CHRONIC TOXICITY EVALUATION OF
SIMULATED DWPF EFFLUENT TO CERIODAPHNIA DUBIA

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Report To:

WESTINGHOUSE SAVANNAH RIVER COMPANY

Savannah River Site
Aiken, South Carolina

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Submitted By:

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I. INTRODUCTION

A 7-Day Full Series Chronic Toxicity test was conducted April 13 - 20, 1990, for the Savannah River Site to assess the chronic toxicity of Simulated DWPF effluent to Ceriodaphnia dubia.

II. METHODS

Dilution water for the toxicity test was collected April 11, 1990 (Lab I.D. No. 90-1601) from McQueens Branch on the Savannah River Site by SHEALY ENVIRONMENTAL SERVICES, INC. personnel and transported iced to the laboratory. The water was filtered with a plankton net (37 mm) prior to use in the test. Ceriodaphnia for the chronic test had been cultured in Upper Three Runs Creek water since October 25, 1988. Water from Upper Three Runs Creek for the cultures was collected at the northside of a bridge on Road 2-1 on the Savannah River Site by SHEALY ENVIRONMENTAL SERVICES, INC. personnel.

All organisms used in the toxicity tests were from SHEALY ENVIRONMENTAL SERVICES, INC.'s in-house cultures which were obtained from the USEPA Newton Laboratory April 20, 1987, Lab I.D. No. 87-271. Ceriodaphnia from in-house cultures are identified and preserved monthly. Standard toxicant tests with the EPA reference toxicant cadmium chloride and laboratory reagent grade cadmium chloride are performed monthly on Ceriodaphnia cultured in water from Upper Three Runs Creek and in conjunction with the chronic toxicity tests. The results of these tests (48-Hour Central Tendency at 20°C was 0.23 ppm)

demonstrated that the condition of the cultures were within the acceptable range for the test organisms (± 2 S.D.).

Test methods conformed to those described in USEPA (1989; see Table 1). The 7-day chronic toxicity bioassay was performed April 13 - 20, 1990, as six treatments exposing 20 test organisms each. The first treatment was the control (100% filtered McQueens Branch water). The Simulated DWPF solutions were 10%, 20%, 40%, 70%, and 100%. All test solutions were prepared from DETF solutions prepared daily by dosing the dilution water with appropriate aliquots of Simulated DWPF (Table 2). The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

Dissolved oxygen, water temperature, pH and conductivity measurements were made daily in conjunction with the test. Temperature was maintained at $25^{\circ} \pm 1^{\circ}\text{C}$ in all test chambers during the test.

The test organisms were placed singly in the test vessels each containing 15 ml of solution. The organisms were between 16 - 24 hours old at the start of the test. Transfer of the neonates was accomplished using an eye dropper where the organisms were never removed from solution. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at a rate of approximately 250,000 cells per ml. test solution per day and 0.1 ml Yeast-Cerophyl-Trout chow mixture. Selenastrum cultures were obtained from

Table 1: Summary of Test Conditions for the Chronic Toxicity Bioassay on DWPF Simulated Effluent with Ceriodaphnia dubia.

1. Temperature:	25 $\pm$ 1°C
2. Light Intensity:	Ambient Laboratory Levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	1 ounce SOLO plastic disposable cups
5. Volume of test solution:	15 ml
6. Age of test organisms:	2-24 hour neonates and all released within the same 8 hour period
7. No. animals per test vessel:	1
8. No. replicate test vessels per concentration:	20
9. Total no. organisms per concentration:	20
10. Feeding regime:	<u>Selenastrum capricornutum</u> at the rate of Approx. 250,000 cells per ml test solution per day and 0.1 ml YCT mixture
11. Aeration:	None
12. Dilution water:	McQueens Branch
13. Test duration:	Until 60% Control females have produced 3 broods
14. Effects measured:	Mortality - no movement of appendages on gentle prodding and number of offspring produced
15. Test acceptability:	80% or greater survival and average of 15 or more young/surviving female in the control solutions. At least 60% of surviving females in control should have produced their third brood.

