Reproductive Success and Mortality Rates of Ceriodaphnia Dubia Maintained in Water from Upper Three Runs, Pen Branch, and Fourmile Branch

by

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**REPRODUCTIVE SUCCESS AND MORTALITY RATES OF
CERIODAPHNIA DUBIA MAINTAINED IN WATER FROM
UPPER THREE RUNS, PEN BRANCH, AND FOURMILE BRANCH**

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SUMMARY

It is anticipated that the new SRS NPDES permit will require toxicity testing of at numerous outfalls and receiving streams, using the standard test species, *Ceriodaphnia dubia*. Because SRS surface waters differ markedly from the standard culture water that is used for *Ceriodaphnia*, studies were undertaken to determine if unimpacted SRS surface waters will support this species. Three SRS surface waters were evaluated: Upper Three Runs at Road 8-1, Pen Branch at Road B, and Fourmile Branch at Road F. Toxicity tests were performed monthly on each water source for eleven months. All three water sources exhibited varying degrees of toxicity to *Ceriodaphnia*, with Pen Branch being the least toxic and Fourmile Branch being the most toxic. These results indicate that if in-stream toxicity testing is required, it may not be possible to separate the naturally occurring toxic effects of the receiving water from possible toxic effects of SRS effluents.

INTRODUCTION

In 1985, the U.S. Environmental Protection Agency (EPA) first issued a methods manual for conducting chronic toxicity tests on the cladoceran, *Ceriodaphnia dubia* (Weber et al, 1985). Since that time, *Ceriodaphnia dubia*, has become the species of choice for conducting toxicity tests on industrial effluents, primarily because it can be easily cultured in the laboratory, it has a relatively short generation time, and it is relatively sensitive to a broad range of toxicants.

The chemical composition of surface waters in the United States varies widely with respect to water hardness and trace mineral content. In order to provide a consistent source of culture water, the EPA (Weber et al., 1989) and the South Carolina Department of Health and Environmental Control (SCDHEC, 1989) recommend that *Ceriodaphnia* be cultured in 20% DMW (dilute mineral water), which is a mixture of 80% deionized water and 20% Perrier mineral water. Perrier mineral water was chosen because the chemical composition of the water is very consistent among lots, it is readily available throughout the country, and it provides the essential trace minerals that are needed for *Ceriodaphnia* to thrive.

The standard mixture of 80% deionized water and 20% Perrier has a hardness of approximately 200 mg/l, which is considered to be moderately hard water. In contrast, SRS streams contain water that is extremely soft, with hardnesses ranging from approximately 2 to 30 mg/l. It is very possible that these very soft waters do not contain sufficient calcium and/or trace minerals to permit long-term survival of *Ceriodaphnia*. It is also possible that naturally occurring conditions of low pH and/or the relatively high concentrations of dissolved organic matter, in the form of humates and fulvates could provide less than ideal conditions for *Ceriodaphnia*.

It is anticipated that the new SRS NPDES permit will contain a requirement for toxicity testing at numerous outfalls. If an outfall fails a toxicity test, the site must either eliminate the toxicity at the outfall, or demonstrate that there is no impact to the receiving stream. Because SRS surface waters differ markedly from the standard culture water that is used for *Ceriodaphnia*, this study was undertaken to determine if

Ceriodaphnia will survive and reproduce in SRS surface waters at locations that do not receive SRS effluents. If reproduction or survival are reduced in unimpacted SRS surface waters, then clearly any results from *Ceriodaphnia* toxicity tests conducted downstream from SRS outfalls must be interpreted with extreme caution, since it may not be possible to separate any toxic effects of outfall contributions from the naturally toxic effects of SRS receiving waters. For that reason, part of this study examined the feasibility of establishing long-term cultures of *Ceriodaphnia* in water from the three streams. If cultures can be maintained, and if the organisms do not differ in their sensitivity to toxicants, then it may be legitimate to use organisms from these long-term cultures to toxicity tests on samples collected downstream from NPDES outfalls and compare the results to controls from the same long-term cultures.

The objectives of this study were 1) to determine if *Ceriodaphnia dubia* is adversely affected by SRS stream waters that do not receive NPDES discharges, 2) to determine if *Ceriodaphnia* can be cultured for extended periods of time in SRS stream waters, and 3) to determine if *Ceriodaphnia* that are cultured in stream waters differ in sensitivity to a reference toxicant, when compared to organisms that are cultured in 20% DMW.

METHODS

Water Collection - Water was collected from three SRS streams at locations that are upstream from any SRS NPDES outfalls. Water was collected from Upper Three Runs at Road 8-1; from Fourmile Branch at Road F, and from Pen Branch at Road B. Collectively, these three streams and their tributaries are receiving streams for approximately 70% of SRS's NPDES outfalls. Sufficient water was collected from each location at biweekly intervals to maintain *Ceriodaphnia* cultures and perform routine chemical analyses (pH, dissolved oxygen, specific conductance, total hardness, and total alkalinity) on the water.

Culturing of Organisms - Cultures of *Ceriodaphnia* were established in water from each of the three water sources in mid-December 1993. In order to gradually acclimate the organisms to the stream waters, the initial culture solutions were a mixture of 20% stream water and 80% DMW. Culture solutions were renewed at 72 hours using a mixture of 40% stream water and 60% DMW, after which time the organisms were cultured in unaltered stream water. Some of the early results indicated that longer acclimation was needed, and cultures were restarted and acclimated at a slower rate. Details of culture methods can be found in Appendices 1 through 3, in the monthly reports for December through February.

After the cultures were established, test organisms were transferred to fresh culture water from the most recently collected batch of stream water on each Monday, Wednesday, and Friday. Each Friday, ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms and all organisms that were two weeks old were discarded. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed a standard diet of 0.05 ml of 6.2×10^7 cells/ml of *Selenastrum capricornutum* suspension and 0.10 ml of YCT (yeast, cerophyll, and trout chow) diet on a daily basis.

Toxicity Tests - Once each month, a toxicity test was performed to compare the percent survival and mean number of young produced by each female in three broods with the results for organisms that had been cultured in standard 20% DMW. Twenty organisms were used for each water source.

Reference Toxicity Tests - As part of a toxicity testing program, toxicity tests are routinely performed on a reference toxicant (usually sodium chloride or cadmium chloride) in order to document the relative sensitivity of the test organisms to a known toxicant. Changes in the sensitivity of the organisms to a reference toxicant are usually indicative of a change in the overall vigor of the culture. In this study, reference toxicant tests, using sodium chloride, were performed monthly on organisms cultured in 20% DMW as well as on organisms from each of the cultures maintained in the stream waters, so that the sensitivities of the cultures to a known toxicant could be compared. Endpoints include NOEC's for survival and reproduction and LC50 values.

RESULTS

Water Chemistry

Water chemistry data are summarized in Table 1 and presented in detail in Appendix 12. The data confirm that SRS streams are very soft, with total hardness averaging 4.4 mg/l in Upper Three Runs, 12.9 mg/l in Fourmile Branch, and 19.6 mg/l in Pen Branch. Alkalinity was also low in all three streams, ranging from 2.4 mg/l in Upper Three Runs to 16.6 mg/l in Pen Branch. Dissolved oxygen concentrations in Pen Branch and Upper Three Runs averaged 9.1 and 9.0 mg/l, respectively, and never fell below 6 mg/l, but Fourmile Branch often had very low dissolved oxygen levels, averaging 4.3 mg/l and ranging from 1.2 to 7.1 mg/l. When dissolved oxygen concentrations were less than 5 mg/l, the water was aerated prior to use. The mean pH of the streams ranged from 5.3 in Fourmile Branch to 6.72 in Pen Branch. *Ceriodaphnia* generally does poorly in water that has a pH of less than 5. Fourmile Branch often had a pH of less than 5 on several occasions, but neither Pen Branch nor Upper Three Runs did. Dissolved organic carbon (DOC), which includes humates, fulvates, and other naturally occurring forms of organic carbon that leach from leaves and other decaying organic matter are relatively high in coastal plain blackwater streams such as those that occur on the SRS. DOC was generally lower in Upper Three Runs, averaging 3.7 mg/l than in Pen Branch (5.6 mg/l) or Fourmile Branch (12.1 mg/l). Specific conductance was extremely low in Upper Three Runs, averaging just 17.4 μ mhos/cm, but was also relatively low in Fourmile Branch (36.9 μ mhos/cm) and Pen Branch (51.7 μ mhos/cm).

Reproductive Success and Mortality

The results of the toxicity tests conducted on water from Pen Branch, Upper Three Runs and Fourmile Branch are presented in Tables 2, 3, and 4, respectively.

Pen Branch - Water from Pen Branch was suitable for long-term culture of *Ceriodaphnia* and was never acutely toxic to the organisms. Percent survival of both the control populations and the populations of *Ceriodaphnia* that were cultured in water from Pen Branch ranged from 90 to 100 percent during the eleven months of testing (Table 2). However, reproduction was significantly lower in the organisms cultured in

TABLE 1. WATER QUALITY DATA FOR PEN BRANCH, UPPER THREE RUNS AND FOURMILE BRANCH

Parameter	Pen Branch		Upper Three Runs		Fourmile Branch	
	Mean	Range	Mean	Range	Mean	Range
D. Oxygen (mg/l)	9.1	6.6 - 11.8	9.0	6.4 - 12.0	4.30	1.2 - 7.1
Hardness (mg/l)	19.6	12.0 - 30.9	4.4	1.96 - 8.0	12.90	8.0 - 23.3
Alkalinity (mg/l)	16.6	6.0 - 26.9	2.4	1.0 - 6.7	4.00	2.0 - 11.0
DOC (mg/l)	5.6	2.9 - 16.9	3.7	1.1 - 11.0	12.10	5.1 - 18.0
Conductivity (umhos/cm)	51.7	37.7 - 66.0	17.4	13.4 - 34.3	36.90	28.5 - 49.0
pH	6.72	6.1 - 7.42	5.7	5.23 - 7.45	5.30	4.55 - 5.93

