0.0000	0.0414	0.0391	0.0432	4.205	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8731666	0.868	0.9181076	2.3295426
Bartlett's Test indicates unequal variances (p = 6.62E-07)	34.250607	13.276704		
The control means are not significantly different (p = 0.92)	0.1009613	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	1.7692	1.7238	1.8159
10.0%	1.7419	1.6951	1.7900
20.0%	1.7068	1.6632	1.7515
Auto-1.6%	1.7902	1.7465	1.8350

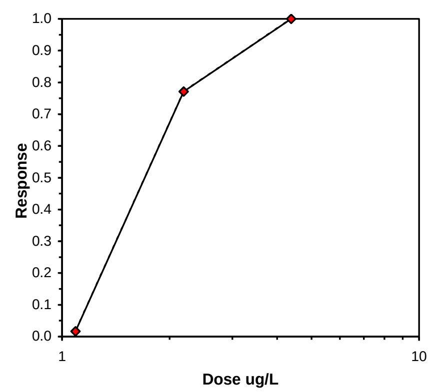

Figure 63. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 5 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date: 11/1/2010	Test ID:	24922	Sample ID:	Mill B WBL Spike 5 Unsettled	
End Date: 11/3/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft	
Sample Dat 10/28/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments: Mill B WBL spike 5 unsettled					

Conc-ug/L	1	2	3	4
B-Control	0.9281	0.9249	0.9317	0.8836
D-Control	0.8935	0.9268	0.9301	0.9337
1.09	0.9172	0.9097	0.9188	0.9101
2.19	0.2536	0.2569	0.2109	0.1250
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9170	0.9957	1.2803	1.2226	1.3063	3.036	4				
D-Control	0.9210	1.0000	1.2872	1.2383	1.3104	2.567	4			51	642
1.09	0.9140	0.9923	1.2732	1.2656	1.2818	0.661	4	14.00	10.00	57	664
*2.19	0.2116	0.2298	0.4745	0.3614	0.5316	16.730	4	10.00	10.00	452	573
*4.38	0.0000	0.0000	0.0417	0.0414	0.0418	0.489	4	10.00	10.00	576	576
*8.75	0.0000	0.0000	0.0398	0.0378	0.0427	6.090	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.7585536	0.868	-1.743603	6.2448789
Bartlett's Test indicates unequal variances (p = 1.19E-09)	47.519878	13.276704		
The control means are not significantly different (p = 0.80)	0.2702006	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	1.7760	1.7293	1.8239
10.0%	1.7484	1.7002	1.7980
20.0%	1.7129	1.6678	1.7592
Auto-0.7%	1.8032	1.7589	1.8486

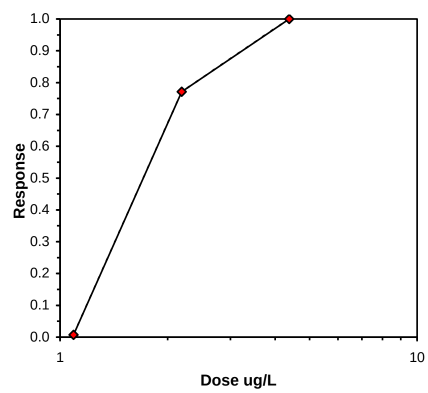

Figure 64. ToxCalc summary sheet including dose-response curve for Mill B weak black liquor spike 5 simulated effluent sample (unsettled).

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/4/2010	Test ID:	24924	Sample ID:	Mill C Mill Effluent
End Date:	11/6/2010	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Dat	11/2/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill C mill-treated effluent				

Conc-%	1	2	3	4
B-Control	0.8836	0.9180	0.9139	0.8929
D-Control	0.9114	0.9016	0.3275	0.9463
1.09	0.9281	0.8865	0.8610	0.8659
2.19	0.9118	0.9126	0.9091	0.8800
4.38	0.8333	0.8539	0.8736	0.8757
8.75	0.1867	0.2649	0.1749	0.2704
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9021	1.1690	1.2533	1.2226	1.2804	2.219	4				
D-Control	0.7717	1.0000	1.1166	0.6093	1.3370	30.471	4			155	661
1.09	0.8854	1.1473	1.2278	1.1887	1.2994	4.122	4	16.00	10.00		
2.19	0.9034	1.1706	1.2553	1.2171	1.2706	2.044	4	19.00	10.00		
4.38	0.8591	1.1133	1.1867	1.1503	1.2105	2.373	4	14.00	10.00	106	747
*8.75	0.2242	0.2906	0.4914	0.4314	0.5469	12.366	4	10.00	10.00	538	693
*17.5	0.0000	0.0000	0.0375	0.0360	0.0407	5.767	4	10.00	10.00	716	716
*35	0.0000	0.0000	0.0386	0.0347	0.0400	6.744	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.6567013	0.896	-2.823194	13.730284
Bartlett's Test indicates unequal variances (p = 6.40E-12)	64.160378	16.811893		
The control means are not significantly different (p = 0.45)	0.8008982	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	7.4922	7.3182	7.6705
5.0%	7.3797	7.1921	7.5722
10.0%	7.2767	7.0777	7.4813
20.0%	7.1139	6.9013	7.3330
Auto-0.0%	7.4922	7.3182	7.6705

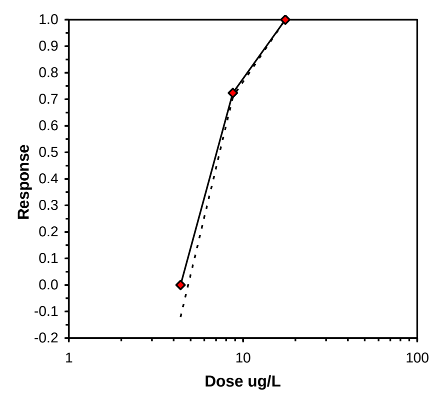

Figure 65. ToxCalc summary sheet including dose-response curve for weak black liquor mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	11/17/2010	Test ID:	24986	Sample ID:	Mill C Reactor Control
End Date:	11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill C reactor control				

Conc-%	1	2	3	4
B-Control	0.9787	0.9627	0.9471	0.9403
D-Control	0.9457	0.9588	0.9529	0.9560
1.09	0.9341	0.9579	0.9512	0.9278
2.19	0.9604	0.9063	0.9527	0.9315
4.38	0.9104	0.9476	0.9171	0.9133
8.75	0.7624	0.8282	0.8233	0.8090
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9572	1.0041	1.3659	1.3240	1.4244	3.284	4				
D-Control	0.9533	1.0000	1.3534	1.3355	1.3665	0.983	4			34	727
1.09	0.9428	0.9889	1.3306	1.2988	1.3641	2.302	4	14.00	10.00		
2.19	0.9377	0.9836	1.3219	1.2596	1.3705	3.748	4	15.00	10.00		
4.38	0.9221	0.9672	1.2893	1.2669	1.3399	2.642	4	11.00	10.00	61	782
*8.75	0.8057	0.8452	1.1151	1.0617	1.1434	3.333	4	10.00	10.00	143	737
*17.5	0.0000	0.0000	0.0393	0.0362	0.0423	6.680	4	10.00	10.00	656	656
*35	0.0000	0.0000	0.0380	0.0355	0.0397	4.960	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.962661	0.896	-0.252498	0.6366211
Bartlett's Test indicates unequal variances (p = 1.51E-04)	26.903662	16.811893		
The control means are not significantly different (p = 0.61)	0.5351905	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	11.277	11.043	11.516
10.0%	11.511	11.210	11.819
20.0%	11.615	11.466	11.766
Auto-3.3%	11.176	10.951	11.405

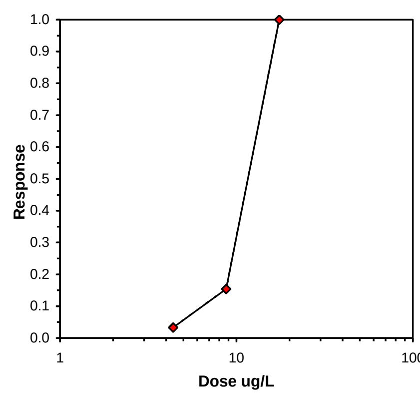

Figure 66. ToxCalc summary sheet including dose-response curve for weak black liquor reactor control simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date: 11/17/2010	Test ID:	24987	Sample ID:	Mill C WBL Spike 1	
End Date: 11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent	
Sample Dat 11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments: Mill C WBL spike 1					

Conc-%	1	2	3	4
B-Control	0.9255	0.9565	0.9427	0.9548
D-Control	0.9581	0.9249	0.9341	0.9733
1.09	0.9321	0.9179	0.9538	
2.19	0.9163	0.9200	0.8956	0.8846
4.38	0.9147	0.9211	0.9113	0.8814
8.75	0.2488	0.1475	0.1779	0.1737
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9449	0.9971	1.3352	1.2943	1.3607	2.295	4					
D-Control	0.9476	1.0000	1.3439	1.2931	1.4065	3.846	4				39	757
1.09	0.9346	0.9863	1.3139	1.2803	1.3543	2.850	3	1.115	2.613	0.0702	40	611
*2.19	0.9041	0.9541	1.2568	1.2242	1.2840	2.273	4	3.499	2.613	0.0650	73	766
*4.38	0.9071	0.9573	1.2620	1.2191	1.2860	2.336	4	3.292	2.613	0.0650	75	819
*8.75	0.1870	0.1973	0.4454	0.3942	0.5222	12.194	4	36.117	2.613	0.0650	635	782
*17.5	0.0000	0.0000	0.0361	0.0340	0.0393	6.513	4	52.573	2.613	0.0650	776	776
*35	0.0000	0.0000	0.0376	0.0354	0.0398	5.496	4	52.510	2.613	0.0650		

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9542512		0.894		0.5057123	0.607708
Bartlett's Test indicates unequal variances (p = 1.62E-04)					26.746212		16.811893			
The control means are not significantly different (p = 0.78)					0.2904817		2.4469118			
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	1.09	2.19	1.5450243		0.0322039	0.0339208	1.4709212	0.0012376	1.9E-24	6, 20
Treatments vs D-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.5417558	0.7931601	7.987162	11.09635	0.0515192	7.0000751	7.8147278	0.07	0.8545344	0.1048025	8
Intercept	-3.153758	0.7359133	-4.596148	-1.711368							
TSCR	0.0714087	0.005538	0.0605541	0.0822632							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	4.0806302	3.5253648	4.536921
EC05	3.355	4.8100496	4.2876578	5.2297983
EC10	3.718	5.2507885	4.7584071	5.6424783
EC15	3.964	5.5707482	5.1042668	5.9398727
EC20	4.158	5.8388905	5.3964435	6.187995
EC25	4.326	6.0791958	5.6598474	6.4097297
EC40	4.747	6.7295022	6.3779023	7.0088583
EC50	5.000	7.1537598	6.8476371	7.4017647
EC60	5.253	7.6047645	7.3433883	7.8258295
EC75	5.674	8.4182646	8.2045031	8.6308842
EC80	5.842	8.7647266	8.5493239	8.9983455
EC85	6.036	9.186608	8.9505071	9.4665969
EC90	6.282	9.7463985	9.458839	10.11539
EC95	6.645	10.639449	10.236379	11.191817
EC99	7.326	12.541269	11.827274	13.580075

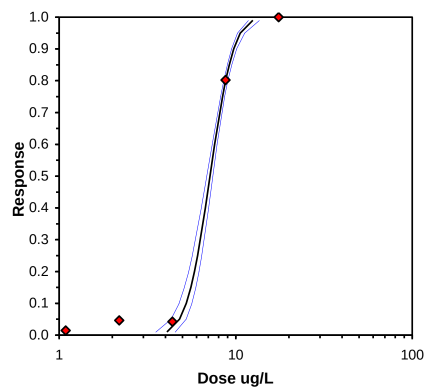

Figure 67. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 1 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date: 11/17/2010	Test ID:	24988	Sample ID:	Mill C WBL Spike 2	
End Date: 11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent	
Sample Date: 11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments: Mill C WBL spike 2					

Conc-%	1	2	3	4
B-Control	0.9519	0.9312	0.9572	0.9895
D-Control	0.9290	0.9400	0.9487	0.9558
1.09	0.9457	0.9181	0.9524	0.9419
2.19	0.8945	0.9385	0.9175	0.9502
4.38	0.8838	0.9022	0.9121	0.8901
8.75	0.0051	0.0061	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9575	1.0149	1.3714	1.3054	1.4680	5.023	4				
D-Control	0.9434	1.0000	1.3314	1.3010	1.3590	1.875	4			43	759
1.09	0.9395	0.9959	1.3235	1.2806	1.3508	2.285	4	17.00	10.00		
2.19	0.9252	0.9807	1.2964	1.2399	1.3459	3.588	4	14.00	10.00	59	785
*4.38	0.8970	0.9509	1.2446	1.2229	1.2698	1.676	4	10.00	10.00	87	832
*8.75	0.0028	0.0029	0.0562	0.0377	0.0779	37.996	4	10.00	10.00	710	712
*17.5	0.0000	0.0000	0.0359	0.0344	0.0378	4.102	4	10.00	10.00	777	777
*35	0.0000	0.0000	0.0355	0.0340	0.0365	3.222	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9743636	0.896	-0.370844	0.7358703
Bartlett's Test indicates unequal variances (p = 4.11E-05)	29.897276	16.811893		
The control means are not significantly different (p = 0.32)	1.0915716	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	2.19	4.38	3.0971277	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.918608	0.9119543	13.131177	16.706038	0.0566535	1.0529334	5.9914646	0.59	0.7578791	0.0670304	5
Intercept	-6.306501	0.6617541	-7.60354	-5.009463							
TSCR	0.0660622	0.0063214	0.0536723	0.0784521							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	3.9989238	3.7801422	4.1946
EC05	3.355	4.4424643	4.2384053	4.6311457
EC10	3.718	4.6986839	4.5002943	4.8872426
EC15	3.964	4.8798514	4.6835847	5.0706747
EC20	4.158	5.0288065	4.8328924	5.2231685
EC25	4.326	5.1602152	4.963469	5.3590516
EC40	4.747	5.5067729	5.3023687	5.723798
EC50	5.000	5.7263662	5.5129894	5.9597168
EC60	5.253	5.9547163	5.7288099	6.2087894
EC75	5.674	6.3546323	6.099822	6.6532699
EC80	5.842	6.5206864	6.2516168	6.8405663
EC85	6.036	6.7197273	6.4320641	7.0669347
EC90	6.282	6.9788201	6.6647839	7.3643539
EC95	6.645	7.3813242	7.0221792	7.8318202
EC99	7.326	8.2000241	7.7369808	8.7994473

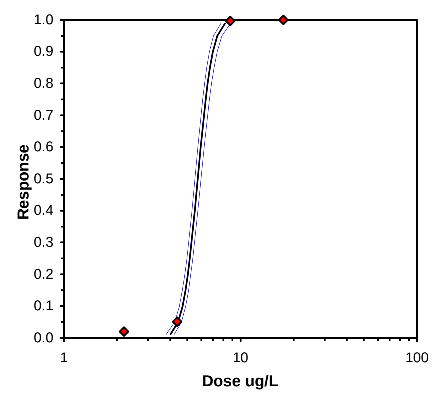

Figure 68. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 2 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal							
Start Date:	11/17/2010	Test ID:	24989	Sample ID:	Mill C WBL Spike 3		
End Date:	11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent		
Sample Dat	11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis		
Comments:	Mill C WBL spike 3						

Conc-%	1	2	3	4
B-Control	0.9572	0.4330	0.9213	0.9607
D-Control	0.9639	0.9402	0.9535	0.9184
1.09	0.9096	0.9115	0.9339	0.9206
2.19	0.9394	0.8783	0.9254	0.9622
4.38	0.5123	0.5865	0.5412	0.4709
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.8180	0.8666	1.1846	0.7182	1.3712	26.443	4				
D-Control	0.9440	1.0000	1.3345	1.2810	1.3797	3.171	4			44	789
1.09	0.9189	0.9734	1.2825	1.2654	1.3108	1.618	4	12.00	10.00		
2.19	0.9263	0.9813	1.3014	1.2145	1.3750	5.146	4	15.00	10.00	59	800
*4.38	0.5277	0.5590	0.8133	0.7562	0.8724	6.011	4	10.00	10.00	392	828
*8.75	0.0000	0.0000	0.0386	0.0359	0.0449	10.970	4	10.00	10.00	687	687
*17.5	0.0000	0.0000	0.0378	0.0355	0.0415	7.380	4	10.00	10.00		

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9324862	0.884	-0.3128	1.4652625
Bartlett's Test indicates unequal variances (p = 1.64E-04)					24.638517	15.086272		
The control means are not significantly different (p = 0.38)					0.9486278	2.4469118		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**				
Steel's Many-One Rank Test	2.19	4.38	3.0971277					
Treatments vs D-Control								

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	4.5480	4.4281	4.6712
10.0%	4.5702	4.4348	4.7097
20.0%	4.6145	4.4292	4.8075
Auto-1.9%	4.5343	4.4220	4.6495

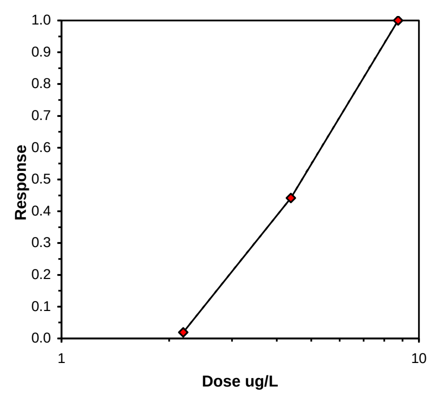

Figure 69. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 3 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	11/17/2010	Test ID:	24990	Sample ID:	Mill C WBL Spike 4
End Date:	11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill C WBL spike 4				

