Results of Toxicity Identification Evaluations (TIE'S) Conducted on the A-01 Outfall
and Its Contributory Waste Streams, July 1996 - February 1997

by

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1.0 Executive Summary

Toxicity tests were conducted at nine locations during the summer of 1996. The results indicated that A-01B, A-01C, A-03, A-04, A-05 and A-01 were toxic to the test species, *Ceriodaphnia dubia*, while A-01A, A-06, and WE-01 were not toxic. Beginning in August 1996, Toxicity Identification Evaluations (TIE's) were initiated on all toxic outfalls in order to identify the toxicants responsible for the observed toxicity. A complete TIE was performed on A-01 because it is the regulatory compliance point for all of the combined waste streams that were tested. Only the portions of a TIE that are related to metal and chlorine toxicity were performed on the remaining locations because existing data indicated that metals and chlorine were present in potentially toxic quantities at these locations, and there was no evidence that other toxicants would be expected to be present in toxic amounts.

The results of the TIE's indicate that metals are responsible for most of the toxicity at all of the outfalls that were toxic and that chlorine contributed to the toxicity at two of the outfalls. Specifically, the toxicity at A-01B, A-01C, and A-01 was due to copper; the toxicity at A-03 was due to primarily to copper, although zinc also contributed to the toxicity; the toxicity at A-04 was due primarily to copper, with residual chlorine and zinc contributing to the toxicity; and the toxicity at A-05 was due primarily to copper, with residual chlorine contributing to the toxicity. A-03 was the most toxic outfall, with 100% mortality occurring at concentrations as low as 12.5% effluent. A-03 was found to have concentrations of copper, lead, and zinc that exceeded EPA water quality criteria by approximately two orders of magnitude.

The metal concentrations at A-01 and WE-01, which is located approximately 0.5 miles downstream from A-01 were similar. However, A-01 was toxic, while WE-01 was not. These results suggest that the bioavailability of metals decreases with increasing distance from the outfall, probably due to binding by naturally occurring humic and fulvic acids and/or clay particles.

2.0 Introduction

In anticipation of possible toxicity testing requirements in SRS's new (1996) NPDES permit, toxicity tests were performed at selected A-Area NPDES outfalls during June-August 1996 in order to determine if the outfalls were toxic. Of the nine locations that were tested, six were toxic and three were not. In order to identify the toxicants responsible for the observed toxicity, Toxicity Identification Evaluations (TIE's) were performed on water collected from each of the locations that was toxic. This report summarizes the results of the TIE's.

3.0 Sampling Locations/Methods

Toxicity Identification Evaluations (TIE's) were conducted on samples collected from all of the locations that exhibited toxicity when the chronic definitive toxicity tests were conducted in June-August 1996 (Specht, 1996). These locations included A-01, A-01B, A-01C, A-03, A-04, and A-05. In the supporting appendices of this report, A-01 is identified as AT-001 and the remaining locations are identified with an additional zero in the name (e.g. A-03 is identified as A-003). TIE's were performed following the U.S.

EPA methods developed for chronically toxic effluents (Norberg-King et al., 1991; Durhan et al., 1993).

Phase I TIE's use a series of chemical and physical treatments of the effluent to identify the class of toxicants that are likely to be causing the toxicity (Table 1). After each treatment, a toxicity test is performed on the treated and untreated water to determine if the toxicity of the sample has been altered. Once the class of toxicants has been identified, additional toxicity testing and chemical analyses are performed in Phase II to identify the specific chemicals that are responsible for the toxicity. In Phase III of a TIE, confirmation procedures are conducted to verify that the toxicity is due to the toxicant that was identified in Phase II. The most commonly used Phase III procedure is the spiking approach, in which the suspected toxicant is removed from an effluent sample, and a toxicity test is performed to demonstrate that the toxicity has been eliminated. The toxicant is then spiked back into the sample to the original concentration and another toxicity test is performed to demonstrate that the toxicity has returned to the original level.

Table 1. Chemical and Physical Treatment Used in TIE's

<u>Treatment</u>	<u>Potential Toxicants Removed</u>
Adjust to pH 3	Insoluble at low pH
Adjust to pH 11	Insoluble at high pH
Aerate at initial pH	Volatile; oxidizable
Aerate at pH 3	Volatile; oxidizable at low pH
Aerate at pH 11	Volatile; oxidizable at high pH
Filter at initial pH	Total suspended solids
Filter at pH 3	Insoluble at low pH
Filter at pH 11	Cationic metals; insoluble at high pH
SPE C-18 column at initial pH	Non-polar organics; metals
SPE C-18 column at pH 3	Non-polar organics
SPE C-18 column at pH 9	Non-polar organics; metals
Sodium thiosulfate addition	Residual chlorine; oxidizers; some metals (Cu, Cd, Hg, Ag)
EDTA chelation	Some metals (Cu, Cd, Hg, Zn, Pb, Ni, Fe); surfactants
Graduated pH (6.0 to 8.5)	Ammonia; some metals; sulfide

Source: Norberg-King et al. (1991).

An examination of the water chemistry data for the sampling locations (Table 2), when compared to U.S. EPA Water Quality Criteria (Table 3; U.S. EPA, 1986), strongly suggested that metals were the most likely cause of the observed toxicity. Potentially toxic concentrations of copper were found at all sampling locations; elevated levels of lead, zinc, aluminum, iron and mercury were also measured at some locations. Of particular note were the very high concentrations of copper, lead, and zinc found at A-03, where in some instances, the EPA criteria were exceeded by more two orders of magnitude. Aluminum and iron concentrations at all sampling locations frequently exceeded the EPA criteria, due to particulates in the effluent. Since iron and aluminum are major constituents of soils, these metals were present primarily as soil particulates and not biologically available.

Since A-01 is the new regulatory compliance point for the combined outfalls previously designated as A-001, A-03, A-04, and A-05, a complete TIE was performed at this location to ensure that no class of toxicants would be erroneously excluded. However,

for the remaining locations, partial TIE's were performed using only the portions of a TIE that focus on metals (EDTA chelation, sodium thiosulfate addition, and pH adjustment/filtration) and chlorine. If toxicants other than metals and chlorine were found to contribute to the toxicity of A-01, additional TIE tests would have been performed at any other locations that were likely to contain these identified toxicants, to determine if these toxicants also contributed to the toxicity at these locations.

Table 2. Metal Concentrations ($\mu\text{g/l}$) at Toxicity Testing Locations

<u>Location</u>	<u>Aluminum</u>	<u>Copper</u>	<u>Iron</u>	<u>Lead</u>	<u>Magnesium</u>	<u>Mercury</u>	<u>Zinc</u>
A-01A	<50 - 274	<5	<20 - 150	<3 - <20	476 - 690	0.4 - 1	<5 - 40
A-01B	<50 - 83	51 - 81	<20 - 184	<20	149 - 221	<0.1	41 - 51
A-01C	<50	87 - 130	<20 - 106	19 - 32	191 - 197	<0.1	22 - 41
A-03	<50 - 396	161 - 214	208 - 408	77 - 120	139 - 177	<0.1	161 - 213
A-04	<50 - 1170	23 - 140	31 - 1550	<3 - 6	140 - 415	<0.1	<5 - 94
A-05	<50 - 1160	<5 - 25	<20 - 322	<3	181 - 301	<0.1 - 0.1	<5 - 37
A-06	283 - 989	<5 - 27	403 - 957	<3	198 - 369	<0.1	<5 - 24
A-01	<50 - 214	<5 - 62	<20 - 142	<3 - 5	<50 - 440	<0.1 - 0.1	<5 - 49
WE-1	208 - 5380	21 - 66	458 - 5910	<3 - 8	146 - 570	<0.1 - 0.1	6 - 36

Table 3. U.S. EPA Freshwater Chronic Water Quality Criteria ($\mu\text{g/l}$) for Selected Elements (U.S. EPA, 1986)

<u>Metal</u>	<u>Criterion</u>
Aluminum	87 ^a
Cadmium	0.19 ^b
Chlorine	11
Chromium	11
Copper	1.65 ^b
Iron	1000
Lead	0.17 ^b
Mercury	0.012
Zinc	15 ^b

^a U.S. EPA chronic screening value (U.S. EPA, 1995)

^b Criterion is based on a water hardness of 10 mg/l

4.0 Results

4.1 A-01B

The cationic metal portions of a TIE were initiated on July 8, 1996. The sample had residual chlorine levels below detection (<0.01 mg/l), low hardness (4 mg/l) and was slightly alkaline (pH of 7.82). The metals data for A-01B (Table 2) indicate that the effluent contained potentially toxic concentrations of copper (51 to 81 $\mu\text{g/l}$) and zinc (41 to 51 $\mu\text{g/l}$).

The TIE results indicate that filtration at pH 11 and sodium thiosulfate reduced, but did not eliminate toxicity; EDTA chelation eliminated the toxicity; and the sample was found to be less toxic at a pH of 8.5 than at pH 6.5 or 7.5 (Table 4; see Appendix A-1 for details).

Table 4. Results of Metals Treatments on A-01B

Treatment	Result
Baseline Toxicity Test	Sample toxic; LC ₅₀ <20%
Filtered at pH 11	Toxicity reduced
Sodium thiosulfate addition	Toxicity reduced
EDTA chelation	Toxicity removed
Graduated pH	Sample less toxic at pH 8.5

Filtration at pH 11 will often reduce the toxicity of cationic metals, which often precipitate as hydroxides at high pH. Sodium thiosulfate is most effective at treating oxidizers such as residual chlorine, but is also effective at binding with copper, cadmium, mercury and silver, and will also somewhat reduce the toxicity of zinc, lead, nickel, and manganese (Hockett and Mount, 1996). EDTA is effective in binding surfactants and cationic metals, especially copper, cadmium, mercury, lead, zinc, and nickel (Hockett and Mount, 1996). However, ETT has found that EDTA does not usually eliminate lead toxicity (see Appendix A-1). Finally, the sample was more toxic at pH 6.5 than at 8.5. Metals which are more toxic at pH 6.5 than at 8.5 include copper and lead. The overall pattern of treatment responses suggests that most of the toxicity is due to copper. The Phase I results also did not eliminate the possibility of surfactants as a possible class of toxicants, since the toxicity of surfactants is also removed by EDTA.

Additional Phase I/II testing was initiated in September 1996 to determine if surfactants were present in toxic amounts. The outfall was less toxic in September (LC₅₀ = 52.5%) than in July (LC₅₀ <20%). A sublation test was performed on an undiluted portion of the A-01B sample (Table 5; see Appendix A-2 for details). A sublation test involves vigorously aerating a portion of sample in a glass vessel such that foam produced by the aeration overflows into an outer glass vessel. If surfactants are present, most of them will be present in the foam. Toxicity tests are then performed on untreated and sublated samples and the results are compared to determine if sublation reduced the toxicity of the sample. The results of the sublation test indicate that the treated and untreated portions of the sample were equally toxic, which indicated that surfactants were not present in toxic quantities. Therefore the Phase I results suggested that copper was responsible for the observed toxicity.

Table 5. Results of Additional TIE Treatments on A-01B

<u>Treatment</u>	<u>Result</u>
Baseline Toxicity Test Sublation	Sample toxic; LC ₅₀ = 52.5% Toxicity not removed

The Phase III TIE on A-01B was started in September 1996 using the spiking approach (see Appendix A-3 for details). Simulated effluent was prepared by diluting A-01B water to a concentration of 50% with moderately hard synthetic fresh water and then diluting the mixture to the same hardness as A-01B (4 mg/l as CaCO₃). Copper was spiked into simulated effluent at concentrations ranging from 5 to 80 µg/l, which when combined with the copper concentrations already present in the diluted effluent, resulted in copper concentrations ranging from 30 to 105 µg/l. All of the dilutions were toxic, resulting in 100% mortality in less than 48 hours. Since copper concentrations in the effluent ranged from 51 to 81 µg/l (Table 2), the copper concentrations present in A-01B easily account for the noted acute toxicity. Based upon the EPA water quality criterion for copper at a hardness of 4 mg/l, the maximum concentration of copper that will avoid acute toxicity is $e^{(0.9422 \ln(\text{hardness}) - 1.464)}$, or 0.85 µg/l (U.S. EPA, 1986). Although not all of the copper present in A-01B effluent is likely to be biologically available, the concentrations of copper present in the effluent were up to two orders of magnitude higher than the calculated criteria. Copper levels at A-01B would have to be reduced by approximately 95% to eliminate acute toxicity, or alternatively, hardness would need to be increased to moderately hard levels (80 to 100 mg/l) and copper levels reduced by 50% to eliminate copper toxicity.

4.2 A-01C

The cationic metal portions of a TIE were initiated on July 8, 1996 (see Appendix B-1 for details). The sample had residual chlorine levels below detection (<0.01 mg/l), low hardness (10 mg/l) and was slightly alkaline (pH of 7.76). The metals data for A-01C (Table 2) indicate that the effluent contained potentially toxic concentrations of copper (87 to 130 µg/l), lead (19 to 32 µg/l), and zinc (22 to 41 µg/l).

The TIE results indicate that neither filtration at pH 11 or the addition of sodium thiosulfate reduced the toxicity of the sample (Table 6). The results of the graduated pH tests indicated that the toxicity was not affected by pH, but because the effluent was very toxic (LC₅₀ < 20%), graduated pH tests conducted on a diluted sample might provide more useful results. The only treatment that removed the toxicity was EDTA chelation. These results suggest that the toxicant was a metal, or more likely, a combination of metals. The results also did not eliminate the possibility of surfactants as a possible class of toxicants, since the toxicity of surfactants is also removed by EDTA.

Table 6. Results of Metals Treatments on A-01C

Treatment	Result
Baseline Toxicity Test	Sample toxic; LC ₅₀ < 20%
Filtered at pH 11	No reduction in toxicity
Sodium thiosulfate addition	No reduction in toxicity
EDTA chelation	Toxicity removed
Graduated pH	Sample equally toxic at all pH's

Additional tests were conducted in September 1996 (see Appendix B-2 for details). The sample collected in September (LC₅₀ = 28.35%) was less toxic than as the sample collected in July (LC₅₀ < 20%). Since EDTA removes metal toxicity and also surfactant toxicity, a sublation test was performed to determine if surfactants contributed to the toxicity. In addition, graduated pH tests were performed at effluent concentrations of 5%, 10%, and 20% (Table 7). The results indicate that surfactants did not contribute to the toxicity and that the sample was less toxic at pH 8.5. These results, when combined with the earlier Phase I/II results suggested that the toxicity of A-01C was due to copper. This conclusion is further supported by the water chemistry data, which indicate that copper concentrations at A-01C ranged from 87 to 130 µg/l. These values greatly exceed the EPA water quality criterion for copper at a hardness of 10 mg/l (1.65 µg/l).

Table 7. Results of Additional Phase I/II Treatments on A-01C

Treatment	Result
Baseline Toxicity Test	Sample toxic; LC ₅₀ = 28.3%
Graduated pH	Less toxic at pH 8.5
Sublation	Toxicity not removed

The Phase III TIE on A-01C was started in September 1996 using the spiking approach (see Appendix B-3 for details). Baseline toxicity testing indicated acute toxicity at effluent concentrations of 40% and greater. Because this meant that the effluent would have to be diluted to a 30% concentration or less, and the extent of the dilution would fundamentally change the chemical characteristics of the effluent, it was decided that A-01C could best be simulated by preparing synthetic freshwater to a hardness of 6 mg/l. Copper was then spiked into the synthetic water at concentrations of 1, 2, 5, and 20 µg/l. The results indicate that copper concentrations of 2 µg/l and higher were acutely toxic at the hardness of the effluent (Table 8). Since copper concentrations in the effluent ranged from 87 to 130 µg/l (Table 2), the copper concentrations present in A-01C easily account for the noted acute toxicity. Based upon the EPA water quality criterion for copper at a hardness of 6 mg/l, the maximum concentration of copper that will avoid toxicity is $e^{(0.9422 \ln(\text{hardness}) - 1.464)}$, or 1.25 µg/l. Although not all of the copper present in A-01C effluent is likely to be biologically available, the concentrations of copper present in the effluent were up to two orders of magnitude higher than the calculated criteria. Copper levels at A-01C would have to be reduced by more than

95% to eliminate acute toxicity, or alternatively, hardness would need to be increased to moderately hard levels (80 to 100 mg/l) and copper levels reduced by 75% to eliminate copper toxicity.

Table 8 Results of Phase III TIE for A-01C

<u>Copper Concentration</u>	<u>48 hour % Mortality</u>
0 µg/l	30%
1 µg/l	40%
2 µg/l	100%
5 µg/l	100%
20 µg/l	100%

4.3 A-01

A Phase I full TIE was initiated on A-01 on July 31, 1996 (see Appendix C-1 for details). The sample had relatively low, but detectable residual chlorine levels (0.08 mg/l), low hardness (10 mg/l) and was relatively neutral in pH (pH of 7.57). The metals data for A-01 (Table 2) indicate that the effluent contained potentially toxic concentrations of copper (up to 62 µg/l) and zinc (up to 49 µg/l).

The TIE treatment results are shown in Table 9. Treatments that reduced, but did not eliminate the toxicity included C-18 SPE column at initial pH, sodium thiosulfate, EDTA chelation, and graduated pH at 8.5. Treatments that increased toxicity included all treatments that were performed at pH 3 and aeration at the initial pH. The only treatments that eliminated the toxicity were filtration at pH 11 and the use of a C-18 SPE column at pH 11.

Table 9. Results of Phase I TIE on A-01

<u>Treatment</u>	<u>Result</u>
Baseline	Sample toxic; LC ₅₀ =41%
Adjust to pH 3	Toxicity increased
Adjust to pH 11	Toxicity unchanged
Aerate at initial pH	Toxicity increased
Aerate at pH 3	Toxicity increased
Aerate at pH 11	Toxicity unchanged
Filter at initial pH	Toxicity unchanged
Filter at pH 3	Toxicity increased
Filter at pH 11	Toxicity removed
SPE C-18 column at initial pH	Toxicity slightly reduced
SPE C-18 column at pH 3	Toxicity increased
SPE C-18 column at pH 11	Toxicity removed
Sodium thiosulfate addition	Toxicity slightly reduced
EDTA chelation	Toxicity slightly reduced
Graduated pH (6.0 to 8.5)	Sample less toxic at higher pH (8.5)

The results suggest that the toxicant became insoluble at pH 11, and also that the toxicant was partially removed by the C-18 SPE column and partially bound by EDTA and sodium thiosulfate. In general, the Phase I results suggest that the toxicant was probably a cationic metal. Specifically, the metals cadmium, copper, iron, manganese, nickel, lead and zinc can be chelated by EDTA resulting in toxicity reduction. Of these metals, copper and cadmium are also treatable by sodium thiosulfate. Copper tends to be more toxic at a lower pH (6.5), while cadmium is more toxic at a higher pH (8.5). The Phase I results therefore point to copper as the probable toxicant. However, zinc could not be ruled out as a possible toxicant, since it was found at concentrations up to 49 µg/l, which is well above the EPA water quality criterion for zinc at a hardness of 10 mg/l (15 µg/l). The reduction in toxicity resulting from treatment by the C-18 SPE column extraction at initial pH and pH 11 is probably a result of physical removal of copper associated with particulates. However, the presence of non-polar organics could not be ruled out in Phase I.

In the follow-up Phase I studies that were initiated in September 1996 (see Appendix C-2 for details), a methanol elution of the column was performed in order to confirm that non-polar organics are not present in A-01 in toxic quantities. However, results of the elution test were inconclusive, methanol concentrations in the eluted fractions were high enough to cause unacceptable levels of mortality in the controls (Table 10). The methanol elution test was repeated in December 1996, using a larger volume of effluent run through the SPE column in order to reduce the concentration of methanol after elution and dilution (see Appendix C-3 for details). These results indicate that none of the methanol-extracted fractions had significant toxicity (Table 11); therefore non-polar organics were not a factor in the toxicity of A-01. The effectiveness of the C-18 SPE column in reducing the toxicity of the effluent sample was due to physical removal of metals by the column.

Table 10. Results of Methanol Elution Tests on A-01

<u>Treatment</u>	<u>Result</u>
Baseline	Sample was toxic
C-18 SPE column at initial pH	Toxicity reduced
Methanol elution	Toxicity in both methanol controls and methanol fractions

Table 11. Results of Second Round of Methanol Elution Tests on A-01

<u>Treatment</u>	<u>Result</u>
Baseline	Sample toxic; LC ₅₀ =17.2%
C-18 SPE column at initial pH	Sample toxicity reduced; LC ₅₀ =35.4%
Methanol elution	No significant toxicity in extracted fractions

The Phase III TIE on A-01 was conducted using a spiking approach with copper and zinc. Due to the very low hardness of the A-01 effluent, it was not deemed feasible to

remove copper and zinc from the effluent without fundamentally changing its characteristics which relate to toxicity. Thus it was decided to prepare a simulated effluent, by adding essential anions and cations to ultra-pure deionized water to match the water chemistry of the effluent sample that was collected on December 9, 1996. Details of the preparation of the simulated effluent can be found in Appendix C-4. The resulting simulated effluent had a hardness of 4 mg/l, which was the same as that of the A-01 effluent. Copper (as copper sulfate) and zinc (as zinc sulfate) were spiked into the simulated effluent separately and together in variable amounts. The concentration range for the metals was chosen to encompass the highest concentration that was measured in the A-01 effluent. Acute definitive toxicity tests were then conducted on the following combinations of spiked samples:

Copper variable (0-30 µg/l)/No zinc
 Copper variable (0-30 µg/l)/Zinc constant (30 µg/l)
 Zinc variable (0-60 µg/l)/No copper
 Zinc variable (0-60 µg/l)/Copper constant (20 µg/l)

The results of the copper spiked tests (Table 12) indicate that copper was acutely toxic at the lowest concentration that was tested (1 µg/l). Thus, copper in the range of concentrations that were measured in the effluent (up to 62 µg/l) easily accounts for the acute toxicity that was observed in the effluent. The results are consistent with the U.S. EPA hardness-based water quality criterion for copper, which specifies a concentration of 0.76 µg/l total copper at a water hardness of 4.0 mg/l (U.S. EPA, 1986).