TABLE 2. REPRODUCTIVE RATES AND MORTALITY IN PEN BRANCH WATER

1994	Reprod. Rate		Chronic Toxicity	% Survival		Acute Toxicity
	Control	Creek		Control	Creek	
January	23.3	25.3	No	100	95	No
February	21.1	25.1	No	100	90	No
March	20.4	19.7	No	90	95	No
April	21.9	20.1	No	100	95	No
May	25.8	15.7	Yes	100	95	No
June	28.8	18.7	Yes	100	100	No
July	25.8	25.0	No	100	100	No
August	27.2	18.6	Yes	100	95	No
September	29.1	21.0	Yes	95	100	No
October	24.6	16.6	Yes	95	95	No
November	20.8	26.3	No	100	100	No

TABLE 3. REPRODUCTIVE RATES AND MORTALITY IN UPPER THREE RUNS WATER

1994	Reprod. Rate		Chronic Toxicity	% Survival		Acute Toxicity
	Control	Creek		Control	Creek	
January	24.1	8.5	Yes	100	90	No
February				100	85	Yes
March	20.4	6.7	Yes	90	65	Yes
April	21.9	12.1	Yes	100	85	Yes
May	25.8	15.3	Yes	100	95	No
June	28.8	16.4	Yes	100	90	No
July	23.0	12.5	Yes	100	80	Yes
August	27.2	12.4	Yes	100	45	Yes
September	29.0	17.6	Yes	100	90	No
October	24.6	15.4	Yes	95	100	No
November	20.8	5.2	Yes	100	15	Yes

TABLE 4. REPRODUCTIVE RATES AND MORTALITY IN FOURMILE BRANCH WATER

1994	Reprod. Rate		Chronic Toxicity	% Survival		Acute Toxicity
	Control	Creek		Control	Creek	
January	24.1	6.6	Yes	100	80	Yes
February						
March						
April						
May						
June						
July						
August	27.2	22.1	Yes	100	90	No
September	28.1	20.4	Yes	100	95	No
October	18.6	6.5	Yes	95	10	Yes
November	20.8	0.0	Yes	100	0	Yes

water from Pen Branch in five of the eleven tests. Overall, the mean number of young produced in the control in three broods was 24.4 young per female, while the mean number produced in the Pen Branch water was 21.1 young per female (Table 2).

Upper Three Runs - *Ceriodaphnia* cultured in water from Upper Three Runs exhibited some degree of acute toxicity (percent survival of less than 90%) in six of the eleven tests. Percent survival in water from Upper Three Runs ranged from 15 to 100% and averaged 76%, while percent survival in the control populations was always 90 to 100% (Table 3). In the chronic tests, organisms cultured in water from Upper Three Runs had significantly lower rates of reproduction in all eleven of the tests. Overall, the mean number of young produced in the control in three broods was 24.6 young per female, while the mean number produced in the Upper Three Runs water was 13.8 young per female (Table 3).

Fourmile Branch - Cultures of *Ceriodaphnia* that were established in water from Fourmile Branch in December 1993 had declined in vigor to the point that they were not sustainable by February. No further culturing was attempted in water from Fourmile Branch until August. Of the five month's results for cultures maintained in Fourmile Branch, the water was acutely toxic in three of the five months, with percent survival ranging from 0 to 95 percent and averaging 55% (Table 4). In the chronic tests, organisms cultured in water from Fourmile Branch had significantly lower rates of reproduction in all five of the tests. Overall, the mean number of young produced in the control in three broods was 23.8 young per female, while the mean number produced in the Fourmile Branch water was 11.1 young per female (Table 3). Reproductive rates showed a continuous decline between August (22.1 young per female) and November, when no young were produced. These data indicate that Fourmile Branch is not capable of sustaining *Ceriodaphnia* on a long-term basis.

Reference Toxicity Tests

The results of the toxicity tests conducted using sodium chloride as a reference toxicant are presented in Table 5. The LC50's for control organisms that were cultured in 20% DMW were consistently between 2.08 and 2.2 g/l. LC50's in Pen Branch water were somewhat lower than the control, ranging from 1.11 to 2.07 g/l. LC50's in Pen Branch water were initially about 1.2 g/l, gradually increased to as much as 2.07 g/l, and then showed a gradual decline to 1.11 g/l. LC50's in the Upper Three Runs cultures were consistently much lower than those of the 20% DMW cultures, ranging from <0.0625 to 0.18 g/l. These results indicate that the Upper Three Runs cultures were severely stressed, and therefore were much more sensitive to the reference toxicant. LC50's in the Fourmile Branch cultures (0.2 to 0.62 g/l) were also much lower than the 20% DMW cultures, indicating that the Fourmile Branch cultures were also severely stressed.

The results of the reference toxicant survival tests followed the same general patterns of the LC50 tests. The concentration of sodium chloride that resulted in a significant reduction in the survival of the organisms cultured in 20% DMW was always 1.5 g/l. Survival in the Pen Branch cultures was initially impacted by somewhat lower concentrations of sodium chloride (0.4 or 0.8 g/l), but then increased to 1.5 g/l, followed by a decrease to 0.8 g/l during the last two months of the tests. Survival in water from Upper Three Runs and Fourmile Branch was always impacted by extremely low

TABLE 5. RESULTS OF REFERENCE TOXICANT TOXICITY TESTS CONDUCTED WITH SODIUM CHLORIDE

	LC50 (g NaCl /liter)				SURVIVAL NOEC (g NaCl/liter)				REPROD. NOEC (g NaCl/liter)			
	Control	Pen Br.	UTR	Fourmile	Control	Pen Br.	UTR	Fourmile	Control	Pen Br.	UTR	Fourmile
1994												
January	2.08	1.22	<0.2	<0.2	1.5	0.8		<0.2	0.4	0.4	<0.2	<0.2
February	2.08	1.13			1.5	0.8			0.4	0.8		
March	2.08	1.22			1.5	0.4			0.4	0.4		
April	2.08	1.19	<0.0625		1.5	0.8	<0.0625		0.4	0.8	<0.0625	
May	2.08	2.07	0.12		1.5	1.5	0.13		0.4	0.8	0.03	
June	2.08	1.92	0.18		1.5	1.5	0.032		0.4	0.8	0.032	
July	2.08	1.55	<0.03		1.5	1.5	0.06		0.4	0.8	0.06	
August	2.08	1.43	<0.03	<1.0	1.5	1.5		<0.02	0.4	0.8		<1
September	2.11	1.43	>0.25	0.26	1.5	1.5	0.25	<0.06	0.4	0.4	0.0125	0.26
October	2.2	1.41	0.11	0.62	1.5	0.8	>0.02		0.8	0.8	>0.02	0.62
November	2.2	1.11	0.16		1.5	0.8	0.01		0.4	0.8	<0.005	

Some fields contain no data because a toxicity test could not be performed, due to high mortality.

concentrations of the reference toxicant (<0.0625 to 0.25 g/l), indicating that the organisms from these cultures were severely stressed.

The results of the reference toxicant reproduction tests differed somewhat from the other tests. the concentration of sodium chloride that impaired reproduction in the 20% DMW cultures was almost always 0.4 g/l. In contrast, in the Pen Branch cultures, the concentration that most often impaired reproduction was 0.8 g/l, which indicates that reproduction in the Pen Branch cultures was less impaired by sodium chloride than the 20% DMW cultures. Reproduction in the Upper Three Runs and Fourmile Branch cultures was generally impaired by extremely low concentrations of sodium chloride, indicating that the cultures were severely stressed.

CONCLUSIONS

Pen Branch - The results of this study indicate that water collected from Pen Branch at Road B is not acutely toxic to *Ceriodaphnia*, but significantly impairs its reproduction approximately 50% of the time. *Ceriodaphnia* can be successfully maintained for extended periods of time in water from Pen Branch, and the response of organisms reared in the stream water to a reference toxicant reasonably approximate the response of organisms that are reared in 20% dilute mineral water. If toxicity assessments are needed for outfalls that discharge into Pen Branch, cultures of *Ceriodaphnia* could be established in water collected from Pen Branch at Road B. Toxicity tests could then be performed on water samples collected from Pen Branch downstream from outfalls using the Road B culture as the control for the tests.

Upper Three Runs - Water from Upper Three Runs at Road 8-1 is capable of maintaining cultures of *Ceriodaphnia* for extended periods of time, but the water resulted in reduced survival of the organisms approximately 50% of the time and consistently resulted in reduced rates of reproduction, when compared to organisms that were reared in 20% DMW. The results of the reference toxicity tests also indicate that *Ceriodaphnia* from the Upper Three Runs cultures are much more sensitive to the reference toxicant than are organisms reared in 20% DMW. These results indicate that it would not be advisable to use cultures from water collected from Upper Three Runs at Road 8-1 as controls for toxicity tests conducted on water collected from Upper Three Runs downstream from NPDES outfalls. Additional studies are needed to determine whether the observed toxicity is due to low pH or the presence of toxicants in the water, or whether essential trace minerals are lacking in the water.

Fourmile Branch - Water from Fourmile Branch at Road F is not capable of maintaining cultures of *Ceriodaphnia* for extended periods of time. Water from Fourmile Branch was often acutely toxic and always chronically toxic to *Ceriodaphnia*. Clearly, water from Fourmile Branch at Road E is not suitable for culturing *Ceriodaphnia*. Additional testing is needed to determine why *Ceriodaphnia* is adversely affected by water from Fourmile Branch, but low pH is probably at least partially responsible for the observed toxicity.

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APPENDIX 1

DECEMBER 1993

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Cultures were established in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Four Mile Creek (sampled at Road F bridge), and 3) Pen Branch (sampled at Road B bridge). Culture water was sampled on December 16, 1993 and December 30, 1993.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 1. Chemical parameter results analyzed in the laboratory are presented in Table 2.

Culture Initiation

Cultures were initiated on December 17, 1993 with receiving water samples collected on December 16, 1993. In order to gradually acclimate the neonates obtained from diluted mineral water (DMW) stocks, the initial culture solutions were set at 20% receiving water and 80% DMW. Culture solutions were renewed at 72 hours using a proportion of 40% receiving water and 60% DMW. After five days some low level acute toxicity was noted in the stocks kept in receiving water from Upper Three Runs Creek (10% mortality) and Four Mile Creek (30% mortality). As a result it was decided to slow the acclimation of stocks in those two receiving waters. Thus at 120 hours (Day 5) cultures were renewed again in 40% receiving water for both Upper Three Runs Creek and Four Mile Creek. Cultures in receiving water from Pen Branch were renewed with 60% receiving water. The renewals are summarized as follows:

<u>Renewal</u>	<u>% Receiving Water</u>	<u>% Dilution Water</u>	<u>Receiving Water</u>
Initiation	20%	80%	Upper Three Runs
	20%	80%	Four Mile Creek
	20%	80%	Pen Branch
Day 3	40%	60%	Upper Three Runs
	40%	60%	Four Mile Creek
	40%	60%	Pen Branch
Day 5	40%	60%	Upper Three Runs
	40%	60%	Four Mile Creek
	60%	40%	Pen Branch

After seven days the initial culture trays had completed producing three broods of young. Means for young per female were 21.1 for Upper Three Runs Creek, 15.9 for Four Mile Branch, and 22.3 for Pen Branch. Mean reproduction for DMW stock trays during the same week ranged from 17.9 to 25.2. Both Upper Three Runs and Pen Branch means fell within this range. Mean reproduction for Four Mile Creek was comparatively low.