Conc-%	1	2	3	4
B-Control	0.9356	0.9607	0.9614	0.9256
D-Control	0.9524	0.9353	0.9442	0.9171
1.09	0.8896	0.9216	0.9119	0.9280
2.19	0.8857	0.8580	0.8915	0.8507
4.38	0.0159	0.0140	0.0233	0.0000
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9458	1.0091	1.3382	1.2945	1.3730	2.984	4				
D-Control	0.9372	1.0000	1.3189	1.2788	1.3508	2.330	4			49	789
1.09	0.9128	0.9739	1.2719	1.2320	1.2991	2.298	4	12.00	10.00	68	793
*2.19	0.8715	0.9298	1.2049	1.1741	1.2352	2.502	4	10.00	10.00	102	799
*4.38	0.0133	0.0142	0.1111	0.0464	0.1531	41.017	4	10.00	10.00	723	734
*8.75	0.0000	0.0000	0.0361	0.0351	0.0367	1.891	4	10.00	10.00	770	770
*17.5	0.0000	0.0000	0.0339	0.0302	0.0379	9.842	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9364768	0.884	-0.796883	0.8290195
Bartlett's Test indicates unequal variances (p = 4.28E-05)	27.641438	15.086272		
The control means are not significantly different (p = 0.47)	0.7687951	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.330043	0.5700284	11.212788	13.447299	0.0621039	1.6104042	5.9914646	0.45	0.4679262	0.0811027	5
Intercept	-0.76955	0.2861345	-1.330374	-0.208727							
TSCR	0.073957	0.0065797	0.0610608	0.0868531							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.9021863	1.791733	2.0028054
EC05	3.355	2.1603499	2.055587	2.2564898
EC10	3.718	2.3120056	2.2106373	2.4058535
EC15	3.964	2.4202968	2.3211461	2.5128863
EC20	4.158	2.5099694	2.4124183	2.6018599
EC25	4.326	2.5895436	2.4931664	2.6811389
EC40	4.747	2.8014253	2.7066443	2.8941201
EC50	5.000	2.9371504	2.8419201	3.0322872
EC60	5.253	3.0794512	2.9823021	3.1788135
EC75	5.674	3.3314181	3.2270197	3.4426448
EC80	5.842	3.437035	3.3281633	3.5548663
EC85	6.036	3.564378	3.4490668	3.6913693
EC90	6.282	3.7313285	3.6059953	3.8721534
EC95	6.645	3.9932663	3.8491001	4.1594503
EC99	7.326	4.5352303	4.3428893	4.765118

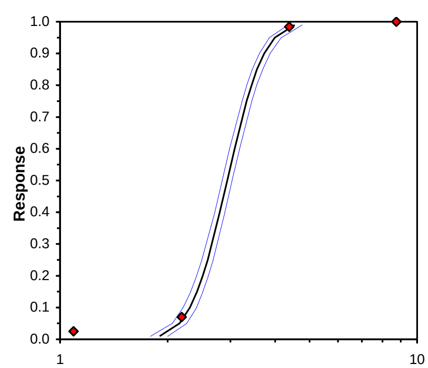

Figure 70. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 4 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date: 11/17/2010	Test ID:	24991	Sample ID:	Mill C WBL Spike 5	
End Date: 11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent	
Sample Dat 11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis	
Comments: Mill C WBL spike 5					

Conc-%	1	2	3	4
B-Control	0.9643	0.9453	0.9369	0.9630
D-Control	0.9409	0.9534	0.9538	0.9352
1.09	0.9124	0.9481	0.9318	0.9256
2.19	0.7414	0.7978	0.8173	0.7608
3.28	0.1006	0.1371	0.1461	0.1878
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9524	1.0069	1.3523	1.3169	1.3807	2.334	4				
D-Control	0.9458	1.0000	1.3365	1.3134	1.3543	1.530	4			44	807
1.09	0.9295	0.9827	1.3031	1.2703	1.3410	2.259	4	12.00	10.00	59	841
*2.19	0.7793	0.8240	1.0827	1.0374	1.1291	3.848	4	10.00	10.00	191	852
*3.28	0.1429	0.1511	0.3856	0.3227	0.4482	13.361	4	10.00	10.00	656	767
*4.38	0.0000	0.0000	0.0376	0.0293	0.0505	24.129	4	10.00	10.00	790	790
*8.75	0.0000	0.0000	0.0356	0.0346	0.0362	1.998	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.967466	0.884	0.0438535	0.9837569
Bartlett's Test indicates unequal variances (p = 1.55E-04)	24.760139	15.086272		
The control means are not significantly different (p = 0.43)	0.8423149	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.73319	0.7531169	8.4927898	14.973591	0.0545229	6.1630175	5.9914646	0.05	0.4236857	0.0852283	6
Intercept	0.028815	0.3420899	-1.443079	1.5007092							
TSCR	0.0632311	0.0105104	0.0180084	0.1084538							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.680409	1.3624229	1.9037832
EC05	3.355	1.9208684	1.6321089	2.1229376
EC10	3.718	2.0628195	1.7950152	2.2524689
EC15	3.964	2.1644734	1.9126161	2.3460167
EC20	4.158	2.2488255	2.0103657	2.424559
EC25	4.326	2.3238072	2.0970577	2.4953898
EC40	4.747	2.5240233	2.3250796	2.6916891
EC50	5.000	2.6526852	2.4664807	2.8257611
EC60	5.253	2.7879056	2.6086066	2.9754662
EC75	5.674	3.0281078	2.842473	3.2657371
EC80	5.842	3.1290728	2.9342356	3.3964369
EC85	6.036	3.2510166	3.0409018	3.5601237
EC90	6.282	3.4112235	3.1755189	3.7834211
EC95	6.645	3.6633111	3.3781239	4.1501555
EC99	7.326	4.1875157	3.7769728	4.9585319

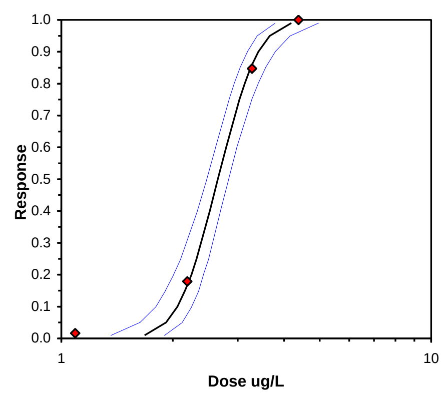

Figure 71. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 5 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/17/2010	Test ID:	24992	Sample ID:	Mill C WBL Spike 5 Unsettled
End Date:	11/19/2010	Lab ID:	NABF	Sample Type:	Simulated effluent
Sample Dat	11/15/2010	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis

Comments: Mill C WBL spike 5 unsettled

Conc-%	1	2	3	4
B-Control	0.9367	0.9695	0.9558	0.9399
D-Control	0.9251	0.9571	0.9270	0.9466
1.09	0.9466	0.9474	0.9309	0.9278
2.19	0.8381	0.8537	0.8564	0.8341
3.28	0.2071	0.2038	0.2536	0.1951
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9505	1.0123	1.3485	1.3164	1.3954	2.703	4				
D-Control	0.9390	1.0000	1.3227	1.2936	1.3621	2.496	4			55	899
1.09	0.9382	0.9992	1.3201	1.2988	1.3393	1.616	4	19.50	10.00	50	816
*2.19	0.8456	0.9005	1.1671	1.1513	1.1821	1.316	4	10.00	10.00	127	820
*3.28	0.2149	0.2289	0.4815	0.4575	0.5277	6.530	4	10.00	10.00	623	794
*4.38	0.0000	0.0000	0.0340	0.0320	0.0359	5.728	4	10.00	10.00	871	871
*8.75	0.0000	0.0000	0.0358	0.0321	0.0392	8.070	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94957	0.884	0.7180785	0.494982
Bartlett's Test indicates unequal variances (p = 8.13E-04)	20.992403	15.086272		
The control means are not significantly different (p = 0.33)	1.0500079	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	12.581311	1.1485792	7.6393738	17.523249	0.0611791	10.70952	5.9914646	4.7E-03	0.4504923	0.079483	6
Intercept	-0.667784	0.5643568	-3.096015	1.7604472							
TSCR	0.0635392	0.0135321	0.0053153	0.1217632							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.8432628	1.2977404	2.1667771
EC05	3.355	2.0881152	1.5865616	2.3803681
EC10	3.718	2.2316742	1.7639049	2.5056187
EC15	3.964	2.334068	1.8932242	2.5957474
EC20	4.158	2.4187877	2.0015358	2.6712808
EC25	4.326	2.4939162	2.0982177	2.739351
EC40	4.747	2.6937391	2.3551864	2.9284257
EC50	5.000	2.8215796	2.5160102	3.0589206
EC60	5.253	2.9554873	2.6775649	3.2074634
EC75	5.674	3.1922931	2.937355	3.5083793
EC80	5.842	3.291447	3.0353525	3.649801
EC85	6.036	3.4109167	3.1459277	3.8313164
EC90	6.282	3.5674165	3.280712	4.0851022
EC95	6.645	3.8126782	3.4756438	4.5125797
EC99	7.326	4.3191409	3.842137	5.4825976

Significant heterogeneity detected (p = 4.73E-03)

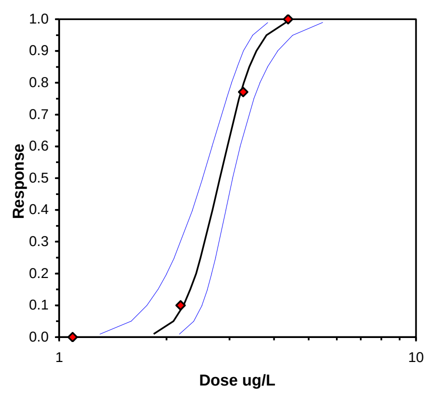

Figure 72. ToxCalc summary sheet including dose-response curve for Mill C weak black liquor spike 5 simulated effluent sample (unsettled).

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	2/9/2011	Test ID:	25128	Sample ID:	Mill D Mill Effluent
End Date:	2/11/2011	Lab ID:	NABF	Sample Type:	Bleached Kraft
Sample Dat	2/8/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill D mill-treated effluent				

Conc-%	1	2	3	4
B-Control	0.9527	0.9551	0.9516	0.9753
D-Control	0.9682	0.9706	0.9508	0.9470
1.09	0.9423	0.9253	0.9557	0.9447
2.19	0.9141	0.9257	0.9602	0.9318
4.38	0.7377	0.7320	0.8129	0.8000
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.9587	0.9995	1.3677	1.3490	1.4130	2.221	4				
D-Control	0.9591	1.0000	1.3689	1.3385	1.3984	2.221	4			27	661
1.09	0.9420	0.9821	1.3286	1.2939	1.3587	2.007	4	12.00	10.00		
2.19	0.9330	0.9727	1.3112	1.2734	1.3700	3.171	4	12.00	10.00	44	663
*4.38	0.7706	0.8035	1.0726	1.0266	1.1234	4.647	4	10.00	10.00	169	733
*8.75	0.0000	0.0000	0.0396	0.0388	0.0404	1.958	4	10.00	10.00	639	639
*17.5	0.0000	0.0000	0.0380	0.0363	0.0394	3.949	4	10.00	10.00		

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.941799	0.884	0.3248715	-0.188729
Bartlett's Test indicates unequal variances (p = 4.25E-06)					32.735325	15.086272		
The control means are not significantly different (p = 0.96)					0.0535111	2.4469118		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test	2.19	4.38	3.0971277					
Treatments vs D-Control								

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	5.4689	5.3440	5.5966
10.0%	5.5773	5.4250	5.7338
20.0%	5.6845	5.5953	5.7751
Auto-2.7%	5.4110	5.2933	5.5313

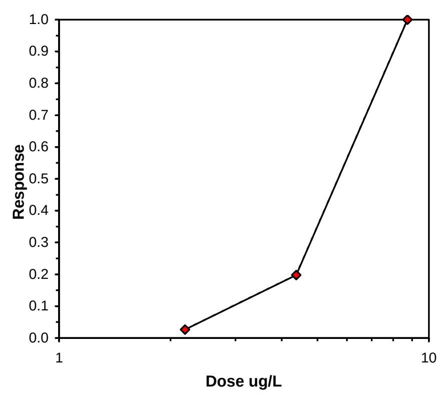

Figure 73. ToxCalc summary sheet including dose-response curve for Mill D mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal				
Start Date: 2/21/2011	Test ID: 25133		Sample ID:	Mill D Reactor Control
End Date: 2/23/2011	Lab ID:	NABF	Sample Type:	Simulated Effluent
Sample Dat 2/17/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments: Mill D Reactor Control				

Conc-%	1	2	3	4
B-Control	0.8545	0.8811	0.8671	0.9205
D-Control	0.8481	0.9071	0.8917	0.9453
1.09	0.8813	0.8514	0.8784	0.9149
2.19	0.9211	0.8807	0.8981	0.8686
4.38	0.8247	0.8833	0.8457	0.8679
8.75	0.7556	0.7862	0.8170	0.8385
17.5	0.0000	0.0000	0.0000	0.0000
35	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.8808	0.9808	1.2202	1.1795	1.2850	3.775	4				
D-Control	0.8981	1.0000	1.2505	1.1704	1.3348	5.433	4			61	583
1.09	0.8815	0.9815	1.2208	1.1750	1.2748	3.361	4	16.00	10.00		
2.19	0.8921	0.9934	1.2374	1.1998	1.2860	3.030	4	17.00	10.00		
4.38	0.8554	0.9525	1.1817	1.1388	1.2222	3.087	4	12.00	10.00	94	655
*8.75	0.7993	0.8900	1.1074	1.0536	1.1573	4.082	4	10.00	10.00	129	639
*17.5	0.0000	0.0000	0.0390	0.0378	0.0410	3.595	4	10.00	10.00	660	660
*35	0.0000	0.0000	0.0384	0.0338	0.0417	8.615	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9658684	0.896	0.1480683	0.6919059
Bartlett's Test indicates unequal variances (p = 8.77E-05)	28.159826	16.811893		
The control means are not significantly different (p = 0.49)	0.7361273	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.38	8.75	6.1907189	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	11.642	11.416	11.873
10.0%	11.857	11.516	12.209
20.0%	11.863	11.736	11.991
Auto-4.3%	11.594	11.369	11.824

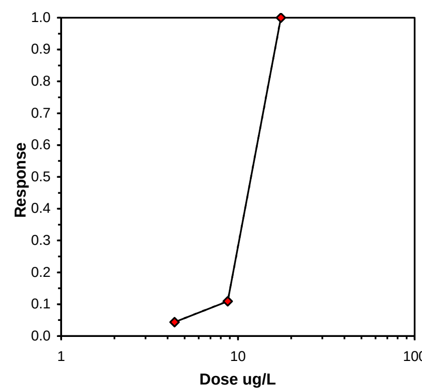

Figure 74. ToxCalc summary sheet including dose-response curve for Mill D reactor control simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal						
Start Date: 2/21/2011	Test ID:	25134	Sample ID:	Mill D WBL Spike 1		
End Date: 2/23/2011	Lab ID:	NABF	Sample Type:	Simulated Effluent		
Sample Dat 2/17/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis		
Comments: Mill D WBL spike 1						

Conc-%	1	2	3	4
B-Control	0.8599	0.8571	0.8987	0.9568
D-Control	0.9137	0.9512	0.9320	0.9091
1.09	0.9221	0.8701	0.8610	0.8561
2.19	0.8701	0.8855	0.8509	0.8511
4.38	0.7208	0.7772	0.7517	0.7803
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.8931	0.9640	1.2447	1.1832	1.3614	6.677	4				
D-Control	0.9265	1.0000	1.2980	1.2645	1.3481	2.935	4			45	599
1.09	0.8773	0.9469	1.2151	1.1817	1.2879	4.053	4	12.00	10.00	78	634
*2.19	0.8644	0.9330	1.1942	1.1744	1.2257	2.068	4	10.00	10.00	84	622
*4.38	0.7575	0.8176	1.0564	1.0141	1.0830	3.030	4	10.00	10.00	154	637
*8.75	0.0000	0.0000	0.0399	0.0378	0.0432	6.563	4	10.00	10.00	634	634
*17.5	0.0000	0.0000	0.0402	0.0388	0.0426	4.215	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9375674	0.884	0.9105699	1.2517056
Bartlett's Test indicates unequal variances (p = 1.19E-04)	25.353884	15.086272		
The control means are not significantly different (p = 0.29)	1.1677493	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%	5.6197	5.4661	5.7775
20.0%	5.7374	5.6482	5.8281
Auto-5.2%	5.4470	5.2920	5.6064

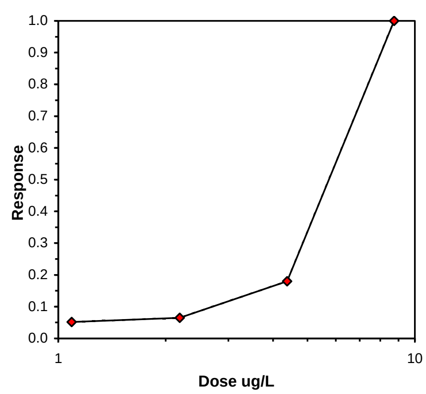

Figure 75. ToxCalc summary sheet including dose-response curve for Mill D weak black liquor spike 1 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal							
Start Date:	2/21/2011	Test ID:	25135	Sample ID:	Mill D WBL Spike 2		
End Date:	2/23/2011	Lab ID:	NABF	Sample Type:	Simulated Effluent		
Sample Date:	2/17/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis		
Comments:	Mill D WBL spike 2						

Conc-%	1	2	3	4
B-Control	0.8675	0.8456	0.8014	0.8909
D-Control	0.9127	0.8852	0.8667	0.9078
1.09	0.8608	0.8773	0.8217	0.8255
2.19	0.8075	0.8061	0.7891	0.8182
4.38	0.6605	0.6645	0.6497	0.5625
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.8514	0.9533	1.1771	1.1089	1.2342	4.510	4				
D-Control	0.8931	1.0000	1.2388	1.1970	1.2709	2.761	4			58	539
1.09	0.8463	0.9476	1.1690	1.1349	1.2129	3.246	4	11.00	10.00	91	599
*2.19	0.8052	0.9016	1.1138	1.0937	1.1303	1.357	4	10.00	10.00	122	627
*4.38	0.6343	0.7102	0.9218	0.8481	0.9530	5.382	4	10.00	10.00	225	618
*8.75	0.0000	0.0000	0.0395	0.0374	0.0410	3.982	4	10.00	10.00	645	645
*17.5	0.0000	0.0000	0.0415	0.0408	0.0423	1.429	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9297811	0.884	-0.922649	1.4944803
Bartlett's Test indicates unequal variances (p = 1.35E-06)	35.243931	15.086272		
The control means are not significantly different (p = 0.10)	1.95585	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	4.8840	4.7203	5.0533
10.0%	5.0770	4.9133	5.2462
20.0%	5.2934	5.0524	5.5459
Auto-5.0%	4.8822	4.7186	5.0514

