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REPRODUCTION RESPONSES OF ADULT FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO PULP OR PAPER MILL EFFLUENTS DURING SPAWNING

TECHNICAL BULLETIN NO. 762

AUGUST 1998

Acknowledgments

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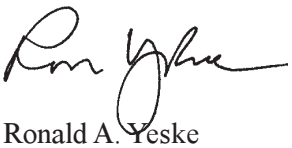
PRESIDENT'S NOTE

Several recent studies in the U.S., Canada, and Scandinavian countries have raised concerns about the reproductive fitness of fish exposed to effluents from pulp and paper manufacturing facilities. NCASI, using a fathead minnow (*Pimephales promelas*) model, chiefly with full life-cycle (20- to 24-week) exposures, has shown via extensive laboratory studies that such effects should not be expected to occur at concentrations typical of in-stream concentrations of effluents in the United States. Nonetheless, NCASI's Aquatic Biology Program has placed additional emphasis on examining the compatibility of forest products manufacturing effluents with fish reproduction.

NCASI and the American Forest & Paper Association sponsored a workshop in June of 1997, attended by industry, regulatory agency, and academic scientists to aid in the development of a strategy for addressing the fish reproduction issues. Two of the conclusions deriving from this workshop were that: (1) the fathead minnow is a useful model for assessing effects of mill effluents on fish reproduction; and (2) a short-term assay, for example, reflective of the endpoints observed during full life-cycle exposures of fathead minnows to pulp mill effluents, is desirable and probably necessary to enable testing of process streams and components which may give rise to the effects observed in the high concentration effluent exposures of fathead minnows in full life-cycle testing.

The investigation described in this bulletin was designed to examine the utility of a short-term test based upon use of the fathead minnow, but exposing fish to effluents only during the relatively short (20- to 24-day) spawning period. Effluents from four mills that were previously used in fathead minnow full life-cycle tests were reevaluated in an abbreviated exposure during the spawning period. Chemical analyses and short-term bioassay test responses evaluated during the previous testing were also re-performed to assure that these effluent samples were similar to those used in the earlier life-cycle tests. Results of the chemical analyses and short-term bioassays indicated the effluents were expected to have effects on fish reproduction that were similar to or possibly greater than the previous life-cycle tests. However, the 30% to 100% v/v effluent concentrations, which caused a 50% reduction in eggs produced per female in previous tests, did not reduce egg production in this abbreviated exposure during the spawning phase of the life cycle. Thus, this abbreviated method was not predictive of life-cycle responses and will probably not be useful as a short-term test. These tests also indicate that short duration exposures of fish to effluent during the spawning phase of the life cycle are unlikely to reduce reproduction. This information is important in understanding effects which have been reported in wild fish, and may be useful in developing mechanism-based assays predictive of effects on fish reproduction.

Additional short-term tests are being performed with these samples of effluents. Similar to the comparisons reported in this bulletin, the results of those methods will be compared to the previous life-cycle tests, and their predictive capability will be evaluated. Additional technical bulletins describing those results are anticipated as other short-term tests are evaluated.



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August 1998

REPRODUCTION RESPONSES OF ADULT FATHEAD MINNOWS (*Pimephales promelas)* EXPOSED TO PULP OR PAPER MILL EFFLUENTS DURING SPAWNING

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ABSTRACT

Recent laboratory investigations have indicated that pulp mill effluents at high concentrations may alter reproductive responses of fish. Short-term tests that accurately predict reproduction responses of fish or other aquatic organisms during life-cycle exposures would be valuable tools, allowing more effluents, individual compounds, or treatment options to be evaluated. The results of an investigation of an abbreviated exposure of fish to effluents during the spawning period of their life cycles are described in this bulletin. A fathead minnow (*Pimephales promelas*) abbreviated life-cycle bioassay, a *Ceriodaphnia dubia* 7-d bioassay, a marine bivalve embryo/larval test, an echinoderm sperm/egg bioassay, and chemical characterization were completed with four pulp or paper mill effluents. Three of the effluents were from kraft pulp mills with (1) OD+ECF bleaching, (2) ECF bleaching, and (3) no bleaching; the fourth effluent was from a deinking recycle fiber mill. The results of the *C. dubia* 7-d tests, bivalve test, echinoderm test, and chemical characteristics were compared to earlier investigations with effluents from the same mill to evaluate changes in effluent quality between the two testing periods. Based on these measurements, similar biological and chemical characteristics were found for Mill B, Mill C, and Mill D for the two testing periods. However, based on both the biological and chemical characteristics, effluent from Mill A exhibited some degradation compared to earlier testing when life-cycle studies were completed. This mill was undergoing process changes which probably altered the effluent quality compared to earlier testing.

During the abbreviated fathead minnow reproduction tests, all fish were maintained as controls throughout their maturation and first three weeks of spawning to provide a reproductive history. Three of these five spawning groups were then exposed to an effluent concentration that had reduced egg production by 50% during earlier life-cycle testing. Another spawning group was exposed to 100% v/v of effluent from the recycle mill which had not produced adverse effects during the earlier life-cycle test. Analysis of covariance for egg production before and after treatment found no significant differences in egg production between the groups due to effluent effects. The fathead minnows from control as well as Mill A, Mill B, and Mill C effluent exposures produced more eggs during the effluent exposure period than during the preexposure period. This increase was probably due to the age of the fish and not related to the effluent. Egg production was also not significantly altered from the well water exposure to the effluent exposure period for each of the four mill effluents tested. These results indicate that (1) exposure during only a portion of the spawning period is unlikely to affect egg production or produce effects on egg production similar to life-cycle exposures, and (2) this approach is not a reliable predictor of life-cycle fathead minnow egg production results.

KEYWORDS

OD, ECF, unbleached, recycled, deink, bleached kraft pulp, paper mill effluent, biological treatment, fathead minnow, *Pimephales*, *Ceriodaphnia*, bioassay, reproduction, echinoderm, bivalve

RELATED NCASI PUBLICATIONS

Technical Bulletin No. 475 (December 1985). *Effects of biologically-treated bleached kraft mill effluent during early life-stage and full life-cycle studies with fish.*

Technical Bulletin No. 722 (August 1996). *Effects of biologically-treated bleached kraft mill effluent on the early life-stage and life-cycle of fathead minnow (Pimephales promelas) and Ceriodaphnia dubia: A comparison before and after conversion to oxygen delignification and ECF bleaching.*

Technical Bulletin No. 732 (March 1997). *Effects of biologically-treated elemental chlorine free bleached kraft mill effluent on early life-stages and life-cycles of the fathead minnow (Pimephales promelas) and Ceriodaphnia dubia.*

Technical Bulletin No. 756 (1998). *Effects of biologically-treated unbleached kraft pulp mill effluent on early life stages and life cycles of fathead minnow (Pimephales promelas) and Ceriodaphnia dubia*.

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REPRODUCTION RESPONSES OF ADULT FATHEAD MINNOWS (*Pimephales promelas)* EXPOSED TO PULP OR PAPER MILL EFFLUENT DURING SPAWNING

1.0 INTRODUCTION

Recently, concerns about the compatibility of pulp mill effluents with the quality of aquatic communities have focused on fish reproduction effects (NCASI 1985, 1996, 1997, 1998a, 1998b, 1998c; Kovacs et al. 1995a, 1995b, Kovacs et al. 1996; Robinson 1994; Owens 1991; Adams et al. 1992; Bortone and Davis 1994; Munkittrick et al. 1991, 1992, 1994; McMaster et al. 1991, 1992; Förlin et al. 1991; Sandström 1994; Swanson et al. 1992). Frequently, investigations addressing these concerns have used indicators of reproductive processes to measure potential effects on fish reproduction (Owens 1991; Adams et al. 1992; Bortone and Davis 1994; Munkittrick et al. 1991, 1992, 1994; McMaster et al. 1991; Förlin et al. 1991; Sandström 1994; Swanson et al. 1992), but a few studies have measured egg production and larval survival of fish during controlled exposures to pulp mill effluents (NCASI 1985, 1996, 1997a, 1998a, 1998b,1998c; Kovacs et al. 1995a, 1995b; Kovacs et al. 1996; Robinson 1994). These investigators used fathead minnow life-cycle tests to determine effects on pulp mill effluents because laboratory methods were readily available (Benoit 1982) or because this test had frequently been used as a chronic test to develop water quality criteria by USEPA (USEPA 1986). Life-cycle tests with the fathead minnow require approximately 20 to 24 weeks to complete or longer if USEPA methods are closely followed (Benoit 1982), which contributes to the low number of studies completed. Thus, there is a need for an abbreviated test that is predictive of life-cycle results while reducing the time required to produce reliable results.