On December 24, 1993 neonates from the third brood of the initial test trays were used to set new culture trays. Culture water was prepared at 40% dilution for Four Mile Creek stocks, 60% dilution for Upper Three Runs Creek stocks and 80% for Pen Branch stocks. On Day 3 the new culture solutions were set at 60% for Four Mile Creek, 80% for Upper Three Runs Creek, and 100% for Pen Branch. Finally, on Day 5 the culture solutions were set at 100% for Pen Branch and Upper Three Runs and at 80% for Four Mile Creek. The renewals are summarized as follows:

<u>Renewal</u>	<u>% Receiving Water</u>	<u>% Dilution Water</u>	<u>Receiving Water</u>
Initiation	60%	40%	Upper Three Runs
	40%	60%	Four Mile Creek
	80%	20%	Pen Branch
Day 3	80%	20%	Upper Three Runs
	60%	40%	Four Mile Creek
	100%	0%	Pen Branch
Day 5	100%	0%	Upper Three Runs
	80%	20%	Four Mile Creek
	100%	0%	Pen Branch

Mean reproduction after seven days was 22.7 young per female for Upper Three Runs, 18.4 for Four Mile Creek, and 21.2 for Pen Branch. All three values were within the range of mean reproduction of stock organisms in DMW. It should be noted, however, that Four Mile Creek stock organisms had yet to be transferred to 100% receiving water.

SUMMARY: Initial results indicated that it would be possible to culture Ceriodaphnia dubia in all three receiving waters. It appeared that cultures in Four Mile Creek water might not reproduce as well as cultures in other receiving waters.

Pass/Fail Receiving Water Tests

In as much as December was the first month of receiving water stock organism initiation, no pass/fail tests were set.

TABLE 1
Field Water Chemistry Measurements (12/16/93)

Parameter	Upper Three Runs	Four Mile Creek	Pen Branch
Sampling Date	12/16/93	12/16/93	12/16/93
Sampling Time	13:30	14:30	15:30
D.O. (ppm)*	9.8	9.5	10.0
Conductivity (μ mhos)*	14.0	31.0	43.0
pH (units)*	5.68	5.38	6.76
Temperature ($^{\circ}$ C)	ND	ND	ND

* On the first sample field measurements were not taken. On all subsequent samples these are field measurements. ND = no data.

TABLE 1
Field Water Chemistry Measurements (12/30/93)

Parameter	Upper Three Runs	Four Mile Creek	Pen Branch
Sampling Date	12/30/93	12/30/93	12/30/93
Sampling Time	12:45	11:30	12:00
D.O. (ppm)	10.0	13.4	11.0
Conductivity (μ mhos)	14.0	33.2	46.2
pH (units)	5.55	4.49	6.73
Temperature ($^{\circ}$ C)	10.6	6.9	7.4

APPENDIX 2

JANUARY 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Four Mile Creek (sampled at Road F bridge), and 3) Pen Branch (sampled at Road B bridge). Culture water was sampled on January 14, 1994 and January 27, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 1. Chemical parameter results analyzed in the laboratory are presented in Table 2.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of January 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 16.5 during the week of December 31 to January 7, 14.6 during the week of January 7-14, 13.1 during the week of January 11-18, 11.7 during the week of January 14-21, and 15.3 during the week of January 21-28. There was no mortality among the stock organisms. This level of reproduction was substantially lower than the level noted for stocks maintained in 20% diluted mineral water (DMW) or receiving water from Pen Branch. The size of the stock organisms was also smaller than the size of those maintained in 20% DMW and Pen Branch water.

Stock Organism Summary - Upper Three Runs Creek - January 1994

Culture Tray	Reproduction	Mortality
December 31-January 7, 1994	16.5	0%
January 7-14, 1994	14.6	0%
January 11-18, 1994	13.1	0%
January 14-21, 1994	11.7	0%
January 21-28, 1994	15.3	0%

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 14.0 during the week of December 31-January 7, 11.6 during the week of January 7-14, 15.1 during the week of January 11-18, 11.7 during the week of January 14-21, and 17.9 during the week of January 21-28. There was also substantial mortality among the stock organisms; 20% (December 31-January 7), 40% (January 7-14), 10% (January 11-18), 10% (January 14-21), and 20% (January 21-28). This level of reproduction was similar to that noted for Upper Three Runs Creek, but substantially lower than the level noted for stocks maintained in 20% diluted mineral water (DMW) or receiving water from Pen Branch. The size of the stock organisms was also smaller than the size of those maintained in 20% DMW and Pen Branch water.

Stock Organism Summary - Four Mile Branch - January 1994

Culture Tray	Reproduction	Mortality
December 31-January 7, 1994	14.0	20%
January 7-14, 1994	11.6	40%
January 11-18, 1994	15.1	10%
January 14-21, 1994	11.7	10%
January 21-28, 1994	17.9	20%

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 20.0 during the week of December 31-January 7, 24.3 during the week of January 7-14, 24.9 during the week of January 11-18, 21.4 during the week of January 14-21, and 26.7 during the week of January 21-28. There was no mortality among the stock organisms. This level of reproduction was similar to that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek and Four Mile Branch. The size and color of the stock organisms was optimal.

Stock Organism Summary - Pen Branch - January 1994

Culture Tray	Reproduction	Mortality
December 31-January 7, 1994	20.0	0%
January 7-14, 1994	24.3	0%
January 11-18, 1994	24.9	0%
January 14-21, 1994	21.4	0%
January 21-28, 1994	26.7	0%

Receiving Water Culture Reproduction

January 1994

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was set January 25, 1994. It was not possible to set the test earlier in the month due to the relatively small broods produced by the stock organisms. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	24.1	
Pen Branch Water	90%	8.5	13.7 (Fail)

The results clearly indicate that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was set January 25, 1994. It was not possible to set the test earlier in the month due to the relatively small broods produced by the stock organisms. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	24.1	
Pen Branch Water	80%	6.6	14.4 (Fail)

The results clearly indicate that stock organisms maintained in Four Mile Branch water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set January 18, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	23.3	
Pen Branch Water	95%	25.3	-1.5 (Pass)

The results clearly indicate that stock organisms maintained in Pen Branch water reproduced as well as those maintained in 20% DMW.

TABLE 1
Field Water Chemistry Measurements (1/14/94)

Parameter	Upper Three Runs	Four Mile Creek	Pen Branch
Sampling Date	1/14/94	1/14/94	1/14/94
Sampling Time	13:00	14:00	12:40
D.O. (ppm)	10.2	10.2	10.9
Conductivity (μ mhos)	14.9	33.7	57.7
pH (units)	5.57	5.88	6.85
Temperature ($^{\circ}$ C)	7.4	8.2	9.3

TABLE 1
Field Water Chemistry Measurements (1/27/94)

Parameter	Upper Three Runs	Four Mile Creek	Pen Branch
Sampling Date	1/27/94	1/27/94	12/30/94
Sampling Time	13:40	13:15	12:40
D.O. (ppm)	12.0	10.6	11.2
Conductivity (μ mhos)	18.8	34.7	39.6
pH (units)	5.73	5.44	7.10
Temperature ($^{\circ}$ C)	11.9	9.0	10.2

Reference Toxicant Testing

Stock organisms cultured in each of the three receiving waters were used in reference toxicant tests to determine the relative health and sensitivity of each of the cultures. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 3.0, 2.5, 2.0, 1.5, 1.0, and 0.0 g/L of reference toxicant. Insufficient neonates were produced by Upper Three Runs Creek and Four Mile Branch stock organisms to provide for setting definitive acute tests. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.22 g/L. This value was substantially more than two standard deviation units below the mean 48 Hour LC_{50} of 2.08 g/L NaCl noted for testing using 20% DMW.

Chronic reference toxicant tests used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Reproduction by controls in the chronic reference toxicant tests for Upper Three Runs Creek and Four Mile Branch was well below 15 young per female, and mortality exceeded 20%. As a result, these cannot be considered valid tests. Mortality was high at all NaCl concentrations down to 0.2 g/L. The Pen Branch chronic reference toxicant test indicated a NOEC for survival of 0.8 g/L NaCl, and a NOEC for reproduction of 0.4 g/L. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW.

Chemical Analyses

Analysis for aluminum in receiving water samples collected on December 16, 1993 showed the following levels to be present:

	Dissolved Aluminum
Upper Three Runs Creek	0.34 mg/L
Four Mile Branch	0.12 mg/L
Pen Branch	0.078 mg/L

APPENDIX 3

FEBRUARY 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Four Mile Creek (sampled at Road F bridge), and 3) Pen Branch (sampled at Road B bridge). Cultures in Four Mile Creek water were discontinued on February 23, 1994. Culture water was sampled at all three sites on February 11, 1994. Due to a storm event, no sample was collected during the week of February 21-25, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 1. Chemical parameter results analyzed in the laboratory are presented in Table 2.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of February 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 9.1 during the week of January 28 to February 4, 4.4 during the week of February 4-11, 12.8 during the week of February 11-18, and 13.2 during the week of February 18-25. There was no mortality among the stock organisms except on the week of February 18-25, when there was 10%

mortality. The noted level of reproduction, which reached a low during the early part of the month, was substantially lower than the level noted for stocks maintained in 20% diluted mineral water (DMW) or receiving water from Pen Branch. The size of the stock organisms was also smaller than the size of those maintained in 20% DMW and Pen Branch water.