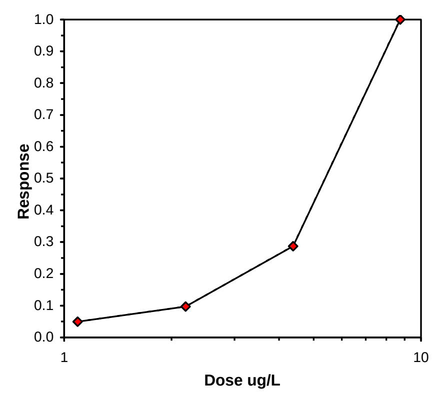

Figure 76. ToxCalc summary sheet including dose-response curve for Mill D weak black liquor spike 2 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	2/21/2011	Test ID:	25136	Sample ID:	Mill D WBL Spike 3
End Date:	2/23/2011	Lab ID:	NABF	Sample Type:	Simulated Effluent
Sample Dat	2/17/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill D WBL spike 3				

Conc-%	1	2	3	4
B-Control	0.8489	0.8344	0.8367	0.8993
D-Control	0.9384	0.8652	0.8562	0.8705
1.09	0.8500	0.8947	0.8733	0.8506
2.19	0.8400	0.8077	0.8256	0.7843
4.38	0.2356	0.2828	0.4088	0.2878
8.75	0.0000	0.0000	0.0000	0.0000
17.5	0.0000	0.0000	0.0000	

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.8549	0.9686	1.1815	1.1517	1.2479	3.819	4					
D-Control	0.8826	1.0000	1.2248	1.1819	1.3199	5.221	4				68	579
1.09	0.8672	0.9825	1.1986	1.1731	1.2404	2.671	4	0.796	2.567	0.0847	80	597
*2.19	0.8144	0.9227	1.1260	1.0878	1.1593	2.735	4	2.997	2.567	0.0847	117	631
*4.38	0.3037	0.3441	0.5818	0.5068	0.6936	13.612	4	19.489	2.567	0.0847	417	595
*8.75	0.0000	0.0000	0.0426	0.0420	0.0435	1.667	4	35.833	2.567	0.0847	552	552
*17.5	0.0000	0.0000	0.0397	0.0386	0.0417	4.264	3	33.255	2.567	0.0915		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8796091	0.881	1.2477988	2.4841122
Bartlett's Test indicates unequal variances (p = 3.17E-06)	33.372753	15.086272		
The control means are not significantly different (p = 0.31)	1.1069226	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	1.09	2.19	1.5450243		0.0644635	0.072839	1.1784406	0.0021771	4.1E-18	5, 17
Treatments vs D-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.2633669	0.5493289	6.1866823	8.3400516	0.1174439	4.0704977	5.9914646	0.13	0.5806793	0.1376772	10
Intercept	0.7823133	0.3532266	0.0899892	1.4746375							
TSCR	0.1323967	0.0093957	0.1139811	0.1508123							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.8213534	1.5522711	2.0531728
EC05	3.355	2.2605829	1.9975551	2.4817771
EC10	3.718	2.5365233	2.2840838	2.7468432
EC15	3.964	2.741486	2.4995903	2.9423062
EC20	4.158	2.9161323	2.6846506	3.108254
EC25	4.326	3.0748039	2.8536424	3.2588102
EC40	4.747	3.513979	3.3230564	3.6769673
EC50	5.000	3.8078451	3.6351803	3.961221
EC60	5.253	4.1262867	3.9668782	4.2779307
EC75	5.674	4.7156453	4.5496345	4.90089
EC80	5.842	4.9722314	4.7898166	5.1878441
EC85	6.036	5.2889874	5.0774961	5.5526948
EC90	6.282	5.7163616	5.4544716	6.0591462
EC95	6.645	6.414135	6.0520929	6.9110322
EC99	7.326	7.9609405	7.3312127	8.8744468

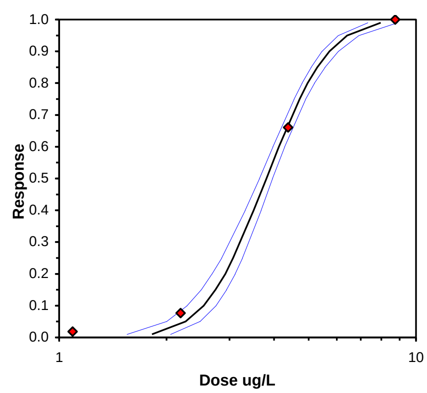

Figure 77. ToxCalc summary sheet including dose-response curve for Mill D weak black liquor spike 3 simulated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	2/21/2011	Test ID:	25138	Sample ID:	Mill D WBL Spike 5
End Date:	2/23/2011	Lab ID:	NABF	Sample Type:	Simulated Effluent
Sample Dat	2/17/2011	Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilus galloprovincialis
Comments:	Mill D WBL spike 5				

Conc-%	1	2	3	4
B-Control	0.8750	0.9295	0.8235	0.8611
D-Control	0.8993	0.8623	0.8933	0.8661
1.09	0.8523	0.8037	0.7917	0.8758
2.19	0.4260	0.3563	0.3221	0.3913
3.28	0.0280	0.0268	0.0417	0.0313
4.38	0.0000	0.0000	0.0000	0.0000
8.75	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
B-Control	0.8723	0.9909	1.2094	1.1373	1.3020	5.690	4				
D-Control	0.8803	1.0000	1.2182	1.1906	1.2478	2.379	4			70	583
1.09	0.8309	0.9439	1.1489	1.0968	1.2106	4.675	4	12.00	10.00	104	617
*2.19	0.3739	0.4248	0.6575	0.6036	0.7112	7.049	4	10.00	10.00	413	662
*3.28	0.0319	0.0363	0.1790	0.1646	0.2056	10.380	4	10.00	10.00	554	572
*4.38	0.0000	0.0000	0.0401	0.0379	0.0418	4.222	4	10.00	10.00	624	624
*8.75	0.0000	0.0000	0.0402	0.0388	0.0426	4.215	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9610446	0.884	0.1589783	0.3001926
Bartlett's Test indicates unequal variances (p = 1.40E-05)	30.116537	15.086272		
The control means are not significantly different (p = 0.82)	0.2353453	2.4469118		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1.09	2.19	1.5450243	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.462409	0.6218092	8.243663	10.681155	0.1200686	3.2989215	5.9914646	0.19	0.3238903	0.1056813	6
Intercept	1.9352171	0.2427926	1.4593435	2.4110907							
TSCR	0.1431215	0.010329	0.1228766	0.1633664							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	1.1968475	1.074678	1.3014875
EC05	3.355	1.4127328	1.2988198	1.5088349
EC10	3.718	1.5433139	1.4364776	1.6329603
EC15	3.964	1.6381689	1.537253	1.7227091
EC20	4.158	1.7176975	1.6221413	1.797791
EC25	4.326	1.788996	1.6984679	1.8650556
EC40	4.747	1.9820577	1.9054444	2.0477091
EC50	5.000	2.1080958	2.039823	2.1683205
EC60	5.253	2.2421487	2.1806809	2.2991921
EC75	5.674	2.4841129	2.4248736	2.5467932
EC80	5.842	2.5872238	2.5242057	2.6575369
EC85	6.036	2.7128265	2.6419018	2.7961341
EC90	6.282	2.8795618	2.7939387	2.9849739
EC95	6.645	3.1457243	3.0302126	3.294338
EC99	7.326	3.7131452	3.5193593	3.9742029

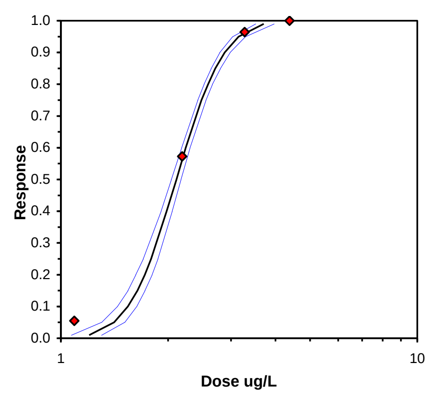

Figure 78. ToxCalc summary sheet including dose-response curve for Mill D weak black liquor spike 5 simulated effluent sample.

APPENDIX J

ToxCalc Summary Sheets for *Mytilus galloprovincialis* Reference Toxicant Tests

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/1/2010	Test ID:	A-RT-ME	Sample ID:	A-RT-ME
End Date:	9/3/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis

Comments: Mill A reference toxicant test for mill-treated using copper chloride

Conc-ug/L	1	2	3	4
D-Control	0.9689	0.9549	0.9781	0.9318
3	0.9433	0.9533	0.9620	0.9708
4.5	0.9534	0.9589	0.9133	0.9429
6.6	0.9355	0.9689	0.9562	0.9057
9.6	0.7961	0.8759	0.7292	0.7589
13.8	0.1000	0.0137	0.0942	0.0299
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	0.9584	1.0000	1.3698	1.3066	1.4223	3.646	4				26	607
3	0.9573	0.9989	1.3642	1.3303	1.3991	2.157	4	0.131	2.451	0.1047		
4.5	0.9421	0.9830	1.3303	1.2720	1.3667	3.143	4	0.926	2.451	0.1047		
6.6	0.9416	0.9824	1.3315	1.2586	1.3936	4.399	4	0.896	2.451	0.1047	36	612
*9.6	0.7900	0.8242	1.0985	1.0235	1.2107	7.419	4	6.354	2.451	0.1047	122	582
*13.8	0.0594	0.0620	0.2312	0.1173	0.3218	43.978	4	26.662	2.451	0.1047	534	568
*20.4	0.0000	0.0000	0.0507	0.0457	0.0563	8.699	4	30.887	2.451	0.1047	396	396

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98618	0.896	0.04336	-0.109
Bartlett's Test indicates equal variances (p = 0.01)	16.5273	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.6	9.6	7.9599		0.05071	0.05281	1.32219	0.00365	4.4E-20	6, 21
Treatments vs D-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits	
Slope	15.8197	0.71058	14.427	17.2125
Intercept	-11.503	0.74633	-12.966	-10.04
TSCR	0.05073	0.00631	0.03837	0.0631

Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
0.04283	0.79614	5.99146	0.67	1.0432	0.06321	4

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	7.87319	7.57762	8.13487
EC05	3.355	8.69419	8.43781	8.92245
EC10	3.718	9.16631	8.93251	9.37625
EC15	3.964	9.49924	9.28054	9.69752
EC20	4.158	9.77245	9.56514	9.9624
EC25	4.326	10.0131	9.81474	10.197
EC40	4.747	10.6461	10.4641	10.8219
EC50	5.000	11.046	10.8673	11.2245
EC60	5.253	11.4609	11.2786	11.6497
EC75	5.674	12.1854	11.9803	12.4097
EC80	5.842	12.4855	12.2656	12.7301
EC85	6.036	12.8446	12.6038	13.1172
EC90	6.282	13.3111	13.0388	13.6249
EC95	6.645	14.0339	13.7055	14.4201
EC99	7.326	15.4974	15.0364	16.0527

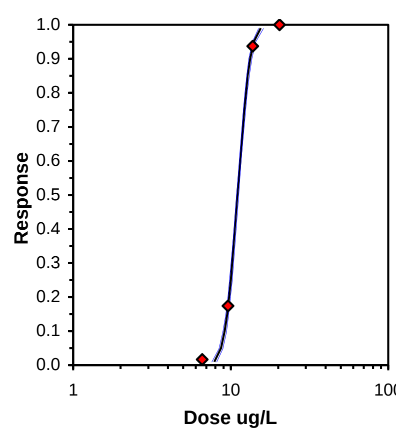

Figure 79. ToxCalc summary sheet including dose-response curve for Mill A reference toxicant test using copper chloride run alongside mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	9/9/2010	Test ID:	A-RT-SE	Sample ID:	A-RT-SE
End Date:	9/11/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill A reference toxicant test for simulated effluents using copper ch				

Conc-ug/L	1	2	3	4
D-Control	0.9281	0.9398	0.9565	0.9103
3	0.9194	0.9133	0.8442	0.8906
4.5	0.8758	0.9357	0.9403	0.8571
6.6	0.8976	0.8805	0.8571	0.8692
9.6	0.5390	0.5000	0.5734	0.5667
13.8	0.0000	0.3514	0.0373	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9337	1.0000	1.3125	1.2667	1.3607	3.018	4			38	569
3	0.8919	0.9552	1.2384	1.1650	1.2829	4.302	4	12.00	10.00		
4.5	0.9022	0.9663	1.2581	1.1832	1.3240	5.691	4	15.00	10.00	57	575
*6.6	0.8761	0.9383	1.2117	1.1832	1.2451	2.174	4	10.00	10.00	72	588
*9.6	0.5448	0.5834	0.8303	0.7854	0.8591	4.032	4	10.00	10.00	257	567
*13.8	0.0972	0.1041	0.2282	0.0400	0.6345	122.742	4	10.00	10.00	535	593
*20.4	0.0000	0.0000	0.0444	0.0418	0.0473	5.264	4	10.00	10.00	511	511

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.74208	0.896	2.14901	10.4487
Bartlett's Test indicates unequal variances (p = 1.71E-07)	42.1536	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.5	6.6	5.44977	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.54766	0.47624	8.61422	10.4811	0.06678	2.69225	7.81473	0.44	1.00756	0.10474	5
Intercept	-4.6198	0.49559	-5.5912	-3.6485							
TSCR	0.08438	0.00771	0.06927	0.0995							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.80633	5.39104	6.17552
EC05	3.355	6.84352	6.46087	7.18106
EC10	3.718	7.47018	7.11315	7.78493
EC15	3.964	7.92509	7.58842	8.22256
EC20	4.158	8.30631	7.98727	8.5894
EC25	4.326	8.64795	8.34467	8.91866
EC40	4.747	9.57244	9.30774	9.81601
EC50	5.000	10.1755	9.92828	10.4109
EC60	5.253	10.8167	10.5763	11.0563
EC75	5.674	11.973	11.7073	12.2619
EC80	5.842	12.4654	12.1749	12.7907
EC85	6.036	13.0651	12.7356	13.4445
EC90	6.282	13.8607	13.468	14.3252
EC95	6.645	15.1299	14.6173	15.7534
EC99	7.326	17.8326	17.0156	18.8594

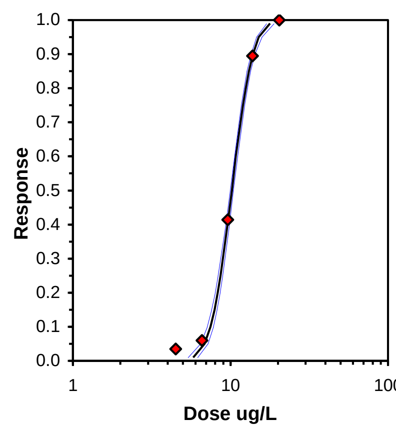

Figure 80. ToxCalc summary sheet including dose-response curve for Mill A reference toxicant test using copper chloride run alongside simulated effluent samples.

Bivalve Larval Survival and Development Test-Proportion Normal						
Start Date:	10/22/2010	Test ID:	B-RT-ME		Sample ID:	B-RT-ME
End Date:	10/24/2011	Lab ID:	NABF		Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast		Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill B reference toxicant test for mill-treated effluent using copper					

Conc-ug/L	1	2	3	4
D-Control	0.9749	0.9589	0.9822	0.9725
3	0.9662	0.9441	0.9492	0.9694
4.5	0.9412	0.9326	0.9719	0.9497
6.6	0.7310	0.7687	0.8133	0.8099
9.6	0.4831	0.4604	0.5206	0.5506
13.8	0.0000	0.0000	0.0000	0.0086
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	0.9721	1.0000	1.4049	1.3667	1.4372	2.075	4				19	696
3	0.9572	0.9847	1.3641	1.3322	1.3949	2.267	4	1.713	2.451	0.0584	31	720
*4.5	0.9489	0.9760	1.3453	1.3083	1.4024	3.039	4	2.507	2.451	0.0584	36	700
*6.6	0.7807	0.8031	1.0845	1.0255	1.1240	4.296	4	13.459	2.451	0.0584	132	597
*9.6	0.5037	0.5181	0.7891	0.7458	0.8361	5.067	4	25.868	2.451	0.0584	331	669
*13.8	0.0022	0.0022	0.0541	0.0380	0.0930	48.359	4	56.743	2.451	0.0584	568	569
*20.4	0.0000	0.0000	0.0420	0.0397	0.0429	3.718	4	57.253	2.451	0.0584	569	569

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97303		0.896		0.10498	-0.6581
Bartlett's Test indicates equal variances (p = 0.02)					15.2287		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	3	4.5	3.67423		0.02218	0.0228	1.44	0.00113	9.2E-26	6, 21
Treatments vs D-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	8.8233	1.69906	4.10595	13.5406	0.0273	87.6809	9.48773	4.1E-18	0.95578	0.11334	7
Intercept	-3.4331	1.66698	-8.0614	1.19517							
TSCR	0.04277	0.0213	-0.0164	0.10191							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	4.92174	2.23337	6.36206
EC05	3.355	5.87972	3.24595	7.20316
EC10	3.718	6.46446	3.95157	7.71629
EC15	3.964	6.89148	4.50412	8.09782
EC20	4.158	7.2509	4.98988	8.42796
EC25	4.326	7.57415	5.43945	8.73583
EC40	4.747	8.45405	6.68826	9.66535
EC50	5.000	9.03188	7.48251	10.3967
EC60	5.253	9.64921	8.25787	11.3367
EC75	5.674	10.7702	9.41809	13.5227
EC80	5.842	11.2503	9.83416	14.6331
EC85	6.036	11.8371	10.2984	16.1115
EC90	6.282	12.619	10.8646	18.2682
EC95	6.645	13.8739	11.6937	22.1345
EC99	7.326	16.5744	13.2965	32.0325

Significant heterogeneity detected (p = 4.09E-18)

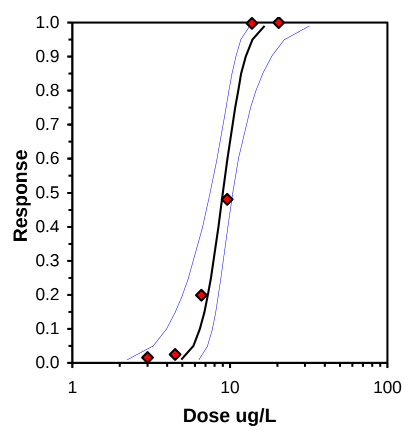

Figure 81. ToxCalc summary sheet including dose-response curve for Mill B reference toxicant test using copper chloride run alongside mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/1/2010	Test ID:	B-RT-SE	Sample ID:	B-RT-SE
End Date:	11/3/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill B reference toxicant test for simulated effluents using copper ch				