Table 12 Results of A-01 Phase III TIE for Copper and Zinc

<u>% Mortality at 48 hours</u>		
	<u>Cu Only</u>	<u>Cu and 30 µg/l Zn</u>
0 µg/l Cu	0%	0%
1 µg/l Cu	100%	100%
2 µg/l Cu	100%	100%
5 µg/l Cu	100%	100%
10 µg/l Cu	100%	100%
30 µg/l Cu	100%	100%

<u>% Mortality at 48 hours</u>		
	<u>Zn Only</u>	<u>Zn and 20 µg/l Cu</u>
0 µg/l Zn	0%	0%
2 µg/l Zn	0%	100%
5 µg/l Zn	0%	100%
10 µg/l Zn	0%	100%
30 µg/l Zn	0%	100%
60 µg/l Zn	50%	100%

The results of the zinc spiked tests (Table 12) indicate that zinc concentrations in the range measured in the A-01 effluent (<5-49 µg/l) do not account for the observed acute

toxicity in the effluent. The U.S. EPA hardness-based water quality criterion for zinc at a water hardness of 4 mg/l is 22.2 µg/l (U.S. EPA, 1986).

The results of the TIE for A-01 indicate that copper is the cause of toxicity in the A-01 effluent. Based on the results with simulated effluent, at a water hardness of 4 mg/l, copper concentrations would need to be reduced to less than 1 µg/l to eliminate acute toxicity. It is likely that in actual effluent, less of the copper would be biologically available, and that somewhat higher copper concentrations could be tolerated without resulting in acute toxicity. However, chronic toxicity testing, rather than acute testing is required at A-01. Because chronic responses occur at lower concentrations of toxicants than acute responses, it is obvious that a substantial reduction in the copper concentration of A-01 will be needed.

Other A-01 Toxicity Investigations

Additional toxicity tests were conducted on A-01 in November 1996 and in February 1997 (Appendices C-5-1 and C-5-2). The November toxicity test was performed during a period when the A-Area air stripper was not running, and therefore A-01 was not receiving effluent from A-01A. A-01 was much more toxic in the November toxicity test (NOEC of 1% effluent) than in previous tests, when the NOEC was generally 25% effluent. The results of the February test, when the air stripper was running, indicated that the NOEC was 25%. Therefore, it appears that dilution from A-01A greatly reduces the toxicity of A-01. Copper and zinc concentrations in the November sample were 500 and 67 µg/l, respectively, which were much higher than the copper and zinc levels measured during previous tests (Table 2). In the February test, copper concentrations ranged from 17 to 48 µg/l and zinc concentrations ranged from 14 to 20 µg/l (Appendix C-5-2).

In order to examine whether an increase in hardness would reduce copper toxicity at the A-01 outfall, toxicity tests were conducted on portions of the November 1996 sample from A-01 at ambient hardness (16 mg/l) and at a hardness of 28 mg/l (see Appendices C-5-1 and C-5-3 for details). The results of the toxicity tests indicated that both tests had NOEC's of 1%. Therefore, it appears that increasing the hardness of the effluent from 16 to 28 mg/l did not result in a reduction in metal toxicity. In theory, the concentration of copper that would elicit a toxic response at a hardness of 16 mg/l should have been about half the concentration that would be needed to elicit a toxic response at a hardness of 28 mg/l. There is no ready explanation for why an increase in hardness did not result in a reduction in toxicity.

An additional investigation was conducted to investigate the effect of hardness on A-01 toxicity (see Appendix C-5-4 for details). In this study, moderately hard synthetic water was prepared by diluting Perrier mineral water with nanopure demineralized water to a hardness of 96 mg/l. The synthetic water was then spiked with the same concentrations of copper and zinc as the November sample (500 and 67 µg/l, respectively), and a dilution series was prepared on the spiked water using dilutions of 0%, 1%, 3%, 10%, 30%, and 100%. The NOEC of the spiked water was 1%, which equates to 5 µg/l copper and 0.67 µg/l zinc. The EPA hardness-based chronic water quality criteria for copper and zinc at a hardness of 96 mg/l are 11.4 and 102.4 µg/l, respectively. The NOEC of 1% from the spiked synthetic water was the same as the

NOEC's from the A-01 and A-01 hardness adjusted tests that were performed in November. These results further suggest that increasing the hardness of A-01 will not result in a substantial reduction in metal-induced toxicity.

4.4 A-03

The cationic metal portions of a Phase I TIE were initiated on July 11, 1996 (see Appendix D-1 for details). The sample had relatively low, but detectable residual chlorine levels (0.07 mg/l), relatively high hardness (90.6 mg/l) and was slightly alkaline (pH of 8.27). The relatively high hardness is due to evaporative losses from the cooling tower, which concentrate ions, including calcium, in the cooling tower blowdown. The metals data for A-03 (Table 2) indicate that the effluent contained very high levels of copper (161 to 214 µg/l), lead (77 to 120 µg/l), and zinc (161 to 213 µg/l).

The Phase I TIE results indicate that EDTA chelation reduced, but did not eliminate the toxicity of the sample (Table 13). None of the other treatments were successful in reducing the toxicity. However, because the effluent was acutely toxic at the lowest concentration that was tested (20%), it is possible that some of the other treatments may have reduced the toxicity if lower concentrations of effluent had been tested.

Table 13. Results of Metals Treatments on A-03

<u>Treatment</u>	<u>Result</u>
Baseline Toxicity Test	Sample toxic; LC ₅₀ <20%
Filtered at pH 11	No reduction in toxicity
Sodium thiosulfate addition	No reduction in toxicity
EDTA chelation	Sample toxicity reduced
Graduated pH	Sample toxic at all pH values

Additional testing on outfall A-03 was initiated on September 18, 1996 (see Appendix D-2 for details). Treatments included sublation and graduated pH at concentrations of 40% and 100% (Table 14). The effluent was less toxic in September (LC₅₀ = 45.4%) than in July (LC₅₀ <20%). The sublation treatment did not reduce the toxicity of the A-03 sample, which indicates that surfactants are not present in toxic concentrations. The results of the graduated pH test indicate that the sample was less toxic at pH 8.5 than at lower pH's, which when combined with the earlier Phase I test results, suggests that the toxicity was probably due to copper.

Table 14. Results of Additional Phase I/II Treatments on A-03

<u>Treatment</u>	<u>Result</u>
Baseline Toxicity Test	Sample toxic; LC ₅₀ = 45.4%
Graduated pH	Sample less toxic at pH 8.5
Sublation	Toxicity not reduced

The Phase III TIE on A-03 was started in September 1996 (see Appendix D-3 for details). Due to the presence of very high concentrations of multiple cationic metals in the effluent that would be difficult to remove without fundamentally changing the chemistry of the effluent, moderately hard synthetic fresh water (with a hardness of 80-100 mg/l) was chosen to simulate the A-03 effluent. Treatments included spiking with copper only; copper, lead, and zinc (Table 15); zinc only; zinc, copper, and lead (Table 16); lead only; and lead, copper, and zinc (Table 17). The results indicate that concentrations of copper as low as 50 µg/l (Table 15) and concentrations of zinc as low as 150 µg/l (Table 16) resulted in 100% mortality in the simulated effluent. Lead did not produce a toxic response at the highest concentration that was tested (100 µg/l; Table 17). Both copper and zinc were measured in the A-03 effluent at concentrations that were greater than the concentrations that resulted in 100% mortality in the simulated effluent. Therefore, it is likely that both copper and zinc contribute to the toxicity of A-03, while lead does not.

Table 15. Results of Copper and Copper/Lead/Zinc Spiking Tests on A-03

<u>Concentration of Copper</u>	<u>%Mortality</u>	
	<u>Cu Only</u>	<u>Cu with Pb and Zn^a</u>
0 µg/l	0%	0%
50 µg/l	100%	100%
100 µg/l	100%	100%
150 µg/l	100%	100%
200 µg/l	100%	100%
250 µg/l	100%	100%

^aLead and zinc were added to the copper dilutions at concentrations of 80 µg/l and 200 µg/l, respectively.

Table 16. Results of Zinc and Zinc/Lead/Copper Spiking Tests on A-03

<u>Concentration of Zinc</u>	<u>%Mortality</u>	
	<u>Zn Only</u>	<u>Zn with Pb and Cu^a</u>
0 µg/l	0%	0%
100 µg/l	30%	100%
150 µg/l	100%	100%
200 µg/l	100%	100%
250 µg/l	100%	100%
300 µg/l	100%	100%

^aLead and copper were added to the zinc dilutions at concentrations of 80 µg/l and 200 µg/l, respectively.

Table 17. Results of Lead, and Lead/Copper/Zinc Spiking Tests on A-03

<u>Concentration of Lead</u>	<u>%Mortality</u>	
	<u>Pb Only</u>	<u>Pb with Cu and Zn^a</u>
0 µg/l	0%	0%
20 µg/l	0%	100%
40 µg/l	0%	100%
60 µg/l	0%	100%
80 µg/l	0%	100%
100 µg/l	0%	100%

^aCopper and zinc were added to the lead dilutions at concentrations of 200 µg/l each.

4.5 A-04

The cationic metal portions of a TIE were initiated on August 1, 1996 (see Appendix E-1 for test details). The sample had high residual chlorine levels (0.29 mg/l), very low hardness (4 mg/l) and was slightly alkaline (pH of 8.23). The metals data for A-04 (Table 2) indicate that the effluent contained potentially toxic concentrations of copper (23 to 140 µg/l) and zinc (<5 to 94 µg/l).

The results indicated that both sodium thiosulfate and EDTA chelation reduced, but did not eliminate the toxicity and that the sample was less toxic at pH 8.5 (Table 18). These results suggested that copper was a probable toxicant. However, total residual chlorine in the sample was 0.29 mg/l, which indicated that chlorine was also present at potentially toxic concentrations in A-04. Since neither EDTA nor sodium thiosulfate eliminated the toxicity, it was also possible that other toxicants were present.

Table 18. Results of Phase I Treatments on A-04

<u>Treatment</u>	<u>Result</u>
Baseline Toxicity Test	Sample toxic; LC ₅₀ = 69.1%
Filtered at pH 11	No reduction in toxicity
Sodium thiosulfate addition	Toxicity reduced
EDTA chelation	Toxicity reduced
Graduated pH	Sample less toxic at pH 8.5

Additional Phase I testing on outfall A-04 was initiated on September 22 1996 to determine if toxicants other than metals were present (see Appendix E-2 for details). Treatments included aeration at pH 3, 11, and initial pH; C-18 SPE column at pH 3, 11, and initial pH; sodium thiosulfate addition; EDTA chelation; and a graduated pH test. (Table 19). The residual chlorine concentration in the sample on the day that the sample was received was 0.35 mg/l. The results of the TIE tests indicate that aeration

at pH 11 was the only treatment that removed the toxicity, while EDTA chelation, sodium thiosulfate addition, and the C-18 SPE column at pH 11 all slightly reduced the toxicity of the sample.

Table 19. Additional Phase I Tests on A-04

Baseline	Sample toxic; LC ₅₀ =16.2%
Baseline; 48 hour delay^a	Toxicity reduced
Adjust to pH 3	Toxicity unchanged
Adjust to pH 11	Toxicity unchanged
Aerate at initial pH	Toxicity unchanged
Aerate at pH 3	Toxicity unchanged
Aerate at pH 11	Toxicity removed
Filter at initial pH	Toxicity unchanged
Filter at pH 3	Toxicity unchanged
Filter at pH 11	Toxicity unchanged
SPE C-18 column at initial pH	Toxicity unchanged
SPE C-18 column at pH 3	Toxicity unchanged
SPE C-18 column at pH 11	Toxicity slightly reduced
Sodium thiosulfate addition	Toxicity slightly reduced
EDTA chelation	Toxicity slightly reduced
Graduated pH (6.0 to 8.5)	Sample less toxic at higher pH (8.5)

^aAll tests subsequent to the baseline test were initiated after a 48 hour delay, to allow time for total residual chlorine concentrations to decline.

The results of the follow-up TIE tests for A-04 were somewhat unexpected. The effects of EDTA, sodium thiosulfate, and graduated pH all paralleled the earlier results, with toxicity being reduced, but not eliminated. However, aeration at pH 11 completely removed the toxicity, which is a treatment that generally does not remove metal or chlorine toxicity. Increasing a sample to pH 11 will often precipitate cationic metals, which can then be removed by filtration. However, this treatment did not reduce the toxicity of the sample. Similarly, aeration can remove volatile toxicants, such as chlorine, ammonia and volatile organic compounds, but aeration at a neutral pH did not reduce the toxicity.

Further investigations were conducted in December 1996 in order to investigate the effect of aeration at pH 11 in eliminating toxicity in effluent from A-04 (see Appendix E-3 for details). A portion of a sample of A-04 effluent that was collected on December 8, 1996 was aerated at pH 11 and another portion of the sample was sparged with nitrogen at pH 11. After aeration or sparging, the pH was readjusted to the initial pH with H₂SO₄. Toxicity tests were run on the sample on the day it was received (December 9) and the aerated and sparged sample after treatment. In addition, a toxicity test was run on the untreated sample concurrently with the treated samples to determine if the toxicity of the sample had declined over time. The results indicate that neither aeration at pH 11 nor sparging with nitrogen at pH 11 reduced the toxicity of the A-04 effluent (Table 20). It was concluded that the aeration at pH 11 results from September were anomalous, and that the toxicity at A-04 was most likely due to one or more cationic metals and/or total residual chlorine.

Table 20. Results of Aeration and Nitrogen Sparging at pH 11 on the Toxicity of A-04

Treatment	Start Date	Result
Baseline	12/9/96	Sample toxic; LC ₅₀ <20%
Baseline	12/16/96	Sample toxic; LC ₅₀ =21.4%
Baseline	1/2/97	Sample toxic; LC ₅₀ =20%
Aeration at pH 11	1/2/97	Toxicity not removed; LC ₅₀ =22.9%
N ₂ sparging at pH 11	1/2/97	Toxicity not removed; LC ₅₀ =24.6%

The Phase III TIE on A-04 was started in October 1996 using the spiking approach for copper and zinc (see Appendix E-4 for details). Baseline toxicity testing indicated acute toxicity at effluent concentrations of 80 to 100%. Based on this information, a simulated effluent was prepared using 40% effluent and 60% moderately hard synthetic fresh water diluted to a hardness of 4 mg/l. In order to determine if zinc was contributing to the toxicity, a series of toxicity tests were conducted on samples that had: 1) variable copper concentrations and no zinc; 2) variable copper concentrations and maximum zinc; and 3) maximum zinc and no copper. The highest concentrations of copper and zinc that were spiked were the highest concentrations that were measured during the July testing. Copper was spiked as copper sulfate and zinc as zinc sulfate. The amounts of copper that were spiked into the simulated effluent ranged from 0 to 100 µg/l. However, the simulated effluent had a baseline copper concentration of 23 µg/l, which resulted in copper concentrations ranging from 23 to 123 µg/l (Table 21). All of the exposures had 100% mortality.

Table 21. Results of Copper and Copper/Zinc Spiking Tests on A-04

<u>Nominal Cu Conc.^a</u>	<u>Actual Cu Conc.</u>	<u>% Mortality</u>	
		<u>Cu Only</u>	<u>Cu/Zn (94 µg/l)</u>
October Testing			
0	23	100%	100%
5	28	100%	100%
10	33	100%	100%
25	48	100%	100%
50	73	100%	100%
100	123	100%	100%
December Testing			
0	100	100%	100%
1	101	100%	100%
2	102	100%	100%
5	105	100%	100%
10	110	100%	100%

^aAll concentrations are in µg/l.

Additional spiking tests were conducted on A-04 effluent in December, using the same procedures described for the October tests, but much lower concentrations of copper (0 to 10 µg/l) were added to the simulated effluent. The December tests again exhibited 100% mortality at all concentrations, including the control. When the concentration of copper was measured in the A-04 effluent, it was found to be 100 µg/l, which resulted in exposure concentrations that ranged from 100 to 110 µg/l Cu (Table 21).

Copper concentrations and toxicity have both been found to vary to a substantial degree in the A-04 effluent (Table 22). Total copper concentrations on five sampling dates ranged from 23 to 249 µg/l, while the 48 hour LC₅₀ values of three tests ranged from 17.4% to 89%. These data can also be compared on the basis of "toxic units". A toxic unit is calculated by taking the reciprocal of the LC₅₀ (1/LC₅₀). The larger the toxic unit, the more toxic the effluent. Based on the toxic units for copper in both the September and December samples, it can be predicted that the A-04 effluent should cease to be acutely toxic when copper concentrations are less than 35-40 µg/l. However in the September spiking tests, when total copper was only 23 µg/l, none of the test organisms survived. The additional toxicity may be due to zinc, to the additive effects of copper and zinc, or to variable water chemistry (pH and/or hardness).

Table 22. Copper Concentrations and LC₅₀ Values for A-04

Date	Total Copper (µg/l)	48 hour LC ₅₀	Toxic Units
7/8/96	140		
7/10/96	23		
7/12/96	45		
7/24/96	not measured	74.8%	1.34
9/19/96	57	89%	1.12
12/8/96	249	17.4%	5.74

4.6 A-05

The cationic metal portions of a Phase I TIE were initiated on July 31, 1996 (see Appendix F-1 for test details). The sample had relatively high residual chlorine levels (0.15 mg/l), low hardness (8 mg/l) and was slightly alkaline (pH of 8.16). The metals data for A-05 (Table 2) indicate that the effluent contained potentially toxic concentrations of copper (<5 to 25 µg/l).

The results indicate that sodium thiosulfate removed all of the toxicity and also that the toxicity was reduced, but not eliminated, by EDTA chelation and by filtration at pH 11 (Table 23). Both sodium thiosulfate and EDTA are effective at removing metal toxicity. Metals effectively chelated by EDTA include cadmium, copper, lead, nickel, and zinc. Of the metals treatable by EDTA, only copper and cadmium are also treatable by sodium thiosulfate. However, cadmium is typically more toxic at pH 8.5 than at 6.5, while copper is more toxic at pH 6.5 than at 8.5. These results suggest that copper is responsible for the observed toxicity. It should be noted that potentially toxic concentrations of total residual chlorine (0.15 mg/l) were measured in the A-05 effluent (Appendix F-1). In addition to removing metal toxicity, sodium thiosulfate is also very

effective at removing chlorine and other oxidizers. Thus, sodium thiosulfate may have been more effective than EDTA in removing the toxicity because it removed the chlorine, as well as binding the metals. Therefore the initial Phase I TIE results for A-05 suggest that most of the toxicity is due to copper, although chlorine may also have contributed to the toxicity. However, since EDTA also removes toxicity due to surfactants, the possibility of surfactants contributing to the toxicity could not be ruled out without further testing.

Table 23. Results of Metals Treatments on A-05

Treatment	Result
Baseline Toxicity Test	Sample toxic; LC ₅₀ = 74.8%
Filtered at pH 11	Toxicity reduced
Sodium thiosulfate addition	Toxicity completely removed
EDTA chelation	Toxicity reduced
Graduated pH	Sample less toxic at pH 8.5

Additional Phase I tests were conducted in September 1996 to differentiate between the residual chlorine and copper toxicity and to determine if surfactants might also contribute to the toxicity (see Appendix F-2 for details). Upon receipt of the effluent sample on September 13, the total residual chlorine concentration in the sample was 0.10 mg/l. A sample of the effluent was exposed to air until chlorine levels were not detectable (<0.05 mg/l). A series of treatments, which included filtration at pH 10, EDTA chelation, and sublation were then performed on the sample. The results indicate that filtration at pH 10 and sublation did not reduce the toxicity, but that EDTA chelation rendered the sample not toxic (Table 24). These results suggest that the toxicity is not due to surfactants, but that the toxicity is due primarily to copper, with residual chlorine also contributing to the toxicity.

Table 24. Results of Additional Phase I TIE Treatments on A-05

Treatment	Result
Baseline Toxicity Test	Sample toxic; LC ₅₀ = 89%
Filtered at pH 11	Toxicity not reduced
EDTA chelation	Toxicity completely removed
Sublation	Toxicity not reduced

The Phase III TIE on A-05 was initiated on October 10, 1996 using the spiking approach with copper (see Appendix F-3 for details). Simulated effluent was prepared using 60% effluent and 40% moderately hard synthetic dilution water diluted to a hardness of 8 mg/l (the hardness of the effluent). The simulated effluent had a baseline concentration of 10 µg/l copper. Copper was spiked into this simulated effluent at concentrations of 2 to 20 µg/l, yielding copper concentrations of 7, 10, 15, 20 and 25 µg/l when mixed with the simulated effluent (Table 25). The results indicate that there was significant mortality even in the control, which suggests that even after

dilution to 60%, the A-05 effluent remained toxic. The addition of copper to a concentration of 10 µg/l in the simulated effluent resulted in 100% mortality, which indicates that the concentrations of copper present in the A-05 effluent (up to 25 µg/l) were toxic.

As shown in Table 25, the A-005 simulated effluent was toxic (80% mortality) when no copper was spiked into it. Therefore, it was difficult to determine whether the toxicity in the spiked dilutions was due to copper or toxicity of the simulated effluent. It was not clear why the non-spiked simulated effluent was toxic, because the baseline toxicity test suggested that it would not be toxic. Water that is extremely soft can result in toxicity, but ETT Environmental has found that hardnesses of greater than 5 mg/l are generally not toxic. This suggests that the unspiked simulated effluent should not have been toxic, since it had a hardness of 8 mg/l. There is no obvious explanation for the toxicity in the unspiked simulated effluent.