Table 2. Daily Preparations of Simulated DWPF Effluent

g/l	Initial	1	2	3	4	5	6
Sodium nitrate	0.010	0.011	0.010	0.011	0.010	0.010	0.011
Sodium oxalate	0.0292	0.0310	0.0295	0.0305	0.0291	0.0309	0.0298
Sodium formate	0.0450	0.0453	0.0449	0.0455	0.0450	0.0449	0.0452
Corros. Inib.	0.0391	0.0384	0.0385	0.0390	0.0385	0.0382	0.0394

Sodium hypochlorite - weighed 0.01 g/l then diluted 100 times
1 ml ---> 100 ml volumetric flask.

Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged Erlenmeyer flasks and maintained under bright fluorescent lighting for 6 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and order of feeding was established based on random number tables.

The results of the 7-day chronic toxicity test are given in Table 3. Mortality of the adult females occurred in the 10% (0% mortality), 20% (5% mortality), 40% (5% mortality), 70% (10% mortality), and 100% (5% mortality) simulated DETF effluent concentrations. Five percent 5% mortality occurred in the control.

Average reproduction and mortality of the adult females in the control and Simulated DWPF solutions was as follows:

	Offspring per <u>Female</u>	<u>% Mortality</u>
<u>Control</u>	= 20.6	5%
<u>DWPF Solutions</u>		
10%	= 20.7	0%
20%	= 19.0	5%
40%	= 18.6	5%
70%	= 19.8	10%
100%	= 22.9	5%

Table 3. Reproduction/Mortality Data recorded in conjunction with the 7-Day chronic toxicity test
with Ceriodaphnia Dubia for Westinghouse Savannah River Company on Simulated DWPF effluent.

Control

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
4	3	3	5	3	6	4	5	5	2	2	4	5	4	3	5	3	5	3	5	5
5	5	0	0	6	0	4	6	0	3	0	0	D/O	0	8	6	3	4	0	0	6
6	10	9	8	0	9	11	5	5	10	7	10	-	11	10	9	0	11	12	3	6
7	4	10	9	12	12	0	5	14	8	11	10	-	3	0	0	12	3	5	4	9
TOTAL	22	22	22	21	27	19	21	24	27	20	24	5	18	21	20	18	23	20	12	26
ADULT	L	L	L	L	L	L	L	L	L	L	L	D	L	L	L	L	L	L	L	L

L=Live

D=Dead

$$\bar{X} = 20.6$$

Treatment 1 (10%)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	5	4	4	5	3	4	4	5	4	6	4	4	3	5	0	4	5	6	4	4
5	0	4	3	8	0	3	5	6	6	5	0	0	0	4	5	9	6	0	4	5
6	11	10	9	10	3	14	10	0	0	9	7	10	11	10	4	12	0	9	9	8
7	0	10	9	0	11	0	0	3	10	0	11	3	5	0	9	0	3	10	8	10
TOTAL	16	28	25	23	17	21	19	14	20	20	22	17	19	19	18	25	14	25	25	27
ADULT	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

L=Live

D=Dead

$$\bar{X} = 20.7$$

Table 3. Reproduction/Mortality Data recorded in conjunction with the 7-Day chronic toxicity test with *Ceriodaphnia Dubia* for Westinghouse Savannah River Company on Simulated DWP effluent.