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 7.0 during the week of January 28-February 4, 8.3 during the week of February 4-11, and 2.0 during the week of February 11-18, 1994. There was also substantial mortality among the stock organisms; 30% (February 4-11), 60% (February 11-18). The noted level of reproduction was initially similar to that noted for Upper Three Runs Creek, but dropped sharply during the month. Due to the poor reproduction and survival, the WSRC STR instructed that culturing using Four Mile Branch Receiving water be discontinued.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 30.0 during the week of January 28-February 4, 22.7 during the week of February 4-11, 21.2 during the week of February 11-18, 18.4 during the week of February 18-25, and 22.1 during the week of February 25-March 4. There was no significant mortality among the stock organisms. The noted level of reproduction was similar to that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek and Four Mile Branch. The size and color of the stock organisms were optimal.

Figure 1

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on February 22, 1994. It was not possible to set the test earlier in the month due to the relatively small broods produced by the stock organisms. Due to contamination of the effluent sample on Day 4, the test had to be terminated. The test results at the point of test termination (prior to sample contamination) are summarized as follows:

	Survival	Average 1st Brood
20% DMW Control	100%	4.5
Upper Three Runs Cr.	85%	0.5

The results suggest that the test would have been a failure, if completed, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW. Due to the date of the test, a re-test could not be completed within the month of February.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was not set due to the small broods produced by the stock organisms.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set February 22, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	90%	21.1	
Pen Branch Water	100%	25.1	-2.3 (Pass)

The results clearly indicate that stock organisms maintained in Pen Branch water reproduced as well (or better) as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (2/11/94 Sample)

Parameter	Upper Three Runs	Four Mile Creek	Pen Branch
Sampling Date	2/11/94	2/11/94	2/11/94
Sampling Time	11:15	11:45	12:20
D.O. (ppm)	11.8	10.4	10.6
Conductivity (μ mhos)	21.2	40.2	42.1
pH (units)	6.11	5.29	6.58
Temperature ($^{\circ}$ C)	11.0	9.7	10.4

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. Insufficient neonates were produced by Upper Three Runs Creek and Four Mile Branch stock organisms to provide for setting definitive acute tests. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.13 g/L. This value was substantially more than two standard deviation units below the mean 48 Hour LC_{50} of 2.08 g/L NaCl noted for testing using 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for both survival and reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW.

Chemical Analyses

No additional chemical analyses were conducted during February 1994.

APPENDIX 4

MARCH 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in two surface waters from the Savannah River Site. The two surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge) and 2) Pen Branch (sampled at Road B bridge). Culture water was sampled at both sites on March 3 and March 11, 1994. Due to a storm event, no sample was collected during the final week of March, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 1. Chemical parameter results analyzed in the laboratory are presented in Table 2.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of March 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 7.6 during the week of February 25 to March 4, 12.0 during the week of March 9-16, 5.9 during the week of March 11-18, 16.0 during the week of March 18-25, and 15.5 during the week of March 25 - April 1. Mortality among the stock organisms was limited to 10% on the week of March 11-18, and 20% during the week of March 25 - April 1, 1994. The noted level of reproduction, which reached a low during the middle part of the month, was substantially lower than the level noted for stocks maintained in 20% diluted mineral water (DMW) or receiving water from Pen Branch.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 22.1 during the week of February 25 - March 4, 24.8 during the week of March 4-11, 13.9 (2 broods; 3 broods estimated at 17.0) during the week of March 11-18, 22.8 during the week of March 21-28, and 20.2 during the week of March 25 - April 1, 1994. There was no significant mortality among the stock organisms. The noted level of reproduction was similar to that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Figure 1

SRS RECEIVING WATER STUDY 7 Day Reproduction

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on March 21, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	90%	20.4	
Upper Three Runs Cr.	65%	6.7	7.15 (Fail)

The results show the test to be a failure, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set February 22, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	90%	20.4	
Pen Branch Water	95%	19.7	-0.33 (Pass)

The results clearly indicate that stock organisms maintained in Pen Branch water reproduced as well (or better) as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (3/3/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	3/3/94		3/3/94
Sampling Time	14:35		15:15
D.O. (ppm)	9.8		10.2
Conductivity (μ mhos)	31.3		56.1
pH (units)	5.23		6.31
Temperature ($^{\circ}$ C)	13.6		13.1

Table 2
Field Water Chemistry Measurements (3/11/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	3/11/94		3/11/94
Sampling Time	11:45		10:30
D.O. (ppm)	10.4		10.4
Conductivity (μ mhos)	15.0		36.0
pH (units)	5.83		6.49
Temperature ($^{\circ}$ C)	16.0		13.0

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. Insufficient neonates were produced by Upper Three Runs Creek stock organisms to provide for setting definitive acute tests. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.22 g/L. This value was substantially more than two standard deviation units below the mean 48 Hour LC_{50} of 2.08 g/L NaCl noted for testing using 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for both survival and reproduction of 0.4 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. It was also lower than the NOEC values noted in February 1994.

Chemical Analyses

No additional chemical analyses were conducted during February 1994.

APPENDIX 5

APRIL 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in two surface waters from the Savannah River Site. The two surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge) and 2) Pen Branch (sampled at Road B bridge). Culture water was sampled at both sites on April 8 and April 21, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of April 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 16.7 during the week of April 1-8, 19.0 during the week of April 8-15, 18.4 during the week of April 15-22, and 11.5 during the week of April 22-29 (Table 1; Figure 1). Mortality among the stock organisms was limited to 10% on the week of April 1-8, and 30% during the week of April 22-29, 1994. The noted level of reproduction, which remained moderately high during most of the month, dropped during the last week of the period.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 22.0 during the week of April 1-8, 25.3 during the week of April 8-15, 21.9 during the week of April 15-22, and 25.3 during the week of April 22-29. There was no mortality among the stock organisms. The noted level of reproduction was similar to that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Figure 1: SRS Receiving Water Study

7 Day Reproduction

red arrows indicate new water sample

■ Upper 3 Runs ■ Pen Branch

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on April 22, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	21.9	
Upper Three Runs Cr.	85%	12.1	883 (Fail)

The results show the test to be a failure, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set April 22, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	21.9	
Pen Branch Water	95%	20.1	1.1 (Pass)

The results clearly indicate that stock organisms maintained in Pen Branch water reproduced as well as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (4/6/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	4/6/94		4/6/94
Sampling Time	14:30		13:45
D.O. (ppm)	9.0		8.9
Conductivity (μ mhos)	12.7		37.7
pH (units)	5.99		7.18
Temperature ($^{\circ}$ C)	19.5		20.1

Table 2
Field Water Chemistry Measurements (4/21/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	4/21/94		4/21/94
Sampling Time	14:00		13:00
D.O. (ppm)	9.2		9.0
Conductivity (μ mhos)	18.7		47.2
pH (units)	6.05		6.99
Temperature ($^{\circ}$ C)	20.3		21.0

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. Insufficient neonates were produced by Upper Three Runs Creek stock organisms to provide for setting definitive acute tests. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.19 g/L. This value was in the same range as noted in previous months (January 1.22 g/L; February 1.13 g/L; March 1.22 g/L) but well below the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set April 22-29, 1994 used a dilution series of 0.0625, 0.125, 0.25, 0.5 and 1.0 g/L NaCl. The control for the test showed a mean reproduction of 12.9 young per female, which is less than the minimum reproduction of 15.0 necessary for a valid test. Results indicated a NOEC for both survival and reproduction of <0.0625 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results indicate that organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity than those cultured in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on April 22-29, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for both survival and reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that although organisms cultured in Pen Branch Receiving water are more acutely sensitive to toxicity than organisms cultured in 20% DMW, they are no more chronically sensitive, i.e., they reproduce equally well.

APPENDIX 6

MAY 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in two surface waters from the Savannah River Site. The two surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge) and 2) Pen Branch (sampled at Road B bridge). Culture water was sampled at both sites on May 3 and May 19, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of May 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 15.3 during the week of April 29-May 5, 16.3 during the week of May 6-13, 15.1 during the week of May 13-20, and 17.6 during the week of May 20-27 (Table 1; Figure 1). Mortality among the stock organisms ranged from 10% to 20%. The noted level of reproduction, which remained moderately high during most of the month, rose slightly during the last week of the period.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 22.1 during the week of April 29 - May 5, 25.3 during the week of May 6-13, 26.1 during the week of May 13-20, and 20.0

during the week of May 20-27. There was no mortality among the stock organisms except during the last week, when there was 10% mortality. The noted level of reproduction was similar to that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Figure 1

SRS Receiving Water Study

7 Day Reproduction

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on May 20, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	25.8	
Upper Three Runs Cr.	95%	15.3	11.5 (Fail)

The results show the test to be a failure, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set May 20, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	25.8	
Pen Branch Water	95%	15.7	9.7 (Fail)

The results indicate that stock organisms maintained in Pen Branch water did not reproduce as well as those maintained in 20% DMW. This result was dissimilar to previous monthly tests, which showed equally good reproduction in 20% DMW and Pen Branch water.

Table 2
Field Water Chemistry Measurements (5/3/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	5/3/94		5/3/94
Sampling Time	14:00		13:15
D.O. (ppm)	7.8		8.4
Conductivity (μ mhos)	18.8		51.5
pH (units)	5.93		6.81
Temperature ($^{\circ}$ C)	17.0		18.1

Table 2
Field Water Chemistry Measurements (5/19/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	5/19/94		5/19/94
Sampling Time	13:15		12:40
D.O. (ppm)	9.5		9.2
Conductivity (μ mhos)	34.3		52.8
pH (units)	5.57		7.42
Temperature ($^{\circ}$ C)	19.0		18.9

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 2.07 g/L. This value was higher than previous monthly values (1.13 - 1.19 g/L) and approximately the same as the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are similarly acutely sensitive to toxicity as test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.13, 0.06, 0.03 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 0.12 g/L. This value was much lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW or Pen Branch water.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set May 17-25, 1994 used a dilution series of 0.016, 0.030, 0.060, 0.130 and 0.250 g/L NaCl. The control for the test showed a mean reproduction of 18.1 young per female. Reproduction declined to 17.2 young per female in 0.016 g/L, 15.8 g/L in 0.03 g/L, 12.0 in 0.06 g/L, 6.9 in 0.13 g/L and 0.0 in 0.25 g/L. The reduction was significant at 0.06 g/L (the LOEC for reproduction) and above, resulting in a NOEC for reproduction of 0.03 g/L. Survival results indicated a NOEC for survival of 0.13 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L

for test organisms in 20% DMW. Results indicate that organisms cultured in Upper Three Runs Creek receiving water are far more chronically sensitive to toxicity than those cultured in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on May 17-25, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

Chemical Analyses

No additional chemical analyses were conducted during May 1994.