The primary objectives of the research reported in this bulletin were (1) to determine if reproduction of adult fathead minnows would be altered by 20–24 days of exposure to pulp or paper mill effluents during the spawning period, (2) to determine if 20–24 days of exposure produces similar results to those found in previous life-cycle tests with these effluents, (NCASI 1996, 1997, 1998b, 1998c), and (3) to determine if this type of exposure could be used as an abbreviated test to predict the effects of effluents, effluent components, or other compounds on fish reproduction. Effluents used for the fathead minnow studies described in this bulletin were also evaluated with *Ceriodaphnia* 7-d reproduction tests (USEPA 1989), marine bivalve larval development tests (Cherr et al 1990; NCASI 1992b), and echinoderm sperm/egg tests (Cherr et al. 1987; NCASI 1992a). Results from these tests allow comparisons of biological endpoints of the effluents used in these investigations with effluents from the same mills used in earlier life-cycle tests (NCASI 1996, 1997, 1998b, 1998c). Concentrations of chemical components in the effluents were also measured to allow comparisons with effluents from the same mills used in earlier testing. The use of the bioassays and chemical analyses allowed greater assurance that the effluent samples used in the investigation reported herein remained similar to effluent samples used in the earlier life-cycle tests (NCASI 1996, 1997, 1998b, 1998c).

This report describes the results of exposures of adult fathead minnows to effluents from (1) a kraft mill with OD + ECF bleaching, (2) a kraft mill with ECF bleaching, (3) an unbleached kraft mill, and (4) a deinking recycle mill, during 20–24 days of the spawning period. The results of one *Ceriodaphnia dubia* 7-d survival and reproduction bioassay, one marine bivalve larval development bioassay, and one marine echinoderm sperm/egg fertilization test with effluent from each of the four mills, as well as chemical characterization of each effluent are also reported. The fathead minnow reproductive response, other bioassays, and chemical concentrations are compared to similar responses or measurements from previous tests with effluents from the same mills.

2.0 TEST FACILITY AND EXPERIMENTAL METHODS

2.1 Facility Description and Dilution Water

All fathead minnow egg production tests were performed in a mobile bioassay laboratory. The laboratory was equipped with a proportional solenoid valve diluter (USEPA 1978), and exposure tanks. Temperature in the laboratory was maintained at 25±2°C at all times. Dissolved oxygen concentrations of the exposure tanks were maintained above 6 mg/L by an aeration blower equipped with a pressure switch and backup. Light levels in the lab were maintained between 90–100 ft-candles by banks of fluorescent lights equipped with broad spectrum bulbs (Durotest Vitalight). An electrical timer controlled the photoperiod to 16 hours of light and 8 hours of darkness per day.

The dilution water for the fathead minnow tests was provided by a 50-m deep well located at the site. Characteristics of the well water are shown in Table 1. Metals analyses of the well water from an earlier study using the same well head are reported in NCASI Technical Bulletin No. 722. The well water was pumped from a 6000-L polyethylene outdoor holding tank equipped with level control to an 850-L polyethylene holding tank inside the mobile laboratory, where the water was aerated and heated to the desired test temperature. The holding tank was equipped with a float switch, which provided a continuous supply of new well water to the tank as needed. Diluent was pumped to the proportional diluter through PVC tubing connected to a centrifugal pump.

Table 1. Characteristics of the Dilution Water for Fathead Minnow Tests with Four Mill Effluents

Parameter	N[a]	Units	Average (SD)
pH	12	units	8.3 (0.2)[b]
Conductivity	12	µmhos/cm	359 (11)
Hardness	3	mg/L	148 (28)
Alkalinity	3	mg/L	197 (4)

[a] number of samples
[b] standard deviation

2.2 Effluent Sources

Effluents from two bleached kraft pulp mills, one unbleached kraft pulp mill, and one deinking recycle mill were used during these studies. The mills have been designated as Mill A, Mill B, Mill C, and Mill D respectively in this bulletin.

Table 2. Description of Mills Providing Effluent

Mill Type/ Bleaching	Mill Designation/ Location	Effluent Treatment	Wood Furnish	Water Use M^3/ADMT
Bleached Kraft O-W-D-Eop-[a] W-D-Eo D	Mill A Southeast USA	Clarifier 14-d ASB	25% Hardwood 75% Pines	150
Bleached Kraft D-Eop-D-Ep-D	Mill B Southeast USA	Clarifier 5-d ASB	10% Hardwood 90% Pines	60
Unbleached Kraft	Mill C Southeast USA	2 Clarifiers 30-d ASB	100% Pines	48
Recycled Deink	Mill D Southeast USA	2 Clarifiers 20-d ASB	Recycled Paper	68

[a] O - oxygen delignification, W - wash, Eop - extraction with oxygen and peroxide, D - chlorine dioxide

Mill A produced bleached kraft fluff market pulp with 86–90% brightness. Major fiber processing equipment consisted of eight batch digesters, a medium consistency oxygen delignification system, and a bleach plant. The bleaching sequence of Mill A was O-W-D-Eop-W-D-E_O-D (O-oxygen delignification, W-washing stage, D-chlorine dioxide, E-extraction, o-oxygen, and p-peroxide) (Table 2). The mill produced an average of about 700 air dried metric tons (ADMT) of pulp per day from a furnish consisting of 20% hardwoods and 80% softwoods. Water use was approximately 150 M^3/ADMT of pulp produced. Effluent from the bleach plant combined with primary clarifier effluent consisting of the alkaline sewer from the kraft pulping process before entering the first aerated stabilization basin (ASB) for secondary treatment. The effluent then flowed to polishing and settling basins for completion of the normal 12 to 14-d biological treatment.

Mill B produced approximately 2200 ADMT/d of bleached kraft pulp with 87–89% brightness from a furnish of 10% hardwoods and 90% pines. Water use was approximately 60 M^3/ADMT of pulp produced. Fluff pulp (60%) and market pulp (17%) were produced from the softwood, and the remaining pulp (23%) was used to produce bleached board. Major fiber processing equipment consisted of 19 digesters and three bleach plants, all of which used complete ClO_2 substitution (ECF) for Cl_2 during the bleaching process. The bleaching sequence was D-Eop-D-Ep-D. The acid sewer and acid stage of the bleach plants combined with primary clarifier effluent in a presettling basin. After completion of the normal 5- to 6-d biological treatment, 33 mgd of effluent exited the treatment basin.

Mill C was a kraft mill which produced approximately 1450 ADMT/d of unbleached market pulp from a furnish of 100% pines. Water use was 48 M^3/ADMT of pulp produced. The 18 mgd of effluent was initially treated in an aerated stabilization basin and then held in a larger basin for a total of approximately 30 days of treatment.

Mill D produced tissue and towel products on four paper machines from deinked pulp, which was produced entirely on-site from 100% recovered paper. The mill produced 800-900 ADMT/d of paper with water use ranging from 64 to 72 M^3/ADMT. Effluent secondary treatment was accomplished with activated sludge accompanied by primary and secondary clarification.

Effluent samples used for biological testing were obtained near the final discharge at each of the mill sites. A sample of approximately 6000 liters of each of the four mill effluents were transported to the testing site, and was stored in polyethylene storage tanks for the duration of the tests. Effluent used in fathead minnow tests were pumped from the storage tanks into 200 L polyethylene tanks in the laboratory where the effluent was aerated and the temperature was adjusted to 25±0.2°C. The holding tanks were equipped with stainless steel float switches which allowed new effluent to enter the lab as it was used. Subsamples for *C. dubia*, marine bivalve, and echinoderm bioassays were collected in 3.8-L glass bottles as each of the four effluents arrived.