Conc-ug/L	1	2	3	4
D-Control	0.9379	0.9191	0.9591	0.9124
3	0.9295	0.9006	0.9225	0.9141
4.5	0.8758	0.8299	0.8686	0.8608
6.6	0.9349	0.9521	0.9375	0.9548
9.6	0.0633	0.0503	0.0190	0.0196
13.8	0.0000	0.0000	0.0000	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9321	1.0000	1.3097	1.2703	1.3671	3.321	4			42	626
3	0.9167	0.9834	1.2786	1.2501	1.3020	1.745	4	15.00	10.00	52	622
*4.5	0.8588	0.9213	1.1862	1.1457	1.2107	2.399	4	10.00	10.00	84	595
6.6	0.9448	1.0136	1.3344	1.3128	1.3567	1.661	4	20.00	10.00	36	651
*9.6	0.0380	0.0408	0.1898	0.1382	0.2543	31.280	4	10.00	10.00	623	648
*13.8	0.0000	0.0000	0.0458	0.0423	0.0505	7.534	4	10.00	10.00	483	483
*20.4	0.0000	0.0000	0.0457	0.0434	0.0493	5.517	4	10.00	10.00		

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.96272	0.896	0.19312	0.2521
Bartlett's Test indicates unequal variances (p = 3.23E-04)					25.1301	16.8119		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**				
Steel's Many-One Rank Test	3	4.5	3.67423					
Treatments vs D-Control								

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	7.9728	7.9388	8.0068
10.0%	7.9728	7.9388	8.0068
20.0%	7.9728	7.9388	8.0068
Auto-1.8%	7.9168	7.8375	7.9969

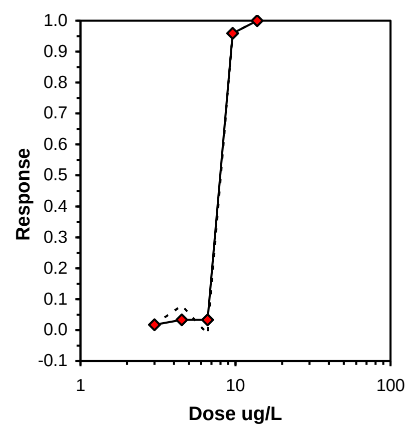

Figure 82. ToxCalc summary sheet including dose-response curve for Mill B reference toxicant test using copper chloride run alongside simulated effluent samples.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/4/2010	Test ID:	C-RT-ME	Sample ID:	C-RT-ME
End Date:	11/6/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill C reference toxicant test for mill-treated effluent using copper				

Conc-ug/L	1	2	3	4
D-Control	0.8916	0.9221	0.9075	0.8907
3	0.8768	0.8947	0.9139	0.9060
4.5	0.8870	0.8867	0.8125	0.8481
6.6	0.8160	0.7884	0.8528	0.8736
9.6	0.1637	0.2313	0.2222	0.2404
13.8	0.0000	0.0000	0.0000	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9030	1.0000	1.2547	1.2339	1.2879	2.040	4			66	676
3	0.8979	0.9944	1.2462	1.2122	1.2730	2.113	4	17.00	10.00	62	609
*4.5	0.8586	0.9508	1.1872	1.1230	1.2280	4.264	4	10.00	10.00	94	661
*6.6	0.8327	0.9222	1.1511	1.0928	1.2074	4.430	4	10.00	10.00	117	697
*9.6	0.2144	0.2375	0.4804	0.4166	0.5125	9.043	4	10.00	10.00	559	712
*13.8	0.0000	0.0000	0.0431	0.0413	0.0444	3.087	4	10.00	10.00	539	539
*20.4	0.0000	0.0000	0.0440	0.0414	0.0493	8.181	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95169	0.896	-0.5129	0.13787
Bartlett's Test indicates unequal variances (p = 1.31E-04)	27.2272	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	3	4.5	3.67423	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.227	0.91744	12.4288	16.0252	0.09763	6.75421	7.81473	0.08	0.93223	0.07029	7
Intercept	-8.2628	0.88656	-10	-6.5252							
TSCR	0.11506	0.00716	0.10102	0.1291							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.87101	5.47682	6.19948
EC05	3.355	6.55563	6.2095	6.84203
EC10	3.718	6.95265	6.63808	7.21274
EC15	3.964	7.23402	6.94296	7.47508
EC20	4.158	7.46574	7.19447	7.69117
EC25	4.326	7.67044	7.41675	7.88232
EC40	4.747	8.21149	8.00256	8.3908
EC50	5.000	8.55518	8.37076	8.71887
EC60	5.253	8.91326	8.74755	9.06843
EC75	5.674	9.54198	9.38236	9.71116
EC80	5.842	9.80361	9.63526	9.99069
EC85	6.036	10.1176	9.93143	10.3342
EC90	6.282	10.5271	10.3084	10.7924
EC95	6.645	11.1646	10.8817	11.5215
EC99	7.326	12.4665	12.024	13.0472

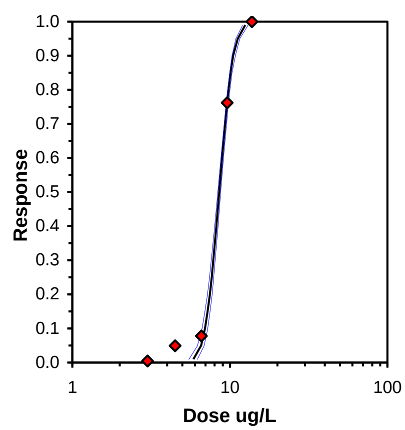

Figure 83. ToxCalc summary sheet including dose-response curve for Mill C reference toxicant test using copper chloride run alongside mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	11/17/2010	Test ID:	C-RT-SE	Sample ID:	C-RT-SE
End Date:	11/19/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill C reference toxicant test for simulated effluents using copper ch				

Conc-ug/L	1	2	3	4
D-Control	0.9518	0.9231	0.9581	0.9327
3	0.9689	0.9347	0.9434	0.9482
4.5	0.9211	0.9404	0.9246	0.9624
6.6	0.9220	0.9055	0.9297	0.8688
9.6	0.0000	0.9307	0.0000	0.0000
13.8	0.0000	0.0000	0.0000	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9414	1.0000	1.3281	1.2898	1.3647	2.628	4			49	846
3	0.9488	1.0078	1.3444	1.3123	1.3936	2.593	4	20.00	10.00		
4.5	0.9371	0.9954	1.3196	1.2860	1.3756	3.096	4	17.00	10.00		
6.6	0.9065	0.9629	1.2622	1.2001	1.3025	3.587	4	11.00	10.00	78	825
9.6	0.2327	0.2472	0.3532	0.0350	1.3044	179.541	4	11.00	10.00	590	778
*13.8	0.0000	0.0000	0.0354	0.0346	0.0368	2.858	4	10.00	10.00	800	800
*20.4	0.0000	0.0000	0.0369	0.0363	0.0381	2.213	4	10.00	10.00	734	734

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.53915	0.896	3.15815	15.7231
Bartlett's Test indicates unequal variances (p = 6.32E-18)	93.2738	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	9.6	13.8	11.51	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	15.3864	1.00433	13.4179	17.3548	0.05792	0.95656	5.99146	0.62	0.93904	0.06499	4
Intercept	-9.4483	0.9733	-11.356	-7.5407							
TSCR	0.05998	0.0079	0.04449	0.07546							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	6.13537	5.7444	6.45854
EC05	3.355	6.79411	6.45353	7.07359
EC10	3.718	7.17372	6.86566	7.42617
EC15	3.964	7.44175	7.15779	7.67467
EC20	4.158	7.6619	7.3982	7.87874
EC25	4.326	7.85596	7.61028	8.05877
EC40	4.747	8.36701	8.16778	8.53529
EC50	5.000	8.69032	8.51742	8.84084
EC60	5.253	9.02613	8.8747	9.1649
EC75	5.674	9.61331	9.47399	9.75891
EC80	5.842	9.85678	9.71105	10.0174
EC85	6.036	10.1484	9.98728	10.3352
EC90	6.282	10.5275	10.3371	10.7586
EC95	6.645	11.1158	10.8666	11.4307
EC99	7.326	12.3092	11.9146	12.8275

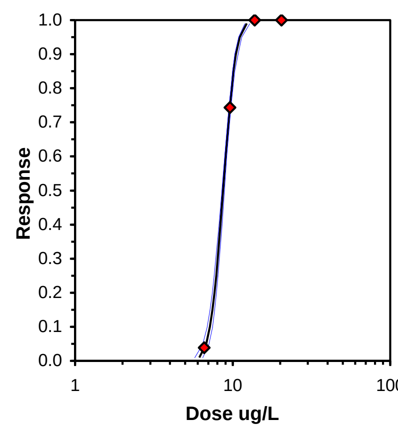

Figure 84. ToxCalc summary sheet including dose-response curve for Mill C reference toxicant test using copper chloride run alongside simulated effluent samples.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	2/9/2010	Test ID:	D-RT-ME	Sample ID:	D-RT-ME
End Date:	2/11/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill D reference toxicant test for mill-treated effluent using copper				

Conc-ug/L	1	2	3	4
D-Control	0.9219	0.9639	0.9252	0.9325
3	0.9110	0.9337	0.9461	0.9304
4.5	0.9456	0.9133	0.9448	0.9382
6.6	0.8815	0.8947	0.8795	0.8868
9.6	0.2313	0.2287	0.3015	0.2000
13.8	0.0000	0.0000	0.0000	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9359	1.0000	1.3172	1.2875	1.3795	3.219	4			48	735
3	0.9303	0.9940	1.3046	1.2678	1.3365	2.172	4	18.00	10.00		
4.5	0.9355	0.9996	1.3151	1.2719	1.3353	2.255	4	19.00	10.00	43	661
*6.6	0.8856	0.9463	1.2259	1.2163	1.2404	0.878	4	10.00	10.00	74	650
*9.6	0.2404	0.2568	0.5113	0.4636	0.5813	9.727	4	10.00	10.00	535	707
*13.8	0.0000	0.0000	0.0388	0.0360	0.0403	5.018	4	10.00	10.00	669	669
*20.4	0.0000	0.0000	0.0448	0.0410	0.0495	8.545	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91009	0.896	0.79772	1.89395
Bartlett's Test indicates unequal variances (p = 2.07E-04)	26.1736	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.5	6.6	5.44977	
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	14.4775	0.87088	12.7706	16.1844	0.06531	1.36307	5.99146	0.51	0.93669	0.06907	4
Intercept	-8.561	0.84251	-10.212	-6.9096							
TSCR	0.06652	0.00658	0.05363	0.0794							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.97042	5.60848	6.27549
EC05	3.355	6.65392	6.3374	6.91917
EC10	3.718	7.04972	6.76266	7.29027
EC15	3.964	7.32998	7.06471	7.55277
EC20	4.158	7.56065	7.3136	7.76893
EC25	4.326	7.76431	7.53334	7.9601
EC40	4.747	8.30219	8.11153	8.46841
EC50	5.000	8.64355	8.4743	8.79617
EC60	5.253	8.99894	8.84517	9.14499
EC75	5.674	9.62234	9.47059	9.78388
EC80	5.842	9.88155	9.72052	10.0603
EC85	6.036	10.1925	10.0139	10.399
EC90	6.282	10.5977	10.388	10.8492
EC95	6.645	11.2281	10.9581	11.5636
EC99	7.326	12.5135	12.0949	13.0527

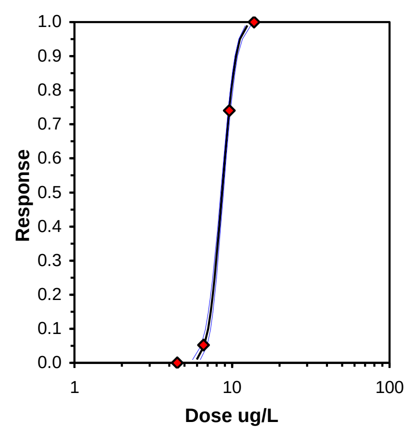

Figure 85. ToxCalc summary sheet including dose-response curve for Mill D reference toxicant test using copper chloride run alongside mill-treated effluent sample.

Bivalve Larval Survival and Development Test-Proportion Normal					
Start Date:	2/21/2010	Test ID:	D-RT-SE	Sample ID:	D-RT-SE
End Date:	2/23/2011	Lab ID:	NABF	Sample Type:	Reference Toxicant
Sample Date:		Protocol:	EPAW 95-EPA West Coast	Test Species:	MG-Mytilis galloprovincialis
Comments:	Mill D reference toxicant test for simulated effluents using copper ch				

Conc-ug/L	1	2	3	4
D-Control	0.9161	0.8889	0.9236	0.9068
3	0.8846	0.8421	0.8521	0.8526
4.5	0.8765	0.8908	0.9156	0.9040
6.6	0.7284	0.8583	0.7808	0.7984
9.6	0.2919	0.3386	0.3529	0.2857
13.8	0.0000	0.0000	0.0000	0.0000
20.4	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Resp	Number
D-Control	0.9089	1.0000	1.2648	1.2310	1.2908	2.033	4			56	610
*3	0.8578	0.9439	1.1848	1.1622	1.2242	2.290	4	10.00	10.00	90	633
4.5	0.8967	0.9866	1.2444	1.2118	1.2760	2.225	4	14.00	10.00	63	612
*6.6	0.7915	0.8709	1.0991	1.0226	1.1849	6.102	4	10.00	10.00	119	557
*9.6	0.3173	0.3491	0.5980	0.5639	0.6361	6.018	4	10.00	10.00	418	612
*13.8	0.0000	0.0000	0.0422	0.0385	0.0449	6.521	4	10.00	10.00	568	568
*20.4	0.0000	0.0000	0.0416	0.0385	0.0437	5.453	4	10.00	10.00		

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9484	0.896	0.32714	2.50452
Bartlett's Test indicates unequal variances (p = 1.23E-04)	27.3725	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<3	3		
Treatments vs D-Control				

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	11.6719	1.58293	6.63426	16.7095	0.0918	16.8872	7.81473	7.5E-04	0.94336	0.08568	8
Intercept	-6.0107	1.54673	-10.933	-1.0884							
TSCR	0.11724	0.01751	0.0615	0.17298							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	5.54684	3.62602	6.6163
EC05	3.355	6.34502	4.57595	7.29579
EC10	3.718	6.81647	5.17454	7.69459
EC15	3.964	7.15418	5.61787	7.98181
EC20	4.158	7.43449	5.99339	8.22289
EC25	4.326	7.68369	6.33162	8.44067
EC40	4.747	8.34934	7.24186	9.05159
EC50	5.000	8.77723	7.81523	9.48381
EC60	5.253	9.22706	8.38597	9.99358
EC75	5.674	10.0264	9.2701	11.0885
EC80	5.842	10.3625	9.59082	11.6224
EC85	6.036	10.7685	9.94721	12.3161
EC90	6.282	11.302	10.3782	13.2945
EC95	6.645	12.1418	11.0021	14.9561
EC99	7.326	13.889	12.1882	18.7873

Significant heterogeneity detected (p = 7.45E-04)

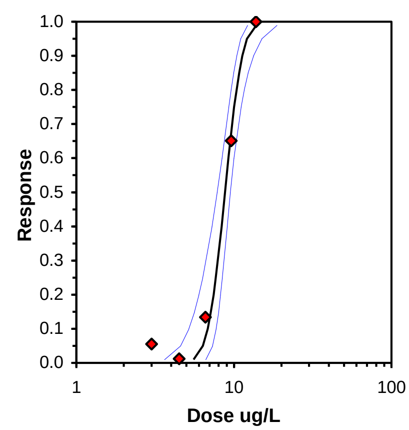

Figure 86. ToxCalc summary sheet including dose-response curve for Mill D reference toxicant test using copper chloride run alongside simulated effluent samples.

APPENDIX K

***Mytilus galloprovincialis* Reference Toxicant Control Chart**

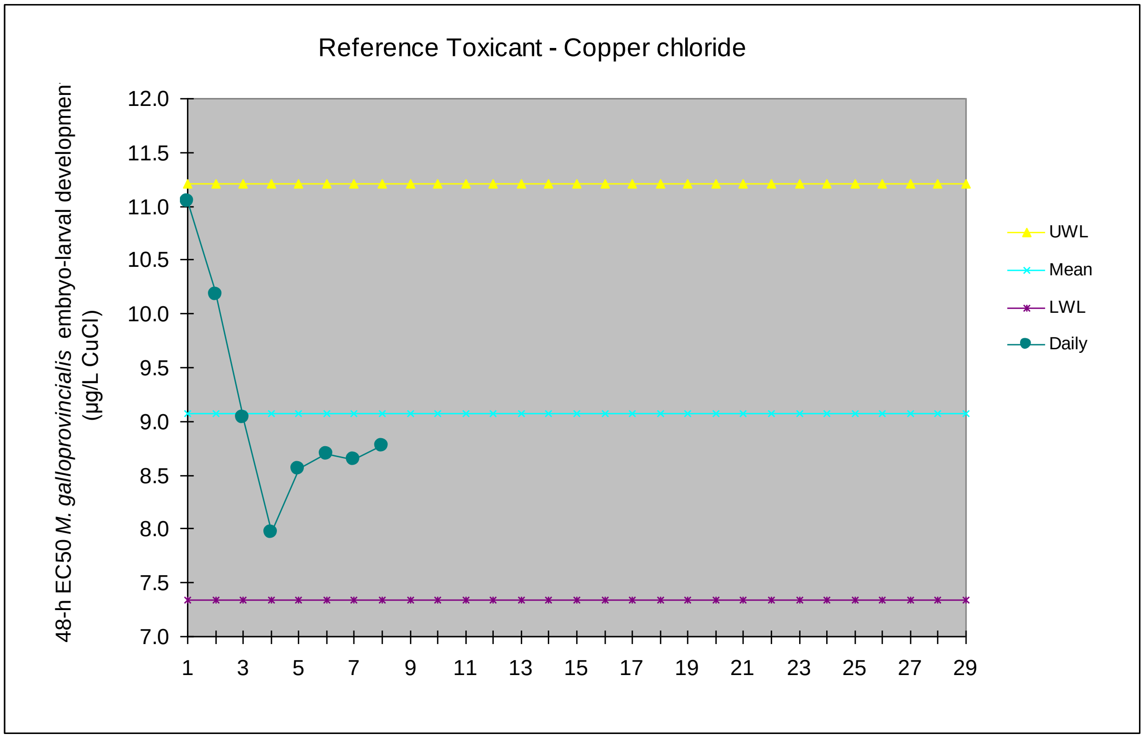

Figure 87. Control chart for 48-h EC50 *Mytilus galloprovincialis* embryo-larval development toxicity tests with reference toxicant (CuCl). Methods for control charting followed guidance provided by Environment Canada (2005).