Table 25. Results of Phase III Copper Spiking Tests on A-05

<u>Copper Concentration</u>	<u>% Mortality</u>
0 µg/l	80%
7 µg/l	90%
10 µg/l	100%
15 µg/l	100%
20 µg/l	100%
25 µg/l	100%

Because of the high mortality in the unspiked A-005 simulated effluent (Table 25), a final series of tests was conducted in November 1996 in which copper was spiked into reconstituted water with a hardness of 5 mg/l at concentrations of 0, 1, 2, 5, and 20 µg/l. Results showed complete mortality of the test organisms at copper concentrations of 2 µg/l and higher (Table 26). Minimal mortality was seen at copper concentrations of 1 µg/l and less. These results indicate that in A-05 effluent, the acute toxic copper concentration can be expected to be between 2 µg/l (the value in reconstituted water of similar hardness) and 10 µg/l (the simulated effluent concentration in which 100% mortality was noted). These results confirm that copper is responsible for the observed toxicity in A-05. However, total residual chlorine concentrations were also high enough to contribute to the observed toxicity.

Table 26. Results of Additional Phase III Copper Spiking Tests on A-05

<u>Copper Concentration</u>	<u>% Mortality</u>
0 µg/l	30%
1 µg/l	40%
2 µg/l	100%
5 µg/l	100%
20 µg/l	100%

5.0 Discussion/Conclusions

The results of the TIE's indicate that all of the locations that failed the toxicity tests had toxic concentrations of copper. In addition some locations had toxic levels of total residual chlorine and/or zinc. Specifically, the toxicity at A-01B, A-01C and, A-01 was due to copper; the toxicity at A-03 was due to primarily to copper, although zinc also contributed to the toxicity; the toxicity at A-04 was due primarily to copper, with residual chlorine and possibly zinc contributing to the toxicity; and the toxicity at A-05 was due primarily to copper, with residual chlorine contributing to the toxicity (Table 27). Clearly, copper is responsible for the toxicity at A-01 as well as most of the toxicity in the effluent streams that contribute to A-01.

Table 27. Summary Results of Toxicity Identification Evaluations

<u>Location</u>	<u>Toxicants</u>
A-001	Copper
A-01B	Copper
A-01C	Copper
A-03	Copper, zinc
A-04	Copper, chlorine, zinc
A-05	Copper, chlorine

Total residual chlorine will dissipate with time and distance and should not be a toxicity issue at A-01, if the concentrations of chlorine in the contributing waste streams are relatively low (<0.1 mg/l). Cationic metals, such as copper and zinc will not dissipate, but will tend to become less biologically available as the effluent flows downstream, due to binding with naturally occurring humates, fulvates, and clay particles. The metals and toxicity data for A-01 and WE-01 indicate that metal bioavailability decreases with increasing distance from the outfall. The metal concentrations at the two locations are fairly similar, but A-01 is toxic, while WE-01, which is located approximately 0.5 miles downstream from A-01 is not.

The toxicity of many cationic metals, including copper and zinc, is inversely related to water hardness. At a hardness values of 0, 10, and 100 mg/l, the models used to predict metal toxicity indicate that the water quality criteria for copper are 0, 1.65, and 11.82 µg/l (Figure 1). Metal toxicity is also influenced by pH. The free metal ion form of many cationic metals is generally the most biologically available form of a metal, and therefore is the most toxic form. At acidic pH values more of the cationic metal exists as free ions, rather than as hydroxides or carbonates. Most of the effluents that are discharged from A Area are comprised primarily of groundwater that has a very low hardness (<10 mg/l) and pH values of less than 7. Under these conditions, cationic metals, such as copper, can be toxic at extremely low concentrations.

Of the outfalls that were tested A-03, was the most toxic outfall, with 100% mortality occurring at concentrations as low as 12.5%. A-03 was also found to have very high concentrations of copper, lead, and zinc. The water from A-03 is much higher

hardness (about 90 mg/l) than the other outfalls. The higher hardness undoubtedly reduces the toxicity of this outfall to some extent. However, when A-03 mixes with the other low-hardness waste streams that merge to form A-01, the metals present in A-03 will become more biologically available, and therefore more toxic. The A-01C and A-04 waste streams also have relatively high concentrations of copper. Clearly, one of the strategies to consider in reducing the toxicity of A-01 is to reduce the metal concentrations in the waste streams that have the highest copper concentrations.

Figure 1. Relationship of Copper Toxicity to Water Hardness

If it is assumed that most of the observed toxicity is due to copper, and that copper concentrations greater than the EPA hardness-based water quality criterion for copper will result in toxicity, then the maximum concentration of copper at A-01 would need to be reduced by approximately 97% in order to render the effluent non-toxic. However, based on toxicity data, a 75% reduction in copper contributions to A-01 should eliminate toxicity when the air stripper is operating.

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APPENDIX A-1

A-001B, PHASE I RESULTS

**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-001B

July 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-001B effluent using the water flea *Ceriodaphnia dubia* was conducted on June 25 - July 3, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 50%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

This report presents the results of a Metals-Focused Phase I Toxicity Identification Evaluation (TIE) conducted on the A-001B effluent on July 8-10, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on July 2nd, 1996 and used for conducting a partial Phase I Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would remove toxicity. Each treated sample was used in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test	7/8/96	T6016	-
Filtered at pH 11	7/8/96	T6016	metals; insoluble at pH 10
Na Thiosulfate Addition	7/8/96	T6016	oxidizers; residual chlorine; metals
EDTA Chelation	7/8/96	T6016	some metals; surfactants
Graduated pH	7/8/96	T6016	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Filtration at pH 11

A 300 mL sample aliquot which had been adjusted to $\text{pH } 11 \pm 0.05$ with NaOH was filtered through a 0.45μ membrane filter. After filtration the pH was readjusted to the initial pH with H_2SO_4 .

2.2.2 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 1.0, 5.0, and 10.0 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to equilibrate for two hours.

2.2.3 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 0.5, 3.0, and 8.0 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to equilibrate for two hours and were pH adjusted back to the initial pH.

2.2.4 Graduated pH Test

Aliquots of effluent at 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. After 2 hours and 18 hours the survival of test organisms at each pH was checked.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 20, 40, 60, 80 and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test which had two replicates of five organisms) . A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was below detectable levels, at <0.01 mg/L. Hardness was very low, at 4.0 mg/L. The effluent was slightly alkaline (7.82) in pH when received. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	7/8/96	T6016	Sample toxic; LC ₅₀ <20%
Filtered at pH 11	7/8/96	T6016	Sample toxicity reduced
Na Thiosulfate Addition	7/8/96	T6016	Sample toxicity reduced
EDTA Chelation	7/8/96	T6016	Sample toxicity removed
Graduated pH	7/8/96	T6016	Sample less toxic at pH 8.5

3.2 Discussion

Toxicity in the effluent was reduced by filtration at pH 11. This is a pattern typical of metals, which can precipitate out in the hydroxide form at high pH. Sodium thiosulfate addition was also effective at reducing effluent toxicity. Sodium thiosulfate is most effective at treating oxidizers such as residual chlorine, but also binds with silver, cadmium, copper, and mercury, reducing the bioavailability of these metals. EDTA was the most effective treatment, removing all effluent toxicity. EDTA is only effective at removing or reducing metal toxicity and surfactant toxicity. Metals effectively chelated by EDTA include cadmium, copper, nickel, and zinc. Chromium, mercury, lead, and selenium are not effectively removed from bioavailability by EDTA. Finally, metals which are more toxic at pH 6.5 than at pH 8.5 include copper and lead. The only metal which fits the pattern of being treatable by filtration at pH 11, EDTA, and sodium thiosulfate, and which is more toxic at pH 6.5 than at pH 8.5 is copper. This makes copper the likely toxicant. Due to the particularly low hardness, it may be anticipated that copper at concentrations as low as 1 µg/L would be toxic to *Ceriodaphnia dubia*.

3.3 Recommendations

Copper concentrations in the effluent should be verified to confirm that sufficient copper is present to cause toxicity. The possibility of surfactant toxicity should be investigated through conducting a sublation treatment with the associated toxicity tests.

4.0 CONCLUSIONS

- ▶ The toxicant in A-001B Effluent is likely to be a cationic metal, although the possibility of surfactants cannot be excluded.
- ▶ The metal which best fits the pattern of the data is copper. Copper is the likely toxicant.

SUMMARY OF RESULTS - ACUTE PHASE I TIE **WSRC Effluent A-001B** **July 1996**

Baseline Testing		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		30%	100%
40%		100%	100%
60%		100%	100%
80%		100%	100%
100%	7.51	100%	100%

Filtration at pH 11		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		0%	0%
40%		0%	0%
60%		0%	20%
80%		0%	40%
100%	7.55	0%	100%

Discussion:

The effluent was acutely toxic at concentrations as low as 20%.
Filtration at pH 11 was effective at reducing effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-001B
July 1996

EDTA Chelation - 18 Hr. Mortality

Effluent Conc.	EDTA Conc. (mg/L)					0.5 Control
	0 Untreated	0.5	3.0	8.0		
0%	0%	0%	0%	0%		
20%	100%	0%	0%	0%		
40%	100%	0%	0%	0%		
60%	100%	0%	0%	0%		
80%	100%	0%	0%	0%		
100%	100%	0%	0%	80%		0%
Control pH						8.26
Effluent pH	7.51	7.55	7.00	7.56		

Sodium Thiosulfate Addition - 18 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)					1.0 Control
	0.0	1.0	5.0	10.0		
0%	0%	0%	0%	0%		
20%	100%	60%	80%	0%		
40%	100%	100%	80%	20%		
60%	100%	100%	20%	0%		
80%	100%	100%	100%	0%		
100%	100%	100%	100%	100%		100%
Control pH						7.90
Effluent pH		8.37	8.4	8.42

Discussion:

EDTA at 0.5 to 3.0 mg/L removed effluent toxicity.

Sodium thiosulfate addition of 10 mg/L reduced effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-001B
July 1996

Graduated pH Test

Effluent Conc.	2 Hour Mortality at Nominal pH (w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.50	0%			8.66	0%
20%		100%				0%
40%		100%				0%
60%		100%				0%
80%		100%				60%
100%	6.51	100%			8.54	100%

Discussion:

The sample was less toxic at pH 8.5 than at pH 6.5.

APPENDIX A-2

A-001B, ADDITIONAL PHASE I RESULTS

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TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only
Follow-up Tests

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-001B

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-001B effluent using the water flea *Ceriodaphnia dubia* was conducted on June 25 - July 3, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 50%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-001B effluent on July 11-13, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. The results indicated that a cationic metal was the cause of toxicity, and that the pattern of toxicity was most consistent with copper toxicity. Measured concentrations of copper in the effluent ranged from 61-81 µg/L, a level which may be expected to be toxic in very soft water such as A-001B effluent.

Due to the effectiveness of EDTA chelation in removing some surfactant toxicity, a sublation treatment and test was conducted on A-001B effluent to confirm that no toxicants other than copper were present. This report presents the results of the sublation test conducted as a follow-up to the original Phase I testing conducted in July 1996, along with a baseline test run in parallel.

2.0 METHODS

2.1 *Sampling*

A grab sample of effluent was collected on September 5, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 *Treatment Methods*

The sample was subjected to a single treatment (sublation). The treated sample as well as the untreated sample was used in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	9/10/96	T6357	-
Sublation	9/10/96	T6357	surfactants

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

Sublation Test

An 80 mL aliquot of 100% effluent was vigorously aerated in glass vessel such that foam produced by the aeration overflowed into an outer glass vessel. The foam which overflowed was collected and labelled as the "sublate." The effluent remaining in the inner vessel after foam was removed was also collected and labelled as the "residual."

2.3 *Toxicity Testing Methods*

Acute toxicity tests were set with both untreated and treated effluent samples. The baseline test was set at 1, 10, 20, 40, and 60% effluent concentration, it being assumed that the effluent would be toxic at higher concentrations. The sublation test was set at 20, 40, 60, 80 and 100% effluent concentration. The baseline test was comprised of two replicates of five neonate test organisms at each test concentration. The sublation

test was comprised of one replicate of five neonate test organisms at each test concentration. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be clear in color and slightly alkaline in pH. Residual chlorine was low, at 0.04 mg/L. Hardness was very low, at 8.0 mg/L, as was alkalinity (53.6 µg/L). The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	9/10/96	T6357	Sample toxic; LC ₅₀ =52.5%
Sublation	9/10/96	T6357	Toxicity not removed

3.2 Discussion

The September 5, 1996 sample of A-001B effluent was less toxic than the sample collected in July (Figure 1). The baseline test showed no significant acute toxicity at effluent concentrations of 40% or less. Effluent in the July sample was toxic at concentrations as low as 20%.

The sublation test did not provide any evidence of the presence of surfactants. Typically, if surfactants are present the residual solution should be substantially less toxic than the sublated portion. In this test, however, the reverse was true - the residual solution was more toxic than the sublate. It is not entirely clear why this is so. It is possible that metal concentrations were lower in the surface (foam) layer than in the main portion of the sample. If so, copper may have become more concentrated in the residual than in the untreated sample. The residual solution was more toxic than the untreated sample. The possibility that the residual and sublate solutions may have been switched was considered. However, when the sublation treatment was re-run, the same result was duplicated.

4.0 CONCLUSION

Follow-up treatments/testing conducted in September 1996 showed that surfactants did not play a role in the toxicity of A-001B effluent.

APPENDIX A-3

A-001B, PHASE III RESULTS

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TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification:A-001B

September 1996

1.0 INTRODUCTION

A-001B effluent was identified as acutely toxic to *Ceriodaphnia dubia* in June 1996. A Phase I TIE initiated in July 1996 and completed in September 1996 indicated that copper was the main toxicant in A-001B effluent. No other metals were detected at levels considered to be potentially toxic. In order to confirm copper as the toxicant, simulated A-001B was prepared and copper was spiked into the simulated effluent. This report presents the results of these investigations, identified as Phase III.

2.0 METHODS

Baseline testing conducted on September 10-12, 1996 indicated acute toxicity at effluent concentrations of 60% and greater. Based upon this information simulated effluent was prepared as a formulation of 50% effluent and 50% moderately hard synthetic freshwater (MHSF) diluted to a hardness of 4 mg/L (the hardness of the effluent). The diluted MHSF was prepared by adding 475 mL of ultra-pure Type I water to 25 mL of MHSF at a hardness of 80 mg/L. Copper was spiked into this simulated effluent.

An acute definitive toxicity test was set with five copper concentrations and a control. Copper was spiked as copper sulfate.

3.0 RESULTS

Copper was spiked at a range of 5 - 80 µg/L into the simulated effluent. However, the simulated effluent at 50% dilution had a baseline level of copper of 25 µg/L. The nominal series of copper concentrations was 5, 10, 20, 40, and 80 µg/L. Adding the baseline level of copper provided a series of copper concentrations of 30, 35, 45, 65, and 105 µg/L. The results are summarized as follows;

48 Hr Mortality By Dilution

<u>Cu Concentration</u>	<u>Cu Only</u>
0 µg/L	0%
30 µg/L	100%
35 µg/L	100%
45 µg/L	100%
65 µg/L	100%
105 µg/L	100%

In the range of 30-105 µg/L of total copper all test organisms died in less than 48 hours in all concentrations except the control. Thus, copper in the range measured in the effluent (51 - 81 µg/L) easily accounts for the noted acute toxicity. Data generated at ETT Environmental in association with water effect ratio testing indicates an LC_{50} of approximately 9.0 µg/L for total copper in soft diluted mineral water (DMW - hardness = 50 mg/L). However, A-001B effluent is much softer than soft diluted mineral water. Based upon the EPA water criterion formula for copper at a hardness of 4 mg/L the CMC (maximum concentration to avoid acute toxicity) is $e^{(0.9422 \ln(\text{hardness}) - 1.464)}$ is 0.85 µg/L. This is a conservative estimate of the range at which copper becomes acutely toxic in A-001B effluent.

It should be noted that hardness, if it is too low, can result in toxicity. In order to determine if the soft DMW contributed to toxicity apart from the influence of copper, a series of DMW dilutions was prepared in which hardness was varied. The results indicated that 35% mortality occurred in DMW with a hardness of 2 mg/L, 95% mortality occurred in ultra-pure water with a hardness of <0.1 mg/L, and no significant mortality in DMW with a hardness of 5 mg/L and higher. This suggests that water with a hardness of 4 mg/L should not have been significantly toxic due only to low hardness.

4.0 CONCLUSION

Copper was present at toxic levels in the A-001B effluent. Copper levels must be reduced by 95% to eliminate copper acute toxicity, or alternatively, hardness must be increased to moderately hard levels (80-100 mg/L) and copper levels reduced by 50% to eliminate copper toxicity.

APPENDIX B-1

A-001C, PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-001C

July 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-001C effluent using the water flea *Ceriodaphnia dubia* was conducted on June 25 - July 3, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 20%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

This report presents the results of a Metals-Focused Phase I Toxicity Identification Evaluation (TIE) conducted on the A-001C effluent on July 8-10, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on July 2nd, 1996 and used for conducting a partial Phase I Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would remove toxicity. Each treated sample was used in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test	7/8/96	T6017	-
Filtered at pH 11	7/8/96	T6017	metals; insoluble at pH 10
Na Thiosulfate Addition	7/8/96	T6017	oxidizers; residual chlorine; metals
EDTA Chelation	7/8/96	T6017	some metals; surfactants
Graduated pH	7/8/96	T6017	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Filtration at pH 11

A 300 mL sample aliquot which had been adjusted to $\text{pH } 11 \pm 0.05$ with NaOH was filtered through a 0.45μ membrane filter. After filtration the pH was readjusted to the initial pH with H_2SO_4 .

2.2.2 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 1.0, 5.0, and 10.0 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to equilibrate for two hours.

2.2.3 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 0.5, 3.0, and 8.0 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to equilibrate for two hours and were pH adjusted back to the initial pH.

2.2.4 Graduated pH Test

Aliquots of effluent at 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. After 2 hours and 18 hours the survival of test organisms at each pH was checked.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 20, 40, 60, 80 and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test which had two replicates of five organisms) . A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was below detectable levels, at <0.01 mg/L. Hardness was very low, at 10.0 mg/L. The effluent was slightly alkaline (7.76) in pH when received. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	7/8/96	T6017	Sample toxic; LC ₅₀ < 20%
Filtered at pH 11	7/8/96	T6017	No reduction in toxicity
Na Thiosulfate Addition	7/8/96	T6017	No reduction in toxicity
EDTA Chelation	7/8/96	T6017	Sample toxicity removed
Graduated pH	7/8/96	T6017	Sample equally toxic at all pHs

3.2 Discussion

Toxicity in the effluent was not reduced by filtration at pH 11. Sodium thiosulfate addition was also ineffective at reducing effluent toxicity. Sodium thiosulfate is most effective at treating oxidizers such as residual chlorine, but also binds with silver, cadmium, copper, and mercury, reducing the bioavailability of these metals. EDTA was the most effective treatment, removing all effluent toxicity. EDTA is only effective at removing or reducing metal toxicity and surfactant toxicity. Metals effectively chelated by EDTA include cadmium, copper, lead, nickel, and zinc. Chromium, mercury, lead, and selenium are not effectively removed from bioavailability by EDTA. Metals which are bound by EDTA but not by sodium thiosulfate include nickel, lead, and zinc. Nickel and zinc typically are more toxic at pH 8.5 than at pH 6.5 and lead is typically more toxic at pH 6.5 than at pH 8.5. None of these fit the pattern of the data. Possible explanations include the presence of more than one metal, e.g., zinc and lead, or concentrations of a single metal which are elevated to the degree that pH adjustment is ineffective.

3.3 Recommendations

Metals data for effluent A-001C should be reviewed to determine which metals are present in potentially toxic concentrations. The graduated pH test should be repeated using dilutions of less than 20% concentration to further investigate pH effects. The possibility of surfactant toxicity should be investigated through conducting a sublation treatment with the associated toxicity test.

4.0 CONCLUSIONS

- ▶ The toxicant in A-001C Effluent is likely to be a cationic metal, although the possibility of surfactants cannot be excluded.
- ▶ No one metal which precisely fits the pattern of the data. Lead, nickel and zinc best fit the data pattern.
- ▶ Toxicity may be due to more than one cationic metal.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-001C
July 1996

Baseline Testing		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		100%	100%
40%		100%	100%
60%		100%	100%
80%		100%	100%
100%	8.26	100%	100%

Filtration at pH 11		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		100%	100%
40%		100%	100%
60%		100%	100%
80%		100%	100%
100%	8.26	100%	100%

Discussion:

The effluent was acutely toxic at concentrations as low as 20%.
 Filtration at pH 11 was not effective at reducing effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-001C

July 1996

EDTA Chelation - 18 Hr. Mortality

Effluent Conc.	EDTA Conc. (mg/L)					0.5 Control
	0 Untreated	0.5	3.0	8.0		
0%	0%					
20%	100%	100%	0%	0%		
40%	100%	100%	0%	0%		
60%	100%	100%	0%	0%		
80%	100%	100%	0%	0%		
100%	100%	100%	0%	100%		0%
Control pH						8.27
Effluent pH		8.27	8.37	7.83		

Sodium Thiosulfate Addition - 18 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)					1.0 Control
	0.0	1.0	5.0	10.0		
0%	0%					
20%	100%	100%	100%	100%		
40%	100%	100%	100%	100%		
60%	100%	100%	100%	100%		
80%	100%	100%	100%	100%		
100%	100%	100%	100%	100%		0%
Control pH						8.34
Effluent pH		8.2	8.32	8.27

Discussion:

EDTA at 3.0 to 8.0 mg/L removed effluent toxicity.