Table 3. Chronology of Investigations

Date	Test	Comment
Feb. 97	Juvenile fathead minnows (≈56 days old) were moved to the laboratory diluter system	From laboratory well water cultures
Mar. 97	Fathead minnows (≈70 days old) were divided among the spawning chambers	Exposed to well water only
Apr. 97	Fathead minnow egg production	Exposed to well water only for 20-24 days
May 97	Fathead minnow egg production *C. dubia* 7-d reproduction and survival test Marine bivalve and echinoderm test	Fish exposed to effluent (17-21 days)

2.3 Effluent Characterization

2.3.1 *Chemical Characterization*

Results of analyses for biochemical oxygen demand (BOD) and total suspended solids (TSS) of each effluent were provided by the mill environmental laboratory personnel using methods described in Standard Methods (APHA et al. 1992). The color of the effluent was measured using NCASI (1971) methods. One grab sample of each effluent was collected when the sample arrived and was preserved for organic chemical analyses. Analyses were carried out at the NCASI West Coast Regional Center for chlorinated phenolic compounds, resin acids, and fatty acids (NCASI 1981a, 1981b, 1986). The alkalinity and hardness were determined using the standard titration method (APHA et al. 1992).

2.3.2 *Ceriodaphnia dubia 7-d Survival and Reproduction Bioassay*

C. dubia tests were initiated with neonates <24 hr old and produced within an 8-hr time period from ten randomly selected pretest culture organisms. Diluent and culture media were EPA moderately hard water. Chronic testing followed procedures described in USEPA/600/4-89/001 (1989), (Table 3).

Neonates were individually placed in 30-ml glass beakers with 15 ml of test solution and 0.5 ml of NCASI food mixture in a random block design. There were four replicate beakers per concentration. All beakers were placed in a styrofoam box with a glass top to limit temperature variation and evaporation. Temperature was maintained at 25±1° C inside an environmental chamber. Original individuals were moved into clean test beakers containing new media and food each day when adult survival and numbers of young produced were recorded. Water chemistry was monitored at the

beginning of the test on the initial samples and the following day on the 24-hr old test solutions. Concentrations tested were 0, 10, 30, and 100% v/v of effluent.

Table 4. Summary of *C. dubia* 7-d Bioassay Test Conditions

	Parameter		Test Condition
1.	Temperature	:	25±1° C
2.	Photoperiod	:	16 hr light/8 hr dark
3.	Test vessel	:	30-ml glass beaker
4.	Volume test solution	:	15 ml
5.	Renewal	:	daily
6.	Age at start	:	8 hr age span, <24 hr old
7.	Number/beaker	:	1
8.	Replicates/conc.	:	4
9.	Concentrations	:	0, 10, 30, 100% v/v
10.	Food[a]	:	0.5ml / 15-ml test solution
11.	Food mixture[b]	:	1 part *Selenastrum capricornumtum* concentrate 62 x 10^6 cells/ml 2 parts YTC (yeast, fermented trout chow, cerophyll) 4 parts extra fermented trout chow
12.	Diluent water	:	EPA moderately hard water
13.	End points	:	7 days/3 broods in the controls
14.	Valid test criteria	:	control survival ≥80% average ≥20 young/adult in controls

[a] deviation from standard methods

[b] YTC and *Selenastrum* concentrate was mixed before feeding

2.3.3 *Bivalve and Echinoderm Marine Bioassays*

Marine bioassays with each of the four mill effluents were carried out by personnel at the NCASI West Coast Aquatic Biology Research Station in Anacortes, Washington. The bivalve embryo/larval test with the blue mussel (*Mytilus edulis*) or the pacific oyster (*Crassostrea gigas*) and the echinoderm sperm/egg bioassay with the purple sea urchin (*Strongylocentrotus purpuratus*) or the sand dollar (*Dendraster excentricus*) were based on Cherr *et al.* (1987) with NCASI modifications (NCASI 1992a, 1992b).

The bivalve embryo/larval bioassay involved the exposure of freshly-fertilized bivalve eggs to a series of effluent dilutions (0, 0.3, 1.0, 3.0 10.0, 30.0, and 70% v/v) for a period of time (72 hr) sufficient to allow development to the first shelled stage (veliger or prodissoconch I larvae). Test results were determined based on the number of larvae developing normal shells in the effluent treatments as compared to controls (NCASI 1992b).

Sperm and eggs from echinoderms were used with sperm exposed effluent for a 10-min period followed by a 10-min period egg exposure. The bioassay was scored based on fertilization success determined with a light microscope. Test concentrations were 0.0, 0.3, 1.0, 3.0, 10.0, 30.0, and 70.0% v/v effluent (NCASI 1992a).

Testing of mill effluents, which are typically low in salinity, required the use of hypersaline brine to adjust test solutions to uniform salinity (30 g/kg). Seventy percent was the highest effluent concentration achievable using 100 g/kg brine.

2.4 Fathead Minnow Adult Exposures

The exposure system for the fathead minnow tests consisted of fourteen 61-cm x 31-cm glass aquaria which received well water or effluent mixtures from a proportional solenoid valve diluter (USEPA 1978). The diluter was modified with a solenoid switch to discharge the entire volume of the effluent and diluent mixture of each test concentration alternately into replicate test chambers. Flow rates to each test aquaria averaged approximately 1000ml /5min. cycle. Replicate A and B test chambers were randomly selected for exposure to effluent concentrations. Light intensity at the water surface of each aquaria was maintained at 110 to 150 ft-c using broad spectrum fluorescent lights mounted over the tanks. Dissolved oxygen in each test chamber was maintained above 6 mg/L using an oil-free blower and airstones.

Juvenile fathead minnows (≈56days old) were moved from well water cultures to test aquaria to begin these studies. The fish were held in well water as two groups in replicate aquaria designated as A and B. When males began to show spawning coloration, one male and two females were placed in each of four spawning chambers in each aquaria. Since each concentration had replicate aquaria, a total of eight spawning groups were exposed to each effluent. Spawning fish were fed 3 ml/chamber of a 250 g/L frozen brine shrimp slurry twice daily. An 8-cm half section of 10-cm diameter stainless steel pipe was placed in each chamber as spawning substrate. The number of eggs and spawns along with mortality were recorded daily throughout the spawning period.

Initially all fish moved to the aquaria were exposed only to well water until the spawning groups had produced eggs for 20–24 days. Thus, fish in all tanks were treated as controls before exposure of the fish to effluent began. As effluent from the four mills arrived at the test site, the spawning groups were exposed to the appropriate effluent concentration. The numbers of eggs spawned and adult mortality were recorded daily. Spawning fish were exposed to the mill effluent until the effluent in the holding tank was depleted (17–21 days), at which time the test was terminated.

Only one concentration of each effluent was tested during the effluent exposure phase of the study. One set of A & B replicate tanks received 100% v/v of effluent from Mill A (OD + ECF), another received 30% v/v of effluent from Mill B (ECF), another received 50% v/v of effluent from Mill C (unbleached), and a fourth set of tanks received 100% v/v of effluent from Mill D (recycled deink). The concentrations of Mill A, Mill B, and Mill C effluents were approximately the IC50 (50% reduction from controls) calculated for egg production during the earlier life-cycle tests with effluents from these mills. Mill D effluent had no effect on fathead minnow reproduction during the earlier life-cycle test, thus fish were exposed to 100% of the effluent from Mill D.

2.5 Statistical Evaluations

C. dubia life-cycle data were analyzed for differences in survival among groups using Fisher's Exact Test and the reproduction data were analyzed using both Dunnett's (parametric) and Steel's (nonparametric) tests (Steel and Torrie 1980; Rodgers 1986). The Bootstrap program (Efron 1982) was used to calculate the IC25 and 95% confidence interval for both survival and reproduction (Gulley et al. 1988).

Marine bioassay endpoints were calculated using the methods suggested by the U.S. EPA (1988), including Dunnett's test and the Bootstrap method for determining the 25% inhibition response level (IC25). For the echinoderm test, Abbott's correction was used to standardize responses to 100%

fertilization in the controls (USEPA 1988). The NOEC and IC25 response endpoints were calculated using a computer program (Tidepool 1993).