APPENDIX L

Southern Aquatic Biology Facility Summary Sheets for *Ceriodaphnia dubia* Toxicity Tests

Black Liquor Spiking Study Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/08/10 – 09/14/10
SAMPLE ID: #24667 A-EFF-BLSS

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	32	40	39	36	39	37.2	3.3
6.25%	100	46	41	38	42	40	41.4	3.0
12.5%	100	40	47	43	40	46	43.2	3.3
25%	100	39	45	47	40	46	43.4	3.6
50%	100	40	40	45	43	41	41.8	2.2
100%	100	32	34	38	31	30	33.0	3.2
NaCl (1500mg/L)	80	28	22	19	23	4	19.2*[c]S[d]	9.1

Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%
Reproduction	**NOEC**= 100%	**LOEC**= >100%	**CV**= >100%	**IC25**= >100%

[a] standard deviation
[b] confidence intervals
[c] significant difference from the control (Dunnett's test p?0.05)
[d] significant difference from the control (Steel's test p?0.05)

Figure 88. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill A mill effluent.

Black Liquor Spiking Study Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/08/10 – 09/14/10
SAMPLE ID: #24667 A-EFF-BLSS

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.6	8.8	291	5	25.5	(0.3)	64	78
6.25%	7.8	8.7	399	5	25.5	(0.2)		
12.5%	7.8	9.8	506	5	25.5	(0.1)		
25%	7.8	9.4	704	5	25.5	(0.1)		
50%	7.9	9.1	1134	5	25.5	(0.1)		
100%	7.9	8.8	1934	5	25.5	(0.2)	348	92
NaCl (1500mg/L)	7.8	9.3	3190	5	25.5	(0.1)	60	80

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.9	7.3	330	6	25.5	(0.3)
6.25%	8.0	7.1	405	6	25.4	(0.2)
12.5%	8.1	7.0	513	6	25.4	(0.2)
25%	8.2	6.9	698	6	25.4	(0.2)
50%	8.4	6.7	1136	6	25.3	(0.2)
100%	8.6	6.7	1919	6	25.3	(0.3)
NaCl (1500mg/L)	8.0	6.9	3180	6	25.2	(0.3)

[a] standard deviation

Figure 89. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill A mill effluent.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24668 A-RWW-CTRL

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	33	27	36	33	36	33.0	3.7
12.5%	100	42	42	44	43	37	41.6	2.7
25%	100	41	45	40	38	41	41.0	2.6
50%	100	43	43	35	46	37	40.8	4.6
100%	100	38	34	34	32	35	34.6	2.2

Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%
Reproduction	**NOEC**= 100%	**LOEC**= >100%	**CV**= >100%	**IC25**= >100%

[a] standard deviation

Figure 90. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill A reactor control.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24668 A-RWW-CTRL

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.5	7.7	300	5	25.5	(0.1)	54	82
12.5%	7.6	7.5	373	5	25.4	(0.2)		
25%	7.6	7.4	446	5	25.4	(0.1)		
50%	7.7	7.5	555	5	25.4	(0.1)		
100%	7.8	7.5	904	5	25.4	(0.1)	152	50

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.7	7.3	311	5	25.5	(0.2)
12.5%	7.9	7.1	424	5	25.4	(0.1)
25%	7.8	7.0	454	5	25.3	(0.2)
50%	7.9	7.0	601	5	25.3	(0.2)
100%	8.1	7.1	892	5	25.3	(0.2)

[a] standard deviation

Figure 91. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill A reactor control.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24670 A-RWBL-2

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	33	27	36	33	36	33.0	3.7
12.5%	100	42	36	36	40	40	38.8	2.7
25%	100	34	45	43	38	40	40.0	4.3
50%	100	32	32	35	35	43	35.4	4.5
100%	100	0	2	2	8	4	3.2*[b]S[c]	3.0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 61.6%	(57.6-64.2)

[a] standard deviation
[b] significant difference from the control (Dunnett's test p≤0.05)
[c] significant difference from the control (Steel's test p≤0.05)

Figure 92. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill A weak black liquor spike 2.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24670 A-RWBL-2

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.5	7.7	300	5	25.5	(0.1)	54	82
12.5%	7.6	7.6	395	5	25.4	(0.2)		
25%	7.5	7.5	488	5	25.4	(0.1)		
50%	7.6	7.5	673	5	25.4	(0.1)		
100%	7.8	7.4	1065	5	25.4	(0.1)	170	52

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.7	7.3	311	5	25.5	(0.2)
12.5%	7.7	7.3	407	5	25.4	(0.1)
25%	7.9	7.3	498	5	25.3	(0.2)
50%	8.0	7.1	682	5	25.3	(0.2)
100%	8.1	6.8	1046	5	25.3	(0.2)

[a] standard deviation

Figure 93. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill A weak black liquor spike 2.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24673 A-RWBL-5

	%SURVIVAL	**YOUNG/ADULT REPLICATES** 1	2	3	4	5	**AVERAGE REPRODUCTION**	**SD**[a]
CONTROL	100	33	27	36	33	36	33.0	3.7
12.5%	100	30	32	24	38	35	31.8	5.3
25%	100	8	13	11	10	13	11.0*[b]S[c]	2.1
50%	60	0	0	0	0	0	0.0*S	0.0
100%	60	0	0	0	0	0	0.0*S	0.0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= 35.4	(31.9-56.3)
Reproduction	**NOEC**= 12.5%	**LOEC**= 25%	**CV**= 17.7%	**IC25**= 16.7%	(14.6-17.5)

[a] standard deviation
[b] significant difference from the control (Dunnett's test $p \leq 0.05$)
[c] significant difference from the control (Steel's test $p \leq 0.05$)

Figure 94. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill A weak black liquor spike 5.

TASK 10.2.09
Black Liquor Spiking Study 7-d Support Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 09/14/10 – 09/20/10
SAMPLE ID: #24673 A-RWBL-5

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.5	7.7	300	5	25.5	(0.1)	54	82
12.5%	7.5	7.7	423	5	25.4	(0.2)		
25%	7.6	7.5	546	5	25.4	(0.1)		
50%	7.7	7.5	774	5	25.4	(0.1)		
100%	7.8	7.2	1295	5	25.4	(0.1)	196	50

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.7	7.3	311	5	25.5	(0.2)
12.5%	7.8	7.1	457	5	25.4	(0.1)
25%	7.9	7.1	557	5	25.3	(0.2)
50%	8.0	7.1	810	5	25.3	(0.2)
100%	8.0	6.2	1274	5	25.3	(0.2)

[a] standard deviation

Figure 95. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill A weak black liquor spike 5.

7-d Bioassay BLSS Support
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 10/25/10 – 10/31/10
SAMPLE ID: #24776 B-EFF

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	37	29	19	31	30	29.2	6.5
12.5%	100	34	44	39	37	40	38.8	3.7
25%	100	35	33	33	40	35	35.2	2.9
50%	100	34	34	38	39	37	36.4	2.3
100%	100	26	18	29	32	27	26.4	5.2

Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%
Reproduction	**NOEC**= 100%	**LOEC**= >100%	**CV**= >100%	**IC25**= >100%

[a] standard deviation
[b] confidence intervals

Figure 96. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill B mill-effluent.

7-d Bioassay BLSS Support
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 10/25/10 – 10/31/10
SAMPLE ID: #24776 B-EFF

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.6	7.5	297	6	25.6	(0.4)	55	85
12.5%	7.8	7.7	415	6	25.5	(0.4)		
25%	7.8	7.7	577	6	25.6	(0.4)		
50%	7.9	7.6	931	6	25.6	(0.4)		
100%	8.0	7.4	1511	6	25.5	(0.4)	272	124

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.2	311	6	24.9	(0.2)
12.5%	7.8	7.1	477	6	24.9	(0.2)
25%	8.0	7.0	629	6	24.9	(0.2)
50%	8.1	6.7	951	6	24.9	(0.2)
100%	8.2	6.2	1579	6	24.9	(0.2)

[a] standard deviation

Figure 97. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill B mill-effluent.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: # 24917 (B-RWW-CTRL-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	22	28	21	26	28	25.0	3.3
12.5%	100	36	43	40	40	40	39.8	2.5
25%	100	34	39	39	39	42	38.6	2.9
50%	100	31	28	20	27	29	27.0	4.2
100%	100	0	0	1	6	0	1.4*[b]S[c]	2.6

				Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 52.2% (44.6- 57.7)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test $p \leq 0.05$)
[c] significant difference from the control (Steel's Test $p \leq 0.05$)

Figure 98. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill B reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: # 24917 (B-RWW-CTRL-DN

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.7	7.6	301	6	25.2	(0.3)	55	86
12.5%	7.6	7.3	458	6	25.2	(0.4)		
25%	7.9	7.3	618	6	25.2	(0.4)		
50%	8.1	7.3	932	6	25.2	(0.4)		
100%	8.2	7.2	1547	6	25.2	(0.4)	292	134

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	310	5	25.7	(0.1)
12.5%	7.6	7.7	463	5	25.7	(0.1)
25%	8.0	7.7	619	5	25.6	(0.1)
50%	8.2	7.6	921	5	25.4	(0.1)
100%	8.3	7.0	1526	5	25.3	(0.1)

[a] standard deviation

Figure 99. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill B reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: #24919 (B-WBL-2-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	22	28	21	26	28	25.0	3.3
12.5%	100	42	40	40	43	40	41.0	1.4
25%	100	33	40	43	36	38	38.0	3.8
50%	100	30	29	32	30	2	24.6	12.7
100%	60	0	0	0	0	0	0*[b]S[c]	0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= 81.2%	(NA-NA)[d]
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 46.5%	(35.0 - 58.1)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)
[d] survival IC25 confidence intervals not available

Figure 100. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill B weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: #24919 (B-WBL-2-DN)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.7	7.6	301	6	25.2	(0.3)	55	86
12.5%	7.8	7.5	462	6	25.2	(0.4)		
25%	7.9	7.5	651	6	25.3	(0.4)		
50%	8.2	7.4	983	6	25.2	(0.3)		
100%	8.3	7.2	1693	6	25.2	(0.4)	348	168

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	310	6	25.7	(0.1)
12.5%	8.0	7.8	493	6	25.2	(0.3)
25%	8.1	7.9	658	6	25.1	(0.3)
50%	8.3	7.7	987	6	24.9	(0.3)
100%	8.5	7.2	1612	6	24.7	(0.3)

[a] standard deviation

Figure 101. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill B weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: #24922 (B-WBL-5-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	27	22	20	29	30	25.6	4.4
12.5%	100	38	35	34	41	34	36.4	3.0
25%	100	21	17	26	26	25	23.0	3.9
50%	60	2	4	2	0	2	2.0*[b]S[c]	1.4
100%	80	0	0	0	0	0	0*S	0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= 45.8%	(NA-NA)[d]
Reproduction	**NOEC**= 25%	**LOEC**= 50%	**CV**= 35.4%	**IC25**= 24.6%	(20.9 - 28.4)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)
[d] survival IC25 confidence intervals not available

Figure 102. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill B weak black liquor spike 5.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/02/10 – 11/08/10
SAMPLE ID: #24922 (B-WBL-5-DN)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.5	7.5	297	6	25.1	(0.3)	55	86
12.5%	7.8	7.6	478	6	25.2	(0.3)		
25%	8.0	7.4	651	6	25.2	(0.3)		
50%	8.2	7.3	1055	6	25.2	(0.4)		
100%	8.3	6.9	1811	6	25.2	(0.3)	392	150

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.8	7.8	309	4	25.1	(0.1)
12.5%	8.0	7.7	507	4	25.4	(0.1)
25%	8.2	7.8	7.4	4	25.4	(0.1)
50%	8.4	7.7	1078	4	25.3	(0.1)
100%	8.5	6.6	1827	4	25.2	(0.1)

[a] standard deviation

Figure 103. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill B weak black liquor spike 5.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/11/10 – 11/17/10
SAMPLE ID: #24924 (C-FE)

	%SURVIVAL	YOUNG/ADULT REPLICATES					AVERAGE REPRODUCTION	SD[a]
		1	2	3	4	5		
CONTROL	100	29	28	28	33	31	29.8	2.2
12.5%	100	41	37	42	36	38	38.8	2.6
25%	100	25	26	36	33	27	29.4	4.8
50%	100	25	26	28	23	31	26.6	3.0
100%	80	0	0	0	0	0	0*[b]S[c]	0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 51.6%	(35.8 – 55.5)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)

Figure 104. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill C mill-effluent.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/11/10 – 11/17/10
SAMPLE ID: #24924 (C-FE)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.6	8.0	297	6	25.2	(0.2)	56	84
12.5%	7.9	8.2	690	6	25.2	(0.2)		
25%	8.0	8.1	1075	6	25.1	(0.2)		
50%	8.1	8.1	1787	6	25.2	(0.2)		
100%	8.0	7.8	3410	6	25.2	(0.2)	500	232

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.7	8.0	318	6	25.4	(0.3)
12.5%	8.1	7.9	708	6	25.4	(0.3)
25%	8.3	7.8	1096	6	25.3	(0.3)
50%	8.6	7.8	1842	6	25.3	(0.3)
100%	8.7	7.5	3360	6	25.2	(0.3)

[a] standard deviation

Figure 105. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill C mill-effluent.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24986 (C-RWW-CTRL-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	34	28	23	24	26	27.0	4.4
12.5%	100	44	31	42	44	35	39.2	5.9
25%	100	40	37	29	43	34	36.6	5.4
50%	100	40	35	34	34	30	34.6	3.6
100%	100	11	4	6	3	8	6.4*[b]S[c]	3.2

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 65.4%	(61.6 – 66.8)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)

Figure 106. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill C reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24986 (C-RWW-CTRL-DN)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.7	8.0	297	6	24.9	(0.5)	56	84
12.5%	8.0	8.1	700	6	25.0	(0.5)		
25%	8.2	8.0	1117	6	24.9	(0.5)		
50%	8.4	7.9	1897	6	24.9	(0.5)		
100%	8.6	7.9	3510	6	24.9	(0.5)	350	212

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	309	6	25.1	(0.2)
12.5%	7.9	7.8	716	6	25.2	(0.2)
25%	8.1	7.8	1117	6	25.2	(0.2)
50%	8.4	7.6	1888	6	25.2	(0.1)
100%	8.6	7.4	3490	6	25.0	(0.3)

[a] standard deviation

Figure 107. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill C reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24988 (C-RWBL-2-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	34	28	23	24	26	27.0	4.4
12.5%	100	34	39	31	34	36	34.8	2.9
25%	100	38	32	37	42	35	36.8	3.7
50%	100	32	32	32	33	34	32.6	0.9
100%	100	1	0	0	0	0	0.2*[b]S[c]	0.4

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 62.2%	(59.8 – 62.7)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)

Figure 108. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill C weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24988 (C-RWBL-2-DN)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.7	8.0	297	6	24.9	(0.5)	56	84
12.5%	8.1	8.3	738	6	25.0	(0.5)		
25%	8.3	8.2	1157	6	25.0	(0.5)		
50%	8.6	8.2	1901	6	25.0	(0.5)		
100%	8.7	7.9	3660	6	25.0	(0.5)	392	208

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	309	6	25.1	(0.2)
12.5%	8.1	7.9	750	6	25.0	(0.3)
25%	8.3	7.9	1168	6	24.8	(0.3)
50%	8.5	7.8	1964	6	24.7	(0.4)
100%	8.7	7.6	3630	6	24.7	(0.4)

[a] standard deviation

Figure 109. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill C weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24991 (C-RWBL-5-DN)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD[a]
CONTROL	100	34	28	23	24	26	27.0	4.4
12.5%	100	45	42	39	36	35	39.4	4.2
25%	100	40	39	38	42	8	33.4	14.1
50%	100	36	32	34	27	27	31.2	4.1
100%	80	0	0	0	1	1	0.4*[b]S[c]	0.5

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**CV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**CV**= 70.7%	**IC25**= 60.1%	(24.4 – 62.7)

[a] standard deviation
[b] significant difference from the control (Dunnett's Test p≤0.05)
[c] significant difference from the control (Steel's Test p≤0.05)

Figure 110. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill C weak black liquor spike 5.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 11/17/10 – 11/23/10
SAMPLE ID: #24991 (C-RWBL-5-DN)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.7	8.0	297	6	24.9	(0.5)	56	84
12.5%	8.2	8.1	798	6	25.0	(0.5)		
25%	8.3	8.2	1191	6	25.1	(0.5)		
50%	8.5	8.2	1987	6	25.1	(0.5)		
100%	8.7	7.9	3700	6	25.0	(0.5)	412	200

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	309	6	25.1	(0.2)
12.5%	8.2	7.9	770	6	25.0	(0.3)
25%	8.3	7.8	1206	6	24.8	(0.3)
50%	8.5	7.7	2110	6	24.7	(0.4)
100%	8.7	7.4	3780	6	24.7	(0.4)

[a] standard deviation

Figure 111. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill C weak black liquor spike 5.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/11/11 – 02/18/11
SAMPLE ID: #25128 (D-FE) Mill D Final Effluent

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD
CONTROL	100	23	27	32	25	35	28.4	5.0
12.5%	100	21	35	37	36	32	32.2	6.5
25%	100	29	32	15	30	34	28.0	7.5
50%	100	33	26	25	28	23	27.0	3.8
100%	100	9	4	6	7	7	6.6*	1.8

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**ChV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 50%	**LOEC**= 100%	**ChV**= 70.7%	**IC25**= 60.5%	(50.0 – 66.5)

SD= standard deviation
*=significant difference from the control (Dunnett's Test $p \leq 0.05$)

Normal Distribution of Data
Bartlett's Test- B=6.755, Df=4 (Critical 1% value=13.28) = homogeneous variance

Figure 112. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill D mill-effluent.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/11/11 – 02/18/11
SAMPLE ID: #25128 (D-FE) Mill D Final Effluent

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)	Alkalinity	Hardness
CONTROL	7.5	7.4	307	7	24.8	(0.4)	54	84
12.5%	7.5	7.6	572	7	24.9	(0.4)		
25%	7.7	7.6	811	7	24.9	(0.3)		
50%	7.8	7.5	1320	7	24.9	(0.3)		
100%	7.9	7.3	2310	7	24.9	(0.3)	404	174

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.9	323	7	24.8	(0.2)
12.5%	7.9	7.8	593	7	24.8	(0.1)
25%	8.1	7.8	858	7	24.8	(0.2)
50%	8.3	7.5	1364	7	24.9	(0.1)
100%	8.5	7.1	2310	7	24.7	(0.2)

(SD)= standard deviation

Figure 113. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill D mill-effluent.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25133 (D-RWW-CTRL)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD
CONTROL	100	28	27	26	29	32	28.4	2.3
12.5%	100	31	30	33	32	39	33.0	3.5
25%	100	33	24	30	24	30	28.2	4.0
50%	100	26	23	18	27	19	22.6*	4.0
100%	100	4	5	7	0	5	4.2*	2.6