Sodium thiosulfate addition did not reduce effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-001C
July 1996

Graduated pH Test

Effluent Conc.	2 Hour Mortality at Nominal pH (w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.50	0%	7.58	0%	8.66	0%
20%		100%		100%		100%
40%		100%		100%		100%
60%		100%		100%		100%
80%		100%		100%		100%
100%	6.58	100%	7.60	100%	8.54	100%

Discussion:

The sample was toxic at all three pH values.

APPENDIX B-2

A-001C, ADDITIONAL PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only
Follow-up Tests**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-001C

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-001C effluent using the water flea *Ceriodaphnia dubia* was conducted on June 25 - July 3, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 25%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-001C effluent on July 8-10, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. The results indicated that a cationic metal was the cause of toxicity, but the pattern of toxicity was not consistent with any particular metal. Copper toxicity is typically reduced by sodium thiosulfate addition and EDTA chelation. Phase I results indicated that effluent toxicity was not reduced by sodium thiosulfate addition, although EDTA chelation was effective. The graduated pH test was inconclusive due to the high level of toxicity. Metals analysis data showed that copper (measured at levels as high as 130 µg/L) was the metal most likely to cause toxicity.

Due to the results of the Phase I treatments and tests, additional testing was conducted in September 1996. The testing focused on two items; 1) a sublation treatment was conducted to confirm that the effectiveness of EDTA treatment was not due to surfactants, and 2) a graduated pH test was conducted at dilute effluent concentrations to obtain additional information on the pH sensitivity of the effluent. This report presents the results of these tests conducted as a follow-up to the original Phase I testing conducted in July 1996.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on September 5, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 Treatment Methods

The sample was subjected to two treatments. The baseline test and the sublation test were tested for acute toxicity using test dilutions of 20, 40, 60, 80, and 100%. The graduated pH test was set at effluent concentrations of 5, 10, and 20%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	9/10/96	T6358	-
Graduated pH	9/10/96	T6358	ammonia; metals; sulfide
Sublation	9/10/96	T6358	surfactants

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

2.2.1 Graduated pH Test

Aliquots (80 mL) of effluent at 5%, 10% and 20% concentration were adjusted to pH 6.5, 7.5 and 8.5. No buffers were used. At two hours, five hours, eighteen hours, 24 hours, and 48 hours the pH of each aliquot was recorded. The test organisms were temporarily removed from the solution to a holding vessel while the solution was readjusted to the original pH. The test organisms were then transferred back to the original aliquot. Mortality was recorded at each interval.

2.2.2 Sublation Test

An 80 mL aliquot of 100% effluent was vigorously aerated in glass vessel such that foam produced by the aeration overflowed into an outer glass vessel. The foam which overflowed was collected and labelled as the "sublate." The effluent remaining in the inner vessel after foam was removed was also collected and labelled as the "residual."

2.3 *Toxicity Testing Methods*

Acute toxicity tests were set with all untreated and treated effluent samples. The baseline test and the sublation test were set at 20, 40, 60, 80 and 100% effluent concentration. The graduated pH test was set only at concentrations of 5%, 10%, and 20% effluent. The baseline test was comprised of two replicates of five neonate test organisms at each test concentration. The graduated pH test was comprised of one replicate of ten neonate test organisms at each pH value. The sublation test was comprised of one replicate of five neonate test organisms at each test concentration. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 *Summary*

Upon arrival the sample was observed to be have a clear color. Residual chlorine was low, at 0.05 mg/L. Hardness was very low, at 6.0 mg/L, as was alkalinity (49.4 µg/L). The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	9/10/96	T6358	Sample toxic; LC ₅₀ =28.3%
Graduated pH	9/10/96	T6358	Sample less toxic at pH 8.5
Sublation	9/10/96	T6358	Toxicity not removed

3.2 *Discussion*

The September 5, 1996 sample of A-001C effluent was less toxic than the sample collected in July (Figure 1). The baseline test showed no significant acute toxicity at effluent concentrations of less than 40%. Effluent in the July sample was toxic at concentrations as low as 20%.

The graduated pH test showed that toxicity was reduced at pH 8.5 as compared to pH 6.5 and pH 7.5. This result is consistent with copper as a toxicant, but not zinc. In the July Phase I treatments the graduated pH test could not distinguish the pH sensitivity of the toxicant due to the rapid mortality of the test organisms. Less toxicity in the September sample allowed for the pH sensitivity to be assessed.

The sublation test did not provide any evidence of the presence of surfactants. Typically, if surfactants are present the residual solution should be substantially less toxic than the sublated portion. In this test, however, both solutions were equally toxic.

Follow-up treatments/testing conducted in September 1996 indicated that copper was the main toxicant of concern in A-001C effluent. The primary piece of evidence suggesting that copper was not the primary toxicant in the July testing was the failure of sodium thiosulfate treatment to affect toxicity. This could reflect either insufficient sodium thiosulfate to mitigate the effects of the relatively high levels of copper in the July sample, or synergistic effects of other metals.

Figure 1
WSRC A-001C Effluent
Phase 1 TIE Follow-Up Testing Results
September 1996

BASELINE TESTING

Effluent Concentration	Mortality	
	July 96	Sept 96
0%	0%	0%
20%	100%	0%
40%	100%	100%
60%	100%	100%
80%	100%	100%
100%	100%	100%
48 Hr LC50	< 20.0%	28.4%
Toxic Units	5.00	3.52
Hardness (mg/L)	10	6

GRADUATED pH TEST (September 1996)

Effluent Concentration	Mortality at 24 Hours			Effluent Concentration	Mortality at 48 Hours		
	pH 6.5	pH 7.5	pH 8.5		pH 6.5	pH 7.5	pH 8.5
5%	20%	10%	0%	5%	20%	20%	0%
10%	0%	40%	0%	10%	10%	40%	0%
20%	50%	10%	0%	20%	80%	10%	0%
Lowest pH (100%)	6.50	7.20	7.28				
Highest pH (100%)	7.22	7.48	8.55				

SUBLATION TEST (September 1996)

Effluent Concentration	Mortality at 48 Hours		
	Untreated	Residual	Sublate
0%	0%	0%	0%
20%	0%	100%	100%
40%	100%	100%	100%
60%	100%	100%	100%
80%	100%	100%	100%
100%	100%	100%	100%

APPENDIX B-3

A-001C, PHASE III RESULTS

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TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification:A-001C

September 1996

1.0 INTRODUCTION

A-001C effluent was identified as acutely toxic to *Ceriodaphnia dubia* in June 1996. A Phase I TIE initiated in July 1996 and completed in September 1996 indicated that copper was the main toxicant in A-001C effluent. No other metals were detected at levels considered to be potentially toxic. In order to confirm copper as the toxicant, simulated A-001C was prepared and copper was spiked into the simulated effluent. This report presents the results of these investigations, identified as Phase III.

2.0 METHODS

Baseline testing conducted on September 10-12, 1996 indicated acute toxicity at effluent concentrations of 40% and greater. Because this meant that effluent would have to be diluted to a 30% concentration or less, and the extent of such dilution would fundamentally change the characteristics of the effluent, it was decided that A-001C could best be simulated by preparing synthetic freshwater at the appropriate hardness. Although the hardness of the effluent was 6 mg/L, the synthetic effluent was prepared with a slightly lower hardness of 5 mg/L. The diluted MHSF was prepared by adding 470 mL of ultra-pure Type I water to 30 mL of MHSF at a hardness of 80 mg/L. Copper was spiked into this simulated effluent.

An acute definitive toxicity test was set with five copper concentrations and a control. Copper was spiked as copper sulfate.

3.0 RESULTS

Copper was spiked at a range of 1-20 µg/L into the simulated effluent. Hardness of the effluent was measured at 6 mg/L. Copper has been measured in untreated effluent at 87 - 130 µg/L. The results are summarized as follows;

48 Hr Mortality By Dilution	
<u>Cu Concentration</u>	<u>Cu Only</u>
0 µg/L	30%
1 µg/L	40%
2 µg/L	100%
5 µg/L	100%
20 µg/L	100%

Although there was some mortality in the control (probably due to the low hardness and small size of the neonates), it was clear that copper concentrations of 2 µg/L and higher were acutely toxic at this hardness. Based upon the EPA water criterion formula for copper at a hardness of 6 mg/L the CMC (maximum concentration to avoid acute toxicity) is $e^{(0.9422 \ln(\text{hardness}) - 1.464)}$ is 1.25 µg/L. This is a conservative estimate of the range at which copper becomes acutely toxic in A-001C effluent, although various other ligands in the effluent may mitigate the effects of copper to some degree.

It should be noted that hardness, if too low, can result in toxicity. In order to determine if the soft DMW contributed to toxicity apart from the influence of copper, a series of DMW dilutions was prepared in which hardness was varied. The results indicated that 35% mortality occurred in DMW with a hardness of 2 mg/L, 95% mortality occurred in ultra-pure water with a hardness of <0.1 mg/L, and no significant mortality in DMW with a hardness of 5 mg/L and higher. This suggests that water with a hardness of 4 mg/L should not have been significantly toxic due only to low hardness. However, this test was conducted with neonates of 16-24 hours in age whereas the testing in copper spiked solutions of simulated effluent was conducted with neonates of 0-8 hours in age, which were likely more sensitive to toxicity.

4.0 CONCLUSION

Copper was present at toxic levels in the A-001C effluent. Copper levels must be reduced by over 95% to eliminate copper acute toxicity, or alternatively, hardness must be increased to moderately hard levels (80-100 mg/L) and copper levels reduced by 75% to eliminate copper toxicity.

APPENDIX C-1

AT-001, PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I**

Client: Westinghouse Savannah River Company

Sample ID: AT-001

August 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of AT-001 water using the water flea *Ceriodaphnia dubia* was conducted on July 16-24, 1996. The test results showed the presence of acute toxicity at sample concentrations as low as 50%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

This report presents the results of a Phase I Toxicity Identification Evaluation (TIE) conducted on a AT-001 sample on July 31 - August 2, 1996. Sample treatments including pH adjustment, filtration, aeration, C-18 SPE column, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed.

2.0 METHODS

2.1 Sampling

A composite sample of effluent was collected on July 31, 1996 and used for conducting Phase I of the Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would reduce or remove toxicity. Each treated sample was used in an acute toxicity test (at a series of dilutions) to determine the effectiveness of the treatment. The treatments and results of the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline	8/6/96	TI6203	-
Adjusted to pH 3	8/6/96	TI6203	pH sensitive
Adjusted to pH 11	8/6/96	TI6203	pH sensitive
Filtered at Initial pH	8/6/96	TI6203	bound to solids; insoluble
Filtered at pH 3	8/6/96	TI6203	bound to solids; insoluble
Filtered at pH 11	8/6/96	TI6203	metals; insoluble at pH 10
Aerated at Initial pH	8/6/96	TI6203	volatile;oxidizable
Aerated at pH 3	8/6/96	TI6203	volatile;oxidizable
Aerated at pH 11	8/6/96	TI6203	volatile;oxidizable
Spe C18 at initial pH	8/6/96	TI6203	non-polar organics; metals
Spe C18 at pH 3	8/6/96	TI6203	non-polar organics; metals
Spe C18 at pH 11	8/6/96	TI6203	non-polar organics; metals
Na Thiosulfate Addition	8/6/96	TI6203	oxidizers; residual chlorine;metals
EDTA Chelation	8/6/96	TI6203	some metals; surfactants
Graduated pH	8/6/96	TI6203	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Adjustment to pH 3

A 350 mL aliquot of sample was adjusted to pH 3.21 with 1N H₂SO₄. After pH adjustment the sample aliquot was re-adjusted to pH 7.56.

2.2.2 Adjustment to pH 11

A 350 mL aliquot of sample was adjusted to pH 11.01 with NaOH. After pH adjustment the sample aliquot was re-adjusted to pH 7.57.

2.2.3 Filtration at Initial pH

A 350 mL sample aliquot was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water.

2.2.4 Filtration at pH 3

A 350 mL sample aliquot which had been adjusted to pH 3.21 was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water. After filtration the pH was re-adjusted to pH 7.56 with NaOH.

2.2.5 Filtration at pH 11

A 350 mL sample aliquot which had been adjusted to pH 11.01 was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water. After filtration the pH was 10.90, and was readjusted to a pH of 7.57 with H₂SO₄.

2.2.6 Aeration at Initial pH

A 100 mL sample aliquot was vigorously aerated for a period of one hour with a diaphragm type aerator. After aeration the pH was 7.66.

2.2.7 Aeration at pH 3

A 100 mL sample aliquot which had been adjusted to pH 3.21 was vigorously aerated for a period of one hour with a diaphragm type aerator. After aeration the pH was 3.10, and was re-adjusted to pH 7.56 with NaOH.

2.2.8 Aeration at pH 11

A 100 mL sample aliquot which had been adjusted to pH 11.01 was vigorously aerated for a period of one hour with a diaphragm type aerator. After aeration the pH was 10.90 and was re-adjusted to pH 7.57 with H₂SO₄.

2.2.9 C-18 SPE Column at Initial pH

A 200 mL aliquot of filtered sample was run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.10 C-18 SPE Column at pH 3

A 200 mL aliquot of sample which had been adjusted to pH 3.10 and filtered was run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. After extraction the pH was re-adjusted to pH 7.57. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.11 C-18 SPE Column at pH 3

A 200 mL aliquot of sample which had been adjusted to pH 11.01 and filtered was run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. After extraction the pH was re-adjusted to pH 7.57. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.12 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 0.03, 0.06, 0.12, 0.25 and 0.5 g/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to sit for two hours.

2.2.13 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 0.045, 0.09, 0.19, 0.37, and 0.75 g/L of EDTA. After addition of the EDTA, the test solutions were allowed to sit for two hours.

2.2.11 Graduated pH Test

Aliquots of effluent were adjusted to pH 6.5, 7.5 and 8.5. At intervals 1, 2, 4 and 36 hours toxicity was monitored and the pH was re-adjusted as necessary.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 12.5, 25, 50, and 100% effluent concentrations. The graduated pH test was run with test concentrations of 0, 50 and 100% at each pH. Dilution water and effluent were both pH adjusted to all three pH values prior to preparation of dilutions. Each test dilution was comprised one replicates of five neonate test organisms (except for the baseline test, which had two replicates of five test organisms. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the seep samples. Several treatments were conducted at more than one pH.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be clear in color. Residual chlorine was relatively low, at 0.08 mg/L. The effluent was relatively neutral in pH (7.57) when received.

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	8/6/96	TI6203	Sample toxic; LC ₅₀ =41%
Adjusted to pH 3	8/6/96	TI6203	Toxicity increased
Adjusted to pH 11	8/6/96	TI6203	Toxicity not reduced
Filtered at Initial pH	8/6/96	TI6203	Toxicity not reduced
Filtered at pH 3	8/6/96	TI6203	Toxicity increased
Filtered at pH 11	8/6/96	TI6203	Toxicity removed
Aerated at Initial pH	8/6/96	TI6203	Toxicity increased
Aerated at pH 3	8/6/96	TI6203	Toxicity increased
Aerated at pH 11	8/6/96	TI6203	Toxicity not reduced
C-18 SPE at Initial pH	8/6/96	TI6203	Toxicity slightly reduced
C-18 SPE at pH 3	8/6/96	TI6203	Toxicity increased
C-18 SPE at pH 11	8/6/96	TI6203	Toxicity removed
Na Thiosulfate Add.	8/6/96	TI6203	Toxicity slightly reduced
EDTA Chelation	8/6/96	TI6203	Toxicity slightly reduced
Graduated pH	8/6/96	TI6203	Sample less toxic at high pH (8.5)

3.2 Discussion

Toxicity in the effluent could be removed or reduced by filtration at pH 11, extraction by C-18 SPE column, EDTA chelation and sodium thiosulfate addition. Aeration was not effective. Toxicity was found to be pH sensitive, such that effluent was less toxic at pH 8.5 and more toxic at pH 6.5. Several potential toxicants can be eliminated from consideration based upon the results of Phase I testing. Residual chlorine measured in the sample was not high enough to account for sample toxicity. Ammonia would be expected to be more toxic at pH 8.5 than at pH 6.5, opposite of the trend noted in the data. The ineffectiveness of aeration at any pH indicates that volatiles were not a factor in toxicity.

Reduction in toxicity through EDTA chelation suggests that cationic metals are involved in sample toxicity. Specifically, the metals cadmium, copper, iron, manganese, nickel, lead, and zinc can be chelated resulting in toxicity reduction. Of these metals cadmium and copper are also treatable by sodium thiosulfate. Copper tends to be more toxic at a lower pH (6.5) whereas cadmium is more toxic at a higher pH (8.5). The data, then point to copper as the toxicant of concern. At pH 11, it may be concluded, copper in the form of copper hydroxide was filtered out as a precipitate.

The effectiveness of C-18 SPE column extraction at initial pH and pH 11 could indicate the presence of toxicity due to non-polar organics. However, in this case it more likely reflects a physical removal of particulate associated copper within the C-18 SPE column.

3.3 Recommendations

A repeat of the C-18 SPE treatment at initial pH is needed followed by a methanol elution to confirm if the effectiveness of the SPE column was due to removal of metals or due to removal of non-polar organics.

4.0 Conclusions

- ▶ Toxicity in the AT-001 effluent is likely due to the presence of a cationic metal.
- ▶ The cationic metal which best fits the pattern of the data is copper.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent AT-001

August 1996

Baseline Test		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0.0%	8.34	0%	0%
12.5%		0%	0%
25.0%		0%	0%
50.0%		80%	90%
100.0%	7.57	100%	100%

pH Adjustment / Aeration / Filtration / C-18 SPE

48 Hr LC50	pH 3	pH 7.57	pH 11
Baseline	20.3%	40.6%	35.4%
Aeration	17.7%	23.3%	35.3%
Filtration	15.3%	40.6%	100.0%
C-18 SPE Column	12.5%	70.7%	100.0%

Discussion: Baseline testing showed the AT-001 sample to be acutely toxic at dilutions as low as 50%. Acute toxicity was not removed by pH adjustment alone. Any samples adjusted to pH 3 and then readjusted became increasingly toxic. Filtration at pH 11 removed toxicity. The C-18 SPE column used at pH 7.57 removed more toxicity than filtration alone.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent AT-001

August 1996

Sodium Thiosulfate Addition - 48 Hr. Mortality

EDTA Chelation - 48 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (g/L)					
	0	0.03	0.06	0.12	0.25	0.5
0%	0%	0%	0%	0%	0%	0%
12.5%	0%	0%	0%	0%	0%	0%
25%	0%	0%	0%	0%	0%	0%
50%	90%	0%	80%	40%	0%	0%
100%	100%	100%	100%	100%	80%	100%

Effluent Conc.	EDTA Conc. (g/L)					
	0	0.045	0.09	0.19	0.37	0.75
0%	0%	0%	0%	0%	100%	100%
12.5%	0%	0%	0%	0%	100%	0%
25%	0%	0%	0%	0%	40%	0%
50%	90%	0%	0%	0%	0%	100%
100%	100%	100%	100%	60%	100%	100%

Discussion:

EDTA reduced toxicity at concentrations from 0.045 to 0.37 g/L. The most effective treatment was at 0.19 g/L EDTA. Sodium thiosulfate reduced toxicity at concentrations greater than 0.06 g/L.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent AT-001
August 1996

Graduated pH Test

Effluent Conc.	2 Hour Mortality at Nominal pH (w/ and w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.58	0%	7.51	0%	8.53	0%
50%	6.52	80%	7.53	60%	8.51	0%
100%	6.53	100%	7.50	40%	8.52	60%

Discussion: The pH of the effluent was found to have an effect on the toxicity. Toxicity was found to be lower at pH 8.5 than at pH 7.5 or pH 6.5.

APPENDIX C-2

AT-001, ADDITIONAL PHASE I RESULTS,
SEPTEMBER 1996

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TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only
Follow-up Tests

Client: Westinghouse Savannah River Company

Sample ID: Effluent AT-001

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of AT-001 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 16 - 24, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 50%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Phase I Toxicity Identification Evaluation (TIE) was conducted on the AT-001 effluent on July 31 - August 2, 1996. Effluent treatments including pH adjustment, filtration, aeration, C-18 SPE column, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed or manipulated. The results indicated that a cationic metal was the cause of toxicity, and the pattern of toxicity was consistent with copper. Copper toxicity is typically reduced by sodium thiosulfate addition and EDTA chelation, and toxicity is less at pH 8.5 than at pH 6.5-7.5. Metals analysis data showed that copper (measured at levels as high as 62 µg/L) was the metal most likely to cause toxicity.

Due to the results of the Phase I treatments and tests, additional testing was conducted in September 1996. The testing focused on two items; 1) a repeat of the C-18 SPE column treatment at initial pH, and 2) a methanol elution of the C-18 SPE column. This report presents the results of these tests conducted as a follow-up to the original Phase I testing conducted in July-August 1996.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on September 19, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 Treatment Methods

The sample was subjected to two treatments. The C-18 SPE column treatment was set at 100% effluent concentration. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	9/22/96	T6428	-
C-18 SPE at Initial pH	9/22/96	T6428	organics; metals
Methanol Elution	9/24/96	T6428	organics

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

2.2.1 C-18 SPE at Initial pH

A 200 mL aliquot of effluent was filtered through a 0.45 μ membrane filter and then extracted through a 6 mL Baker C-18 SPE column at a rate of 1 mL per minute. The column was first used with methanol and a dilution water rinse. Effluent was pulled through the column with a chemical metering pump.