Fathead minnow egg production of most of the groups including the controls increased during the exposure period, thus egg production results were compared before and after effluent exposure by analysis of covariance (Dataxiom 1997). Additional testing of means for egg production with students t test was performed if analysis of covariance indicated means could be significantly different.

3.0 RESULTS

3.1 Comparisons of Effluent Characterization Results with Previous Life-Cycle Testing

Summaries of characteristics measured in the four effluent samples used for these tests are presented in Tables 5, 6, 7, and 8. The concentrations of 61 individual compounds in each sample of effluent are provided in Appendix A.

3.1.1 *Comparison of Mill A Chemical and Biological Parameters to Previous Life-Cycle Tests*

The amounts of TOC, BOD, TSS, COD, conductivity, aromatic acids, chlorinated resin acids, and phytosterols in Mill A effluent during this study were more than 1 standard deviation higher than the average of the same components measured during the previous life-cycle test period (Table 5). No component was reduced by more than one standard deviation. Thus, chemical concentrations found in Mill A effluent used in this study were nearly always greater than average chemical concentrations found during the previous life-cycle testing period. Two of the three biological tests also indicated increases in the biological effects of this effluent, compared to earlier testing, that were greater than 1 standard deviation. The IC25 for *C. dubia* reproduction averaged 35% v/v of effluent for four tests during the previous life-cycle testing period, however, during this study the IC25 for *C. dubia* reproduction was reduced to 7% v/v of effluent (Table 5). Four marine bivalve bioassays from the previous life-cycle studies had an average IC25 for larval development of 9.7% effluent but the IC25 for bivalve larval development was 4.8 % v/v of effluent used in this study. Only the echinoderm egg fertilization test demonstrated decreased effects compared to the previous study. Four echinoderm fertilization tests during the previous study had an average IC25 of 7.8% effluent, and during this study, one test had an IC25 of 17.1% v/v of effluent, indicating less effect of the effluent used in this study. Generally, both the concentrations of chemical components found in this effluent sample from Mill A and bioassay responses suggest that this effluent should have been at least as potent in producing biological effects as during earlier testing (NCASI 1996) and had the potential to produce greater effects than found in previous life-cycle testing.

3.1.2 *Comparison of Mill B Chemical and Biological Parameters to Previous Life-Cycle Tests*

Most of the chemical components of the effluent sample from Mill B (ECF) (Table 6) used in this study had similar or reduced concentrations compared to those found during the previous life-cycle study (NCASI 1997). BOD and chloraldehydes were more than one standard deviation greater in the effluents used from Mill B compared to earlier studies, but conductivity, color and fatty acids were more than one standard deviation less than the mean measured in earlier studies. The IC25 for reproduction from four *C. dubia* bioassays averaged 50% v/v of effluent during previous life-cycle testing and one test during this study had an IC25 48% v/v of effluent. Marine bivalve tests during the previous life-cycle study with effluent from Mill B had an average IC25 from two tests of 4.8% v/v of effluent and during this study the IC25 was 9.9% v/v of the ECF BKME. Marine echinoderm bioassay IC25 from four tests during the previous study averaged 19.8% v/v of effluent and during this study the IC25 from one test was 9.2% v/v of effluent. The chemical concentrations and

biological responses of the effluent sample used during this study indicates the biological effects are not expected to be greatly altered compared to effluent from Mill B used in earlier studies (NCASI 1997).

Table 5. Comparison of Effluent Quality Parameters from Mill A (OD+ECF) to Previous Studies

		Previous study			This study	
Parameter/Compound	Units	N[a]	Mean Concentration	(SD)[b]	N	Concentration (SD)
AOX	(mg/L)	29	1.3	(0.3)	1	1.5
TOC	(mg/L)	25	76.3	(9.9)	1	133
BOD	(mg/L)	445	14.7	(5.6)	1	46
COD	(mg/L)	8	337	(27)	1	455
Color	(color units)	8	614	(272)	1	726
Conductivity	(µmhos/cm)	247	1593	(150)	1	2130
Tannin/Lignin	(mg/L)	4	31.8	(7.4)	1	39
TSS	(mg/L)	56	11.1	(5.5)	1	47.5
Phenols[d]	(µg/L)	56	ND[c][2.2]		1	ND[2.2]
Chlorinated phenols[e]	(µg/L)	56	ND[2.1]		1	ND[2.1]
Chlorinated guaiacols[f]	(µg/L)	56	ND[2.0]		1	ND[2.0]
Chlorinated catechols[g]	(µg/L)	56	ND[2.0]		1	ND[2.0]
Chloraldehydes[h]	(µg/L)	56	ND[2.0]		1	ND[2.0]
Aromatic acids[i]	(µg/L)	56	ND[1.5]		1	1.8
Fatty acids[j]	(µg/L)	56	3.5	(3.8)	1	6.2
Resin acids[k]	(µg/L)	56	65.7	(82.1)	1	34
Chlorinated resin acids[l]	(µg/L)	56	ND[0.7]		1	1.3
Phytosterols[n]	(µg/L)	17[m]	67	(48)	1	198
Ceriodaphnia dubia	(IC25)[o]	4	35	(18)	1	7
Marine Bivalve	(IC25)	3	9.7	(4.6)	1	4.8
Marine Echinoderm	(IC25)	4	7.8	(7.6)	1	17.1

[a] number of samples
[b] standard deviation
[c] not detected [detection limit]
[d] total of 6 phenols
[e] total of 7 chlorinated phenols
[f] total of 10 chlorinated guaiacols
[g] total of 9 chlorinated catechols
[h] total of 9 chloraldehydes
[i] total of 3 aromatic acids
[j] total of 3 fatty acids
[k] total of 7 resin acids
[l] total of 3 chlorinated resin acids
[m] Samples within 6 months of life-cycle test
[n] total of 4 phytosterols
[o] 25% reduction from the control as % v/v effluent

Table 6. Comparison of Effluent Quality Parameters from Mill B (ECF) to Previous Studies

		Previous study			This study	
Parameter/Compound	Units	N[a]	Mean Concentration	(SD)[b]	N	Concentration (SD)
AOX	(mg/L)	27	5.1	(0.6)	1	5.3
TOC	(mg/L)	27	219	(22.7)	1	170
BOD	(mg/L)	185	9.5	(3.0)	1	19
COD	(mg/L)	185	691	(138)	1	558
Color	(color units)	4	1330	(64)	1	1095
Conductivity	(µmhos/cm)	52	5028	(817)	1	5310
Tannin/Lignin	(mg/L)	4	53.5	(8.2)	1	46
TSS	(mg/L)	185	12.4	(3.2)	1	12.8
Phenols[d]	(µg/L)	27	4.2	(9.1)	1	ND[2.2]
Chlorinated phenols[e]	(µg/L)	27	ND[c][2.1]		1	ND[2.1]
Chlorinated guaiacols[f]	(µg/L)	27	ND[2.0]		1	ND[2.0]
Chlorinated catechols[g]	(µg/L)	27	ND[2.0]		1	ND[2.0]
Chloraldehydes[h]	(µg/L)	27	2.7	(3.1)	1	6.7
Aromatic acids[i]	(µg/L)	27	4.2	(5.3)	1	1.6
Fatty acids[j]	(µg/L)	27	26.3	(24.2)	1	ND[1.0]
Resin acids[k]	(µg/L)	27	114.1	(148.3)	1	60.5
Chlorinated resin acids[l]	(µg/L)	27	1.4	(1.4)	1	ND[0.7]
Phytosterols[m]	(µg/L)	19	150.8	(108)	1	44.0
Ceriodaphnia dubia	(IC25)[n]	4	50	(17)	1	48
Bivalve Bioassay	(IC25)	2	4.8	(0.3)	1	9.9
Echinoderm Bioassay	(IC25)	4	19.8	(12.8)	1	9.2

[a] number of samples
[b] standard deviation
[c] not detected [detection limit]
[d] total of 6 phenols
[e] total of 7 chlorinated phenols
[f] total of 10 chlorinated guaiacols
[g] total of 9 chlorinated catechols
[h] total of 9 chloraldehydes
[i] total of 3 aromatic acids
[j] total of 3 fatty acids
[k] total of 7 resin acids
[l] total of 3 chlorinated resin acids
[m] total of 4 phytosterols
[n] 25% reduction from the control as % v/v effluent