APPENDIX 7

JUNE 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in two surface waters from the Savannah River Site. The two surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge) and 2) Pen Branch (sampled at Road B bridge). Culture water was sampled at both sites on June 1, June 14, and June 30, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of May 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 24.2 during the week of May 27 - June 2, 20.1 during the week of June 3-10, 16.9 during the week of June 10-17, 16.9 during the week of June 17-24, and 18.0 during the week of June 24-30 (Table 1; Figure 1). Mortality among the stock organisms ranged from 0% to 30% per week. The noted level of reproduction fell slightly during the latter part of the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 28.5 during the week of May 27 - June 2, 26.3 during the week of June 3-10, 25.0 during the week of June 10-17, 20.2 during the week of June 17-24, and 25.3 during the week of June 24-30. There was no mortality among the stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Figure 1

SRS Receiving Water Study

Pass Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on June 17, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	25.8	
Upper Three Runs Cr.	90%	16.4	(Fail)

The results show the test to be a failure, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set June 17, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	28.8	
Pen Branch Water	100%	18.7	6.6 (Fail)

The results indicate that stock organisms maintained in Pen Branch water did not reproduce as well as those maintained in 20% DMW. This result was similar to that noted in May, but dissimilar to previous monthly tests, which showed equally good reproduction in 20% DMW and Pen Branch water.

Table 2
Field Water Chemistry Measurements (6/1/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	6/1/94		6/1/94
Sampling Time	14:00		13:20
D.O. (ppm)	9.4		9.0
Conductivity (µmhos)	13.4		58.1
pH (units)	5.75		7.08
Temperature (°C)	21.3		24.3

Table 2

Field Water Chemistry Measurements (6/14/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	6/14/94		6/14/94
Sampling Time	13:30		13:00
D.O. (ppm)	8.4		8.7
Conductivity (µmhos)	16.5		66.0
pH (units)	5.45		7.18
Temperature (°C)	22.3		26.2

Table 2
Field Water Chemistry Measurements (6/30/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	6/30/94		6/30/94
Sampling Time	12:35		11:45
D.O. (ppm)	8.8		8.6
Conductivity (µmhos)	14.2		53.7
pH (units)	5.43		6.65
Temperature (°C)	20.7		21.5

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.92 g/L. This value was similar to the value noted in May but higher than previous monthly values (1.13 - 1.19 g/L) and approximately the same as the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are similarly acutely sensitive to toxicity as test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.13, 0.06, 0.03 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 0.18 g/L. This value was much lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW or Pen Branch water.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set June 14-21, 1994 used a dilution series of 0.032, 0.0625, 0.125, 0.250 and 0.50 g/L NaCl. The control for the test showed a mean reproduction of 16.9 young per female. Reproduction declined to 14.0 young per female in 0.032 g/L, 6.1 g/L in 0.0625 g/L, 0.40 in 0.125 g/L, 0.0 in 0.25 g/L and 0.0 in 0.50 g/L. The reduction was significant at 0.0625 g/L (the LOEC for reproduction) and above, resulting in a NOEC for reproduction of 0.032 g/L. Survival results indicated a NOEC for survival of 0.032 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction

of 0.4 g/L for test organisms in 20% DMW. Results indicate that organisms cultured in Upper Three Runs Creek receiving water are far more chronically sensitive to toxicity than those cultured in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on June 14-21, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

Chemical Analyses

No additional chemical analyses were conducted during May 1994.

APPENDIX 8

JULY 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three Surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Pen Branch (sampled at Road B bridge) and 3) Four Mile Branch (sampled at Road F). Culture water for Pen Branch and Upper Three Runs Creek was sampled at both sites on July 12 and July 28, 1994. Culture Water for Four Mile Branch was sampled on July 22 and July 28, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of July 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994 and reestablished on July 29, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 9.4 during the week of July 1-7 1994 (a third brood was not released due to mortality of the stock organisms caused by acidic contamination of the sample), 23.4 during the week of July 8-15, 25.6 during the week of July 15-22, and 12.4 during the week of July 22-29, (Table 1; Figure 1). With the exception of stock organisms during the week of July 1-7, no mortality among the stock organisms was noted. The noted level of reproduction fell substantially during the last week of the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 25.0 during the week of July 1-7, 20.9 during the week of July 8-15, 25.5 during the week of July 15-22, and 27.6 during the week of July 22-29. There was no mortality among the stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Figure 1

SR5 Receiving Water Study

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on July 22, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	23.0	
Upper Three Runs Cr.	80%	12.5	6.5 (Fail)

The results show the test to be a fail, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set July 1, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	25.8	
Pen Branch Water	100%	25.0	0.61 (Pass)

The results indicate that stock organisms maintained in Pen Branch water did reproduce as well as those maintained in 20% DMW. This result was similar to previous monthly tests, which showed equally good reproduction in 20% DMW and Pen Branch water.

Table 2
Field Water Chemistry Measurements (6/30/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	6/30/94		6/30/94
Sampling Time	12:35		11:45
D.O. (ppm)	8.8		8.6
Conductivity (μ mhos)	14.2		53.7
pH (units)	5.43		6.65
Temperature ($^{\circ}$ C)	20.7		21.5

Table 2
Field Water Chemistry Measurements (7/12/94 Sample)

Parameter	Upper Three Runs		Pen Branch
Sampling Date	7/12/94		7/12/94
Sampling Time	10:30		12:00
D.O. (ppm)	8.4		8.7
Conductivity (μ mhos)	16.5		66.0
pH (units)	7.45		7.42
Temperature ($^{\circ}$ C)	22.3		26.2

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.55 g/L. This value was higher than most previous monthly values (1.13 - 1.19 g/L) and substantially lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.13, 0.06, 0.03 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of <0.03 g/L. This value was much lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW or Pen Branch water.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set July 21-28, 1994 used a dilution series of 0.03, 0.06, 0.125, 0.25 and 0.5 g/L NaCl. The control for the test showed a mean reproduction of 14.6 young per female. Reproduction declined to 8.1 young per female in 0.03 g/L, 11.4 g/L in 0.06 g/L, 1.7 in 0.125 g/L, 0.0 in 0.25 g/L and 0.0 in 0.5 g/L. The reduction was significant at 0.125 g/L (the LOEC for reproduction) and above, resulting in a NOEC for reproduction of 0.06 g/L. Survival results also indicated a NOEC of 0.06 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L.

for test organisms in 20% DMW. Results indicate that organisms cultured in Upper Three Runs Creek receiving water are far more chronically sensitive to toxicity than those cultured in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on July 21-28, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

Chemical Analyses

Two effluent samples were analyzed for dissolved heavy metals during July 1994. The results of these analyses are summarized below:

<u>Metal</u>	<u>Outfall A-001</u>	<u>Outfall A-003</u>
Ag	<3.0 µg/L	Not analyzed
As	<5.0 µg/L	14.0 µg/L
Cd	<0.3 µg/L	Not analyzed
Cr	<3.0 µg/L	4.3 µg/L
Cu	42.0 µg/L	160 µg/L
Fe	12.0 µg/L	Not analyzed
Hg	0.23 µg/L	Not analyzed
Mn	<3.0 µg/L	Not analyzed
Ni	<3.0 µg/L	Not analyzed
Pb	16.0 µg/L	10 µg/L
Zn	22.0 µg/L	15.0 µg/L

APPENDIX 9

AUGUST 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Pen Branch (sampled at Road B bridge) and 3) Four Mile Branch (sampled at Road F). Culture water for Pen Branch, Upper Three Runs Creek and Four Mile Branch was sampled on August 11, and August 25, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of August 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994 and reestablished on July 29, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Stocks set on August 5, 1994 for Pen Branch were changed to stocks for Upper Three Runs Creek on August 8, due to the mortality of the Upper Three Runs Creek stocks set on August 5, 1994. Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 22.1 during the week of August 5-12 1994, 15.8 during the week of August 12-19, 14.8 during the week of August 19-26. (Table 1; Figure 1). Stock organisms during the week of August 5-12 had 10% mortality and 30% during the week of August 19-26. The noted level of reproduction fell substantially during the middle of the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 22.5 during the week of August 5-12, 21.6 during the week of August 12-19, 20.6 during the week of August 19-26. There was no mortality among the stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW) and substantially higher towards the end of the month than that noted for stocks maintained in receiving water from Upper Three Runs Creek. The size and color of the stock organisms were optimal.

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 17.5 during the week of August 5-12, 21.9 during the week of August 12-19, and 20.9 during the week of August 19-26. Mortality among the test organisms was 10% or less during the period. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water and approximately the same as noted for stocks maintained in Pen Branch water.

Figure 1

MONTHLY STOCK CULTURE

Mean Reproduction (C. dubia)

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on August 19, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	27.2	
Upper Three Runs Cr.	45%	12.4	935 (Fail)

The results show the test to be a fail, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set August 19, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	27.2	
Pen Branch Water	95%	18.6	6.41 (Fail)

The results show the test to be a fail, and that stock organisms maintained in Pen Branch water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was set August 19, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	27.2	
Pen Branch Water	90%	22.1	3.45 (Fail)

The results show the test to be a fail, and that stock organisms maintained in Four Mile Branch water did not reproduce as well as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (8/11/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	8/11/94	8/11/94	8/11/94
Sampling Time	12:50 PM	11:45 AM	11:10 AM
D.O. (ppm)	8.6	1.2	7.8
Conductivity (μ mhos)	15.8	49.0	65.9
pH (units)	5.67	5.75	6.71
Temperature ($^{\circ}$ C)	20.8	23.4	25.3

Table 2
Field Water Chemistry Measurements (8/25/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	8/25/94	8/25/94	8/25/94
Sampling Time	3:30 PM	2:45 PM	2:15 PM
D.O. (ppm)	8.0	4.0	7.6
Conductivity (µmhos)	137	36.1	52.9
pH (units)	5.69	5.36	7.03
Temperature (°C)	22.0	27.3	25.0

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.43 g/L. This value was within the range of most previous monthly values (1.19 - 1.55 g/L) and substantially lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.13, 0.06, 0.03 and 0.0 g/L of NaCl. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of <0.03 g/L. This value was much lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW or Pen Branch water.