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**ChV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 25%	**LOEC**= 50%	**ChV**= 35.4%	**IC25**= 48.1%	(37.0% - 57.2%)

SD=standard deviation
*=significant difference from the control (Dunnett's Test $p \leq 0.05$)

Bartlett's Test – B=1.8, Df=4 (Critical 1% value=13.28) = homogeneous variance (Dunnett's Test)

Figure 114. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill D reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25133 (D-RWW-CTRL)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)[a]	Alkalinity	Hardness
CONTROL	7.5	8.0	303	6	25.1	(0.5)	55	80
12.5%	7.9	8.0	485	6	25.1	(0.5)		
25%	8.0	7.8	668	6	25.1	(0.5)		
50%	8.3	8.2	1024	6	25.2	(0.5)		
100%	8.5	8.0	1718	6	25.1	(0.5)	274	138

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.4	320	5	24.9	(0.5)
12.5%	7.8	7.3	498	5	24.9	(0.4)
25%	7.9	7.4	679	5	24.9	(0.4)
50%	8.2	7.5	1036	5	24.9	(0.5)
100%	8.4	7.4	1712	5	24.9	(0.5)

[a] standard deviation

Figure 115. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill D reactor control.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25135 (D-RWBL-2)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD
CONTROL	100	28	27	26	29	32	28.4	2.3
12.5%	100	33	30	29	24	32	29.6	3.5
25%	100	25	32	32	32	34	31.0	3.5
50%	100	12	7	13	1	25	11.6 S	8.9
100%	100	0	0	0	0	2	0.4 S	0.9

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**ChV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 25%	**LOEC**= 50%	**ChV**= 35.4%	**IC25**= 35.2%	(32.5% - 41.3%)

SD=standard deviation
S=significant difference from the control (Steel's Test $p \leq 0.05$)

Bartlett's Test – B=16.8, Df=4 (Critical 1% value=13.28) = heterogeneous variance (Steel's Test)

Figure 116. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill D weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25135 (D-RWBL-2)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)	Alkalinity	Hardness
CONTROL	7.5	8.0	303	5	25.0	(0.5)	55	80
12.5%	7.8	7.8	515	5	25.2	(0.5)		
25%	8.0	8.0	725	5	25.1	(0.5)		
50%	8.4	7.9	1131	5	25.1	(0.5)		
100%	8.6	7.9	1917	5	25.2	(0.5)	316	141

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.4	320	5	24.8	(0.4)
12.5%	7.8	7.7	529	5	25.0	(0.4)
25%	8.0	7.6	726	5	24.9	(0.5)
50%	8.2	7.4	1131	5	24.9	(0.5)
100%	8.5	7.3	1904	5	24.9	(0.4)

Figure 117. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill D weak black liquor spike 2.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25138 (D-RWBL-5)

	%SURVIVAL	YOUNG/ADULT REPLICATES 1	2	3	4	5	AVERAGE REPRODUCTION	SD
CONTROL	100	28	27	26	29	32	28.4	2.3
12.5%	100	36	32	30	28	28	30.8	3.3
25%	100	27	27	28	22	25	25.8	2.4
50%	100	6	10	6	4	9	7.0*	2.4
100%	100	0	0	0	0	0	0.0*	0.0

					Confidence Intervals
Survival	**NOEC=** 100%	**LOEC**= >100%	**ChV=** >100%	**IC25**= >100%	
Reproduction	**NOEC**= 25%	**LOEC**= 50%	**ChV**= 35.4%	**IC25**= 29.8%	(27.1% - 32.0%)

SD=standard deviation
*=significant difference from the control (Dunnett's Test $p \leq 0.05$)

Bartlett's Test – B=0.71, Df=3 (Critical 1% value=11.34) = homogeneous variance (Dunnett's Test)

Figure 118. Summary sheet for *Ceriodaphnia dubia* toxicity test with Mill D weak black liquor spike 5.

BLSS Support 7-d Bioassay
TEST ORGANISM: *Ceriodaphnia dubia*
DATE: 02/21/11 – 02/27/11
SAMPLE ID: #25138 (D-RWBL-5)

INITIAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)	Alkalinity	Hardness
CONTROL	7.5	8.0	303	6	25.1	(0.5)	55	80
12.5%	8.1	8.1	555	6	25.1	(0.5)		
25%	8.2	8.1	793	6	25.1	(0.5)		
50%	8.5	7.9	1274	6	25.1	(0.5)		
100%	8.6	7.9	2180	6	25.1	(0.5)	378	138

FINAL WATER CHEMISTRY

	pH	DO	Cond.	N	Temp.	(SD)
CONTROL	7.6	7.4	320	5	24.9	(0.5)
12.5%	7.9	7.5	561	5	24.9	(0.4)
25%	8.1	7.4	793	5	25.0	(0.4)
50%	8.3	7.4	1271	5	24.9	(0.4)
100%	8.6	7.3	2150	5	25.0	(0.4)

Figure 119. Summary sheet of test chamber chemistry for *Ceriodaphnia dubia* toxicity test with Mill D weak black liquor spike 5.

APPENDIX M

Shapiro-Wilk's Tests for Normality

Table 64. Results of Shapiro-Wilks test for normality showing test statistic (W) and p-values.WBL: weak black liquor; TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Parameter	W	p-value
WBL solids	0.9444	0.223
48-h EC50 *M. galloprovincialis* embryo-larval development	0.8046	0.000
7-d IC25 *C. dubia* reproduction	0.9150	0.140
7-d IC25 *C. dubia* survival	0.4859	0.000
pH	0.9396	0.119
Color	0.9599	0.367
Conductivity	0.8491	0.001
Turbidity	0.9029	0.016
TSS	0.9309	0.073
Polyphenols	0.9664	0.510
Hardness	0.8925	0.009
Alkalinity	0.9479	0.191
Salinity	0.6605	0.000
BOD	0.9231	0.053
DCOD	0.9761	0.766
DOC	0.9642	0.459
Campesterol	0.6494	0.000
Stigmastanol	0.8846	0.006
Beta-sitosterol	0.7155	0.000
Stigmasterol	0.8622	0.002
Pimaric acid	0.2686	0.000
Sandracopimaric acid	0.2631	0.000
Isopimaric acid	0.4073	0.000
Palustric acid	0.4674	0.000
Dehydroabietic acid	0.3731	0.000
Abietic acid	0.2970	0.000
Neoabietic acid	0.3794	0.000

APPENDIX N

Spearman's rho Correlation Analysis

Figure 120. Spearman's rho correlation analysis of effluent chemical parameters showing correlation coefficients. Data from all four mills were pooled together for correlation analysis. Bold numbers indicate significant correlations.TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Spearman's rho												
Parameter	pH	Color (PCU)	Conductivity (µs/cm)	Turbidity (ntu)	TSS (mg/L)	Polyphenols (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	Salinity (ppt)	BOD (mg/L)	DCOD (mg/L)	DOC (mg/L)
pH												
Color (PCU)	0.361											
Conductivity (µs/cm)	**0.597**	**0.600**										
Turbidity (ntu)	-0.374	0.202	**-0.548**									
TSS (mg/L)	-0.393	0.135	**-0.607**	**0.926**								
Polyphenols (mg/L)	0.028	**0.736**	0.160	**0.577**	**0.501**							
Hardness (mg/L)	**0.567**	**0.635**	**0.819**	-0.261	-0.383	0.133						
Alkalinity (mg/L)	0.487	**0.615**	**0.952**	-0.464	**-0.585**	0.185	0.857					
Salinity (ppt)	0.061	-0.348	0.062	-0.285	-0.357	-0.242	**-0.144**	0.039				
BOD (mg/L)	-0.151	-0.056	0.188	-0.002	-0.190	-0.022	0.156	0.250	0.354			
DCOD (mg/L)	**0.521**	**0.859**	**0.847**	-0.109	-0.239	**0.552**	**0.767**	**0.832**	-0.074	0.184		
DOC (mg/L)	**0.582**	**0.666**	**0.748**	-0.114	-0.239	0.200	**0.860**	**0.740**	-0.112	0.088	**0.815**	
Campesterol (µg/L)	**-0.619**	-0.071	**-0.578**	**0.603**	**0.687**	0.252	**-0.528**	**-0.496**	-0.461	-0.313	-0.408	-0.436
Stigmastanol (µg/L)	0.254	**0.723**	0.313	0.227	0.282	0.468	0.392	0.276	-0.698	-0.365	0.454	0.437
Beta-sitosterol (µg/L)	-0.451	0.099	-0.333	**0.518**	**0.582**	0.402	-0.363	-0.241	-0.302	-0.306	-0.187	-0.268
Stigmasterol (µg/L)	**-0.655**	0.084	**-0.512**	**0.650**	**0.660**	0.338	-0.387	-0.372	**-0.348**	-0.150	-0.278	-0.341
Pimaric acid (µg/L)	0.345	0.111	**0.622**	-0.447	**-0.572**	-0.099	**0.504**	**0.592**	0.365	0.361	0.442	0.456
Sandracopimaric acid (µg/L)	-0.272	-0.063	0.235	-0.125	-0.272	-0.090	0.331	0.415	0.094	0.347	0.087	0.098
Isopimaric acid (µg/L)	0.201	**0.507**	0.452	0.179	-0.110	0.303	**0.709**	**0.592**	-0.029	0.426	**0.643**	**0.703**
Palustric acid (µg/L)	0.415	**0.607**	**0.676**	-0.051	-0.291	0.370	**0.778**	**0.776**	-0.009	0.248	**0.781**	**0.750**
Dehydroabietic acid (µg/L)	**0.534**	0.430	**0.821**	-0.352	**-0.500**	0.190	**0.774**	**0.804**	0.192	0.411	**0.730**	**0.677**
Abietic acid (µg/L)	0.489	**0.618**	**0.731**	-0.039	-0.278	0.374	**0.822**	**0.805**	0.045	0.352	**0.810**	**0.783**
Neoabietic acid (µg/L)	0.357	**0.547**	**0.581**	0.085	-0.170	0.394	**0.707**	**0.692**	0.075	0.366	**0.745**	**0.716**

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Spearman's rho											
Parameter	Campesterol (µg/L)	Stigmastanol (µg/L)	Beta-sitosterol (µg/L)	Stigmasterol (µg/L)	Pimaric acid (µg/L)	Sandracopimaric acid (µg/L)	Isopimaric acid (µg/L)	Palustric acid (µg/L)	Dehydroabietic acid (µg/L)	Abietic acid (µg/L)	Neoabietic acid (µg/L)
pH											
Color (PCU)											
Conductivity (µs/cm)											
Turbidity (ntu)											
TSS (mg/L)											
Polyphenols (mg/L)											
Hardness (mg/L)											
Alkalinity (mg/L)											
Salinity (ppt)											
BOD (mg/L)											
DCOD (mg/L)											
DOC (mg/L)											
Campesterol (µg/L)											
Stigmastanol (µg/L)	0.279										
Beta-sitosterol (µg/L)	**0.877**	0.292									
Stigmasterol (µg/L)	**0.888**	0.290	**0.798**								
Pimaric acid (µg/L)	**-0.580**	-0.221	-0.323	**-0.541**							
Sandracopimaric acid (µg/L)	-0.081	-0.304	0.063	-0.016	0.482						
Isopimaric acid (µg/L)	-0.277	0.150	-0.127	-0.043	0.425	0.460					
Palustric acid (µg/L)	-0.385	0.191	-0.171	-0.224	**0.610**	0.463	**0.867**				
Dehydroabietic acid (µg/L)	**-0.681**	0.023	-0.394	**-0.612**	**0.818**	0.454	**0.627**	**0.781**			
Abietic acid (µg/L)	-0.465	0.211	-0.213	-0.306	**0.656**	0.454	**0.831**	**0.909**	**0.868**		
Neoabietic acid (µg/L)	-0.354	0.110	-0.088	-0.184	**0.601**	0.466	**0.884**	**0.894**	**0.757**	**0.915**	

Table 65. Spearman's rho correlation analysis of effluent chemical parameters showing p-values. Data from all four mills were pooled together for correlation analysis. Bold numbers indicate significant correlations.WBL: weak black liquor; TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

p-values												
Parameter	pH	Color (PCU)	Conductivity (μs/cm)	Turbidity (ntu)	TSS (mg/L)	Polyphenols (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	Salinity (ppt)	BOD (mg/L)	DCOD (mg/L)	DOC (mg/L)
pH												
Color (PCU)	0.065											
Conductivity (μs/cm)	**0.001**	**0.001**										
Turbidity (ntu)	0.055	0.311	**0.004**									
TSS (mg/L)	0.043	0.501	**0.001**	**0.000**								
Polyphenols (mg/L)	0.891	**0.000**	0.424	**0.002**	**0.008**							
Hardness (mg/L)	**0.002**	**0.000**	**0.000**	0.189	0.049	0.508						
Alkalinity (mg/L)	0.010	**0.001**	**0.000**	0.016	**0.001**	0.354	**0.000**					
Salinity (ppt)	0.763	0.076	0.759	0.149	0.068	0.223	0.472	0.845				
BOD (mg/L)	0.460	0.784	0.357	0.993	0.353	0.917	0.445	0.218	0.076			
DCOD (mg/L)	**0.005**	**0.000**	**0.000**	0.588	0.229	**0.003**	**0.000**	**0.000**	0.715	0.368		
DOC (mg/L)	**0.001**	**0.000**	**0.000**	0.570	0.230	0.317	**0.000**	**0.000**	0.580	0.671	**0.000**	
Campesterol (μg/L)	**0.001**	0.723	**0.002**	**0.001**	**0.000**	0.204	**0.005**	**0.009**	0.016	0.120	0.035	0.023
Stigmastanol (μg/L)	0.201	**0.000**	0.112	0.253	0.154	0.015	0.043	0.163	**0.000**	0.067	0.018	0.023
Beta-sitosterol (μg/L)	0.018	0.624	0.089	**0.006**	**0.001**	0.038	0.063	0.226	0.126	0.129	0.349	0.176
Stigmasterol (μg/L)	**0.000**	0.675	**0.007**	**0.000**	**0.000**	0.085	0.046	0.057	0.075	0.465	0.160	0.081
Pimaric acid (μg/L)	0.078	0.580	**0.001**	0.019	**0.002**	0.623	**0.007**	**0.001**	0.061	0.070	0.021	0.017
Sandracopimaric acid (μg/L)	0.169	0.754	0.238	0.534	0.170	0.655	0.092	0.031	0.643	0.083	0.665	0.626
Isopimaric acid (μg/L)	0.314	**0.007**	0.018	0.372	0.585	0.125	**0.000**	**0.001**	0.886	0.030	**0.000**	**0.000**
Palustric acid (μg/L)	0.031	**0.001**	**0.000**	0.799	0.141	0.058	**0.000**	**0.000**	0.964	0.221	**0.000**	**0.000**
Dehydroabietic acid (μg/L)	**0.004**	0.026	**0.000**	0.073	**0.008**	0.340	**0.000**	**0.000**	0.337	0.037	**0.000**	**0.000**
Abietic acid (μg/L)	0.010	**0.001**	**0.000**	0.847	0.161	0.056	**0.000**	**0.000**	0.825	0.078	**0.000**	**0.000**
Neoabietic acid (μg/L)	0.067	**0.003**	**0.001**	0.675	0.397	0.042	**0.000**	**0.000**	0.710	0.066	**0.000**	**0.000**

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	p-values										
Parameter	Campesterol (µg/L)	Stigmastanol (µg/L)	Beta-sitosterol (µg/L)	Stigmasterol (µg/L)	Pimaric acid (µg/L)	Sandracopimaric acid (µg/L)	Isopimaric acid (µg/L)	Palustric acid (µg/L)	Dehydroabietic acid (µg/L)	Abietic acid (µg/L)	Neoabietic acid (µg/L)
pH											
Color (PCU)											
Conductivity (µs/cm)											
Turbidity (ntu)											
TSS (mg/L)											
Polyphenols (mg/L)											
Hardness (mg/L)											
Alkalinity (mg/L)											
Salinity (ppt)											
BOD (mg/L)											
DCOD (mg/L)											
DOC (mg/L)											
Campesterol (µg/L)											
Stigmastanol (µg/L)	0.158										
Beta-sitosterol (µg/L)	**0.000**	0.139									
Stigmasterol (µg/L)	**0.000**	0.142	**0.000**								
Pimaric acid (µg/L)	**0.002**	0.267	0.101	**0.004**							
Sandracopimaric acid (µg/L)	0.689	0.124	0.754	0.936	0.011						
Isopimaric acid (µg/L)	0.162	0.455	0.529	0.831	0.027	0.016					
Palustric acid (µg/L)	0.047	0.341	0.394	0.262	**0.001**	0.015	**0.000**				
Dehydroabietic acid (µg/L)	**0.000**	0.909	0.042	**0.001**	**0.000**	0.017	**0.000**	**0.000**			
Abietic acid (µg/L)	0.015	0.290	0.286	0.120	**0.000**	0.017	**0.000**	**0.000**	**0.000**		
Neoabietic acid (µg/L)	0.070	0.585	0.662	0.358	**0.001**	0.014	**0.000**	**0.000**	**0.000**	**0.000**	

APPENDIX O

Kmeans Clustering Cluster Means

Table 66. Kmeans cluster means with four clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Run 1	pH	8.556667	8.275	7.963333	8.372857
	Color	1428.333	1328.25	669	581.5714
	Conductivity	3740	1858	1115.333	1856.571
	Turbidity	21.01667	73.45	68.5	34.67143
	TSS	27.8	61.1	86.31667	34.11429
	Polyphenols	44.60083	63.26875	43.84817	30.41714
	Hardness	203.1667	147	43.53333	138.7143
	Salinity	1.983333	2	2	3.142857
	BOD	10.31667	13.85	8.725	16.82857
	DCOD	567.3333	519.25	224.1667	356.2857
	DOC	182.6667	135.25	57.5	118.4286
	Campesterol	3.678333	9.5425	50.46333	3.321429
	Stigmastanol	80.83333	50.665	32.44167	13.80429
	Beta-sitosterol	73.7	38.38	141.0483	17.66857
	Stigmasterol	7.34	17.22	26.92833	6.311429
	Pimaric acid	3.166667	0.92	0.92	2.588571
	Sandracopimaric acid	0.94	0.94	0.94	0.94
	Isopimaric acid	3.2	5.395	1.12	2.6
	Palustric acid	3.175	2.895	0.99	1.771429
	Dehydroabietic acid	12.30667	5.66	2.701667	8.661429
	Abietic acid	14.38667	11.27	3.65	10.66429
	Neoabietic acid	2.015	2.02	0.99	1.577143