2.2.2 Methanol Elution

Methanol concentrations of 25, 50, 75, 80, 85, 90, 95, and 100% methanol were prepared, diluted with deionized water. Two successive 1.5 mL portions of each methanol concentration were collected and run through the column. The two portions of each concentration were collected and combined into a single fraction. This fraction was then brought to a total volume of 200 mL with moderately hard synthetic freshwater (MHSF) and tested for acute toxicity.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all untreated and treated effluent samples. The C-18 SPE column at initial pH test was set with two replicates of 5 test organisms. A single replicate of five test organisms was used to assess each methanol control and effluent fraction.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Hardness was very low, at 6.0 mg/L. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	8/6/96		Sample toxic; $LC_{50}=41\%$
C-18 SPE Column at Init. pH	9/22/96	T6428	Sample toxic reduced; $LC_{50}=95\%$
Methanol Elution	9/24/96	T6428	Toxicity in controls and fractions

3.2 Discussion

Due to the persistence of toxicity in AT-001 effluent a Baseline Test was not run on the September 5, 1996 sample. Assuming similar toxicity in the September 5th sample as in the August sample it can be concluded that the C-18 SPE column reduced but did not remove effluent toxicity. This is consistent with metal toxicity, because metals are typically removed in a physical filtration process by the column. Although methanol was kept below 2% concentration in the diluted samples of each fraction used for testing, the controls for each fraction above 75% methanol demonstrated mortality of the control test organisms as well as the treated effluent test organisms. Thus, it was not possible to determine if the toxicity had been eluted from the column or not.

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I
Follow-up Tests - C-18 SPE and Methanol Elution**

Client: Westinghouse Savannah River Company

Sample ID: Effluent AT-001

December 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of AT-001 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 16 - 24, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 50%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Phase I Toxicity Identification Evaluation (TIE) was conducted on the AT-001 effluent on July 31 - August 2, 1996. Effluent treatments including pH adjustment, filtration, aeration, C-18 SPE column, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed or manipulated. The results indicated that a cationic metal was the cause of toxicity, and the pattern of toxicity was consistent with copper. Copper toxicity is typically reduced by sodium thiosulfate addition and EDTA chelation, and toxicity is less at pH 8.5 than at pH 6.5-7.5. Metals analysis data showed that copper (measured at levels as high as 62 µg/L) was the metal most likely to cause toxicity.

Due to the results of the Phase I treatments and tests, which left open the possibility that non-polar organics might play a role in AT-001 toxicity, additional testing was conducted in September 1996. The testing focused on two items; 1) a repeat of the C-18 SPE column treatment at initial pH, and 2) a methanol elution of the C-18 SPE column. Methanol elution was conducted with the same C-18 SPE column used for treatment at initial pH in Phase I, which was shown to be effective in reducing toxicity. However, the results of the methanol elution tests in September were confounded by the presence of methanol toxicity in the control blanks. Therefore, the September tests were repeated in December 1996, and the results are presented in this report. The volume of effluent run through the SPE column was increased in December testing in order to reduce the concentration of methanol after elution and dilution.

2.0 METHODS

2.1 Sampling

A grab sample of AT-001 effluent was collected on December 8, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 Treatment Methods

The sample was subjected to two treatments. The C-18 SPE column treatment was set at 100% effluent concentration. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	12/9/96	T6805	-
C-18 SPE at Initial pH	12/9/96	T6805	organics; metals
Methanol Elution	12/11/96	T6805	organics

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

2.2.1 C-18 SPE at Initial pH

A 500 mL aliquot of effluent was filtered through a 0.45 μ membrane filter and then extracted through a 6 mL Baker C-18 SPE column at a rate of 5 mL per minute. The column was first used with methanol and a dilution water rinse. Effluent was pulled through the column with a chemical metering pump.

2.2.2 Methanol Elution

Methanol concentrations of 25, 50, 75, 80, 85, 90, 95, and 100% methanol were prepared, diluted with deionized water. Two successive 1.5 mL portions of each methanol concentration were collected and run through the column. The two portions of each concentration were collected and combined into a single fraction. This fraction was then brought to a total volume of 500 mL with moderately hard synthetic freshwater (MHSF) and tested for acute toxicity. By bringing the final volume to 500 mL, the concentration of any eluted toxicants should have been restored to the pre-extraction concentration.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent AT-001

December 1996

Baseline Test and C-18 SPE Test

Concentration	24 Hr Mortality		48 Hr Mortality	
	Baseline	C-18 SPE	Baseline	C-18 SPE
0.0%	0%	0%	0%	0%
2.0%	0%		0%	
5.0%	0%		0%	
10.0%	30%		30%	
20.0%	40%		50%	
25.0%		0%		0%
50.0%	80%	50%	100%	100%
100.0%		100%	100%	100%

Methanol Elution

Methanol Conc.	Mortality by Effluent Conc.		
	Control	50.0%	100.0%
0%	0.0%	0.0%	0.0%
25%	0.0%	0.0%	0.0%
50%	0.0%	0.0%	0.0%
75%	0.0%	0.0%	0.0%
80%	0.0%	0.0%	0.0%
85%	0.0%	0.0%	0.0%
90%	0.0%	0.0%	0.0%
95%	0.0%	0.0%	0.0%
100%	0.0%	0.0%	30.0%

Baseline Toxicity vs. C-18 SPE

Methanol Elution Results

APPENDIX C-4
AT-001, PHASE III RESULTS

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TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification:AT-001

December 1996

1.0 INTRODUCTION

AT-001 effluent was identified as acutely toxic to *Ceriodaphnia dubia* in July 1996. A Phase I TIE initiated in July 1996 and completed in August 1996 indicated that copper was the main toxicant in AT-001 effluent. Copper levels were measured at 57 - 62 µg/L. Zinc was found to be present at concentrations as great as 33 µg/L. In order to confirm the relative contribution to total toxicity of the copper and zinc, simulated AT-001 effluent was prepared and the two metals were spiked into the effluent individually and together. This report presents the results of these investigations, identified as Phase II.

2.0 METHODS

2.1 Preparation of Simulated Effluent

Due to the nature of the effluent, it was believed that it was not feasible to remove copper and zinc from the effluent without fundamentally changing its characteristics which relate to toxicity. Thus it was decided to prepared a simulated effluent. The simulated effluent was prepared by adding essential anions and cations to ultra-pure water. These essential ions were first measured in the effluent sample collected on December 9, 1996. Subsequently the ions were added to ultra-pure water in the same proportions, as follows;

<u>Parameter</u>	<u>Measured Conc. In Effluent</u>	<u>Added in the Form of</u>	<u>Simulated Effluent</u>	
			<u>Amount of Salt Added</u>	<u>Amount of Ion Added</u>
Na ⁺	8.92 mg/L	NaHCO ₃	11.6 mg/L	3.17 mg/L
		Na ₂ SO ₄ ·10H ₂ O	30.8 mg/L	4.4 mg/L
		NaCl	3.42 mg/L	1.35 mg/L
K ⁺	1.68 mg/L	K Cl	3.21 mg/L	1.68 mg/L
Mg ²⁺	0.387 mg/L	MgSO ₄	3.91 mg/L	0.387 mg/L
Ca ²⁺	0.984 mg/L	CaSO ₄ ·2H ₂ O	4.24 mg/L	0.984 mg/L
HCO ₃ ⁻	8.3 mg/L	NaHCO ₃	11.6 mg/L	8.3 mg/L
Cl ⁻	3.60 mg/L	NaCl	3.42 m/L	2.07 mg/L
		K Cl	3.21 mg/L	1.53 mg/L

The calculated hardness based upon measured concentrations of calcium and magnesium (for both the actual effluent and simulated effluent) was 4.05 mg/L. Alkalinity was measured as 13.8 mg/L as CaCO₃. Other parameters measured in the December 9 effluent sample included TOC (<1.0 mg/L), sulfate (<5.0 mg/L), zinc (0.0309 mg/L), iron (<0.05 mg/L), and TDS (42 mg/L).

2.2 Spiking of Copper and Zinc into Simulated Effluent

Copper and zinc were spiked separately and together into aliquots of effluent, in variable amounts. Copper was spiked as copper sulfate and zinc was spiked as zinc sulfate. Acute definitive toxicity tests were conducted on the following spiked samples;

Copper Variable / No Zinc
Copper Variable / Zinc constant
Zinc Variable / No Copper
Zinc Variable / Copper constant

When a metal was spiked in variable amounts the range was adjusted to include the highest amount measured in the effluent. From this maximum amount a series of more dilute concentrations were prepared. When both metals were spiked, one metal was variable and the other metal was spiked at a concentration in the upper range of the maximum measured concentration. An acute definitive toxicity test was set on each spiked solution, using two replicates of five test organisms at each dilution.

3.0 RESULTS

3.1 Simulated Effluent

Controls set with simulated effluent with no copper or zinc added demonstrated that simulated effluent was not acutely toxic.

3.2 Copper Spiked Tests

Copper was spiked at a range of 1-30 $\mu\text{g/L}$ into the simulated effluent. No copper was added to the simulated effluent prior to spiking. Copper at all concentrations tested was toxic to *Ceriodaphnia dubia*, with or without the presence of zinc. When present, zinc was at a concentration of 30 $\mu\text{g/L}$. The results are summarized as follows;

% Mortality at 48 Hours By Dilution		
	<u>Cu Only</u>	<u>Cu w/ Zn</u>
0 $\mu\text{g/L}$ Cu	0%	0%
1 $\mu\text{g/L}$ Cu	100%	100%
2 $\mu\text{g/L}$ Cu	100%	100%
5 $\mu\text{g/L}$ Cu	100%	100%
10 $\mu\text{g/L}$ Cu	100%	100%
30 $\mu\text{g/L}$ Cu	100%	100%

All mortality occurred in less than 24 hours in all concentrations (except the control). Thus, copper in the range measured in the effluent (57-62 $\mu\text{g/L}$) easily accounts for the noted acute toxicity. The results are consistent with U.S. EPA data which, using a formula incorporating hardness, provide a water quality criterion of 0.76 $\mu\text{g/L}$ for total copper at a hardness of 4.0 mg/L (EPA/440/5-86/001).

3.3 Zinc Spiked Tests

Zinc was spiked at a range of 2-60 µg/L into the simulated effluent. No zinc was added to the simulated effluent prior to spiking. Zinc was not acutely toxic to *Ceriodaphnia dubia* at any concentration except at the highest concentration (60 µg/L). At 60 µg/L of total zinc one-half the test organisms died in 48 hours. It was not toxic at concentrations of 30 µg/L or less. The presence of 20 µg/L of copper along with any concentration of zinc resulted in copper toxicity. The results are summarized as follows;

% Mortality at 48 Hours By Dilution		
	<u>Zn Only</u>	<u>Zn w/ 20 µg/L Cu</u>
0 µg/L Zn	0%	0%
2 µg/L Zn	0%	100%
5 µg/L Zn	0%	100%
10 µg/L Zn	0%	100%
30 µg/L Zn	0%	100%
60 µg/L Zn	50%	100%

Zinc in the range measured in the effluent (<5-33 µg/L) does not account for the noted acute toxicity in the effluent. U.S. EPA data, using a formula incorporating hardness, provide a water quality criterion of 22.2 µg/L for total zinc at a hardness of 4.0 mg/L (EPA/440/5-86/001).

4.0 CONCLUSION

Copper is confirmed as being the likely cause of toxicity in AT-001 effluent. Zinc, on the other hand, is not present in concentrations high enough to result in acute toxicity in the AT-001 effluent. Copper levels must be reduced to well below 1.0 µg/L to eliminate copper acute toxicity.

APPENDIX C-5

ADDITIONAL TOXICITY STUDIES ON AT-001

P.O. Box 16414, Greenville, SC 29606

(864) 877-6942 • FAX (864) 877-6938

4 Craftsman Court, Greer, SC 29650

7 Day Chronic Definitive Survival and Reproduction Bioassay

Method: EPA/600/4-91/002

Test Organism: *Ceriodaphnia dubia*

APPENDIX C-5-1

Facility: WSRC
Sample ID: AT-001

County: Aiken

ETT#: T6672

Sample Date: November 8, 1996

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST **Survival and Reproduction Results**

Client: **WSRC**
Sample Identification: **AT-001**
Test Date: **November 8, 1996**

County: **Aiken**
NPDES#:
ETT #: **T6672**

Reproduction at 0.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	5	6	9	0	0	20
2	3	1	11	12	0	27
3	4	6	13	0	0	23
4	2	6	11	0	0	19
5	3	0	0	14	0	17
6	4	9	1	0	0	14
7	4	0	0	15	0	19
8	4	6	0	14	0	24
9	5	6	0	10	0	21
10	5	3	0	15	0	23

Reproduction at 1.00% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	6	7	9	0	0	22
2	X	X	X	X	X	0
3	4	6	11	0	0	21
4	6	8	0	11	0	25
5	1	5	0	11	0	17
6	4	6	10	0	0	20
7	6	7	13	0	0	26
8	6	8	0	12	0	26
9	4	10	0	13	0	27
10	5	6	0	13	0	24

Reproduction at 3.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	3	7	0	9	0	19
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	3	6	0	9	0	18
5	0	4	0	9	0	13
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	1	3	0	13	0	17
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 10.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 30.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 100.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Statistical Analyses

Client: **WSRC**
 Sample Identification: **AT-001**
 Test Date: **November 8, 1996**

Test for Normality

Test Used: Shapiro-Wilks W= 0.885
 critical 0.93

The data are not normal in distribution

Test for Homogeneity of Variance

Test Used: Bartlett's Test B= 6.17
 critical 13.28

The data are homogeneous in variance

Test for Differences in Reproduction

Test Type Used: **Fishers Exact Test for Survival**
 Test Type Used: **Wilcoxon's Test for Reproduction**

t Test		Test Concentration					
	Control	1.0%	3.0%	10.0%	30%	100%	
t Value		-0.04	5.63	8.33	N/A	N/A	
critical t		2.33	2.33	2.33	2.33	2.33	
reproduct	20.7	20.8	6.7	0.0	0.0	0.0	
survival	100%	100%	40%	0%	0%	0%	
CV=	18%				MSD=	5.79	

Wilcoxon's Test		Test Concentration				
		1.0%	3.0%	10.0%	30%	100%
Rank Sum					NA	NA
critical					NA	NA

NOEC: Survival: 1% Reproduction: 1.0%
 LOEC: Survival: 3% Reproduction: 3.0%
 Chronic Value: 1.7%

Effluent Conc. vs. Reproduction

Summary

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Water Chemistry

Client: WSRC
 Sample Identification: AT-001
 Test Date: November 8, 1996

pH (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	6.26	6.69				8.38
Day 1						
Day 2	7.25	6.84			7.54	
Day 3	6.99	6.72				
Day 4	7.28	6.81		6.79		
Day 5	6.84	6.74		6.74		
Day 6	6.45	6.58				

pH (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	7.22	7.20			7.58	
Day 2	6.83	6.89			8.15	
Day 3	7.08	6.88	7.27			
Day 4	6.73	6.64	6.89			
Day 5	6.69	6.66	6.72			
Day 6	7.55	7.10	7.10			
Final						

Dissolved Oxygen (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	8.2	8.1				8.5
Day 1						
Day 2	8.6	8.8			8.8	
Day 3	8.1	8.3				
Day 4	8.6	8.6				
Day 5	8.5	8.5	8.4			
Day 6	8.3	8.4	8.3			

Dissolved Oxygen (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	8.6	8.3			8.3	
Day 2	8.2	8.3			8.4	
Day 3	8.6	8.6	8.6			
Day 4	8.4	8.4	8.4			
Day 5	8.3	8.2	8.2			
Day 6	8.1	8.1	8.0			
Final						

Dilution Water & Effluent Parameters

Effluent Sample		Hard.		
Date 11/5/96		mg/L		
Eff. Sample 1		16		

Temperature

	Incubator °C
Initial	25.0
Day 1	25.1
Day 2	24.2
Day 3	25.1
Day 4	24.9
Day 5	24.6
Day 6	24.6
Final	

Test Results Reviewed and Approved By:

L. Kelly

P.O. Box 16414, Greenville, SC 29606

(864) 877-6942 • FAX (864) 877-6938

4 Craftsman Court, Greer, SC 29650

**7 Day Chronic Definitive Survival
and Reproduction Bioassay**
Method: EPA/600/4-91/002

Test Organism: *Ceriodaphnia dubia*

APPENDIX C-5-2

Facility: WSRC
Sample ID: AT-001

County: Aiken
NPDES#: NA
ETT#: T7104

Sample ID: February 4, 1997

Survival and Reproduction Results

Client: **WSRC**
 Sample Identification: **AT-001**
 Test Date: **February 4, 1997**

County: **Aiken**
 NPDES#: **NA**
 ETT #: **T7104**

Reproduction at 0.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	4	6	13	0	0	23
2	13	0	12	0	0	25
3	5	7	11	0	0	23
4	5	7	10	0	0	22
5	5	7	15	0	0	27
6	6	5	11	0	0	22
7	6	8	13	0	0	27
8	3	8	12	0	0	23
9	5	6	13	0	0	24
10	4	6	11	0	0	21

Reproduction at 6.25% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	4	6	13	0	0	23
2	11	0	14	0	0	25
3	5	7	12	0	0	24
4	6	6	16	0	0	28
5	6	7	13	0	0	26
6	6	8	15	0	0	29
7	7	8	14	0	0	29
8	3	6	16	0	0	25
9	5	6	12	0	0	23
10	5	7	12	0	0	24

Reproduction at 12.5% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	7	7	0	0	0	14
2	5	8	14	0	0	27
3	5	7	15	0	0	27
4	5	7	12	0	0	24
5	6	4	13	0	0	23
6	4	6	14	0	0	24
7	5	7	10	0	0	22
8	5	5	12	0	0	22
9	5	5	11	0	0	21
10	4	8	12	0	0	24

Reproduction at 25.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	9	1	15	0	0	25
2	5	8	14	0	0	27
3	6	5	9	0	0	20
4	5	10	12	0	0	27
5	6	7	9	0	0	22
6	3	6	13	0	0	22
7	5	8	12	0	0	25
8	3	8	12	0	0	23
9	5	5	12	0	0	22
10	4	7	15	0	0	26

Reproduction at 50.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	6	N/A	N/A	N/A	N/A	N/A
3	X	X	X	X	X	0
4	6	3	8	0	0	17
5	4	4	14	0	0	22
6	4	6	12	0	0	22
7	5	8	10	0	0	23
8	4	6	11	0	0	21
9	2	7	13	0	0	22
10	5	7	15	0	0	27

Reproduction at 100.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

N/A: organism lost in transfer

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Statistical Analyses

Client: **WSRC**
 Sample Identification: **AT-001**
 Test Date: **February 4, 1997**

Test for Normality

Test Used: Shapiro-Wilks W= 0.877
 critical 0.93

The data are not normal in distribution

Test for Homogeneity of Variance

Test Used: Bartlett's Test B= 38.54
 critical 13.28

The data are not homogeneous in variance

Test for Differences in Reproduction

Test Type Used: **Fishers Exact Test for Survival**
 Test Type Used: **Wilcoxon's Test for Reproduction**

t Test		Test Concentration					
	Control	6.3%	12.5%	25.0%	50%	100%	
t Value		-0.86	0.41	-0.09	2.91	N/A	
critical t		2.33	2.33	2.33	2.33	2.33	
reproduct	23.7	25.6	22.8	23.9	15.4	0.0	
survival	100%	100%	100%	100%	80%	0%	
CV= 9%		MSD= 5.13					

Wilcoxon's Test		Test Concentration				
		6.3%	12.5%	25.0%	50%	100%
Rank Sum		NA	NA	NA	65	NA
critical		NA	NA	NA	74	NA

NOEC: Survival: 50% Reproduction: 25.0%
 LOEC: Survival: 100% Reproduction: 50.0%
 Chronic Value: 35.3%

Effluent Conc. vs. Reproduction

Summary

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Water Chemistry

Client: **WSRC**
 Sample Identification: **AT-001**
 Test Date: **February 4, 1997**

pH (After Renewal)

	Test Concentration					
	Control	6.25%	12.50%	25.00%	50%	100%
Initial	7.65	7.59				7.38
Day 1	7.73	7.92				8.09
Day 2	7.46	7.82			7.99	
Day 3	7.72	8.27			7.99	
Day 4	7.62	8.11			8.12	
Day 5	7.64	8.14			8.16	
Day 6						

pH (Before Renewal)

	Test Concentration					
	Control	6.25%	12.50%	25.00%	50%	100%
Day 1	8.22	8.15				8.03
Day 2	8.29	7.70				8.00
Day 3	8.21	8.27			8.06	
Day 4	8.24	8.26			8.26	
Day 5	8.29	8.31			8.30	
Day 6	8.17	8.21			8.13	
Final						

Dissolved Oxygen (After Renewal)

	Test Concentration					
	Control	6.25%	12.50%	25.00%	50%	100%
Initial	8.5	8.5				8.5
Day 1	8.6	8.6				8.6
Day 2	8.5	8.5				
Day 3	8.6	8.4			8.3	
Day 4	8.6	8.2			8.2	
Day 5	8.5	8.4			8.4	
Day 6						

Dissolved Oxygen (Before Renewal)

	Test Concentration					
	Control	6.25%	12.50%	25.00%	50%	100%
Day 1	8.4	8.5				8.4
Day 2	8.5	8.4				8.4
Day 3	8.4	7.9			7.90	
Day 4	8.6	7.8			7.90	
Day 5	8.4	8.1			8.00	
Day 6	8.3	8.4			8.40	
Final						

Temperature

Incubator °C	
Initial	24.5
Day 1	25.6
Day 2	24.4
Day 3	25.3
Day 4	24.4
Day 5	24.5
Day 6	24.6
Final	25.3

Test Results Reviewed and Approved By: _____

[Signature]

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P.O. Box 16414, Greenville, SC 29606

4 Craftsman Court, Greer, SC 29650

DATE REPORTED: March 7, 1997

CLIENT: Attn: Winona Specht
Westinghouse Savannah River Company
P.O. Box 616
Aiken, SC 29802