An acute definitive reference toxicant test with Four Mile Branch dilution water was set with a dilution series of 3.0, 2.5, 2.0, 1.5 and 1.0 g/L of NaCl. The results of the acute reference toxicant test for Four Mile Branch stock organisms in Four Mile Branch water showed a 48 Hour LC_{50} of <1.0 g/L NaCl. This value was much lower than that of the 48 Hour LC_{50} of 2.08 g/L NaCl noted for the reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Four Mile Branch water are far more acutely sensitive to toxicants as test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water was not a valid test, due to mortality exceeding 10% among the control organisms.

A chronic reference toxicant test set for Pen Branch receiving water, set on August 26-September 2, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

A chronic reference toxicant test set for Four Mile Branch receiving water, set on August 19-26, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of <0.2 g/L and a NOEC for reproduction of <0.2 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Four Mile Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

Chemical Analyses

No samples were analyzed for chemical parameters during August 1994.

APPENDIX 10

SEPTEMBER 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Pen Branch (sampled at Road B bridge) and 3) Four Mile Branch (sampled at Road F). Culture water for Pen Branch, Upper Three Runs Creek and Four Mile Branch was sampled on September 8, and September 22, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of August 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994 and reestablished on July 29, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 19.7 during the week of September 2-10, 19.3 during the week of September 9-17 and 17.6 during the week of September 16-23. (Table 1; Figure 1). Stock organisms during the week of September 2-10 had 30% mortality and there was 10% mortality during the week of September 16-23. The noted level of reproduction fell during the middle of the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 28.6 during the week of September 2-10, 20.8 during the week of September 9-17 and 24.5 during the week of September 16-23. There was no mortality among the stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW).

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 16.4 during the week of September 2-10, 17.7 during the week of September 9-16 and 14.5 during the week of September 16-23. Mortality among the stock organisms was less than 10% during the week of September 2-10. The noted level of reproduction was substantially lower than that noted for stocks maintained in 20% diluted mineral water.

Figure 1

MONTHLY STOCK CULTURE

Mean Reproduction (C. dubia)

■ Upper Three Runs ■ Pen Branch
 ■ Four Mile Branch

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was initiated on September 16, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	29.0	
Upper Three Runs Cr.	90%	17.6	6.18 (Fail)

The results show the test to be a fail, indicating that stock organisms maintained in Upper Three Runs Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set September 9, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	95%	29.1	
Pen Branch Water	100%	21.0	3.15 (Fail)

The results show the test to be a fail, and that stock organisms maintained in Pen Branch water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was set September 23, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	28.1	
Four Mile Branch	95%	20.4	3.96 (Fail)

The results show the test to be a fail, and that stock organisms maintained in Four Mile Branch water did not reproduce as well as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (9/08/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	9/08/94	9/08/94	9/08/94
Sampling Time	1:30 PM	1:00 PM	12:30 PM
D.O. (ppm)	7.6	2.4	7.6
Conductivity (µmhos)	14.2	37.1	52.7
pH (units)	5.07	5.12	6.1
Temperature (°C)	20.5	24.5	24.5

Table 2
Field Water Chemistry Measurements (9/22/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	9/22/94	9/22/94	9/22/94
Sampling Time	9:25 AM	11:00 AM	12:00 PM
D.O. (ppm)	8.2	1.8	8.3
Conductivity (μmhos)	146	46.6	57.5
pH (units)	5.72	5.93	6.75
Temperature (°C)	19.3	19.8	20.2

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water, Upper Three Runs Creek and Four Mile Branch water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.43 g/L. This value was within the range of most previous monthly values (1.19 - 1.55 g/L) and substantially lower than the 48 Hour LC_{50} of 2.11 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.25, 0.125, 0.06, 0.03, 0.015 and 0.0 g/L of NaCl. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of >0.25 g/L. This value was much lower than the 48 Hour LC_{50} of 2.08 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Four Mile Branch dilution water was set with a dilution series of 2.0, 1.5, 0.5, 0.25, 0.125 and 0.0 g/L of NaCl. The results of the acute reference toxicant test for Four Mile Branch stock organisms in Four Mile Branch water showed a 48 Hour LC_{50} of 0.26 g/L NaCl. This value was much lower than that of the 48 Hour LC_{50} of 2.11 g/L NaCl noted for the reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Four Mile Branch water are far more acutely sensitive to toxicants as test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set on September 16-24, 1994, used a dilution series of 0.006, 0.0125, 0.025, 0.05 and 0.1 g/L. Results indicated a NOEC for survival of 0.025 g/L and a NOEC for reproduction of 0.0125 g/L. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on September 9-16, 1994, used a dilution series of 0.4, 0.8, 1.5, 2.0 and 2.5 g/L. Results indicated a NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.4 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

A chronic reference toxicant test set for Four Mile Branch receiving water, set on September 16-24, 1994, used a dilution series of 0.06, 0.125, 0.25, 0.5 and 1.0 g/L. Results indicated a NOEC for survival of <0.06 g/L and a NOEC for reproduction of <0.06 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Four Mile Branch Receiving water are far more chronically sensitive to toxicity than organisms cultured in 20% DMW.,

Chemical Analyses

No samples were analyzed for chemical parameters during September 1994.

APPENDIX 11

OCTOBER 1994

See Appendix 12 (November 1994) for cumulative toxicity and water chemistry data

0 SUMMARY OF PROJECT STATUS

1 Culture Maintenance Program

Sampling

Leiodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Pen Branch (sampled at Road B bridge) and 3) Four Mile Branch (sampled at Road F). Culture water for Pen Branch, Upper Three Runs Creek and Four Mile Branch was sampled on October 6, and October 20, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of October 1994, with the exception of cultures in Four Mile Branch water, which were discontinued on February 23, 1994 and reestablished on July 29, 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.1 mL of Selenastrum capricornutum and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 16.1 during the week of September 30-October 6, 13.5 during the week of October 7-14, 11.45 during the week of October 14-22 and 7.8 during the week of October 21-30. (Table 1; Figure 1). Mortality of stock organisms was 0% during the week of September 30-October 6, 5% during the week of October 7-14, 10% during the week of October 14-22, and 25% during the week of October 21-30. The noted level of reproduction fell gradually during the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 24.0 during the week of September 30-October 6, 18.25 during the week of October 7-14, 13.9 during the week of October 14-22 and 15.3 during the week of October 21-28. There was no mortality among stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW).

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 15.9 during the week of September 30-October 6, 10.2 during the week of October 7-14, 12.05 during the week of October 14-22 and 4.0 during the week of October 21-28. The condition of the cultures was variable, and during the final week of the month all the stock organisms in Four Mile Branch water died. Mortality among the test organisms was less than 10% during the week of September 30-October 6, and 45% during the week of October 7-14. Cultures were reinitiated on 10/28/94. The noted level of reproduction was low during most of the month, considerably lower than stocks maintained in 20% diluted mineral water.

Figure 1

MONTHLY STOCK CULTURE

Mean Reproduction (C. dubia)

■ Upper Three Runs ■ Pen Branch
 ■ Four Mile Branch

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was set on October 11, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	95%	24.6	
Upper Three Runs Water	100%	15.4	Rank Sum=226 (Fail)

The results show the test to be a fail, and that stock organisms in Upper Three Run Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set October 11, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	95%	24.6	
Pen Branch Water	95%	16.6	4.64 (Fail)

The results show the test to be a fail, and that stock organisms maintained in Pen Branch water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was set on October 24, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Water	95%	18.6	
Four Mile Branch Water	10%	6.5	3.71 (Fail)

The test results show the test to be a fail, and that stock organisms maintained in Four Mile Branch water did not reproduce as well as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (10/06/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	10/06/94	10/06/94	10/06/94
Sampling Time	11:20 AM	10:45 AM	10:15 AM
D.O. (ppm)	8.0	5.2	8.4
Conductivity (µmhos)	15.0	37.2	50.0
pH (units)	5.72	5.56	6.81
Temperature (°C)	17.5	18.0	17.0

Table 2
Field Water Chemistry Measurements (10/20/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	10/20/94	10/20/94	10/20/94
Sampling Time	3:00 PM	3:00 PM	3:00 PM
D.O. (ppm)	8.2	6.5	8.1
Conductivity (µmhos)	18.6	31.2	49
pH (units)	5.88	5.4	6.77
Temperature (°C)	16.6	20.1	19.1

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.41 g/L. This value was within the range of most previous monthly values (1.19 - 1.55 g/L) and substantially lower than the 48 Hour LC_{50} of 2.2 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.1, 0.05, 0.01 and 0.0 g/L of NaCl. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 0.11 g/L. This value was much lower than the 48 Hour LC_{50} of 2.2 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Four Mile Branch dilution water was set with a dilution series of 2.0, 1.5, 0.5, 0.25, 0.125 and 0.0 g/L of NaCl. The results of the acute reference toxicant test for Four Mile Branch stock organisms in Four Mile Branch water showed a 48 Hour LC_{50} of 0.62 g/L NaCl. This value was much lower than that of the 48 Hour LC_{50} of 2.2 g/L NaCl noted for the reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Four Mile Branch water are far more acutely sensitive to toxicants as test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set on October 11-20, 1994, used a dilution series of 0.00125, 0.0025, 0.005, 0.01 and 0.02 g/L. Results indicated a NOEC for survival of >0.02 g/L and a NOEC for reproduction of >0.02 g/L. This compared to a mean NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.8 g/L for test organisms in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on October 11-18, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 0.8 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.8 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.

A chronic reference toxicant test set for Four Mile Branch receiving water, set on October 24-November 1, 1994, used a dilution series of 0.06, 0.125, 0.25, 0.5 and 1.0 g/L. The test was invalid due to the toxicity of Four Mile Branch water without addition of the reference toxicant. Results indicated that, had the test been valid, it would have showed a NOEC for survival of 0.06 g/L and a NOEC for reproduction of 0.06 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.8 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Four Mile Branch Receiving water are more chronically sensitive to toxicity than organisms cultured in 20% DMW.

Chemical Analyses

No samples were analyzed for chemical parameters during October 1994.

APPENDIX 12

NOVEMBER 1994

This Appendix contains cumulative toxicity and water chemistry data.