3.1.3 *Comparison of Mill C Chemical and Biological Parameters to Previous Life-Cycle Tests*

Chemical characteristics of the effluent sample from Mill C (unbleached) were usually similar to or lower than those found during earlier life-cycle studies (Table 7). Several chlorinated compounds were not measured but would not be expected in an unbleached kraft mill. Concentrations of BOD, TSS, and conductivity were very similar between the two studies, but effluent color, COD, and phytosterols were more than one standard deviation higher in the effluent used in this study, while TOC, fatty acids and resin acids were more than one standard deviation less than the earlier life-cycle study (Table 7). The three biological tests show similar or slightly greater effects than during the earlier testing. Three *C. dubia* reproduction IC25s from the previous study averaged 88% v/v of effluent while during this study the IC25 for reproduction of one test was 58% v/v of effluent which was greater than one standard deviation lower (greater effect) than during earlier testing. The IC25 for larval development from one marine bioassay during this study was nearly identical to the IC25 for this bioassay during previous life-cycle testing (14.9% and 14.3% v/v of effluent). Three echinoderm sperm/egg tests from the previous study had an average IC25 of 55.5% v/v of effluent

and one test during this study had an IC25 of 38.7% v/v of effluent. The concentrations of effluent chemical components, and biological test responses measured during this study did not consistently increase or decrease compared to those found during the earlier study, therefore biological responses similar to those found during earlier life-cycle testing were expected if this test was predictive of life-cycle responses.

3.1.4 *Comparison of Mill D Chemical and Biological Parameters to Previous Life-Cycle Tests*

Concentrations of chemical components in Mill D (recycled deink) effluent were similar to an earlier study (NCASI 1998c). The concentrations of phenols, chlorinated phenols, guaiacols, catechols, chloraldehydes, and chlorinated resin acids were not detected during both studies (Table 8). The tannin/lignin concentration was 2.2 mg/L during both studies and phytosterols averaged 3.5 µg/L during the earlier testing and was 3.0 µg/L during this test. The IC25 for *C. dubia* reproduction averaged 42% effluent for three tests during the previous study and >100% from one test during this study. Three marine bivalve tests during the previous study had an average IC25 of 64% effluent and during this study the IC25 was >70% effluent (highest concentration tested). IC25 results of the marine echinoderm sperm/egg test were >70% effluent (highest concentration tested) during both studies. Effluent chemical concentrations were similar between the two study periods, and the IC25s were similar to or somewhat greater than the same endpoints during earlier life-cycle testing. The biological and chemical measurements indicate this effluent would be expected to have similar or slightly less effects on fathead minnow egg-production than earlier test responses.

Table 7. Comparison of Effluent Quality Parameters from Mill C (Unbleached) to Previous Studies

		Previous study			This study	
Parameter/Compound	Units	N[a]	Mean Concentration	(SD)[b]	N	Concentration (SD)
AOX	(mg/L)	0	NA[m]		0	NA
TOC	(mg/L)	17	55.9	(6.3)	1	36.3
BOD	(mg/L)	118	32	(11)	1	28
COD	(mg/L)	2	192	(8.5)	1	242
Color	(color units)	4	365	(86)	1	894
Conductivity	(µmhos/cm)	46	1507	(113)	1	1471
Tannin/Lignin	(mg/L)	3	37	(4.6)	1	38
TSS	(mg/L)	118	44	(18)	1	44
Phenols[d]	(µg/L)	28	711	(926)	1	NA
Chlorinated phenols[e]	(µg/L)	28	ND[c][2.1]		1	NA
Chlorinated guaiacols[f]	(µg/L)	28	ND[2.0]		1	NA
Chlorinated catechols[g]	(µg/L)	28	ND[2.0]		1	NA
Chloraldehydes[h]	(µg/L)	28	ND[2.0]		1	NA
Aromatic acids[i]	(µg/L)	28	1.8	(3.8)	1	ND[1.5]
Fatty acids[j]	(µg/L)	28	102	(47)	1	33.3
Resin acids[k]	(µg/L)	27	1385	(363)	1	555.2
Chlorinated resin acids[l]	(µg/L)	27	ND[0.7]		1	ND[0.7]
Phytosterols[n]	(µg/L)	27	143.6	(67.7)	1	345.0
Ceriodaphnia dubia	(IC25)[o]	3	88	(11)	1	58

Bivalve Bioassay	(IC25)	1	14.9	(0)	1	14.3
Echinoderm Bioassay	(IC25)	3	55.5	(16.1)	1	38.7

[a] number of samples
[b] standard deviation
[c] not detected [detection limit]
[d] total of 6 phenols
[e] total of 7 chlorinated phenols
[f] total of 10 chlorinated guaiacols
[g] total of 9 chlorinated catechols
[h] total of 9 chloraldehydes
[i] total of 3 aromatic acids
[j] total of 3 fatty acids
[k] total of 7 resin acids
[l] total of 3 chlorinated resin acids
[m] not available
[n] total of 4 phytosterols
[o] 25% reduction from the control as % v/v effluent

Table 8. Comparison of Effluent Quality Parameters from Mill D (Recycled deink) to Previous Studies

		Previous study			This study	
			Mean			
Parameter/Compound	Units	N[a]	Concentration	(SD)[b]	N	Concentration
AOX	(mg/L)	3	1.25	(0.3)	1	1.09
TOC	(mg/L)	4	22.5	(5.2)	1	20.9
BOD	(mg/L)	210	3.7	(1.2))	1	10
COD	(mg/L)	3	73.7	(9.3)	1	84
Color	(color units)	12	71	(15)	1	46
Conductivity	(µmhos/cm)	24	1663	(109)	1	1848
Tannin/Lignin	(mg/L)	4	2.2	(0)	1	2.2
TSS	(mg/L)	210	5.3	(1.2)	1	9.5
Phenols[d]	(µg/L)	4	ND[c][2.2]		1	ND[2.2]
Chlorinated phenols[e]	(µg/L)	4	ND[2.1]		1	ND[2.1]
Chlorinated guaiacols[f]	(µg/L)	4	ND[2.0]		1	ND[2.0]
Chlorinated catechols[g]	(µg/L)	4	ND[2.0]		1	ND[2.0]
Chloraldehydes[h]	(µg/L)	4	ND[2.0]		1	ND[2.0]
Aromatic acids[i]	(µg/L)	20	1.8	(2.6)	1	1.2
Fatty acids[j]	(µg/L)	20	2.0	(2.9)	1	0.9
Resin acids[k]	(µg/L)	20	0.7	(2.8)	1	ND[1.0]
Chlorinated resin acids[l]	(µg/L)	20	ND[0.7]		1	ND[0.7]
Phytosterols[m]	(µg/L)	20	3.5	(4.4)	1	3.0
Ceriodaphnia dubia	(IC25)[n]	3	42	(43)	1	>100
Bivalve Bioassay	(IC25)	3	64	(10)	1	>70[o]
Echinoderm Bioassay	(IC25)	3	>70[o]	(0)	1	>70[o]

[a] number of samples
[b] standard deviation
[c] not detected (detection limit)
[d] total of 6 phenols
[e] total of 7 chlorinated phenols
[f] total of 10 chlorinated guaiacols
[g] total of 9 chlorinated catechols
[h] total of 9 chloraldehydes
[i] total of 3 aromatic acids
[j] total of 3 fatty acids
[k] total of 7 resin acids
[l] total of 3 chlorinated resin acids
[m] total of 4 phytosterols
[n] 25% reduction from the control as % v/v effluent
[o] highest concentration tested

3.2 Fathead Minnow Survival and Egg Production

Survival of adult males and females during effluent exposure was not significantly reduced from the survival of control fish or between before and after effluent exposure periods in any of the four kraft mill effluents tested (Table 9, Figure 1, Appendix B). Egg production increased after the first three weeks for the control and three of the four spawning groups exposed to effluent (Table 9, Figure 1, Appendix B). However, analysis of covariance (ANCOVA) did not find any slopes of the treatment regressions that were significantly different from the slope of the control exposure (Table 10). This indicates the exposures to effluent did not significantly alter egg production. The mean of egg production from Mill D exposed fish was likely to be significantly changed compared to controls using ANCOVA (Table 10). However additional analysis using t tests found no significant differences among treatment means. Total egg production, from fish exposed to control, Mill A, Mill B, and Mill C effluent increased during the effluent exposure period (Figure 1). This increase was probably due to the age of the spawning fish and not related to the effluent, because egg production tends to gradually increase over the first five weeks of spawning and then begin to slow in both controls and treatments (NCASI 1996, 1997, 1998a, 1998b). A slight reduction in average eggs/f/d during testing with effluent from Mill D was observed but the reduction was not significant.