LAB #: SAMPLE ID

T7104A WSRC- AT-001 (2-3-97)
T7104B WSRC- AT-001 (2-5-97)
T7104C WSRC- AT-001 (2-7-97)

CHEMICAL ANALYSIS REPORT

<u>Sample ID</u>	<u>Sample Date</u>	<u>Parameter</u>	<u>Result</u>	<u>Units</u>	<u>Method</u>
AT-001	2-3-97	Total Copper	0.048	mg/L	200.7
AT-001	2-3-97	Total Lead	<0.015	mg/L	200.7
AT-001	2-3-97	Total Zinc	0.019	mg/L	200.7
AT-001	2-5-97	Total Copper	0.027	mg/L	200.7
AT-001	2-5-97	Total Lead	<0.015	mg/L	200.7
AT-001	2-5-97	Total Zinc	0.014	mg/L	200.7
AT-001	2-7-97	Total Copper	0.017	mg/L	200.7
AT-001	2-7-97	Total Lead	<0.015	mg/L	200.7
AT-001	2-7-97	Total Zinc	0.020	mg/L	200.7

SC Laboratory Certification #23104
Outside Laboratory Certification #02002

Reported By:

Robert W. Kelley, Ph.D., Laboratory Manager

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4 Craftsman Court, Greer, SC 29650

7 Day Chronic Definitive Survival and Reproduction Bioassay

Method: EPA/600/4-91/002

Test Organism: *Ceriodaphnia dubia*

APPENDIX C-5-3

Facility: WSRC

Sample ID: AT-001 (Hardness Adjusted)

County: Aiken

ETT#: T6672

Date: November 8, 1996

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Survival and Reproduction Results

Client: **WSRC**
 Sample Identification: **AT-001 (Hardness Adjusted)**
 Test Date: **November 8, 1996**

County: **Aiken**
 NPDES#:
 ETT #: **T6672**

Reproduction at 0.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	4	7	8	0	0	19
2	6	1	0	14	0	21
3	4	5	10	0	0	19
4	2	5	11	0	0	18
5	7	8	12	0	0	27
6	4	5	11	0	0	20
7	1	4	13	0	0	18
8	4	0	11	13	0	28
9	6	9	12	0	0	27
10	3	5	11	0	0	19

Reproduction at 1.00% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	3	6	9	0	0	18
2	5	11	11	0	0	27
3	4	5	8	0	0	17
4	4	5	11	0	0	20
5	7	7	0	14	0	28
6	2	5	8	0	0	15
7	6	8	0	12	0	26
8	0	6	9	0	0	15
9	4	7	0	13	0	24
10	4	5	9	0	0	18

Reproduction at 3.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	2	5	0	10	0	17
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	2	X	X	X	X	2
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 10.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	2	X	X	X	X	2
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	3	5	0	8	0	16
10	X	X	X	X	X	0

Reproduction at 30.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 100.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Statistical Analyses

Client: **WSRC**
 Sample Identification: **AT-001 (Hardness Adjusted)**
 Test Date: **November 8, 1996**

Test for Normality

Test Used: Shapiro-Wilks W= 0.772
 critical 0.93

The data are not normal in distribution

Test for Homogeneity of Variance

Test Used: Bartlett's Test B= 0.72
 critical 13.28

The data are homogeneous in variance

Test for Differences in Reproduction

Test Type Used: **Fishers Exact Test for Survival**
 Test Type Used: **Wilcoxon's Test for Reproduction**

t Test

	Test Concentration					
	Control	1.0%	3.0%	10.0%	30%	100%
t Value		0.41	10.09	10.14	N/A	N/A
critical t		2.33	2.33	2.33	2.33	2.33
reproduct	21.6	20.8	1.9	1.8	0.0	0.0
survival	100%	100%	10%	10%	0%	0%
CV=	19%		MSD= 4.55			

Wilcoxon's Test

	Test Concentration				
	1.0%	3.0%	10.0%	30%	100%
Rank Sum				NA	NA
critical				NA	NA

NOEC: Survival: 1% Reproduction: 1.0%
 LOEC: Survival: 3% Reproduction: 3.0%
 Chronic Value: 1.7%

Effluent Conc. vs. Reproduction

Summary

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Water Chemistry

Client: **WSRC**
 Sample Identification: **AT-001 (Hardness Adjusted)**
 Test Date: **November 8, 1996**

pH (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	6.26	6.96				8.37
Day 1					7.65	
Day 2	7.25	7.13			8.09	
Day 3	6.99	6.95				
Day 4	7.28	6.73		7.34		
Day 5	6.84	6.77		7.27		
Day 6	6.45	7.15				

pH (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	7.22	7.64			7.76	
Day 2	6.83	7.34				8.25
Day 3	7.08	6.98				8.17
Day 4	6.73	6.84		7.40		
Day 5	6.69	7.10		7.35		
Day 6	7.55	6.98		7.39		
Final						

Dissolved Oxygen (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	8.2	8.0				8.2
Day 1					8.7	
Day 2	8.6	8.5				8.0
Day 3	8.1	8.4				
Day 4	8.6	8.5		8.4		
Day 5	8.5	8.6		8.3		
Day 6	8.3	8.4				

Dissolved Oxygen (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	8.6	8.6			8.6	
Day 2	8.2	8.2				8.3
Day 3	8.6	8.4				8.5
Day 4	8.4	8.4		8.4		
Day 5	8.3	8.2		8.1		
Day 6	8.1	8.1		8.0		
Final						

Dilution Water & Effluent Parameters

Effluent Sample		Hard.		
Date 11/5/96		mg/L		
Pre Adjustment		16		
Post-Adjustment		28		

Temperature

Incubator °C	
Initial	25.0
Day 1	25.1
Day 2	24.2
Day 3	25.1
Day 4	24.9
Day 5	24.6
Day 6	24.6
Final	

Test Results Reviewed and Approved By: _____

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4 Craftsman Court, Greer, SC 29650

7 Day Chronic Definitive Survival and Reproduction Bioassay

Method: EPA/600/4-91/002

Test Organism: *Ceriodaphnia dubia*

APPENDIX C-5-4

Facility: WSRC

Sample ID: Lab Water Spiked to Simulate AT-001

County: Aiken

ETT#: T6672S

Sample Date: November 13, 1996

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Survival and Reproduction Results

Client: **WSRC**
 Sample Identification: **Lab Water Spiked to Simulate AT-001**
 Test Date: **November 13, 1996**

County: **Aiken**
 NPDES#:
 ETT #: **T6672S**

Reproduction at 0.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	1	0	4	6	0	11
2	5	0	9	0	0	14
3	6	4	0	1	7	18
4	6	7	0	6	2	21
5	2	7	0	12	0	21
6	5	0	6	9	0	20
7	0	1	10	0	14	25
8	2	7	10	0	0	19
9	5	9	11	0	0	25
10	3	0	8	0	5	16

Reproduction at 1.00% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	5	0	3	8	0	16
2	3	7	4	0	12	26
3	6	0	2	5	13	26
4	5	7	0	0	0	12
5	6	0	0	8	9	23
6	6	0	8	14	0	28
7	0	0	0	0	0	0
8	5	0	0	9	10	24
9	5	6	12	0	0	23
10	6	0	7	12	0	25

Reproduction at 3.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	0	0	X	X	X	0
2	X	X	X	X	X	0
3	5	0	5	0	11	21
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 10.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 30.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

Reproduction at 100.0% Concentration						
Rep.	Day 3/4	Day 5	Day 6	Day 7	Day 8	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Statistical Analyses

Client: **WSRC**
 Sample Identification: **Lab Water Spiked to Simulate AT-001**
 Test Date: **November 13, 1996**

Test for Normality

Test Used: Shapiro-Wilks W= 0.829
 critical 0.93

The data are not normal in distribution

Test for Homogeneity of Variance

Test Used: Bartlett's Test B= 3.66
 critical 13.28

The data are homogeneous in variance

Test for Differences in Reproduction

Test Type Used: **Fishers Exact Test for Survival**
 Test Type Used: **Wilcoxon's Test for Reproduction**

t Test		Test Concentration				
	Control	1.0%	3.0%	10.0%	30%	100%
t Value		-0.55	7.17	8.06	N/A	N/A
critical t		2.33	2.33	2.33	2.33	2.33
reproduct	19.0	20.3	2.1	0.0	0.0	0.0
survival	100%	100%	10%	0%	0%	0%
CV=	24%		MSD= 5.49			

Wilcoxon's Test		Test Concentration				
		1.0%	3.0%	10.0%	30%	100%
Rank Sum					NA	NA
critical					NA	NA

NOEC: Survival: 1% Reproduction: 1.0%
 LOEC: Survival: 3% Reproduction: 3.0%
 Chronic Value: 1.7%

Effluent Conc. vs. Reproduction

Comments: Laboratory water was spiked with 500 µg/l of copper (as copper sulfate) and 67 µg/l of zinc (as zinc sulfate)

CHRONIC DEFINITIVE SURVIVAL AND REPRODUCTION TEST

Water Chemistry

Client: **WSRC**
 Sample Identification: **Lab Water Spiked to Simulate AT-001**
 Test Date: **November 13, 1996**

pH (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	6.55	6.61				6.64
Day 1	6.45	6.79				
Day 2	7.21	6.98	6.81			
Day 3	7.38	7.13				
Day 4	7.69	7.22	7.22			
Day 5	7.52	7.22	7.07			
Day 6	6.50	7.14	7.05			

pH (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	7.04	6.83				6.72
Day 2	7.10	6.96	6.98			
Day 3	7.18	7.10				
Day 4	7.93	7.56	7.36			
Day 5	7.42	7.11	7.02			
Day 6	7.32	7.23	7.15			
Final	7.21	7.25	7.17			

Dissolved Oxygen (After Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Initial	8.3	8.3				8.4
Day 1	8.1	8.1				
Day 2	8.1	8.1	8.1			
Day 3	8.1	8.1				
Day 4	8.0	8.1	8.2			
Day 5	8.4	8.6	8.6			
Day 6	8.3	8.2	8.3			

Dissolved Oxygen (Before Renewal)

	Test Concentration					
	Control	1.00%	3.00%	10.00%	30%	100%
Day 1	8.2	8.2				8.1
Day 2	8.0	8.1	8.0			
Day 3	8.0	8.0				
Day 4	8.0	8.0	8.0			
Day 5	8.0	8.1	8.3			
Day 6	8.2	8.2	8.3			
Final	8.3	8.3	8.3			

Dilution Water & Effluent Parameters

Effluent Sample			Hard.		
Date 11/5/96			mg/L		
Eff. Sample 1			96		

Temperature

	Incubator °C
Initial	24.6
Day 1	24.6
Day 2	25.8
Day 3	24.1
Day 4	25.8
Day 5	24.9
Day 6	25.8
Final	25.6

Test Results Reviewed and Approved By: _____

R. Kelly

APPENDIX D-1

A-003, PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-003

July 1996

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on June 24th, 1996 and used for conducting a partial Phase I Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would remove toxicity. Each treated sample was used in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test	7/11/96	T6044	-
Filtered at pH 11	7/11/96	T6044	metals; insoluble at pH 10
Na Thiosulfate Addition	7/11/96	T6044	oxidizers; residual chlorine;metals
EDTA Chelation	7/11/96	T6044	some metals; surfactants
Graduated pH	7/11/96	T6044	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Filtration at pH 11

A 300 mL sample aliquot which had been adjusted to $\text{pH } 11 \pm 0.05$ with NaOH was filtered through a 0.45μ membrane filter. After filtration the pH was readjusted to the initial pH with H_2SO_4 .

2.2.2 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 1.0, 5.0, and 10.0 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to equilibrate for two hours.

2.2.3 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 0.5, 3.0, and 8.0 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to equilibrate for two hours and were pH adjusted back to the initial pH.

2.2.4 Graduated pH Test

Aliquots of effluent at 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. After 2 hours and 18 hours the survival of test organisms at each pH was checked.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 20, 40, 60, 80 and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test which had two replicates of five organisms). A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was low, at 0.07 mg/L. Hardness was relatively high, at 90.6 mg/L. The effluent was slightly alkaline (8.27) in pH when received. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	7/11/96	T6044	Sample toxic; LC ₅₀ <20%
Filtered at pH 11	7/11/96	T6044	No reduction in toxicity
Na Thiosulfate Addition	7/11/96	T6044	No reduction in toxicity
EDTA Chelation	7/11/96	T6044	Sample toxicity reduced
Graduated pH	7/11/96	T6044	Sample toxic at all pH values

3.2 Discussion

Toxicity in the effluent was not removed by filtration at pH 11 and was not removed by sodium thiosulfate addition. EDTA was effective at removing most, although not all, effluent toxicity. EDTA is only effective at removing or reducing metal toxicity and surfactant toxicity. Metals effectively chelated by EDTA include cadmium, copper, nickel, and zinc. Chromium, mercury, and selenium are not effectively removed from bioavailability by EDTA. Of the metals treatable by EDTA, copper and cadmium should also be treatable by sodium thiosulfate. This leaves nickel and zinc as potential metal toxicants which fit the pattern of the data.

3.3 Recommendations

The graduated pH treatment test showed no toxicity at 2 hours and mortality of all test organisms at 18 hours. Nickel or zinc aquo ions would be expected to be less toxic at pH 6.5 than at pH 8.5. Therefore, it would be worthwhile repeating the graduated pH test with monitoring of mortality between 2 and 18 hours to determine if pH affects toxicity during this time interval. The possible role of surfactants should be investigated by conducting a sublation treatment with the associated toxicity test.

4.0 CONCLUSIONS

- ▶ The toxicant in A-003 Effluent is likely to be a cationic metal, although the possibility of surfactants cannot be excluded.
- ▶ The metals nickel and zinc best fit the pattern of toxicity noted for the various treatments conducted on the effluent.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-003
July 1996

Baseline Testing		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		100%	100%
40%		100%	100%
60%		100%	100%
80%		100%	100%
100%	8.27	100%	100%

Filtration at pH 11		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%		0%	0%
20%		100%	100%
40%		100%	100%
60%		100%	100%
80%		100%	100%
100%	8.2	100%	100%

Discussion:

The effluent was acutely toxic at concentrations as low as 20%.

Filtration at pH 11 did not remove or reduce effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-003

July 1996

EDTA Chelation - 18 Hr. Mortality

Effluent Conc.	EDTA Conc. (mg/L)				
	0 Untreated	0.5	3.0	8.0	0.5 Control
0%	0%	0%	0%	0%	
20%	100%	100%	0%	0%	
40%	100%	100%	0%	60%	
60%	100%	100%	0%	100%	
80%	100%	100%	100%	100%	
100%	100%	100%	100%	100%	0%
Control pH					7.95
Effluent pH	8.27	8.38	8.34	8.25	

Sodium Thiosulfate Addition - 18 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)				
	0.0	1.0	5.0	10.0	1.0 Control
0%	0%	0%	0%	0%	
20%	100%	100%	100%	100%	
40%	100%	100%	100%	100%	
60%	100%	100%	100%	100%	
80%	100%	100%	100%	100%	
100%	100%	100%	100%	100%	0%
Control pH					7.90
Effluent pH		8.37	8.4	8.42

Discussion:

EDTA at 3 mg/L reduced effluent toxicity.

Sodium thiosulfate addition did not reduce effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-003

July 1996

Graduated pH Test

Effluent Conc.	2 Hour Mortality at Nominal pH (w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.38	0%	7.49	0%	8.57	0%
100%	6.52	100%	7.58	100%	8.51	100%

Discussion:

The sample was toxic at all three pH values.

APPENDIX D-2

A-003, ADDITIONAL PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only
Follow-up Tests**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-003

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-003 effluent using the water flea *Ceriodaphnia dubia* was conducted on June 25 - July 3, 1996. The test results showed the presence of acute toxicity at effluent concentrations as low as 6.25%. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-003 effluent on July 11-13, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. The results indicated that a cationic metal was the cause of toxicity, and that the pattern of toxicity was most consistent with zinc toxicity. Copper toxicity is typically reduced by sodium thiosulfate addition, but zinc toxicity is not. Phase I results indicated that effluent toxicity was not reduced by sodium thiosulfate addition. Metals analysis data contradicted the indication of zinc being the primary toxicant, and showed that copper (measured at levels as high as 214 µg/L) was the metal most likely to cause toxicity.

Due to the results of the Phase I treatments and tests, additional testing was conducted in September 1996. The testing focused on two items; 1) a sublation treatment was conducted to confirm that the effectiveness of EDTA treatment was not due to surfactants, and 2) a graduated pH test was conducted at dilute effluent concentrations to obtain additional information on the pH sensitivity of the effluent. This report presents the results of these tests conducted as a follow-up to the original Phase I testing conducted in July 1996.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on September 11, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 Treatment Methods

The sample was subjected to two treatments. Each treated sample as well as the untreated sample was used in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	9/19/96	T6395	-
Graduated pH	9/18/96	T6395	ammonia; metals; sulfide
Sublation	9/19/96	T6395	surfactants

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

2.2.1 Graduated pH Test

Aliquots (80 mL) of effluent at 40% and 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. No buffers were used. At two hours and at four hours the pH of each aliquot was recorded. The test organisms were temporarily removed from the solution to a holding vessel while the solution was readjusted to the original pH. The test organisms were then transferred back to the original aliquot. Mortality was recorded at two hours, four hours, and at 24 hours.

2.2.2 Sublation Test

An 80 mL aliquot of 100% effluent was vigorously aerated in glass vessel such that foam produced by the aeration overflowed into an outer glass vessel. The foam which overflowed was collected and labelled as the

"sublate." The effluent remaining in the inner vessel after foam was removed was also collected and labelled as the "residual."

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all untreated and treated effluent samples. The baseline test and the sublation test were set at 20, 40, 60, 80 and 100% effluent concentration. The graduated pH test was set only at concentrations of 40% and 100% effluent. The baseline test was comprised of two replicates of five neonate test organisms at each test concentration. The graduated pH test was comprised of one replicate of ten neonate test organisms at each pH value. The sublation test was comprised of one replicate of five neonate test organisms at each test concentration. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was low, at 0.04 mg/L. Hardness was very low, at 16.0 mg/L, as was alkalinity (43.3 mg/L). The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	9/19/96	T6395	Sample toxic; $LC_{50}=45.4\%$
Graduated pH	9/18/96	T6395	Sample less toxic at pH 8.5
Sublation	9/18/96	T6395	Toxicity not removed

3.2 Discussion

The September 11, 1996 sample of A-003 effluent was less toxic than the sample collected in July (Figure 1). The baseline test showed no significant acute toxicity at effluent concentrations of 40% or less. Effluent in the July sample was toxic at concentrations as low as 20%. Copper concentrations in the September sample were substantially lower than in the July sample. Assuming 1.0 toxic units (no toxicity) at a copper concentration of 0 µg/L, a correlation coefficient (r^2) of 0.996 is obtained using the two datapoints to compare toxicity to copper concentration. This suggests that toxicity is related to copper concentration.

The graduated pH test showed that toxicity was reduced at pH 8.5 as compared to pH 6.5 and pH 7.5. This results is consistent with copper as a toxicant, but not zinc. In the July Phase I treatments the graduated pH test could not distinguish the pH sensitivity of the toxicant due to the rapid mortality of the test organisms. Less toxicity in the September sample allowed for the pH sensitivity to be assessed.

The sublation test did not provide any evidence of the presence of surfactants. Typically, if surfactants are present the residual solution should be substantially less toxic than the sublated portion. In this test, however, both solutions were equally toxic.

Follow-up treatments/testing conducted in September 1996 indicated that copper was the main toxicant of concern in A-003 effluent. The primary piece of evidence suggesting that copper was not the primary toxicant in the July testing was the failure of sodium thiosulfate treatment to affect toxicity. This could reflect either insufficient sodium thiosulfate to mitigate the effects of the relatively high levels of copper in the July sample, or synergistic effects of the zinc.

Figure 1
WSRC A-003 Effluent
Phase 1 TIE Follow-Up Testing Results
September 1996

BASELINE TESTING

Effluent Concentration	Mortality	
	July 96	Sept 96
0%	0%	0%
20%	100%	0%
40%	100%	20%
60%	100%	90%
80%	100%	100%
100%	100%	100%
48 Hr LC50	< 20.0%	45.4%
Toxic Units	5.00	2.20
T-Cu (µg/L)	214	77
Hardness (mg/L)	90	16

GRADUATED pH TEST (September 1996)

Effluent Concentration	Mortality at 4 Hours		
	pH 6.5	pH 7.5	pH 8.5
0%	0%	0%	0%
40%	0%	0%	0%
100%	100%	100%	60%
Lowest pH (100%)	6.40	7.50	8.23
Highest pH (100%)	7.03	7.79	8.52

SUBLATION TEST (September 1996)

Effluent Concentration	Mortality at 48 Hours		
	Untreated	Residual	Sublate
0%	0%	0%	0%
20%	0%	20%	20%
40%	20%	80%	80%
60%	90%	80%	80%
80%	100%	60%	60%
100%	100%	100%	100%

APPENDIX D-3

A-003, PHASE III RESULTS

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TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification:A-003

September 1996

1.0 INTRODUCTION

A-003 effluent was identified as acutely toxic to *Ceriodaphnia dubia* in June 1996. A Phase I TIE initiated in July 1996 and completed in September 1996 indicated that copper was the main toxicant in A-003 effluent. Zinc and lead also were detected in A-003 effluent, but it was believed that they would not be toxic at the levels which were measured. In order to confirm the relative contribution to total toxicity of the three metals, simulated A-003 effluent was prepared and the three metals were spiked into the effluent individually and together. This report presents the results of these investigations, identified as Phase III.