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Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Run 2	pH	8.556667	7.963333	8.372857	8.275
	Color	1428.333	669	581.5714	1328.25
	Conductivity	3740	1115.333	1856.571	1858
	Turbidity	21.01667	68.5	34.67143	73.45
	TSS	27.8	86.31667	34.11429	61.1
	Polyphenols	44.60083	43.84817	30.41714	63.26875
	Hardness	203.1667	43.53333	138.7143	147
	Salinity	1.983333	2	3.142857	2
	BOD	10.31667	8.725	16.82857	13.85
	DCOD	567.3333	224.1667	356.2857	519.25
	DOC	182.6667	57.5	118.4286	135.25
	Campesterol	3.678333	50.46333	3.321429	9.5425
	Stigmastanol	80.83333	32.44167	13.80429	50.665
	Beta-sitosterol	73.7	141.0483	17.66857	38.38
	Stigmasterol	7.34	26.92833	6.311429	17.22
	Pimaric acid	3.166667	0.92	2.588571	0.92
	Sandracopimaric acid	0.94	0.94	0.94	0.94
	Isopimaric acid	3.2	1.12	2.6	5.395
	Palustric acid	3.175	0.99	1.771429	2.895
	Dehydroabietic acid	12.30667	2.701667	8.661429	5.66
	Abietic acid	14.38667	3.65	10.66429	11.27
	Neoabietic acid	2.015	0.99	1.577143	2.02
Run 3	pH	8.372857	7.963333	8.275	8.556667
	Color	581.5714	669	1328.25	1428.333
	Conductivity	1856.571	1115.333	1858	3740
	Turbidity	34.67143	68.5	73.45	21.01667
	TSS	34.11429	86.31667	61.1	27.8
	Polyphenols	30.41714	43.84817	63.26875	44.60083
	Hardness	138.7143	43.53333	147	203.1667
	Salinity	3.142857	2	2	1.983333
	BOD	16.82857	8.725	13.85	10.31667
	DCOD	356.2857	224.1667	519.25	567.3333
	DOC	118.4286	57.5	135.25	182.6667
	Campesterol	3.321429	50.46333	9.5425	3.678333
	Stigmastanol	13.80429	32.44167	50.665	80.83333
	Beta-sitosterol	17.66857	141.0483	38.38	73.7
	Stigmasterol	6.311429	26.92833	17.22	7.34
	Pimaric acid	2.588571	0.92	0.92	3.166667
	Sandracopimaric acid	0.94	0.94	0.94	0.94
	Isopimaric acid	2.6	1.12	5.395	3.2
	Palustric acid	1.771429	0.99	2.895	3.175
	Dehydroabietic acid	8.661429	2.701667	5.66	12.30667
	Abietic acid	10.66429	3.65	11.27	14.38667
	Neoabietic acid	1.577143	0.99	2.02	2.015

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Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Run 4	pH	8.372857	8.275	7.963333	8.556667
	Color	581.5714	1328.25	669	1428.333
	Conductivity	1856.571	1858	1115.333	3740
	Turbidity	34.67143	73.45	68.5	21.01667
	TSS	34.11429	61.1	86.31667	27.8
	Polyphenols	30.41714	63.26875	43.84817	44.60083
	Hardness	138.7143	147	43.53333	203.1667
	Salinity	3.142857	2	2	1.983333
	BOD	16.82857	13.85	8.725	10.31667
	DCOD	356.2857	519.25	224.1667	567.3333
	DOC	118.4286	135.25	57.5	182.6667
	Campesterol	3.321429	9.5425	50.46333	3.678333
	Stigmastanol	13.80429	50.665	32.44167	80.83333
	Beta-sitosterol	17.66857	38.38	141.0483	73.7
	Stigmasterol	6.311429	17.22	26.92833	7.34
	Pimaric acid	2.588571	0.92	0.92	3.166667
	Sandracopimaric acid	0.94	0.94	0.94	0.94
	Isopimaric acid	2.6	5.395	1.12	3.2
	Palustric acid	1.771429	2.895	0.99	3.175
	Dehydroabietic acid	8.661429	5.66	2.701667	12.30667
	Abietic acid	10.66429	11.27	3.65	14.38667
	Neoabietic acid	1.577143	2.02	0.99	2.015
Run 5	pH	8.15	8.306667	8.49	8.623333
	Color	604.5455	1124.667	1197	1659.667
	Conductivity	1410	1935	3641.667	3838.333
	Turbidity	54.31818	58.33333	14.43333	27.6
	TSS	62.90909	51.51667	22.4	33.2
	Polyphenols	35.11082	57.14417	33.05	56.15167
	Hardness	88.01818	142	202.3333	204
	Salinity	2.363636	2.666667	1.933333	2.033333
	BOD	11.71364	16.11667	11.13333	9.5
	DCOD	259.6364	510	510	624.6667
	DOC	85.18182	129.6667	164.6667	200.6667
	Campesterol	29.33636	6.916667	2.41	4.946667
	Stigmastanol	25.35455	35.84	76.83333	84.83333
	Beta-sitosterol	85.84727	29.86167	13.26667	134.1333
	Stigmasterol	18.16636	12.46667	6.64	8.04
	Pimaric acid	1.42	1.95	2.113333	4.22
	Sandracopimaric acid	0.94	0.94	0.94	0.94
	Isopimaric acid	1.917273	4.235	2.166667	4.233333
	Palustric acid	1.299091	2.605	2.523333	3.826667
	Dehydroabietic acid	4.563636	8.213333	8.406667	16.20667
	Abietic acid	5.828182	12.92	10.42667	18.34667
	Neoabietic acid	1.179091	2.015	1.316667	2.713333

Table 67. Kmeans cluster means with three clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3
Run 1	pH	8.556667	8.337273	7.963333
	Color	1428.333	853.0909	669
	Conductivity	3740	1857.091	1115.333
	Turbidity	21.01667	48.77273	68.5
	TSS	27.8	43.92727	86.31667
	Polyphenols	44.60083	42.36318	43.84817
	Hardness	203.1667	141.7273	43.53333
	Salinity	1.983333	2.727273	2
	BOD	10.31667	15.74545	8.725
	DCOD	567.3333	415.5455	224.1667
	DOC	182.6667	124.5455	57.5
	Campesterol	3.678333	5.583636	50.46333
	Stigmastanol	80.83333	27.20818	32.44167
	Beta-sitosterol	73.7	25.2	141.0483
	Stigmasterol	7.34	10.27818	26.92833
	Pimaric acid	3.166667	1.981818	0.92
	Sandracopimaric acid	0.94	0.94	0.94
	Isopimaric acid	3.2	3.616364	1.12
	Palustric acid	3.175	2.18	0.99
	Dehydroabietic acid	12.30667	7.57	2.701667
	Abietic acid	14.38667	10.88455	3.65
	Neoabietic acid	2.015	1.738182	0.99
Run 2	pH	7.963333	8.556667	8.337273
	Color	669	1428.333	853.0909
	Conductivity	1115.333	3740	1857.091
	Turbidity	68.5	21.01667	48.77273
	TSS	86.31667	27.8	43.92727
	Polyphenols	43.84817	44.60083	42.36318
	Hardness	43.53333	203.1667	141.7273
	Salinity	2	1.983333	2.727273
	BOD	8.725	10.31667	15.74545
	DCOD	224.1667	567.3333	415.5455
	DOC	57.5	182.6667	124.5455
	Campesterol	50.46333	3.678333	5.583636
	Stigmastanol	32.44167	80.83333	27.20818
	Beta-sitosterol	141.0483	73.7	25.2
	Stigmasterol	26.92833	7.34	10.27818
	Pimaric acid	0.92	3.166667	1.981818
	Sandracopimaric acid	0.94	0.94	0.94
	Isopimaric acid	1.12	3.2	3.616364
	Palustric acid	0.99	3.175	2.18
	Dehydroabietic acid	2.701667	12.30667	7.57
	Abietic acid	3.65	14.38667	10.88455
	Neoabietic acid	0.99	2.015	1.738182

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3
Run 3	pH	8.15	8.306667	8.556667
	Color	604.5455	1124.667	1428.333
	Conductivity	1410	1935	3740
	Turbidity	54.31818	58.33333	21.01667
	TSS	62.90909	51.51667	27.8
	Polyphenols	35.11082	57.14417	44.60083
	Hardness	88.01818	142	203.1667
	Salinity	2.363636	2.666667	1.983333
	BOD	11.71364	16.11667	10.31667
	DCOD	259.6364	510	567.3333
	DOC	85.18182	129.6667	182.6667
	Campesterol	29.33636	6.916667	3.678333
	Stigmastanol	25.35455	35.84	80.83333
	Beta-sitosterol	85.84727	29.86167	73.7
	Stigmasterol	18.16636	12.46667	7.34
	Pimaric acid	1.42	1.95	3.166667
	Sandracopimaric acid	0.94	0.94	0.94
	Isopimaric acid	1.917273	4.235	3.2
	Palustric acid	1.299091	2.605	3.175
	Dehydroabietic acid	4.563636	8.213333	12.30667
	Abietic acid	5.828182	12.92	14.38667
	Neoabietic acid	1.179091	2.015	2.015
Run 4	pH	8.556667	8.15	8.306667
	Color	1428.333	604.5455	1124.667
	Conductivity	3740	1410	1935
	Turbidity	21.01667	54.31818	58.33333
	TSS	27.8	62.90909	51.51667
	Polyphenols	44.60083	35.11082	57.14417
	Hardness	203.1667	88.01818	142
	Salinity	1.983333	2.363636	2.666667
	BOD	10.31667	11.71364	16.11667
	DCOD	567.3333	259.6364	510
	DOC	182.6667	85.18182	129.6667
	Campesterol	3.678333	29.33636	6.916667
	Stigmastanol	80.83333	25.35455	35.84
	Beta-sitosterol	73.7	85.84727	29.86167
	Stigmasterol	7.34	18.16636	12.46667
	Pimaric acid	3.166667	1.42	1.95
	Sandracopimaric acid	0.94	0.94	0.94
	Isopimaric acid	3.2	1.917273	4.235
	Palustric acid	3.175	1.299091	2.605
	Dehydroabietic acid	12.30667	4.563636	8.213333
	Abietic acid	14.38667	5.828182	12.92
	Neoabietic acid	2.015	1.179091	2.015

(Continued on next page)

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2	Cluster 3
Run 5	pH	8.49	8.623333	8.205294
	Color	1197	1659.667	788.1176
	Conductivity	3641.667	3838.333	1595.294
	Turbidity	14.43333	27.6	55.73529
	TSS	22.4	33.2	58.88824
	Polyphenols	33.05	56.15167	42.88729
	Hardness	202.3333	204	107.0706
	Salinity	1.933333	2.033333	2.470588
	BOD	11.13333	9.5	13.26765
	DCOD	510	624.6667	348
	DOC	164.6667	200.6667	100.8824
	Campesterol	2.41	4.946667	21.42353
	Stigmastanol	76.83333	84.83333	29.05529
	Beta-sitosterol	13.26667	134.1333	66.08765
	Stigmasterol	6.64	8.04	16.15471
	Pimaric acid	2.113333	4.22	1.607059
	Sandracopimaric acid	0.94	0.94	0.94
	Isopimaric acid	2.166667	4.233333	2.735294
	Palustric acid	2.523333	3.826667	1.76
	Dehydroabietic acid	8.406667	16.20667	5.851765
	Abietic acid	10.42667	18.34667	8.331176
	Neoabietic acid	1.316667	2.713333	1.474118

Table 68. Kmeans cluster means with two clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2
Run 1	pH	8.205294	8.556667
	Color	788.1176	1428.333
	Conductivity	1595.294	3740
	Turbidity	55.73529	21.01667
	TSS	58.88824	27.8
	Polyphenols	42.88729	44.60083
	Hardness	107.0706	203.1667
	Salinity	2.470588	1.983333
	BOD	13.26765	10.31667
	DCOD	348	567.3333
	DOC	100.8824	182.6667
	Campesterol	21.42353	3.678333
	Stigmastanol	29.05529	80.83333
	Beta-sitosterol	66.08765	73.7
	Stigmasterol	16.15471	7.34
	Pimaric acid	1.607059	3.166667
	Sandracopimaric acid	0.94	0.94
	Isopimaric acid	2.735294	3.2
	Palustric acid	1.76	3.175
	Dehydroabietic acid	5.851765	12.30667
	Abietic acid	8.331176	14.38667
	Neoabietic acid	1.474118	2.015
Run 2	pH	8.205294	8.556667
	Color	788.1176	1428.333
	Conductivity	1595.294	3740
	Turbidity	55.73529	21.01667
	TSS	58.88824	27.8
	Polyphenols	42.88729	44.60083
	Hardness	107.0706	203.1667
	Salinity	2.470588	1.983333
	BOD	13.26765	10.31667
	DCOD	348	567.3333
	DOC	100.8824	182.6667
	Campesterol	21.42353	3.678333
	Stigmastanol	29.05529	80.83333
	Beta-sitosterol	66.08765	73.7
	Stigmasterol	16.15471	7.34
	Pimaric acid	1.607059	3.166667
	Sandracopimaric acid	0.94	0.94
	Isopimaric acid	2.735294	3.2
	Palustric acid	1.76	3.175
	Dehydroabietic acid	5.851765	12.30667
	Abietic acid	8.331176	14.38667
	Neoabietic acid	1.474118	2.015

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2
Run 3	pH	8.205294	8.556667
	Color	788.1176	1428.333
	Conductivity	1595.294	3740
	Turbidity	55.73529	21.01667
	TSS	58.88824	27.8
	Polyphenols	42.88729	44.60083
	Hardness	107.0706	203.1667
	Salinity	2.470588	1.983333
	BOD	13.26765	10.31667
	DCOD	348	567.3333
	DOC	100.8824	182.6667
	Campesterol	21.42353	3.678333
	Stigmastanol	29.05529	80.83333
	Beta-sitosterol	66.08765	73.7
	Stigmasterol	16.15471	7.34
	Pimaric acid	1.607059	3.166667
	Sandracopimaric acid	0.94	0.94
	Isopimaric acid	2.735294	3.2
	Palustric acid	1.76	3.175
	Dehydroabietic acid	5.851765	12.30667
	Abietic acid	8.331176	14.38667
	Neoabietic acid	1.474118	2.015
Run 4	pH	8.556667	8.205294
	Color	1428.333	788.1176
	Conductivity	3740	1595.294
	Turbidity	21.01667	55.73529
	TSS	27.8	58.88824
	Polyphenols	44.60083	42.88729
	Hardness	203.1667	107.0706
	Salinity	1.983333	2.470588
	BOD	10.31667	13.26765
	DCOD	567.3333	348
	DOC	182.6667	100.8824
	Campesterol	3.678333	21.42353
	Stigmastanol	80.83333	29.05529
	Beta-sitosterol	73.7	66.08765
	Stigmasterol	7.34	16.15471
	Pimaric acid	3.166667	1.607059
	Sandracopimaric acid	0.94	0.94
	Isopimaric acid	3.2	2.735294
	Palustric acid	3.175	1.76
	Dehydroabietic acid	12.30667	5.851765
	Abietic acid	14.38667	8.331176
	Neoabietic acid	2.015	1.474118

Kmeans Run	Chemical Parameter	Cluster 1	Cluster 2
Run 5	pH	8.556667	8.205294
	Color	1428.333	788.1176
	Conductivity	3740	1595.294
	Turbidity	21.01667	55.73529
	TSS	27.8	58.88824
	Polyphenols	44.60083	42.88729
	Hardness	203.1667	107.0706
	Salinity	1.983333	2.470588
	BOD	10.31667	13.26765
	DCOD	567.3333	348
	DOC	182.6667	100.8824
	Campesterol	3.678333	21.42353
	Stigmastanol	80.83333	29.05529
	Beta-sitosterol	73.7	66.08765
	Stigmasterol	7.34	16.15471
	Pimaric acid	3.166667	1.607059
	Sandracopimaric acid	0.94	0.94
	Isopimaric acid	3.2	2.735294
	Palustric acid	3.175	1.76
	Dehydroabietic acid	12.30667	5.851765
	Abietic acid	14.38667	8.331176
	Neoabietic acid	2.015	1.474118

APPENDIX P

Riffle Clustering Proportional Reduction in Error Scores

Table 69. Proportional reduction in error scores for Riffle runs with four clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Chemical Parameter	Run 1	Run 2	Run 3	Run 4	Run 5
pH	0.651261	0.764706	0.764706	0.716387	0.588235
Color	0.218487	0.323529	0.323529	0.254202	0.294118
Conductivity	0.810924	0.705882	0.705882	0.810924	0.882353
Turbidity	0.355042	0.470588	0.470588	0.289916	0.323529
TSS	0.535714	0.514706	0.514706	0.46875	0.454044
Polyphenols	0.159664	0.235294	0.235294	0.159664	0.117647
Hardness	0.810924	0.823529	0.823529	0.781513	0.882353
Salinity	0.198413	0.343137	0.343137	0.234127	0.372549
BOD	0.355042	0.588235	0.588235	0.355042	0.588235
DCOD	0.420168	0.411765	0.411765	0.420168	0.382353
DOC	0.485294	0.470588	0.470588	0.55042	0.470588
Campesterol	0.420168	0.529412	0.529412	0.485294	0.588235
Stigmastanol	0.319328	0.441177	0.441177	0.384454	0.5
Beta-sitosterol	0.420168	0.470588	0.470588	0.384454	0.411765
Stigmasterol	0.485294	0.647059	0.647059	0.485294	0.588235
Pimaric acid	0.36039	0.387701	0.387701	0.314935	0.342246
Sandracopimaric acid	NaN	NaN	NaN	NaN	NaN
Isopimaric acid	0.414286	0.498039	0.498039	0.380952	0.560784
Palustric acid	0.5	0.44958	0.44958	0.464286	0.413866
Dehydroabietic acid	0.745798	0.823529	0.823529	0.710084	0.764706
Abietic acid	0.55042	0.705882	0.705882	0.586135	0.588235
Neoabietic acid	0.464286	0.579832	0.579832	0.5	0.478992