**Toxicity Testing of Aqueous Samples
(Subcontract Number AA86036N)
MONTHLY PROGRESS REPORT**

for Month of November 1994

Prepared for Westinghouse Savannah River Company

Submittal Date: December 1, 1994

1.0 SUMMARY OF PROJECT STATUS

1.1 Culture Maintenance Program

Sampling

Ceriodaphnia dubia cultures were maintained in three surface waters from the Savannah River Site. The three surface waters were; 1) Upper Three Runs Creek (sampled at Road 8-1 bridge), 2) Pen Branch (sampled at Road B bridge) and 3) Four Mile Branch (sampled at Road F). Culture water for Pen Branch, Upper Three Runs Creek and Four Mile Branch was sampled on November 3, and November 18, 1994.

Chemical Analyses

Values of chemical parameters measured in the field are shown in Table 2. Chemical parameter results analyzed in the laboratory are presented in Table 3.

Culture Maintenance

Cultures established during the month of December 1993 were maintained during the month of November 1994. Test organisms were transferred to fresh culture water from the most recently collected batch on each monday, wednesday and friday. Each friday ten neonates (<24 hours old) were placed in ten test cups for each receiving water to serve as new stock organisms. Two week old organisms were also disposed of each friday. Reproduction and survival were recorded during the first seven days for each stock organism. Cultures were fed daily with 0.05 mL of 6.2×10^7 cells/mL of Selenastrum capricornutum suspension and 0.10 mL of YCT diet.

Upper Three Runs Creek Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Upper Three Runs Creek water was 14.0 during the week of October 28 - November 4, 10.7 during the week of November 4-11, 15.6 during the week of November 11-18, and 13.0 during the week of November 18-25 (Table 1; Figure 1). Mortality of stock organisms was 25% during the week of October 28 - November 4, 20% during the week of November 4-11, 5% during the week of November 11-18, and 0% during the week of November 18-25. Levels of reproduction remained about the same through the month.

Pen Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Pen Branch water was 16.4 during the week of October 28 - November 4, 16.9 during the week of November 4-11, 26.3 during the week of November 11-18, and 10.1 during the week of November 18-25. There was no significant mortality among stock organisms. The noted level of reproduction was slightly lower than that noted for stocks maintained in 20% diluted mineral water (DMW), and was uncharacteristically low during the final week of the month. It appears that the water quality of the sample collected on November 18 may have been less optimal than noted in previous samples.

Four Mile Branch Cultures:

Mean reproduction (7 Day/3 Brood) of stock organisms maintained in Four Mile Branch water was 14.8 during the week of October 28 - November 4, 10.7 during the week of November 4-11, 10.3 during the week of November 11-18, and 6.0 during the week of November 18-25. As noted periodically during previous months, surface water from Four Mile Branch is intermittently toxic to *Ceriodaphnia dubia*. This pattern continued during November 1993. Mortality among the test organisms was 80% during the week of October 28 - November 4, 60% during the week of November 4-11, 5% during the week of November 11-18, and 45% during the week of November 18-25. Mortality was likely related to the lower pH values of surface water samples collected during November, as compared to previous months. Due to high mortality among stock organisms, it was necessary to set new rows of stock organisms with neonates from Upper Three Runs Creek cultures during the first three weeks of the month. The noted level of reproduction was low during most of the month, considerably lower than stocks maintained in 20% diluted mineral water.

Table 1

Summary of Monthly Stock Culture Mean Reproduction (*C. dubia*)

Date	Upper Three Runs	Pen Branch
12/31/93	16.5	20.0
1/7/94	14.6	24.3
1/11/94	13.1	24.9
1/14/94	11.7	21.4
1/21/94	15.3	26.7
1/28/94	9.1	30.0
2/4/94	4.4	22.7
2/11/94	12.8	21.2
2/18/94	13.2	18.4
2/25/94	7.6	22.1
3/9/94	12.0	17.0
3/11/94	5.9	17.0
3/18/94	16.0	22.8
3/25/94	15.5	20.2
4/1/94	16.7	22.0
4/8/94	19.0	25.3
4/15/94	18.4	21.9
4/22/94	11.5	25.3
4/29/94	15.3	22.1
5/6/94	16.3	25.3
5/13/94	15.1	26.1
5/20/94	17.6	20.1
5/27/94	24.2	26.8

Table 1Summary of Monthly Stock Culture Mean Reproduction (*C. dubia*)

Date	Upper Three Runs	Pen Branch	Four Mile Branch
6/03/94	20.1	20.1	26.3
6/10/94	16.9	16.9	25.0
6/17/94	16.9	16.9	20.2
6/24/94	18.0	18.0	25.3
7/01/94	9.4	9.4	25.0
7/08/94	23.4	23.4	20.9
7/15/94	25.6	25.6	25.5
7/22/94	12.4	12.4	27.6
8/05/94	22.1	22.1	22.5
8/12/94	15.8	15.8	21.6
8/19/94	14.8	14.8	20.6
9/02/94	19.7	28.6	16.4
9/09/94	19.3	20.8	17.7
9/16/94	17.6	24.5	14.5
9/23/94	18.6	16.2	11.0
9/30/94	16.1	24.0	15.9
10/07/94	13.5	18.3	10.2
10/14/94	11.5	13.9	12.1
10/21/94	7.8	15.3	4.0

Table 1

Summary of Monthly Stock Culture Mean Reproduction (*C. dubia*)

Date	Upper Three Runs	Pen Branch	Four Mile Branch
10/28/94	14.0	16.4	14.8
11/4/94	10.7	16.9	10.7
11/11/94	15.6	26.3	10.3
11/18/94	13.0	10.1	6.0
11/25/94			
12/2/94			
12/9/94			
12/16/94			
12/23/94			

Figure 1

MONTHLY STOCK CULTURE

Mean Reproduction (C. dubia)

■ Upper Three Runs ■ Pen Branch
 ■ Four Mile Branch

Pass/Fail Receiving Water Tests

A Pass/Fail chronic toxicity test of Upper Three Runs Creek receiving water, as compared to laboratory water (20% DMW), was set on November 25, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	20.8	
Upper Three Runs Water	15%%	5.2	8.9 (Fail)

The results show the test to be a fail, and that stock organisms in Upper Three Run Creek water did not reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Pen Branch receiving water, as compared to laboratory water (20% DMW), was set November 11, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Control	100%	20.8	
Pen Branch Water	100%	26.3	-3.2 (Pass)

The results show the test to be a pass, and that stock organisms maintained in Pen Branch water did reproduce as well as those maintained in 20% DMW.

A Pass/Fail chronic toxicity test of Four Mile Branch receiving water, as compared to laboratory water (20% DMW), was set on November 25, 1994. The test results are summarized as follows:

	Survival	Mean Reproduction	t Value
20% DMW Water	100%	20.8	
Four Mile Branch Water	0%	0.0	N/A (Fail)

The test results show the test to be a fail, and that stock organisms maintained in Four Mile Branch water did not reproduce as well as those maintained in 20% DMW.

Table 2
Field Water Chemistry Measurements (11/03/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	11/03/94	11/03/94	11/03/94
Sampling Time	1330	1030	1000
D.O. (ppm)	10.0	6.2	10.0
Conductivity (μmhos)	14.9	28.5	42.5
pH (units)	6.26	4.55	6.17
Temperature (°C)	14.7	11.4	12.1

Table 2
Field Water Chemistry Measurements (11/18/94 Sample)

Parameter	Upper Three Runs	Four Mile Branch	Pen Branch
Sampling Date	11/18/94	11/18/94	11/18/94
Sampling Time	1200	1115	1030
D.O. (ppm)	9.2	7.1	9.8
Conductivity (µmhos)	14.6	29.5	46.8
pH (units)	5.67	4.84	5.63
Temperature (°C)	16.8	15.7	15.7

TABLE 3
Laboratory Water Chemistry Measurements

Upper Three Runs Creek

Sample Date	Lab #	Initial or Final	Hardness (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Conduct (umhos)	D.O. (mg/L)	pH
12/16/93	T1900	Initial	1.96	1.92	4.0	14.0	9.8	5.68
12/16/93	T1900	Final	10.0	1.92	spilled	27.8	10.2	5.96
12/30/93	T1937	Initial	8.0	1.92	2.6	14.0	10.0	5.50
12/30/93	T1937	Final	4.0	2.0	3.5	14.7	11.7	5.68
1/14/94	T2008	Initial	4.0	4.0	10.2	14.9	10.2	5.57
1/14/94	T2008	Final	4.0	1.0	4.0	18.1	9.2	6.65
1/27/94	T2069	Initial	4.0	2.0	4.0	18.8	12.0	5.73
1/27/94	T2069	Final	4.0	2.0	3.5	14.2	9.7	6.65
2/11/94	T2123	Initial	4.0	1.0	4.0	21.2	11.8	6.11
2/11/94	T2123	Final	3.6	<1.0	2.1	14.7	8.9	4.32
3/4/94	T2216	Initial	1.8	<1.0	11.0	31.3	9.8	5.23
3/4/94	T2216	Final	6.0	4.0	4.5	22.3	9.0	5.15
3/11/94	T2242	Initial	6.0	2.0	3.8	31.3	9.8	5.23
3/11/94	T2242	Final	4.0	2.0	3.4	33.8	9.0	5.65
4/8/94	T2357	Initial	4.0	2.0	3.5	12.7	9.0	5.99
4/22/94	T2425	Initial	4.0	6.7	2.7	18.7	9.2	6.05
4/22/94	T2425	Final	2.0	2.2	5.4	13.8	9.0	5.84
5/3/94	T2503	Initial	5.9	2.2	2.6	18.8	7.8	5.93
5/3/94	T2503	Final	5.4	2.0	2.5	14.2	8.4	5.81
5/19/94	T2565	Initial	4.2	2.0	2.1	34.3	9.5	5.57
5/19/94	T2565	Final	1.8	2.0	1.6	13.6	8.8	5.90