Figure 2 shows the egg production of fathead minnows for the ten days before effluent exposure and during subsequent five-day time periods when exposed to the effluents. Fish in control water, Mill B effluent, and Mill D effluent show a similar response trend in that the egg production was highest during the first and middle five-day time periods of the exposure, but was reduced during the last five days of the testing period. Fish in effluent from Mill C also demonstrated this trend, except egg production did not decrease during the final five days of exposure. Fish exposed to Mill A effluent had a somewhat different response pattern from all other groups. Egg production of fish in Mill A dropped significantly during the first five days of exposure, but this was followed by a similar response to other groups of fish. This may have been a response to the effluent, but if so, the fish recovered rapidly.

Table 9. Fathead Minnow Egg Production and Adult Survival During Tests With Mill Effluents

Parameter	Control Well Water		Mill A OD+ECF		Mill B ECF		Mill C Unbleached		Mill D Recycle Deink	
			100% V/V		30% V/V		50% V/V		100% V/V	
	Before[a]	After[b]	Before	After	Before	After	Before	After	Before	After
Days of Exp.[c]	21	21	20	17	20	22	23	21	24	20
Total Eggs/Group										
Replicate A	1675	2083	470	328	1298	1870	1663	1740	1432	891
(SD)[d]	(672)	(1125)	(347)	(255)	(163)	(1094)	(778)	(684)	(788)	(652)
Replicate B	1197	1512	1202	1134	1418	1638	1303	1675	1093	907
(SD)	(1112)	(776)	(503)	(426)	(268)	(657)	(571)	(571)	(1142)	(810)
Total	1436	1798	836	731	1408	1754	1483	1708	1263	899
(SD)	(888)	(945)	(560)	(540)	(205)	(845)	(660)	(584)	(919)	(679)
Total Eggs	11488	14380	6689	5848	11262	14032	11864	13660	10426	7190
Total Spawns/ Group	10	9	7	4	10	8	9	7	9	5
(SD)	(2)	(3)	(4)	(3)	(3)	(4)	(3)	(2)	(4)	(3)
Total Spawns	79	74	52	30t[e]	83	63t	75	56	72	38
Eggs/F/Day										
Replicate A	38.9	49.6	11.8	9.7	36.1	59.5	36.2	41.4	29.9	22.3
(SD)	(16)	(27)	(9)	(8)	(7)	(54)	(17)	(16)	(16)	(16)
Replicate B	28.5	36	33.3	41.2	35.5	37.2	28.6	50.5	30.4	30.3
(SD)	(27)	(19)	(13)	(19)	(7)	(15)	(12)	(27)	(24)	(20)
Total (Avg.)[f]	34.2	42.8	22.5	25.4	35.8	48.4	32.4	46.0	30.1	25.7
(SD)	(21)	(23)	(15)	(22)	(6)	(39)	(14)	(21)	(19)	(17)
Avg % Change During Exposure		+25%		+13%		+35%		+42%		-15%
Adult Survival %[g]										
Male (Avg.)	100	100	100	100	100	100	100	100	100	100
Female (Avg.)	100	100	94	100	94	100	94	100	88	100
Total (Avg.)	100	100	95	100	95	100	95	100	95	100

[a] egg production in well water only
[b] egg production after the introduction of effluent
[c] days of exposure
[d] standard deviation
[e] significant difference T-Test ($p \leq 0.05$)
[f] average
[g] survival based on number of males and females in each concentration after separating (8 M, 16 F)

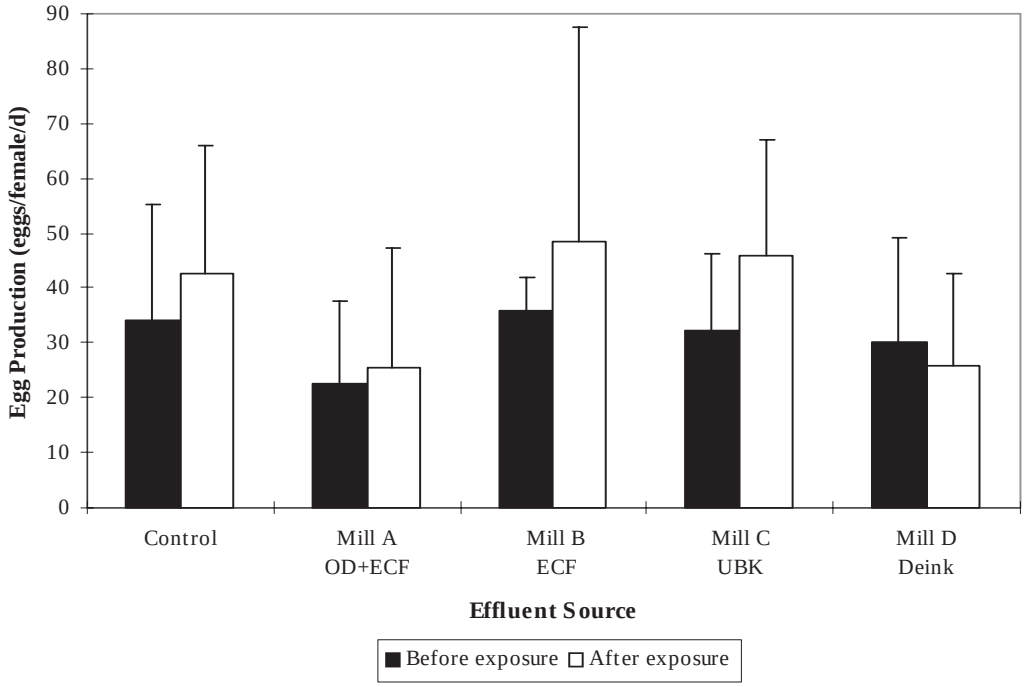

Figure 1. Fathead Minnow Egg Production Before and After Exposure to Effluents

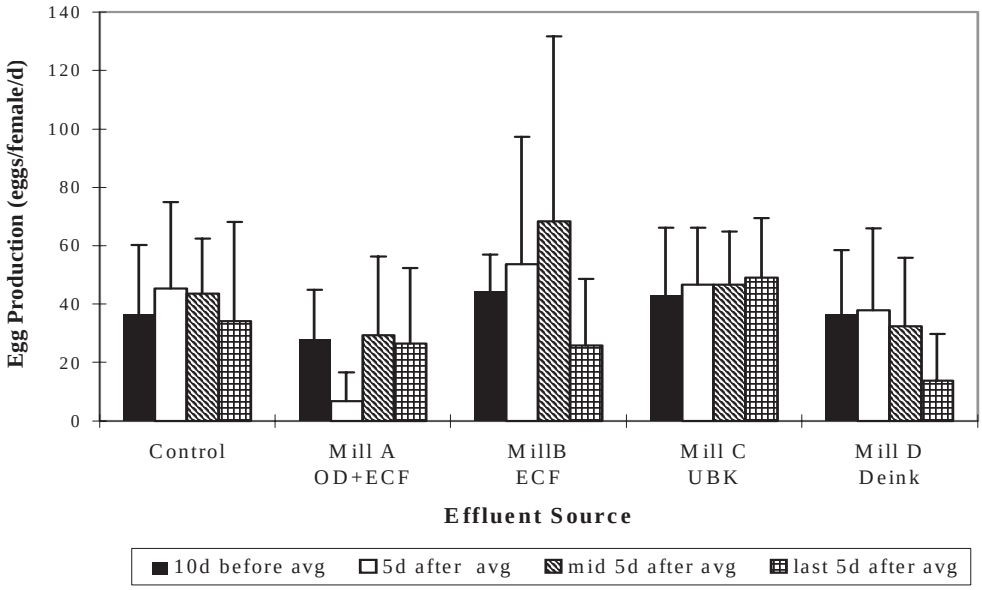

Figure 2. Fathead Minnow Egg Production During the 10 Days Before Effluent Exposure, First Five Days of Effluent Exposure, Middle Five Days of Exposure, and Last Five Days of Exposure