2.0 METHODS

Due to the presence of elevated levels of multiple cationic metals in the effluent, it was thought that it would be difficult to remove metal toxicity without fundamentally changing the effluent. Based upon the hardness measured in the July sample (90.6 mg/L), moderately hard synthetic freshwater (MHSF) was chosen for use as simulated A-003 effluent. To this simulated effluent the following combinations of copper, lead, and zinc were added;

Copper Variable / No Zinc or Lead
Copper Variable / Maximum Zinc and Lead
Zinc Variable / No Copper or Lead
Zinc Variable / Maximum Copper and Lead
Lead Variable / No Copper or Zinc
Lead Variable / Maximum Copper and Zinc

When a metal was spiked in variable amounts the highest amount used was the maximum measured concentration from July 1996 testing. From this maximum amount of series of more dilute concentrations were prepared. When one metal was variable, the other two metals were spiked at the maximum measured amount along with each serial dilution of the variable metal. An acute definitive toxicity test was set on each spiked solution, using two replicates of five test organisms at each dilution. Copper was spiked as copper sulfate, zinc as zinc sulfate, and lead as lead nitrate.

3.0 RESULTS

3.1 Copper Spiked Tests

Copper was spiked at a range of 50 - 250 µg/L into the simulated effluent. The MHSF had <5.0 µg/L of copper before spiking. In one definitive test no zinc or lead were added to the copper dilutions. In the second test 80 µg/L of lead and 200 µg/L of zinc were added to the copper dilutions. The results are summarized as follows;

	% Mortality By Dilution	
	<u>Cu Only</u>	<u>Cu w/ Pb and Zn</u>
0 µg/L Cu	0%	0%
50 µg/L Cu	100%	100%
100 µg/L Cu	100%	100%
150 µg/L Cu	100%	100%
200 µg/L Cu	100%	100%
250 µg/L Cu	100%	100%

In the range of 50-250 µg/L of copper all test organisms died in less than 24 hours in all concentrations except the control. Thus, copper in the range measured in the effluent (161 - 214 µg/L) easily accounts for the noted acute toxicity. Data generated at ETT Environmental in association with water effect ratio testing indicates an LC₅₀ of approximately 15 µg/L for total copper in moderately hard diluted mineral water. This is likely close to the range at which copper becomes acutely toxic in A-003 effluent. Because copper was toxic throughout the range tested, the contribution of zinc and lead could not be determined.

3.2 Zinc Spiked Tests

Zinc was spiked at a range of 100 - 300 µg/L into the simulated effluent. The MHSF had <5.0 µg/L of copper before spiking. In one definitive test no copper or lead were added to the copper dilutions. In the second test 80 µg/L of lead and 200 µg/L of copper were added to the zinc dilutions. The results are summarized as follows;

% Mortality By Dilution

	<u>Zn Only</u>	<u>Zn w/ Pb and Cu</u>
0 µg/L Zn	0%	0%
100 µg/L Zn	30%	100%
150 µg/L Zn	100%	100%
200 µg/L Zn	100%	100%
250 µg/L Zn	100%	100%
300 µg/L Zn	100%	100%

In the range of 100-300 µg/L of zinc all test organisms died in less than 24 hours in all concentrations of 150 µg/L and higher. Partial mortality was noted at 100 µg/L of zinc. If it is assumed that concentrations below 100 µg/L would demonstrate no toxicity, an LC₅₀ of 104 µg/L. However, zinc typically shows a pattern of partial mortality over a wide range of concentrations. Data generated at ETT Environmental in association with water effect ratio testing indicates an LC₅₀ of approximately 60 µg/L for total zinc in moderately hard diluted mineral water. Thus, it is likely that partial acute toxicity due to zinc can be anticipated in A-003 effluent at zinc concentrations of 60-100 µg/L. Zinc in the range measured in the effluent (161 - 213 µg/L) does likely contribute to the noted acute toxicity. At 100 µg/L of zinc, copper toxicity was additive to the zinc toxicity, resulting in 100% mortality.

3.2 Lead Spiked Tests

Lead was spiked at a range of 20 - 100 µg/L into the simulated effluent. The MHSF had <5.0 µg/L of lead before spiking. In one definitive test no copper or zinc were added to the lead dilutions. In the second test 200 µg/L of zinc and 200 µg/L of copper were added to the lead dilutions. The results are summarized as follows;

% Mortality By Dilution

	<u>Pb Only</u>	<u>Pb w/ Zn and Cu</u>
0 µg/L Pb	0%	0%
20 µg/L Pb	0%	100%
40 µg/L Pb	0%	100%
60 µg/L Pb	0%	100%
80 µg/L Pb	0%	100%
100 µg/L Pb	0%	100%

In the range of 20-100 µg/L of lead no test organisms died in 24 hours in any concentration. Data in the EPA Water Quality Criteria database for lead indicate no tests where the LC₅₀ for daphnids was less than 600 µg/L (EPA 440/5-84-027). Lead in the range measured in the effluent (77-120 µg/L) does not likely contribute to the noted acute toxicity. Throughout the range of lead spiked into the effluent, copper and/or zinc toxicity were additive to the lead toxicity, resulting in 100% mortality.

4.0 CONCLUSION

Copper and zinc both contribute to toxicity in the A-003 effluent. Copper levels must be reduced by 95% to eliminate copper acute toxicity. Zinc levels must be reduced by approximately 50% to eliminate zinc acute toxicity. Lead does not play a role in A-003 acute toxicity.

APPENDIX E-1

A-004, PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-004

July 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-004 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 9 - July 17, 1996. The test results showed the presence of acute toxicity in undiluted effluent. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

This report presents the results of a Metals-Focused Phase I Toxicity Identification Evaluation (TIE) conducted on the A-004 effluent on August 1-3, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on July 24th, 1996 and used for conducting a partial Phase I Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would remove toxicity. Each treated sample was used in an acute toxicity test, using test dilutions of 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test	8/1/96	T6139	-
Filtered at pH 11	8/1/96	T6139	metals; insoluble at pH 10
Na Thiosulfate Addition	8/1/96	T6139	oxidizers; residual chlorine; metals
EDTA Chelation	8/1/96	T6139	some metals; surfactants
Graduated pH	8/1/96	T6139	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Filtration at pH 11

A 300 mL sample aliquot which had been adjusted to $\text{pH } 11 \pm 0.05$ with NaOH was filtered through a 0.45μ membrane filter. After filtration the pH was readjusted to the initial pH with H_2SO_4 .

2.2.2 Sodium Thiosulfate Addition

An 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 1.0, 5.0, and 10.0 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to equilibrate for two hours.

2.2.3 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 0.5, 3.0, and 8.0 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to equilibrate overnight and were pH adjusted back to the initial pH.

2.2.4 Graduated pH Test

Aliquots of effluent at 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. After 2 hours and 18 hours the survival of test organisms at each pH was checked.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 60, 80 and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test which had two replicates of five organisms). A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was high, at 0.29 mg/L. The effluent was slightly alkaline (8.23) in pH when received. Hardness was very low, at 4.0 mg/L. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	7/31/96	T6139	Sample toxic; LC ₅₀ =69.1%
Filtered at pH 11	7/31/96	T6139	Sample toxicity not reduced
Na Thiosulfate Addition	7/31/96	T6139	Sample toxicity reduced
EDTA Chelation	8/1/96	T6139	Sample toxicity reduced
Graduated pH	7/31/96	T6139	Sample more toxic at pH 6.5

3.2 Discussion

Toxicity in the effluent was not removed by filtration at pH 11. Toxicity was substantially removed by sodium thiosulfate addition. EDTA also was partially effective at removing effluent toxicity. EDTA typically removes or reduces only metal toxicity and surfactant toxicity. Metals effectively chelated by EDTA include cadmium, copper, lead, nickel, and zinc. Chromium, mercury, and selenium are not effectively removed from bioavailability by EDTA. Of the metals treatable by EDTA, copper and cadmium should also be treatable by sodium thiosulfate. However, cadmium is typically more toxic at pH 8.5 than at

pH 6.5, which is the opposite of the pattern of the data in this study. This leaves copper as the only metal which fits the treatment/toxicity data. It should be noted that moderately high measured residual chlorine in the sample (0.29 mg/L) suggests that residual chlorine may have played a role in toxicity. Thus, some of the effect of the sodium thiosulfate addition may relate to removal of residual chlorine. Toxicity remaining after filtration at pH 11 likely shows the presence of residual chlorine after metal removal. The effectiveness of EDTA chelation, which is not effective for residual chlorine, verifies that residual chlorine is not the only toxicant. Nevertheless, EDTA was not as effective as sodium thiosulfate, possibly because EDTA removes only metals whereas sodium thiosulfate removes metals and residual chlorine.

3.3 Recommendations

Due to the apparent presence of more than one toxicant, that a full Phase I TIE be conducted on A-004 effluent, so that the toxicity can be fully characterized. This would include conducting EDTA chelation and filtration at pH 11 on samples which were first dechlorinated, to separate the effects of the two likely toxicants.

4.0 CONCLUSIONS

- ▶ Two toxicants are present in the effluent; residual chlorine and a cationic metal.
- ▶ Copper is the metal which best fits the pattern of toxicity noted for the various treatments conducted on the effluent.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-004

July 1996

EDTA Chelation - 48 Hr. Mortality

Effluent Conc.	EDTA Conc. (mg/L)					
	0	45	90	180	370	45 Control
0%	0%					
60%	30%	0%	0%	100%	100%	
80%	60%	0%	100%	100%	100%	
100%	100%	100%	100%	100%	100%	0%
Control pH						7.95
Effluent pH	8.23	8.13	8.28	8.16	8.15	

Sodium Thiosulfate Addition - 18 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)					
	0	30	60	120	250	30 Control
0%	0%					
60%	30%	0%	0%	0%	100%	
80%	60%	0%	0%	0%	100%	
100%	100%	40%	40%	60%	100%	0%
Control pH						7.95
Effluent pH		8.23	8.21	8.19	8.15

Discussion:

EDTA at 45 mg/L reduced effluent toxicity.

Sodium thiosulfate at 30-120 mg/L removed most effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-004

July 1996

Baseline Testing		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%	7.91	0%	0%
60%	7.98	0%	30%
80%	8.08	10%	60%
100%	8.23	100%	100%

Filtration at pH 11		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%	7.95	0%	0%
60%	8.04	0%	0%
80%	8.15	60%	60%
100%	8.23	100%	100%

Discussion:

The effluent was significantly acutely toxic at concentrations as low as 80%.

Filtration at pH 11 was not effective at reducing effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE **WSRC Effluent A-004** **July 1996**

Graduated pH Test

Effluent Conc.	4 Hour Mortality at Nominal pH (w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.70	0%	7.54	0%	8.24	0%
60%		100%	7.68	0%	8.50	0%
80%		100%	7.71	0%	8.51	0%
100%	6.50	100%	7.55	100%	8.47	100%

Discussion:

The sample was less toxic at pH 7.5 to 8.5 than at pH 6.5

APPENDIX E-2

**A-004, ADDITIONAL PHASE I RESULTS
SEPTEMBER 1996**

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I
Follow-up Tests**

Client: Westinghouse Savannah River Company

Sample ID: A-004

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-004 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 9-17, 1996. The test results showed the presence of acute toxicity in undiluted effluent. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-004 effluent on July 31- August 2, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. Both EDTA and sodium thiosulfate addition reduced toxicity, and the effluent was noted to be less toxic at pH 8.5 than at pH 6.5. The results indicated that both residual chlorine and copper played a role in effluent toxicity. The measured concentration of residual chlorine at sample receipt was 0.29 mg/L and the measured concentrations of copper were as much as 140 µg/L. Due to the apparent presence of more than one toxicant, it was recommended to broaden the TIE Phase I to include other treatments and repeat the earlier treatments, paying attention to residual chlorine levels. This report presents the results of these follow-up treatments and tests for the Phase I Toxicity Identification Evaluation (TIE) conducted in early August.

2.0 METHODS

2.1 Sampling

A composite sample of effluent was collected on September 19, 1996 and used for conducting the follow-up Phase I tests of the Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would reduce or remove toxicity. Each treated sample was used in an acute toxicity test (at a series of dilutions) to determine the effectiveness of the treatment. The treatments and results of the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline	9/20/96	T6429	-
48 Hour Delay Baseline	9/22/96		unstable compounds
Adjusted to pH 3	9/22/96		pH sensitive
Adjusted to pH 11	9/22/96		pH sensitive
Filtered at Initial pH	9/22/96		bound to solids; insoluble
Filtered at pH 3	9/22/96		bound to solids; insoluble
Filtered at pH 11	9/22/96		metals; insoluble at pH 10
Aerated at Initial pH	9/22/96		volatile;oxidizable
Aerated at pH 3	9/22/96		volatile;oxidizable
Aerated at pH 11	9/22/96		volatile;oxidizable
Spe C18 at initial pH	9/22/96		non-polar organics; metals
Spe C18 at pH 3	9/22/96		non-polar organics; metals
Spe C18 at pH 11	9/22/96		non-polar organics; metals
Na Thiosulfate Addition	9/22/96		oxidizers; residual chlorine;metals
EDTA Chelation	9/22/96		some metals; surfactants
Graduated pH	9/22/96		ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Adjustment to pH 3

A 1000 mL aliquot of sample was adjusted to pH 3.33 with 1N H₂SO₄. After pH adjustment the sample aliquot was re-adjusted to pH 8.53.

2.2.2 Adjustment to pH 11

A 1000 mL aliquot of sample was adjusted to pH 10.76 with NaOH. After pH adjustment the sample aliquot was re-adjusted to pH 8.40.

2.2.3 Filtration at Initial pH

A 400 mL sample aliquot was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water. After filtration the pH was re-adjusted to pH 7.79 with NaOH.

2.2.4 Filtration at pH 3

A 400 mL sample aliquot which had been adjusted to pH 3.33 was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water. After filtration the pH was re-adjusted to pH 8.48 with NaOH.

2.2.5 Filtration at pH 11

A 400 mL sample aliquot which had been adjusted to pH 10.76 was filtered through a 0.45 µm membrane filter which had been pre-rinsed with deionized water. After filtration the pH was 10.90, and was readjusted to a pH of 8.27 with H₂SO₄.

2.2.6 Aeration at Initial pH

A 100 mL sample aliquot was vigorously aerated for a period of one hour with a diaphragm type aerator.

2.2.7 Aeration at pH 3

A 100 mL sample aliquot which had been adjusted to pH 3.33 was vigorously aerated for a period of one hour with a diaphragm type aerator. After aeration the pH re-adjusted to pH 8.72 with NaOH.

2.2.8 Aeration at pH 11

A 100 mL sample aliquot which had been adjusted to pH 10.76 was vigorously aerated for a period of one hour with a diaphragm type aerator. After aeration the pH was re-adjusted to pH 8.33 with H₂SO₄.

2.2.9 C-18 SPE Column at Initial pH

A 200 mL aliquot of filtered sample was run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.10 C-18 SPE Column at pH 3

A 200 mL aliquot of sample which had been adjusted to pH 3.30 and filtered was run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. After extraction the pH was re-adjusted to pH 8.30. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.11 C-18 SPE Column at pH 3

A 200 mL aliquot of sample which had been adjusted to pH 10.76 and filtered was readjusted to pH 9.02 and run through a Baker 6 mL C-18 SPE column at a rate of 5 mL per minute. The column was activated with 10 mL of methanol, followed by a DI water rinse prior to sample extraction. After extraction the pH was readjusted to pH 8.30. The first 30 mL of sample extracted were saved, as were the last 30 mL of sample extracted. Each aliquot was tested.

2.2.12 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 1, 5, 10, 25 and 50 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to sit for two hours.

2.2.13 EDTA Chelation

A 15 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 1, 5, 10, 25, and 50 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to sit for two hours.

2.2.11 Graduated pH Test

Aliquots of effluent were adjusted to pH 6.5, 7.5 and 8.5. At intervals 1 and 2 hours toxicity was monitored and the pH was re-adjusted (as necessary).

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 20, 40, 60, 80, and 100% effluent concentrations. The graduated pH test was only run with test concentrations of 50 and 100% at each pH. Dilution water and effluent were both pH adjusted to all three pH values prior to preparation of dilutions. Each test dilution was comprised one replicates of five neonate test organisms (except for the baseline test, which had two replicates of five test organisms. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the seep samples. Several treatments were conducted at more than one pH.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be clear in color. Residual chlorine was elevated, at 0.35 mg/L. The effluent was slightly alkaline in pH (8.03) when received. Effluent was low in hardness (4.0 mg/L) and alkalinity (52.8 mg/L).

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	9/20/96	T6429	Sample toxic; LC ₅₀ =16.2%
48 Hr Delay Baseline	9/22/96		Toxicity reduced
Adjusted to pH 3	9/22/96		Toxicity not reduced
Adjusted to pH 11	9/22/96		Toxicity not reduced
Filtered at Initial pH	9/22/96		Toxicity not reduced
Filtered at pH 3	9/22/96		Toxicity not reduced
Filtered at pH 11	9/22/96		Toxicity not reduced
Aerated at Initial pH	9/22/96		Toxicity not reduced
Aerated at pH 3	9/22/96		Toxicity not reduced
Aerated at pH 11	9/22/96		Toxicity removed
C-18 SPE at Initial pH	9/22/96		Toxicity not reduced
C-18 SPE at pH 3	9/22/96		Toxicity not reduced
C-18 SPE at pH 11	9/22/96		Toxicity slightly reduced
Na Thiosulfate Add.	9/22/96		Toxicity slightly reduced
EDTA Chelation	9/22/96		Toxicity slightly reduced
Graduated pH	9/22/96		Sample less toxic at high pH (8.5)

3.2 Discussion

The sample upon receipt clearly had elevated levels of residual chlorine (0.35 mg/L) and, when tested, the residual chlorine caused substantial toxicity. In the July-August Phase I tests there was no toxicity at 20-40% effluent concentration, moderate toxicity at 60-80% effluent concentration, and complete mortality at 100% effluent concentration. However, these tests were run on a 7 day delay after residual chlorine was measured. In the September baseline test there was no time delay, so the residual chlorine remained high - resulting in toxicity at effluent concentrations as low as 20%. At a 20% effluent concentration, residual chlorine would have been reduced to 0.07 mg/L. The 48 hour delay test of September 22, 1996 was initiated when the residual

chlorine had been reduced to <0.05 mg/L, a non-toxic level. At this point the effluent was toxic only at 100% concentration, resulting in an LC_{50} of 89%. Thus, the non-residual chlorine toxicity was less than in the early August test.

Both the August test and the September 22nd time-delay test were run with essentially no residual chlorine present. The August test was conducted with total copper concentrations in the effluent as high as 140 $\mu\text{g/L}$. The resulting LC_{50} of 69.1% translates to 1.45 toxic units. The September 22nd time-delay test was conducted with total copper concentrations in the effluent of 45 $\mu\text{g/L}$. The resulting LC_{50} of 89% translates to 1.12 toxic units. If it is assumed that a copper concentration of 0 $\mu\text{g/L}$ was present in the blank, with 1.00 toxic units (no toxicity), a strong correlation between copper concentration and toxicity is observed ($r^2=0.996$).

As noted in the early August Phase I tests, EDTA chelation and sodium thiosulfate addition were effective in reducing sample toxicity. The graduated pH tests also confirmed August results, showing the effluent to be less toxic at pH 8.5 than at lower pH values. At initial pH neither aeration, filtration, nor treatment with a C-18 SPE column were effective. The same was true at pH 3. Unexpectedly, however, aeration at pH 11 was effective at removing toxicity, as was the C-18 SPE column used at pH 11. Certainly the adjustment to pH 11 may have resulted in more copper in the less toxic hydroxide form rather than the more toxic aquo ion form, and this could have been filtered by the C-18 SPE column. Yet the role of aeration is not clear.

In sum, follow-up Phase I testing reports confirm copper and residual chlorine as the toxicants in A-004 effluent.

TABLE 1

SUMMARY OF RESULTS - ACUTE PHASE I TIE WSRC Effluent A-004 September 1996

Baseline Test Eff. Conc.	48 Hour Mortality		
	July 96	September 96	
		Baseline	48 Hr Delay
0.0%	0%	0%	0%
20.0%	0%	80%	0%
40.0%	0%	100%	0%
60.0%	30%	100%	0%
80.0%	60%	100%	0%
100.0%	100%	100%	100%

pH Adjustment / Aeration / Filtration / C-18 SPE

48 Hr LC50	pH 3	pH 8.03	pH 11
Baseline	72.9%	89.0%	76.7%
Aeration	89.0%	89.0%	100.0%
Filtration	80.7%	89.0%	80.7%
C-18 SPE Column	89.0%	89.0%	95.0%

Discussion: Baseline testing showed that A-004 effluent was very toxic when received but lost most of the toxicity over 48 hours. pH manipulation and filtration were ineffective at reducing toxicity. Aeration and solid phase extraction (C-18 SPE) at pH 11 reduced toxicity.

TABLE 2

SUMMARY OF RESULTS - ACUTE PHASE I TIE WSRC Effluent A-004 September 1996

Sodium Thiosulfate Addition - 48 Hr. Mortality

EDTA Chelation - 48 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)					
	0	1	5	10	25	50
0%	0%	0%	0%	0%	0%	0%
20%	0%	0%	0%	0%	0%	0%
40%	0%	0%	0%	0%	0%	0%
60%	0%	0%	0%	0%	0%	0%
80%	0%	0%	0%	0%	0%	0%
100%	100%	100%	100%	100%	60%	60%

Effluent Conc.	EDTA Conc. (mg/L)					
	0	1	5	10	25	50
0%	0%	0%	0%	0%	0%	0%
20%						
40%						
60%						
80%	0%	0%	0%	100%	100%	100%
100%	100%	80%	80%	100%	60%	60%

Discussion:

Sodium thiosulfate and EDTA both slightly reduced toxicity at concentration of 25-50 mg/L.