Table 70. Proportional reduction in error scores for Riffle runs with three clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Chemical Parameter	Run 1	Run 2	Run 3	Run 4	Run 5
pH	0.447619	0.6	0.266667	0.8	0.6
Color	0.654762	0.4	0.333333	0.333333	0.4
Conductivity	0.345238	0.533333	0.333333	0.6	0.533333
Turbidity	0	0.066667	0.4	0.266667	0.066667
TSS	0.240476	0.266667	0.533333	0.333333	0.266667
Polyphenols	0.207143	0.2	0.2	0.066667	0.2
Hardness	0.483333	0.466667	0.266667	0.533333	0.466667
Salinity	0.035714	0.066667	0.566667	0.066667	0.066667
BOD	0.135714	0.266667	0.466667	0.266667	0.266667
DCOD	0.654762	0.8	0.266667	0.6	0.8
DOC	0.72381	0.866667	0.466667	0.6	0.866667
Campesterol	0.447619	0.4	0.8	0.4	0.4
Stigmastanol	0.380952	0.4	0.266667	0.4	0.4
Beta-sitosterol	0.240476	0.2	0.733333	0.4	0.2
Stigmasterol	0.171429	0.133333	0.8	0.233333	0.133333
Pimaric acid	0.152597	0.348485	0.336364	0.393939	0.348485
Sandracopimaric acid	NaN	NaN	NaN	NaN	NaN
Isopimaric acid	0.478022	0.497436	0.353846	0.784615	0.497436
Palustric acid	0.777473	0.497436	0.248718	0.497436	0.497436
Dehydroabietic acid	0.45	0.533333	0.6	0.733333	0.533333
Abietic acid	0.72381	0.733333	0.266667	0.666667	0.733333
Neoabietic acid	0.478022	0.425641	0.248718	0.497436	0.425641

Table 71. Proportional reduction in error scores for Riffle runs with two clusters. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Chemical Parameter	Run 1	Run 2	Run 3	Run 4	Run 5
pH	0.454546	0.181818	0.454546	0.181818	0.727273
Color	0.454546	0.545455	0.454546	0.545455	0
Conductivity	1	0.363636	1	0.545455	0.545455
Turbidity	0.545455	0	0.545455	0.090909	1
TSS	0.727273	0.090909	0.727273	0.272727	0.818182
Polyphenols	0.090909	0.363636	0.090909	0.181818	0.272727
Hardness	0.363636	0.818182	0.363636	0.818182	0
Salinity	0.045455	0.045455	0.045455	0.045455	0.181818
BOD	0.090909	0.181818	0.090909	0.181818	0
DCOD	0.818182	0.545455	0.818182	0.727273	0.363636
DOC	0.636364	0.727273	0.636364	0.909091	0.181818
Campesterol	0.545455	0.090909	0.545455	0.272727	0.636364
Stigmastanol	0.272727	0.363636	0.272727	0.363636	0
Beta-sitosterol	0.363636	0.090909	0.363636	0.272727	0.454546
Stigmasterol	0.545455	0	0.545455	0.090909	1
Pimaric acid	0.59596	0.090909	0.59596	0.090909	0.69697
Sandracopimaric acid	NaN	NaN	NaN	NaN	NaN
Isopimaric acid	0.140909	0.809091	0.140909	0.618182	0.140909
Palustric acid	0.522727	0.809091	0.522727	0.618182	0.045455
Dehydroabietic acid	0.818182	0.181818	0.818182	0.363636	0.727273
Abietic acid	0.454546	0.909091	0.454546	0.909091	0
Neoabietic acid	0.191919	0.69697	0.191919	0.49495	0.045455

APPENDIX Q

Principal Component Analysis Variable Loadings

Table 72. Principal component analysis variable loadings for first 10 principal components for nonrandom data file. TSS: total suspended solids; BOD: biochemical oxygen demand; DCOD: dissolved chemical oxygen demand; DOC: dissolved organic carbon.

Chemical Parameter	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10
pH	-0.268	-0.154	-0.136			-0.189	0.228	-0.165	0.169	0.635
Color	-0.207	0.318			-0.204	0.243				0.132
Conductivity	-0.27		-0.287		-0.177			0.316		
Turbidity	0.153	0.312	0.301	-0.169		-0.215		0.198		0.234
TSS	0.206	0.289	0.183					0.448	-0.263	0.228
Polyphenols		0.374	0.237	0.144	-0.232	0.298	-0.207	-0.323	-0.151	0.112
Hardness	-0.287		-0.13	-0.155	-0.199	-0.174		0.178	0.251	-0.251
Salinity		-0.305	0.337	0.277		-0.249	-0.653		0.231	
BOD		-0.154	0.449		-0.644	-0.277	0.405			
DCOD	-0.276	0.17			-0.252	0.13	-0.264	-0.132		
DOC	-0.279			-0.183		-0.176	-0.199	0.367	-0.376	
Campesterol	0.233	0.223		0.325			0.104	0.216	0.218	-0.123
Stigmastanol	-0.124	0.278	-0.395		-0.19	-0.37				
Beta-sitosterol	0.113	0.247	-0.239	0.436		-0.464		-0.302	-0.172	0.143
Stigmasterol	0.213	0.302		0.124			0.143		0.536	-0.164
Pimaric acid	-0.237			0.427	0.292			0.272		
Isopimaric acid	-0.174	0.227	0.21	-0.39	0.203	-0.321		-0.182	0.157	-0.281
Palustric acid	-0.265	0.17			0.225	0.244			0.394	0.301
Dehydroabietic acid	-0.277			0.333	0.103	0.1		0.107	-0.167	-0.226
Abietic acid	-0.275		0.214	0.166	0.116		0.137			
Neoabietic acid	-0.249	0.145	0.239		0.281		0.346	-0.235	-0.189	-0.316

Table 73. Proportional and cumulative variance for first 10 principal components using simulated effluent chemistry data for nonrandom and random data files.

Data file	Variable	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10
Nonrandom	Standard deviation	3.186489	2.191155	1.484539	1.238598	0.815213	0.704011	0.540579	0.539932	0.405287	0.375475
	Proportion of Variance	0.48351	0.228627	0.104946	0.073054	0.031646	0.023602	0.013915	0.013882	0.007822	0.006713
	Cumulative proportion	0.48351	0.712137	0.817082	0.890136	0.921782	0.945384	0.959299	0.973181	0.981003	0.987716
Random 1	Standard deviation	1.825376	1.685571	1.432647	1.367597	1.299224	1.178038	1.117576	1.076661	0.982413	0.963359
	Proportion of Variance	0.158667	0.135293	0.097737	0.089063	0.08038	0.066084	0.059475	0.0552	0.045959	0.044193
	Cumulative proportion	0.158667	0.293959	0.391696	0.480759	0.561139	0.627224	0.686699	0.741899	0.787858	0.832051
Random 2	Standard deviation	1.761795	1.694014	1.517425	1.505572	1.396044	1.251766	1.2082	1.127558	0.982293	0.841374
	Proportion of Variance	0.147806	0.136652	0.109647	0.10794	0.092807	0.074615	0.069512	0.060542	0.045948	0.03371
	Cumulative proportion	0.147806	0.284457	0.394104	0.502044	0.594851	0.669466	0.738978	0.79952	0.845468	0.879178
Random 3	Standard deviation	1.766231	1.706286	1.560464	1.490701	1.442962	1.233601	1.143874	1.035995	0.947091	0.845782
	Proportion of Variance	0.148551	0.138639	0.115955	0.105819	0.09915	0.072465	0.062307	0.051109	0.042713	0.034064
	Cumulative proportion	0.148551	0.28719	0.403144	0.508963	0.608113	0.680578	0.742885	0.793994	0.836707	0.870771

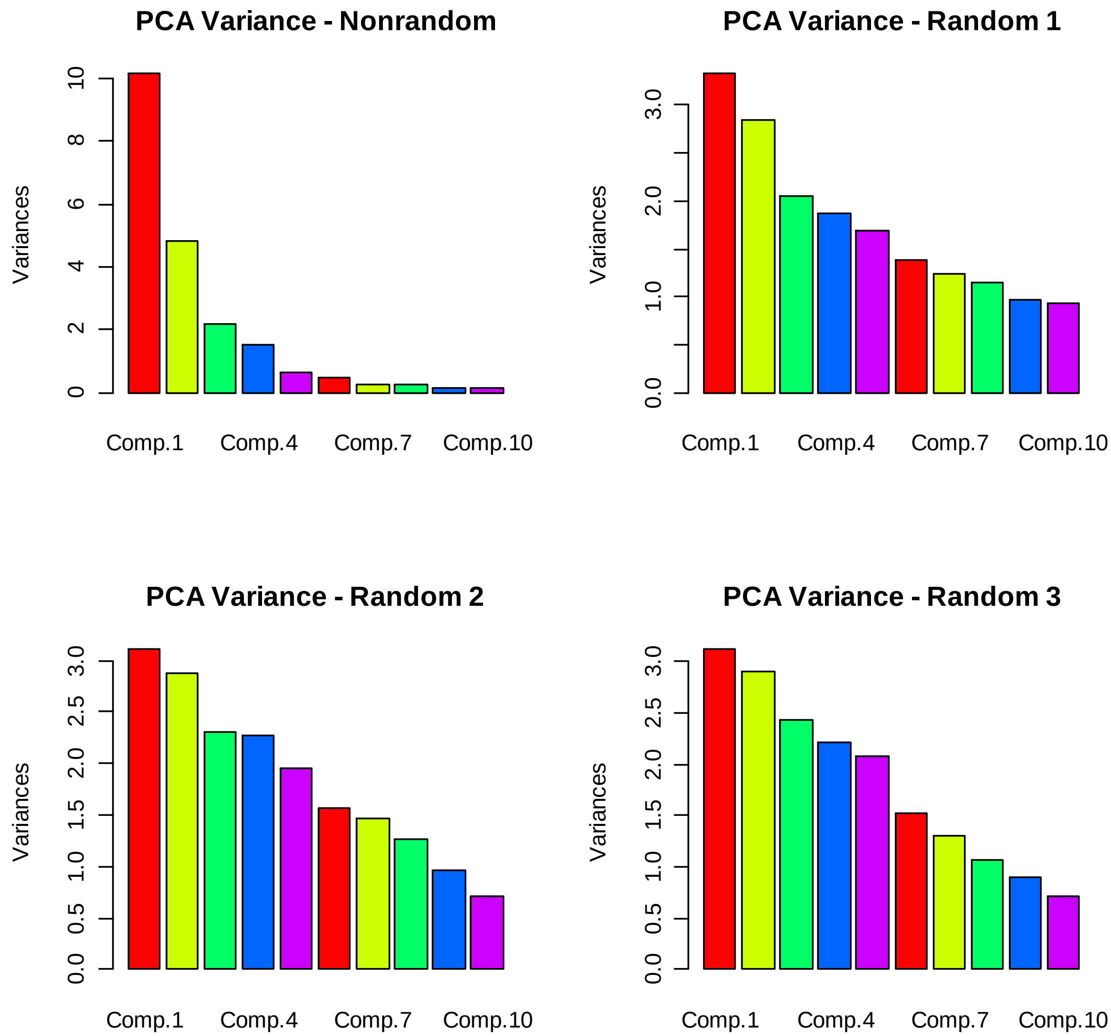

Figure 121. Principal component analysis (PCA) variance of simulated effluent samples with respect to principal component for nonrandom and random data files.

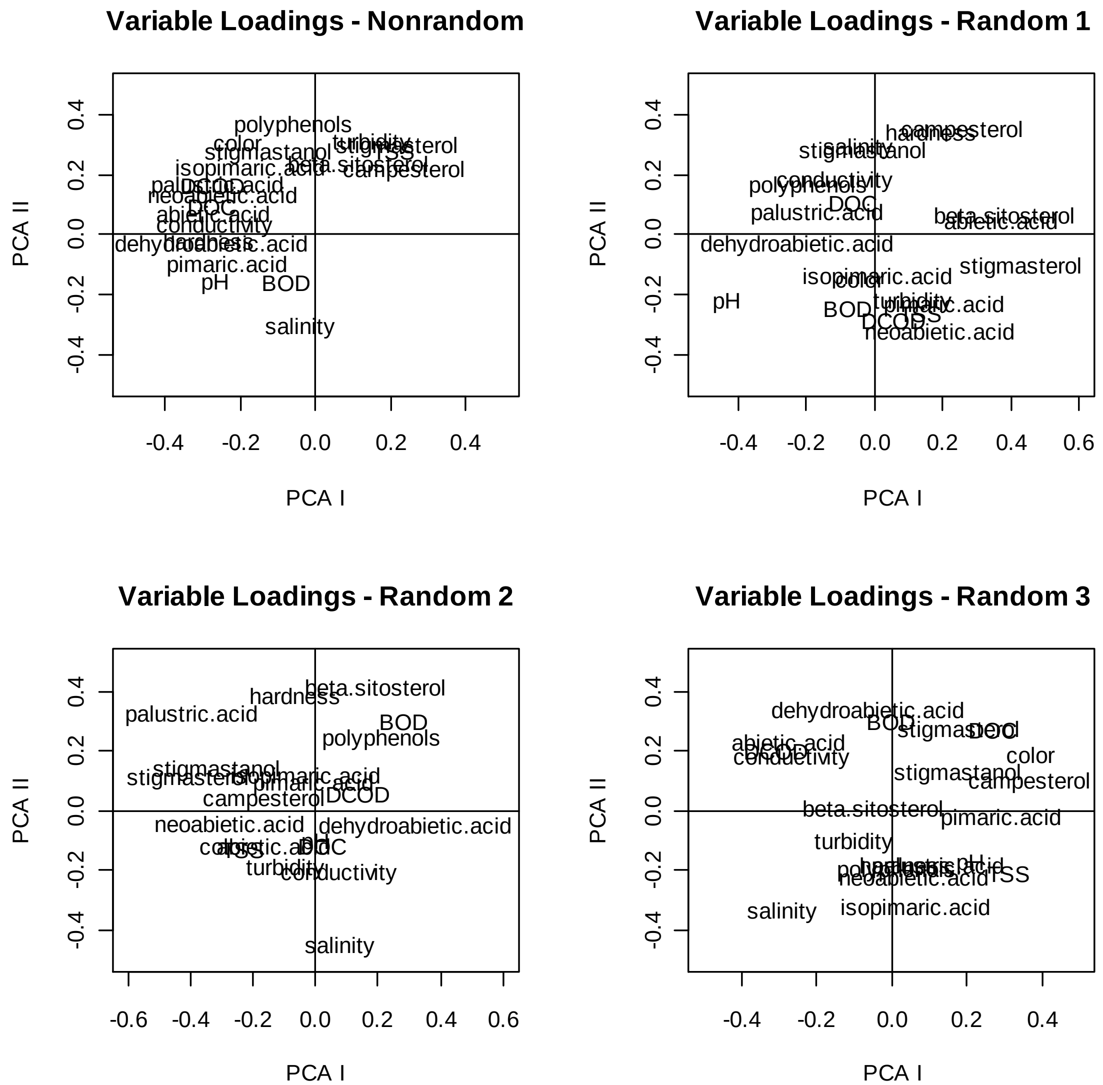

Figure 122. Principal component analysis (PCA) variable loading of simulated effluent samples for first two principal components for nonrandom and random data files.

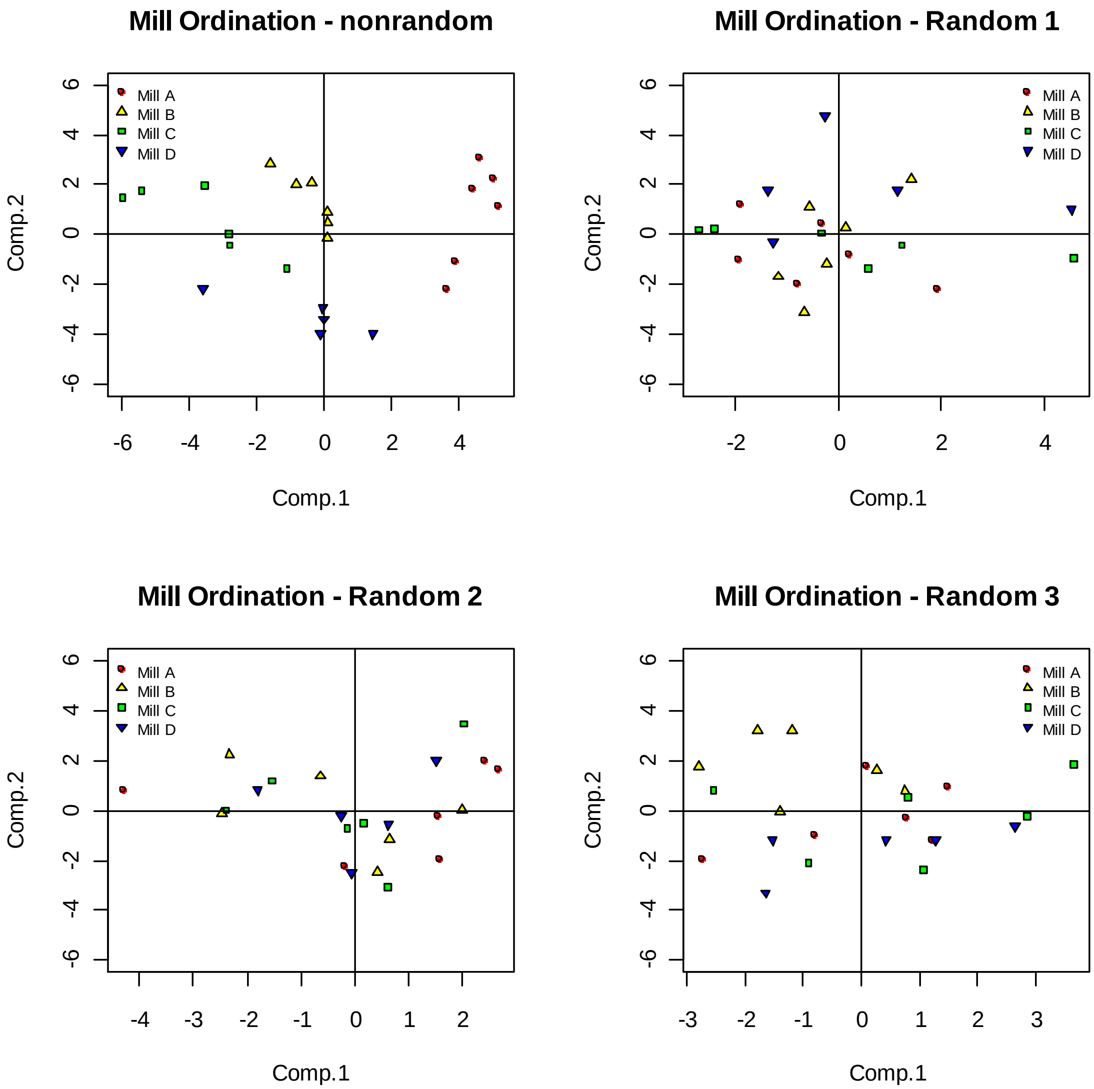

Figure 123. Principal component analysis (PCA) ordination of simulated effluent samples for nonrandom and random data files. Samples coded to denote mill.