Treatment 2 (20%)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
4	3	4	5	4	4	3	8	4	4	4	4	3	5	4	3	4	4	5	3	3
5	4	6	0	0	0	D/0	0	0	9	4	0	3	3	9	6	0	0	0	0	4
6	0	0	11	10	9	-	8	8	0	0	10	12	10	7	0	11	14	0	4	10
7	11	4	10	12	13	-	0	9	11	12	0	0	0	3	14	0	3	13	0	9
TOTAL	18	14	26	26	26	3	16	21	27	20	14	18	18	23	23	15	21	18	7	26
ADULT	L	L	L	L	L	D	L	L	L	L	L	L	L	L	L	L	L	L	L	L

L=Live

D=Dead

X = 19.0

Treatment 3 (40%)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	4	3	3	6	4	4	5	4	4	5	4	3	2	4	3	4	4	4	5	5
5	0	6	3	0	0	4	0	0	5	5	6	9	0	0	3	4	9	8	7	0
6	10	10	8	7	10	9	4	5	5	6	0	0	9	3	3	6	7	D/6	3	3
7	3	0	9	3	10	3	0	10	9	10	10	9	3	10	0	13	0	-	3	4
TOTAL	17	19	23	16	24	20	9	19	23	26	20	21	14	17	9	27	20	18	18	12
ADULT	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	D	L	L

L=Live

D=Dead

X = 18.6

Table 3. Reproduction/Mortality Data recorded in conjunction with the 7-Day chronic toxicity test
with Ceriodaphnia Dubia for Westinghouse Savannah River Company on Simulated DWPF effluent.

Treatment 4 (70%)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	4	4	D/O	0	3	4	6	3	3	4	4	5	2	4	4	2	4	5	4	6
5	0	0	-	4	6	0	3	0	0	6	10	0	8	0	3	3	4	6	9	3
6	4	9	-	3	0	6	7	7	9	8	4	5	8	3	5	5	6	6	0	0
7	13	10	-	17	10	10	8	8	D/O	3	4	9	0	4	10	10	7	6	11	14
TOTAL	21	23	0	24	19	20	24	18	12	21	22	19	18	21	22	20	21	23	24	23
ADULT	L	L	D	L	L	L	L	L	D	L	L	L	L	L	L	L	L	L	L	L

L=Live

D=Dead

X = 19.8

Treatment 5 (100%)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
4	4	2	4	3	5	4	5	5	4	3	3	**	4	6	3	0	0	4	4	6
5	9	3	0	4	6	6	8	7	0	4	3		9	8	7	8	10	3	3	2
6	6	0	10	4	14	0	10	10	9	8	14		0	0	10	13	0	0	11	9
7	12	11	8	14	0	16	0	D/O	0	12	0		10	13	10	3	10	13	0	3
TOTAL	31	16	22	25	25	26	23	22	16	27	20	**	23	27	30	24	20	20	18	20
ADULT	L	L	L	L	L	L	L	D	L	L	L	**	L	L	L	L	L	L	L	L

L=Live

D=Dead

X = 22.9

*Animal lost during transfer - not included in reproduction data.

The reproduction data were tested for normality and homogeneity of variances using the Chi-Square Goodness of Fit Test and Bartlett's Test, respectively ($p=0.01$). The data was found to be not normally distributed (Chi-Square = 17.36, Critical Value = 13.28), therefore, the non-parametric Wilcoxon's Rank Sum Procedure was used. Results from this test indicated no chronic toxicity at any of the concentrations tested at the probability level of 0.05 (Appendix A). The no observed effect concentration (NOEC) was the 100%. Water chemistry data taken in conjunction with the chronic toxicity test are given in Table 4. All parameters monitored were within acceptable limits for bioassay purposes.

Table 4. Water Chemistry Data recorded in conjunction with the 7-Day Chronic Toxicity Test with *Ceriodaphnia dubia* for WESTINGHOUSE SAVANNAH RIVER COMPANY on Simulated DWPF effluent.