Table 3
Laboratory Water Chemistry Measurements
Upper Three Runs Creek

Sample Date	Lab #	Initial or Final	Hardness (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Conduct (µmhos)	D.O. (mg/L)	pH
6/1/94	T2607	Initial	3.6	2.03	2.6	13.4	9.4	5.75
6/1/94	T2607	Final	3.6	2.03	3.1	13.0	8.6	5.71
6/14/94	T2645	Initial	5.46	2.1	4.4	16.5	8.4	5.45
6/14/94	T2645	Final	3.64	-----	3.0	15.5	8.0	6.25
6/30/94	T2704	Initial	-----	2.07	3.6	14.2	8.8	5.43
6/30/94	T2704	Final	5.37	<1	3.9	14.2	9.0	5.19
7/12/94	T2769	Initial	5.37	2.07	2.7	16.5	8.4	7.45
7/12/94	T2769	Final	3.58	2.27	3.7	13.6	8.6	5.65
7/28/94	T2828	Initial	3.58	2.27	6.3	16.6	6.4	5.47
7/28/94	T2828	Final	3.58	2.19	4.1	15.0	8.1	5.27
8/11/94	T2895	Initial	-----	-----	1.6	15.8	8.6	5.67
8/11/94	T2895	Final	9.0	2.27	1.5	13.45	8.4	5.65
8/25/94	T2952	Initial	3.6	2.27	1.1	13.7	8.0	5.69
8/25/94	T2952	Final	5.64	2.27	1.5	18.73	8.0	6.12
9/08/94	T2996	Initial	3.58	2.27	1.7	14.2	7.6	5.07
9/08/94	T2996	Final	5.88	4.4	3.0	35.3	7.8	5.99
9/23/94	T3067	Initial	5.88	2.2	2.4	14.6	8.2	5.72
9/23/94	T3067	Final	7.84	4.4	4.0	17.37	8.4	6.13
10/06/94	T3106	Initial	3.92	2.15	2.5	15.0	8.0	5.72
10/06/94	T3106	Final	4.0	4.0	3.0	20.1	9.2	5.92
10/20/94	T3191	Initial	6.0	2.0	2.6	18.6	8.2	5.88

Upper Three Runs Creek

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TABLE 3
Laboratory Water Chemistry Measurements
Pen Branch

Sample Date	Lab #	Initial or Final	Hardness (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Conduct (umhos)	DO (mg/L)	pH
12/16/93	T1902	Initial	17.6	15.7	5.9	43.0	10.0	6.76
12/16/93	T1902	Final	24.0	7.7	spilled	46.0	10.2	6.87
12/30/93	T1939	Initial	18.0	16.3	4.1	46.2	11.0	6.73
12/30/93	T1939	Final	18.0	16.0	4.0	48.8	11.8	6.65
1/14/94	T2010	Initial	16.0	18.0	3.6	57.7	10.9	6.85
1/14/94	T2010	Final	14.0	12.0	6.1	45.2	9.1	6.75
1/27/94	T2071	Initial	14.0	12.0	4.2	39.6	11.2	7.10
1/27/94	T2071	Final	16.0	16.0	5.1	31.8	9.2	7.07
2/11/94	T2125	Initial	14.0	12.0	7.5	42.1	10.6	6.58
2/11/94	T2125	Final	16.2	14.0	4.3	42.4	9.0	6.99
3/4/94	T2215	Initial	12.6	6.0	10.0	56.1	10.2	6.31
3/4/94	T2215	Final	12.0	20.0	6.0	37.0	8.6	6.56
3/11/94	T2241	Initial	12.0	12.0	5.9	56.1	10.2	6.31
3/11/94	T2241	Final	14.0	14.0	6.2	135	9.2	6.36
4/8/94	T2356	Initial	14.0	14.0	5.7	37.7	8.9	7.18
4/8/94	T2356	Final	12.0	15.6	4.1	40.3	9.0	7.03
4/22/94	T2425	Initial	16.0	15.6	5.4	47.2	9.0	6.99
4/22/94	T2425	Final	19.6	20.1	5.6	46.1	9.0	6.74
5/3/94	T2502	Initial	21.6	22.3	5.3	51.5	8.4	6.81
5/3/94	T2502	Final	23.4	20.3		84.8	8.4	7.08
5/19/94	T2564	Initial	29.4	22.3		52.8	9.2	7.42

Table 3
Laboratory Water Chemistry Measurements
Pen Branch

Sample Date	Lab #	Initial or Final	Hardness (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Conduct. (umhos)	D.O. (mg/L)	pH
6/1/94	T2606	Initial	27	24.4	3.3	58.1	9.0	7.08
6/1/94	T2606	Final	27.3	22.3	4.6	56.3	8.7	7.06
6/14/94	T2644	Initial	25.5	25.5	-----	66.0	8.7	7.18
6/14/94	T2644	Final	25.5	26.9	4.4	64.1	9.0	7.23
6/30/94	T2703	Initial	30.9	18.6	7.9	53.7	8.6	6.65
6/30/94	T2703	Final	21.5	16.6	7.5	50.5	8.7	6.55
7/12/94	T2770	Initial	26.9	26.9	4.5	66.0	8.7	7.42
7/12/94	T2770	Final	25.1	22.7	4.7	62.2	8.3	7.01
7/28/94	T2829	Initial	21.48	15.89	7.4	50.2	7.2	6.05
7/28/94	T2829	Final	16.11	15.33	5.9	43.8	8.2	6.81
8/11/94	T2894	Initial	-----	-----	2.9	65.9	7.8	6.71
8/11/94	T2894	Final	25.2	20.43	2.3	60.6	8.3	6.77
8/25/94	T2951	Initial	19.8	6.81	3.9	52.9	7.6	7.03
8/25/94	T2951	Final	20.68	18.16	4.0	57.3	8.2	6.79
9/08/94	T2995	Initial	19.69	18.16	3.5	52.7	7.6	6.1
9/08/94	T2995	Final	21.56	22.0	5.0	58.2	8.0	7.13
9/22/94	T3066	Initial	25.5	24.2	4.7	57.5	8.3	6.75
9/22/94	T3066	Final	25.5	22.0	6.5	58.6	8.2	7.18
10/06/94	T3105	Initial	17.6	15.05	8.5	50	8.4	6.81
10/06/94	T3105	Final	8.0	10.0	-----	49.9	8.6	7.26
10/20/94	T3192	Initial	16.0	12.0	8.1	49.0	8.1	6.77
10/20/94	T3192	Final	15.68	12.0	5.0	45.5	8.6	6.56

Table 3

Laboratory Water Chemistry Measurements

Pen Branch

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Table 3
Laboratory Water Chemistry Measurements
Four Mile Branch

Sample Date	Lab #	Initial or Final	Hardness (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Conduct (umhos)	D.O. (mg/L)	pH
8/11/94	T2896	Initial	-----	-----	17.0	49.0	1.2	5.75
8/11/94	T2896	Final	43.2	11.35	18.0	44.3	4.6	5.93
8/25/94	T2953	Initial	18.0	2.27	13.0	36.1	4.0	5.36
8/25/94	T2953	Final	18.8	2.27	13.0	35.2	7.4	5.47
9/08/94	T2997	Initial	23.27	6.81	18.0	37.1	2.4	5.12
9/08/94	T2997	Final	17.64	6.6	21.0	63.0	7.3	5.85
9/22/94	T3068	Initial	13.72	11.0	16.0	46.6	1.8	5.93
9/22/94	T3068	Final	11.76	6.6	19.0	51.3	7.2	6.48
10/06/94	T3107	Initial	9.8	2.15	8.0	37.2	5.2	5.56
10/06/94	T3107	Final	10.0	2.0	7.4	35.3	8.4	5.76
10/20/94	T3190	Initial	8.0	2.0	5.1	31.2	6.5	5.40
10/20/94	T3190	Final	9.8	2.0	7.3	40.3	8.2	4.97
11/3/94	T3224	Initial	9.8	2.0	7.7	28.5	6.2	4.55
11/3/94	T3224	Final	6.0	2.1		29.2	7.4	5.30
11/18/94	T3285	Initial	8.0	2.1		29.5	7.1	4.84
11/18/94	T3285	Final						

Reference Toxicant Testing

Stock organisms cultured in Pen Branch water and Upper Three Runs Creek water were used in reference toxicant tests to determine the relative health and sensitivity of the culture. The reference toxicant was sodium chloride (NaCl). An acute definitive reference toxicant test with Pen Branch dilution water was set with a dilution series of 2.5, 2.0, 1.5, 1.0, 0.5 and 0.0 g/L of reference toxicant. The results of the acute reference toxicant test of Pen Branch stock organisms in Pen Branch water showed, upon analysis by the Trimmed Spearman-Kärber method, a 48 Hour LC_{50} of 1.11 g/L. This value was slightly lower than the range of most previous monthly values (1.19 - 1.55 g/L) and substantially lower than the 48 Hour LC_{50} of 2.2 g/L NaCl noted for reference toxicant testing using 20% DMW. Results indicate that test organisms cultured in Pen Branch receiving water are more acutely sensitive to toxicity than test organisms cultured in 20% DMW.

An acute definitive reference toxicant test with Upper Three Runs dilution water was set with a dilution series of 0.5, 0.25, 0.1, 0.05, 0.01 and 0.0 g/L of NaCl. The results of the acute reference toxicant test of Upper Three Runs Creek stock organisms in Upper Three Runs Creek water showed, upon analysis by the Trimmed Spearman-Kärber method, a 24 Hour LC_{50} of 0.16 g/L. This value was slightly higher than the 48 Hour LC_{50} of 0.11 g/L NaCl noted for reference toxicant testing during the previous month. Results indicate that test organisms cultured in Upper Three Runs Creek receiving water are far more acutely sensitive to toxicity as test organisms cultured in 20% DMW.

A chronic reference toxicant test set for Upper Three Runs Creek receiving water, set on November 15-22, 1994, used a dilution series of 0.005, 0.01, 0.05, 0.1 and 0.2 g/L. Results indicated a NOEC for survival of 0.01 g/L and a NOEC for reproduction of <0.005 g/L. This compared to a mean NOEC for survival of 1.5 g/L and a NOEC for reproduction of 0.4 g/L for test organisms in 20% DMW.

A chronic reference toxicant test set for Pen Branch receiving water, set on November 8-15, 1994, used a dilution series of 0.2, 0.4, 0.8, 1.5 and 3.0 g/L. Results indicated a NOEC for survival of 0.8 g/L and a NOEC for reproduction of 0.8 g/L NaCl. This compared to a mean NOEC for survival of 1.5 g/L and a mean NOEC for reproduction of 0.8 g/L for test organisms in 20% DMW. Results suggest that organisms cultured in Pen Branch Receiving water are no more chronically sensitive to toxicity than organisms cultured in 20% DMW.