Table 10. Analysis of Covariance Results for Eggs/f/day

	Control vs. Mill A	Control vs. Mill B	Control vs. Mill C	Control vs. Mill D
F for Means	0.78	0.07	0.22	7.22
Degrees Freedom	1,13	1,13	1,13	1,12
Significant Difference	No	No	No	Yes
F for Slopes	1.09	2.88	1.7	0.05
Degrees Freedom	1,12	1,12	1,12	1,11
Significant Difference	No	No	No	No

4.0 DISCUSSION

Since the primary objective of this study was to determine if a short-term exposure of spawning adults to effluent from pulp or paper mills could predict effects on fish reproduction found during earlier life-cycle tests with the same effluents, a comparison of effluent characteristics between the investigations was needed. As expected, some characteristics varied between tests. The chemical components and biological endpoints measured for Mill A (OD + ECF) effluent suggested this effluent could be expected to have greater biological effects during the life-cycle tests. These changes

were probably the result of the construction and implementation of new pulping and bleaching processes that were being introduced at the time of this testing. Effluent from this mill may have had some initial effect on egg production within the first five days, but over 21 days of exposure egg production was similar to controls. Effluents from Mill B (ECF) and Mill C (unbleached) had chemical or biological characteristics that were similar when compared to earlier testing, but no effects on egg production due to these effluents were noted. Mill D (deink recycle) effluent had very low levels of chemical constituents during both testing periods and the biological effects were similar to or reduced from the earlier life-cycle tests. Since this effluent did not alter egg production during the life-cycle test, no effect on egg production would be expected during the tests described in this bulletin. Although effects on egg production were not significant, fish in effluent from Mill D were the only group with reduced egg production (15% reduction compared to preexposure) during exposure. Overall, exposures to the three effluents where effects were expected (Mills A, B, and C) did not significantly change egg production, and effluent from Mill D, which was not expected to have effects also did not produce significant effects on egg production. However egg production of fish in Mill D effluent was closer to a threshold for effects than fish in effluents from the three mills that caused reduction during life-cycle tests. Thus, this method of abbreviated exposure of spawning adults to effluent was not predictive of effects on egg production during life-cycle tests.

The results presented in this paper suggest that exposure to concentrations of pulp mill effluents that were much greater than concentrations causing a 25% reduction during life-cycle exposures during only the spawning phase of the life-cycle, is unlikely to produce adverse effects on reproduction. At least three possibilities exist for the differences in results between the abbreviated and life-cycle test protocols. These include: (1) exposure during a sensitive life stage may be required to cause an effect on egg production, (2) only a continuous combination of exposures over the entire life-cycle may cause the effects on egg-production found in life-cycle tests or (3) time for one or more effluent components to accumulate may be required to cause the effects noted. Changes in effluent constituents between the abbreviated and life-cycle tests are also possible. However, the chemical and biological tests of the effluent did not show great improvements, and the possibility of all three of the effluents that had effects earlier, now having no effects during a life-cycle test if one had been performed, are remote.

To date, the abbreviated test described in this bulletin, as well as other short-term tests have not demonstrated a significant or strong correlation to life-cycle egg production responses. Additional testing with other time periods or portions of the life cycle may be useful in determining if the length of exposure or timing of the exposure are important causative factors in life-cycle responses.

Fathead minnow egg production increased from the control production period (first 20–24 days spawning) to the effluent exposure period in control water and effluent from Mill A, B, and C and was slightly reduced in effluent from Mill D during this study. These increases were probably due to the age of the fish and not related to the effluent, because: (1) control fish demonstrated this response, and (2) egg production had tended to gradually increase for the first five weeks of spawning during other tests (NCASI 1996, 1997, 1998a, 1998b, 1998c). Therefore no effluent reproduction effects were found even though the chemical composition of the effluents were similar to or greater than in the previous studies. Previous studies with these same effluents found reproduction effects with effluent from Mill A, Mill B, and Mill C. Short term reproduction testing during this study failed to predict life cycle reproduction effects found during the previous studies with these same effluents. Therefore, further research will be needed, to develop a test that will shorten the duration of exposure for the fathead minnow life-cycle test.

5.0 SUMMARY

Fathead minnow (*Pimephales promelas)* were exposed to four biologically treated pulp and paper mill effluents during 17 to 21 days of the spawning period, to determine if biological responses from previous life-cycle tests with the same effluents could be predicted from these abbreviated exposures.

The findings were:

a) Chemical concentrations of the sample of effluent from Mill A (OD+ECF) tended to be greater than or equal to those found during earlier life-cycle testing with this effluent. Bioassays with *C. dubia* and echinoderms tended to have increased effects compared to earlier testing, which was consistent with the increased chemical concentrations. However, no significant effect of the effluent was found on egg-production during the abbreviated tests.

Chemical concentrations and biological endpoints of the effluent from Mill B (ECF) and Mill C (unbleached) were similar to earlier test results and the concentration used would have been expected to reduce egg production by 50% during the abbreviated testing reported herein. However, similar to the results with Mill A effluent, egg production was not significantly different from controls during those tests.

Chemical concentrations and biological endpoints of the effluent from Mill D (recycled deink) were similar to or reduced from earlier tests. This effluent had no effect on reproduction during an earlier life-cycle test and, as expected, did not cause a significant effect on reproduction during this test.

b) Fathead minnow spawning results during this study did not produce effects on reproduction as seen during previous testing with these same effluents (NCASI 1996, 1997, 1998a) even though the chemical concentrations of the effluents were equal to or greater than during previous studies. Methods used in this study to reduce the duration of exposure required to complete reproduction testing with the fathead minnow did not produce results that predicted effects found during life cycle testing with effluents from the same mills. Therefore, this method of testing is not a viable alternative to life-cycle tests. Additional testing during specific life stages or different duration of exposure may provide greater insight into methods to shorten the test duration.

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APPENDIX A

ORGANIC CHEMICAL CHARACTERISTICS OF FOUR EFFLUENT SAMPLES USED FOR ABBREVIATED EXPOSURES DURING SPAWNING

Table A1. Organic Chemical Characteristics of Four Effluent Samples Used for Abbreviated Spawning. 05/02/97–05/08/97

Compound units:	Mill A 05/02/97 ppb	Mill B 05/02/97 ppb	Mill C 05/05/97 ppb	Mill D 05/08/97 ppb
Phenolics				
Phenol	ND[a]	ND	NA[b]	2.1
o-Cresol	ND	ND	NA	ND
p-Cresol	ND	ND	NA	ND
m-Cresol	ND	ND	NA	ND
Guaiacol	ND	ND	NA	ND
Catechol	ND	ND	NA	ND
Resorcinol	ND	ND	NA	ND
Chlorinated Phenols				
4-Chlorophenol	ND	ND	NA	ND
2,6-Dichlorophenol	ND	ND	NA	ND
2,4-Dichlorophenol	ND	ND	NA	ND
2,4,6-Trichlorophenol	ND	ND	NA	ND
2,4,5-Trichlorophenol	ND	ND	NA	ND
2,3,4,6-Tetrachlorophenol	ND	ND	NA	ND
Pentachlorophenol	ND	ND	NA	ND
Chlorinated Guaiacols				
6-Chloroguaiacol	ND	ND	NA	ND
4-Chloroguaiacol	ND	ND	NA	ND
5-Chloroguaiacol	ND	ND	NA	ND
4,6-Dichloroguaiacol	ND	ND	NA	ND
3,4-Dichloroguaiacol	ND	ND	NA	ND
4,5-Dichloroguaiacol	ND	ND	NA	ND
3,4,6-Trichloroguaiacol	ND	ND	NA	ND
3,4,5-Trichloroguaiacol	ND	ND	NA	ND
4,5,6-Trichloroguaiacol	ND	ND	NA	ND
Tetrachloroguaiacol	ND	ND	NA	ND
Chlorinated Catechols				
3-Chlorocatechol	ND	ND	NA	ND
4-Chlorocatechol	ND	ND	NA	ND
3,6-Dichlorocatechol	ND	ND	NA	ND
3,5-Dichlorocatechol	ND	ND	NA	ND
3,4-Dichlorocatechol	ND	ND	NA	ND
4,5-Dichlorocatechol	ND	ND	NA	ND

(Continued on next page. See notes at end of table.)