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
Control	Temp. (deg. C)	24.5		25.0		25.0		25.0		24.5		25.0		24.0		
	D.O. (ppm)	7.20	7.30	7.40	7.40	7.60	7.50	7.40	7.10	7.20	7.10	7.30	7.10	7.50	7.20	
	pH (SU)	6.94	7.11	7.02	7.14	6.99	7.19	7.04	7.21	7.12	7.31	7.10	7.14	7.20	7.36	
	Alk. (ppm CaCO ₃)	10.7		11.9		9.5		10.7		-		10.7		7.1		
	Hard. (ppm CaCO ₃)	15.7		23.5		19.6		15.7		19.6		19.6		15.7		
	Cond. (umhos/cm)	54.0		54.0		54.0		53.0		54.0		53.0		53.0		

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
(10%)	Temp. (deg. C)	24.5		25.0		25.0		25.0		24.5		25.0		24.0		
	D.O. (ppm)	7.40	7.30	7.40	7.50	7.40	7.30	7.50	7.20	7.20	7.10	7.30	7.20	7.40	7.20	
	pH (SU)	6.91	6.93	7.12	7.10	6.95	7.21	6.99	7.21	7.21	7.35	7.15	7.36	7.25	7.42	

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
(20%)	Temp. (deg. C)	24.5		25.0		25.0		25.0		24.5		25.0		24.0		
	D.O. (ppm)	7.40	7.50	7.45	7.40	7.30	7.40	7.50	7.30	7.30	7.20	7.40	7.20	7.40	7.30	
	pH (SU)	6.92	7.12	6.99	7.14	7.10	7.25	7.05	7.22	7.25	7.41	7.22	7.48	7.21	7.52	

Table 4. Water Chemistry Data recorded in conjunction with the 7-Day Chronic Toxicity Test with *Ceriodaphnia dubia* for WESTINGHOUSE SAVANNAH RIVER COMPANY on Simulated DWPF effluent.

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
	Temp. (deg. C)	25.0	25.0	25.0	25.0	25.0	25.0	24.5	25.0	24.0						
(40%)	D.O. (ppm)	7.70	7.60	7.70	7.20	7.60	7.40	7.40	7.20	7.20	7.10	7.30	7.30	7.50	7.40	
	pH (SU)	7.04	7.32	7.71	7.20	7.21	7.38	7.25	7.45	7.10	7.24	7.30	7.28	7.25	7.46	

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
	Temp. (deg. C)	25.0	25.0	25.0	25.0	25.0	24.5	25.0	24.0							
(70%)	D.O. (ppm)	7.90	7.30	7.80	7.20	7.60	7.50	7.20	7.40	7.20	7.30	7.40	7.20	7.50	7.60	
	pH (SU)	6.82	7.25	7.10	7.25	7.29	7.46	7.34	7.39	7.22	7.21	7.43	7.49	7.42	7.36	

Conc.	Parameter	Init.	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
			old	renew	old	renew	old	renew	old	renew	old	renew	old	renew	old	renew
	Temp. (deg. C)	25.0	25.0	25.0	25.0	25.0	24.5	25.0	24.0							
(100%)	D.O. (ppm)	7.90	7.40	7.70	7.30	7.50	7.40	7.30	7.50	7.40	7.30	7.50	7.30	7.60	7.50	
	pH (SU)	6.99	7.21	7.07	7.31	7.35	7.55	7.46	7.35	7.32	7.42	7.52	7.58	7.55	7.61	
	Alk. (ppm CaCO ₃)	78.2	77.0	75.8	74.7	73.5	77.0	74.7								
	Hard. (ppm CaCO ₃)	19.6	15.7	19.6	15.7	15.7	11.8	19.6								
	Cond. (umhos/cm)	176	167	167	171	167	176	171								

APPENDIX A: Summary of statistical Analyses of Survival and
Reproduction Data for the 7-Day Chronic Ceriodaphnia
dubia Test for the Simulated DWPF effluent.

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Mortality Data

Fisher's Exact Test: No significant mortality observed at the
probability level of 0.05.

Reproduction Data

Chi-Square Goodness of Fit Test = 17.36

Bartlett's Test for Homogeneity of Variances = 0.432

WILCOXON'S RANK SUM PROCEDURE

<u>CONCENTRATION</u>	<u>CALCULATED RANK SUM</u>	<u>CRITICAL RANK SUM</u>
10	N/A*	-
20	376.5	327
40	334.0	327
60	391.5	327
80	N/A*	-

*Effluent with greater number of offspring than the control.

**DATE
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5/01/92