Table A1. Continued

Compound units:	Mill A 05/02/97 ppb	Mill B 05/02/97 ppb	Mill C 05/05/97 ppb	Mill D 05/08/97 ppb
3,4,6-Trichlorocatechol	ND	ND	NA	ND
3,4,5-Trichlorocatechol	ND	ND	NA	ND
Tetrachlorocatechol	ND	ND	NA	ND
Chloraldehydes				
5-Chlorovanillin	ND	ND	NA	ND
6-Chlorovanillin	ND	5.4	NA	ND
5,6-Dichlorovanillin	ND	ND	NA	ND
Chlorosyringaldehyde	ND	1.3	NA	ND
2,6-Dichlorosyringaldehyde	ND	ND	NA	ND
3-Chlorosyringol	ND	ND	NA	ND
3,5-Dichlorosyringol	ND	ND	NA	ND
Trichlorosyringol	ND	ND	NA	ND
3,5-Dichloro-4-hydroxyben	ND	ND	NA	ND
Aromatic Acids				
Benzoic Acid	1.8	1.6	ND	1.2
p-Hydroxybenzoic acid	ND	ND	ND	ND
3,5-Dichloro-4-OH benzoic	ND	ND	ND	ND
Fatty Acids				
Linoleic acid	2.9	ND	9.8	0.9
Oleic acid	3.3	ND	23.5	ND
Dichlorostearic acid	ND	ND	ND	ND
Resin Acids				
Pimaric acid	7.0	2.4	120	ND
Sandracopimaric acid	ND	ND	ND	ND
Isopimaric acid	5.1	6.6	43.9	ND
Palustric acid	ND	7.1	28.0	ND
Dehydroabietic acid	14.1	19.5	124	ND
Abietic acid	7.8	21.3	216	ND
Neoabietic acid	ND	3.8	23.3	ND
14-Chlorodehydroabietic acid	ND	ND	ND	ND
12-Chlorodehydroabietic acid	1.3	ND	ND	ND
Dichlorodehydroabietic acid	ND	ND	ND	ND
Phytosterols				
Campersterol	12.1	3.4	25.9	ND
Stigmasterol	18.2	7.8	12.1	2.6
Beta-Sitosterol	137.0	26.7	276.0	ND
Stigmastanol	31.1	5.9	31.2	ND

[a] not detected
[b] not analyzed

APPENDIX B

FATHEAD MINNOW EGG PRODUCTION DURING ABBREVIATED EXPOSURES TO FOUR PULP AND PAPER MILL EFFLUENTS

Table B1. Fathead Minnow Egg Production During Abbreviated Exposures to Four Pulp and Paper Mill Effluents

Repl.	Average Female	Days Exposure	TOTAL Eggs	TOTAL Spawns	Eggs Female/day	Total Eggs Per Conc.	MEAN EGGS female/day
Control (before exposure period-well water)							
0A1	2	21	2664	11	63.4	11488	34.2
0A2	2	21	1477	13	35.2		
0A3	2	21	1387	12	33.0		
0A4	2	21	1171	9	27.9		
0B1	2	21	1189	9	28.3		
0B2	2	21	426	5	10.1		
0B3	2	21	402	8	9.6		
0B4	2	21	2772	13	66.0		
Control (after exposure period-well water)							
0A1	2	21	3684	16	87.7	14380	42.8
0A2	2	21	1645	9	39.2		
0A3	2	21	1928	12	45.9		
0A4	2	21	1075	4	25.6		
0B1	2	21	1764	10	42.0		
0B2	2	21	1320	8	31.4		
0B3	2	21	559	5	13.3		
0B4	2	21	2405	10	57.3		
Mill A Control (before exposure-well water)							
0A1	2	20	679	6	17.0	6689	22.5
0A2	2	20	219	4	5.5		
0A3	2	20	848	6	21.2		
0A4	2	20	135	1	3.4		
0B1	2	20	1861	9	46.5		
0B2	1.4	20	1067	8	39.5		
0B3	2	20	1231	9	30.8		
0B4	2	20	649	9	16.2		
Mill A (OD+ECF effluent exposure)							
100A1	2	17	684	4	20.1	5848	25.4
100A2	2	17	207	1	6.1		
100A3	2	17	325	3	9.6		
100A4	2	17	95	1	2.8		
100B1	2	17	1562	5	45.9		
100B2	1	17	1075	5	63.2		
100B3	2	17	1331	6	39.1		
100B4	2	17	569	3	16.7		

(Continued on next page.)

Table B1. Continued

Repl.	Average Female	Days Exposure	TOTAL Eggs	TOTAL Spawns	Eggs Female/day	Total Eggs Per Conc.	MEAN EGGS female/day
Mill B Control (before exposure-well water)							
0A1	2	20	1297	9	32.4	11262	35.8
0A2	1.7	20	1639	13	45.8		
0A3	2	20	1305	10	32.6		
0A4	2	20	1349	10	33.7		
0B1	2	20	1639	14	41.0		
0B2	2	20	1030	5	25.8		
0B3	2	20	1476	12	36.9		
0B4	2	20	1527	8	38.2		
Mill B (ECF effluent exposure)							
30A1	2	22	2558	10	58.1	14032	48.4
30A2	1	22	2992	14	136.0		
30A3	2	22	1296	7	29.5		
30A4	2	22	633	4	14.4		
30B1	2	22	2411	9	54.8		
30B2	2	22	1958	10	44.5		
30B3	2	22	1106	6	25.1		
30B4	2	22	1078	6	24.5		
Mill C Control (before exposure-well water)							
0A1	2	23	2324	8	50.5	11864	32.4
0A2	2	23	627	7	13.6		
0A3	2	23	2193	12	47.7		
0A4	2	23	1509	9	32.8		
0B1	2	23	2143	10	46.6		
0B2	2	23	968	13	21.0		
0B3	1.9	23	923	10	21.1		
0B4	2	23	1177	6	25.6		
Mill C (unbleached effluent exposure)							
50A1	2	21	1555	8	37.0	13660	46.0
50A2	2	21	1669	7	39.7		
50A3	2	21	2684	8	63.9		
50A4	2	21	1052	6	25.0		
50B1	2	21	2427	9	57.8		
50B2	2	21	1368	8	32.6		
50B3	1	21	1783	6	84.9		
50B4	2	21	1122	7	26.7		

(Continued on next page.)

Table B1. Continued

Repl.	Average Female	Days Exposure	TOTAL Eggs	TOTAL Spawns	Eggs Female/day	Total Eggs Per Conc.	MEAN EGGS female/day
Mill D Control (before exposure-well water)							
0A1	2	24	2582	16	53.8	10101	30.1
0A2	2	24	849	8	17.7		
0A3	2	24	1011	8	21.1		
0A4	2	24	1288	8	26.8		
0B1	2	24	81	2	1.7		
0B2	2	24	2380	12	49.6		
0B3	1.7	24	325	3	7.9[a]		
0B4	2	24	1910	15	39.8		
Mill D (recycled de-ink effluent exposure)							
100A1	2	20	1810	10	45.3	7190	25.7
100A2	2	20	291	4	7.3		
100A3	2	20	833	6	20.8		
100A4	2	20	629	4	15.7		
100B1	2	20	274	2	6.9		
100B2	2	20	1707	7	42.7		
100B3	0						
100B4	2	20	1646	8	41.2		

[a] not used in average, both females died before effluent exposure.