TABLE 3

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-004

September 1996

Graduated pH Test

Effluent Conc.	2 Hour Mortality at Nominal pH					
	pH 6.5		pH 7.5		pH 8.5	
	Baseline	48 Hr Delay	Baseline	48 Hr Delay	Baseline	48 Hr Delay
0%	0%	0%	0%	0%	0%	0%
50%	90%		30%		30%	
100%	100%	100%	100%	100%	100%	0%

Discussion: The pH of the effluent was found to have an effect on the toxicity. Toxicity was found to be lower at pH 8.5 than at pH 7.5 or pH 6.5.

APPENDIX E-3

A-004, ADDITIONAL PHASE I RESULTS
DECEMBER 1996

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I
Follow-up Tests - Group 2**

Client: Westinghouse Savannah River Company

Sample ID: A-004

December 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-004 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 9-17, 1996. The test results showed the presence of acute toxicity in undiluted effluent. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-004 effluent on July 31- August 2, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. Both EDTA and sodium thiosulfate addition reduced toxicity, and the effluent was noted to be less toxic at pH 8.5 than at pH 6.5. The results indicated that both residual chlorine and copper played a role in effluent toxicity. The measured concentration of residual chlorine at sample receipt was 0.29 mg/L and the measured concentrations of copper were as much as 140 µg/L. Due to the apparent presence of more than one toxicant, it was recommended to broaden the TIE Phase I to include other treatments and repeat the earlier treatments, paying attention to residual chlorine levels. This full Phase I TIE was conducted on September 22, 1996. Results confirmed that residual chlorine and copper played a role in effluent toxicity. Unexpectedly, aeration at pH 11 was found to reduce toxicity. This result was considered potentially anomalous. Consequently the aeration at pH 11 test was repeated, along with an aeration with nitrogen at pH 11 test, and the results are presented below.

2.0 METHODS

2.1 Sampling

A composite sample of effluent was collected on December 8, 1996 and used for conducting follow-up aeration treatments and tests.

2.2 Treatment Methods

The sample was subjected to two treatments to determine which would reduce or remove toxicity. Each treated sample was used in an acute toxicity test (at a series of dilutions) to determine the effectiveness of the treatment. The treatments are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline	12/9/96	T6806	-
Baseline	12/16/96		-
Aerated at pH 11 w/ Air	12/16/96		volatile or oxidizable
Aerated at pH 11 w/N ₂	12/16/96		volatile

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Aeration at pH 11 w/ Air

A 100 mL sample aliquot which had been adjusted to pH 11 was vigorously aerated for a period of one hour with room air with a diaphragm type aerator. After aeration the pH was re-adjusted to the initial pH with H₂SO₄.

2.2.2 Aeration at pH 11 w/ Nitrogen

A 100 mL sample aliquot which had been adjusted to pH 11 was vigorously aerated for a period of one hour with pure nitrogen gas. After aeration the pH was re-adjusted to the initial pH with H₂SO₄.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 20, 40, 60, 80, and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test, which had two replicates of five test organisms. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the seep samples.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be clear in color. Effluent was low in hardness (4.0 mg/L).

<u>Treatment</u>	<u>Start Date</u>	<u>Result</u>
Baseline	12/9/96	Sample toxic; LC ₅₀ = <20%
Baseline	12/16/96	Sample toxic; LC ₅₀ = 21.4%
Baseline	1/2/97	Sample toxic; LC ₅₀ = 20.0%
Aerated at pH 11 w/ Air	1/2/97	Toxicity not removed; LC ₅₀ = 22.9%
Aerated at pH 11 w/ N ₂	1/2/97	Toxicity not removed; LC ₅₀ = 24.6%

3.2 Discussion

The results provide no indication that aeration at pH 11 removes or substantially reduces toxicity. It may be concluded that the aeration at pH 11 test results for the September 1996 Phase I TIE were anomalous.

APPENDIX E-4

A-004, PHASE III RESULTS

TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification: A-004

Revision 1

December 1996

1.0 INTRODUCTION

A-004 effluent was identified as acutely toxic to *Ceriodaphnia dubia* in June-July 1996. A Phase I TIE initiated in July 1996 and completed in September 1996 indicated that copper and residual chlorine were the two toxicants in A-004 effluent. Significant levels of zinc also were detected in A-004 effluent. In order to confirm the relative contribution to total toxicity of the two metals, simulated A-004 effluent was prepared and the two metals were spiked into the effluent individually and together. These investigations were initiated on October 9, 1996 and identified as Phase III. Relatively high concentrations of copper were spiked into A-004 effluent in the October studies. In December 1996 additional spiking tests were conducted in which lesser amounts of copper were spiked into A-004 effluent.

2.0 METHODS

Baseline testing conducted on July 31 - August 2, 1996 indicated acute toxicity at concentrations of 80-100% effluent. Based upon this information simulated effluent was prepared as a formulation of 40% effluent and 60% moderately hard synthetic freshwater (MHSF) diluted to a hardness of 4 mg/L (the hardness of the effluent). The diluted MHSF was prepared by adding 570 mL of ultra-pure Type I water to 30 mL of MHSF at a hardness of 80 mg/L. To the simulated effluent the following combinations of copper and zinc were added;

Copper Variable / No Zinc
Copper Variable / Maximum Zinc
Maximum Zinc / No Copper

When a metal was spiked in variable amounts for the October tests the highest amount used was the maximum measured concentration from July 1996 testing. From this maximum amount a series of more dilute concentrations were prepared. When copper was variable, the zinc was spiked at the maximum measured amount along with each serial dilution of the copper. An acute definitive toxicity test was set on each spiked solution, using two replicates of five test organisms at each dilution. Copper was spiked as copper sulfate and zinc as zinc sulfate.

3.0 RESULTS

3.1 Copper Spiked Tests (October Series)

Copper was spiked at a range of 5 - 100 µg/L into the simulated effluent in October and 1-10 µg/L in December. The MHSF had <5.0 µg/L of copper before spiking. However, the simulated effluent at 40% dilution had a baseline level of copper of 23 µg/L in October and 100 µg/L in December. The nominal series of copper concentrations was 5, 10, 25, 50, and 100 µg/L. In one definitive test no zinc was added to the copper dilutions. In the second test 94 µg/L of zinc was added to the copper dilution. The results are summarized as follows;

% Mortality By Dilution			
<u>Nominal Conc.</u>	<u>Actual Conc.</u>	<u>Cu Only</u>	<u>Cu w/ Zn</u>
October Testing			
0 µg/L Cu	23 µg/L Cu	100%	100%
5 µg/L Cu	28 µg/L Cu	100%	100%
10 µg/L Cu	33 µg/L Cu	100%	100%
25 µg/L Cu	48 µg/L Cu	100%	100%
50 µg/L Cu	73 µg/L Cu	100%	100%
100 µg/L Cu	123 µg/L Cu	100%	100%
December Testing			
0 µg/L Cu	100 µg/L Cu	100%	100%
1 µg/L Cu	101 µg/L Cu	100%	100%
2 µg/L Cu	102 µg/L Cu	100%	100%
5 µg/L Cu	105 µg/L Cu	100%	100%
10 µg/L Cu	110 µg/L Cu	100%	100%

All test organisms died in all test concentrations including the control. This indicates that in both sets of tests the simulated effluent was toxic.

Total copper and toxicity have been found to vary to a substantial degree in A-004 effluent. Measured concentrations during 1996 are as follows;

<u>Date</u>	<u>Total Copper</u>	<u>Untreated Effluent</u> <u>48 Hour LC₅₀ / Toxic Units</u>
7/8/96	140 µg/L	
7/10/96	25 µg/L	
7/12/96	45 µg/L	
7/24/96	not measured	74.8% / 1.34
9/19/96	57 µg/L	89% / 1.12
12/8/96	249 µg/L	17.4% / 5.74

Based upon the toxic units for copper in both the September and December samples, it can be predicted that A-004 effluent should cease to be acutely toxic when copper concentrations decline below 35-40 µg/L. However, in the September spiking tests, when total copper was only 23 µg/L, none of the test organisms survived. The additional toxicity may be due to zinc or additive effects of copper and zinc. In testing in October and December residual chlorine was allowed to decrease to <0.05 mg/L prior to testing.

Data generated at ETT Environmental in association with water effect ratio testing indicates an LC₅₀ of approximately 15 µg/L for total copper in moderately hard diluted mineral water. However, A-004 effluent was far softer than moderately hard water, with a hardness of 4.0 mg/L. Based upon the EPA water criterion formula for copper at a hardness of 4 mg/L the CMC (maximum concentration to avoid acute toxicity) is $e^{(0.9422 \ln (\text{hardness}) - 1.464)} = 0.85 \mu\text{g/L}$. The acute toxicity level of copper in A-004 effluent appears to be substantially higher than this value.

It should be noted that hardness itself, if too low, can result in toxicity. In order to determine if the soft DMW contributed to toxicity apart from the influence of copper, a series of DMW dilutions was prepared in which hardness was varied. The results indicated that 35% mortality occurred in DMW with a hardness of 2 mg/L, 95% mortality in ultra-pure water with a hardness of <0.1 mg/L, and no significant mortality in DMW with a hardness of 5 mg/L and higher. This suggests that water with a hardness of 4 mg/L should not have been significantly toxic due only to low hardness.

Due to high background levels of copper and zinc in A-004 effluent it appears that effluent must be highly diluted (to a concentration of 20% or less) to eliminate metal toxicity with the goal of spiking metals back into the effluent. With such a degree of dilution the resultant simulated effluent would be too dissimilar to undiluted effluent to accurately predict the toxicity level of copper and zinc. An alternative strategy of preparing simulated effluent by measuring the concentration of all essential ions and physical parameters (hardness, alkalinity, bicarbonate, Na, K, Mg, Ca, Cl, SO₄, Fe, Mn, TDS, TOC) and adding salts to ultra-pure water to achieve the same concentrations, may achieve a more realistic prediction.

On November 6, 1996 a final series of tests was conducted in which copper was spiked into reconstituted water with a hardness of 5 mg/L. In this case the ions were added according to standard EPA formulas rather than according to measured levels in the effluent. Copper was spiked at concentrations of 0, 1, 2, 5, and 20 µg/L. Results showed complete mortality of test organisms at copper concentrations of 2 µg/L and higher. Minimal mortality was noted at copper concentrations of 1 µg/L and less.

3.2 Zinc Spiked Tests

Zinc was spiked at 94 µg/L into the simulated effluent. Prior to spiking the simulated effluent had 0.4 x 94 µg/L of zinc (37.6). Thus the nominal concentration was 94 µg/L but the actual concentration was 131.6 µg/L. The MHSF had <5.0 µg/L of zinc before spiking. No copper was spiked into the dilutions (although the copper contributed by the 40% untreated effluent contributed >23 µg/L). The results are summarized as follows;

<u>Nominal Conc.</u>	<u>Actual Conc.</u>	% Mortality By Dilution
		<u>Zn Only</u>
0 µg/L Zn	37.6 µg/L Zn	100%
94 µg/L Zn	131.6 µg/L Zn	100%

All test organisms died in all test concentrations including the control. This indicates that the simulated effluent was toxic. Data generated at ETT Environmental in association with water effect ratio testing indicates an LC₅₀ of approximately 60 µg/L for total zinc in moderately hard diluted mineral water. However, A-004 effluent was far softer than moderately hard water, with a hardness of 4.0 mg/L. Based

upon the EPA water criterion formula for zinc at a hardness of 4 mg/L the CMC (maximum concentration to avoid acute toxicity) is $e^{(0.8473 \ln(\text{hardness}) + 0.8604)} = 7.7 \mu\text{g/L}$. It is not unexpected, then, that a zinc concentration of 37.6 $\mu\text{g/L}$ in the simulated effluent would be toxic.

On November 6, 1996 a final series of tests was conducted in which zinc was spiked into reconstituted water with a hardness of 5 mg/L. Essential ions were added according to standard EPA formulas and not according to measured levels in the effluent. Spiked zinc concentrations were 0, 1, 2, 5, and 20 $\mu\text{g/L}$. Results showed no significant mortality at any concentration, giving an LC_{50} of greater than 20 $\mu\text{g/L}$ of zinc. This indicates that in A-004 effluent, the toxic zinc level can be expected to be greater than 20 $\mu\text{g/L}$ (the value in reconstituted water of similar hardness).

4.0 CONCLUSION

Copper is confirmed as being at concentrations high enough to result in effluent toxicity. Zinc may also play a role in toxicity in the A-004 effluent.

APPENDIX F-1

A-005, PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-005

July 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-005 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 9 - July 17, 1996. The test results showed the presence of acute toxicity in undiluted effluent. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

This report presents the results of a Metals-Focused Phase I Toxicity Identification Evaluation (TIE) conducted on the A-005 effluent on July 31 - August 2, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on July 24th, 1996 and used for conducting a partial Phase I Toxicity Identification Evaluation.

2.2 Treatment Methods

The sample was subjected to a series of treatments to determine which would remove toxicity. Each treated sample was used in an acute toxicity test, using test dilutions of 60, 80, and 100%. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test	7/31/96	T6140	-
Filtered at pH 11	7/31/96	T6140	metals; insoluble at pH 10
Na Thiosulfate Addition	7/31/96	T6140	oxidizers; residual chlorine; metals
EDTA Chelation	8/1/96	T6140	some metals; surfactants
Graduated pH	7/31/96	T6140	ammonia; metals; sulfide

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls.

2.2.1 Filtration at pH 11

A 300 mL sample aliquot which had been adjusted to pH 11 ± 0.05 with NaOH was filtered through a 0.45μ membrane filter. After filtration the pH was readjusted to the initial pH with H_2SO_4 .

2.2.2 Sodium Thiosulfate Addition

A 8.5 g/L stock solution of sodium thiosulfate was prepared. This stock solution was used to prepare aliquots of effluent with 30, 60, 120 and 250 mg/L of sodium thiosulfate. After addition of the sodium thiosulfate, the test solutions were allowed to equilibrate for two hours.

2.2.3 EDTA Chelation

A 2.5 g/L stock solution of diSodium EDTA was prepared. This stock solution was used to prepare aliquots of effluent with 45, 90, and 190 mg/L of EDTA. After addition of the EDTA, the test solutions were allowed to equilibrate overnight and were pH adjusted back to the initial pH.

2.2.4 Graduated pH Test

Aliquots of effluent at 100% concentration were adjusted to pH 6.5, 7.5 and 8.5. After 2 hours and 18 hours the survival of test organisms at each pH was checked.

2.3 Toxicity Testing Methods

Acute toxicity tests were set with all treated effluent samples. Most tests were set at 60, 80 and 100% effluent concentrations. Each test dilution was comprised of one replicate of five neonate test organisms (except for the baseline test which had two replicates of five organisms). A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 Summary

Upon arrival the sample was observed to be have a clear color. Residual chlorine was high, at 0.15 mg/L. The effluent was slightly alkaline (8.16) in pH when received. Hardness was very low, at 8.0 mg/L. The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	7/31/96	T6140	Sample toxic; $LC_{50}=74.8\%$
Filtered at pH 11	7/31/96	T6140	Sample toxicity reduced
Na Thiosulfate Addition	7/31/96	T6140	Sample toxicity removed
EDTA Chelation	8/1/96	T6140	Sample toxicity reduced
Graduated pH	7/31/96	T6140	Sample less toxic at pH 8.5

3.2 Discussion

Toxicity in the effluent was largely removed by filtration at pH 11. Toxicity was completely removed by sodium thiosulfate addition. EDTA was partially effective at removing effluent toxicity. EDTA typically removes or reduces only metal toxicity and surfactant toxicity. Metals effectively chelated by EDTA include cadmium, copper, lead, nickel, and zinc. Chromium, mercury, and selenium are not effectively removed from bioavailability by EDTA. Of the metals treatable by EDTA, copper and cadmium should also be treatable by sodium thiosulfate. However, cadmium is typically more toxic at pH 8.5 than at pH 6.5, which is the opposite of the pattern of the data in this study. This leaves copper as the only metal which fits the treatment/toxicity

data. It should be noted that moderately high measured residual chlorine in the sample (0.15 mg/L) suggests that residual chlorine may have played a minor role in toxicity. Thus, some of the effect of the sodium thiosulfate addition may relate to removal of residual chlorine. Toxicity remaining after filtration at pH 11 likely shows the presence of residual chlorine after metal removal. The effectiveness of EDTA chelation, which is not effective for residual chlorine, verifies that residual chlorine is not the only toxicant. Nevertheless, EDTA was not as effective as sodium thiosulfate, possibly because EDTA removes only metals whereas sodium thiosulfate removes metals and residual chlorine.

3.3 Recommendations

It is recommended that EDTA chelation and filtration at pH 11 treatments and tests be repeated on a sample which has been treated with sodium thiosulfate to remove residual chlorine, in order to determine if only copper is contributing toxicity or if copper and residual chlorine are involved. The possibility of surfactant toxicity should be investigated through conducting a sublation treatment with the associated toxicity test.

4.0 CONCLUSIONS

- ▶ The primary toxicant present in the effluent is a cationic metal.
- ▶ Copper is the metal which best fits the pattern of toxicity noted for the various treatments conducted on the effluent.
- ▶ Residual chlorine may also play a role in effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-005
July 1996

Baseline Testing		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%	7.91	0%	0%
60%	7.98	0%	0%
80%	8.06	30%	70%
100%	8.16	90%	100%

Filtration at pH 11		Mortality	
Concentration	Initial pH	24 Hr	48 Hr
0%	7.95	0%	0%
60%	8.09	0%	0%
80%	8.17	0%	0%
100%	8.25	0%	40%

Discussion:

The effluent was significantly acutely toxic at concentrations as low as 80%.

Filtration at pH 11 was effective at reducing effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE

WSRC Effluent A-005

July 1996

EDTA Chelation - 48 Hr. Mortality

Effluent Conc.	EDTA Conc. (mg/L)					
	0	45	90	190	370	45 Control
0%	0%					
60%	0%	0%	0%	100%	100%	
80%	70%	0%	80%	100%	100%	
100%	100%	100%	100%	100%	100%	0%
Control pH						7.95
Effluent pH	7.75	7.87	7.96	7.43	8.08	

Sodium Thiosulfate Addition - 18 Hr. Mortality

Effluent Conc.	Sodium Thiosulfate Conc. (mg/L)					
	0	30	60	120	250	30 Control
0%	0%					
60%	0%	0%	0%	0%	80%	
80%	70%	0%	0%	0%	100%	
100%	100%	20%	0%	40%	100%	0%
Control pH						8.04
Effluent pH	7.79	7.86	7.9	7.91	7.95	

EDTA Addition

Sodium Thiosulfate Addition

Discussion:

EDTA at 45 mg/L reduced effluent toxicity.

Sodium thiosulfate at 60 mg/L removed effluent toxicity.

SUMMARY OF RESULTS - ACUTE PHASE I TIE
WSRC Effluent A-005
July 1996

Graduated pH Test

Effluent Conc.	48Hour Mortality at Nominal pH (w/o Buffer)					
	pH 6.5		pH 7.5		pH 8.5	
	Actual pH	% Mortality	Actual pH	% Mortality	Actual pH	% Mortality
0%	6.49	0%	7.52	0%	8.48	0%
60%	6.47	100%	7.54	0%	8.54	0%
80%	6.53	100%	7.65	60%	8.53	40%
100%	6.48	100%	7.51	100%	8.54	100%

Discussion:

The sample was less toxic at pH 7.5 to 8.5 than at pH 6.5

APPENDIX F-2

A-005, ADDITIONAL PHASE I RESULTS

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**TOXICITY IDENTIFICATION EVALUATION
PHASE I - Metals Only
Follow-up Tests**

Client: Westinghouse Savannah River Company

Sample ID: Effluent A-005

September 1996

1.0 INTRODUCTION

A chronic definitive survival and reproduction test of A-005 effluent using the water flea *Ceriodaphnia dubia* was conducted on July 9 - July 17, 1996. The test results showed the presence of acute toxicity in undiluted effluent. This chronic definitive test was conducted as part of a program of investigation of wastewater toxicity sources within the A- Area at the Savannah River Site.

A Metals-Focused Phase I Toxicity Identification Evaluation (TIE) was conducted on the A-005 effluent on July 31 - August 2, 1996. Effluent treatments including filtration at pH 11, EDTA chelation, sodium thiosulfate addition, and graduated pH were conducted, followed by acute toxicity testing with *Ceriodaphnia dubia* to determine if toxicity had been removed. The results indicated that toxicity was likely due to both residual chlorine and copper. Metals analysis data showed that copper (measured at levels of 10 µg/L) was relatively low, but still high enough to cause toxicity in very soft water such as A-005 effluent.

Due to the results of the Phase I treatments and tests, additional testing was conducted in September 1996. The testing focused on differentiating residual chlorine and copper toxicity. This was done by allowing residual chlorine toxicity to decrease to non-toxic levels with time, followed by repeating the EDTA chelation treatment and the filtration at pH 10 treatment. In addition a sublation treatment was conducted to confirm that the effectiveness of EDTA treatment was not due to surfactants. This report presents the results of these tests conducted as a follow-up to the original Phase I testing conducted in July 1996.

2.0 METHODS

2.1 Sampling

A grab sample of effluent was collected on September 12, 1996 by WSRC personnel and used for conducting treatments and tests.

2.2 Treatment Methods

The sample was subjected to three treatments. The baseline sample as well as the pH 10 filtration sample were assessed in an acute toxicity test, using test dilutions of 20, 40, 60, 80, and 100%. EDTA chelation was conducted at effluent concentrations of 80% and 100%. Sublation was only conducted with 100% effluent. The treatments and the associated toxicity tests are summarized as follows:

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Toxicants Potentially Removed</u>
Baseline Test (untreated)	9/20/96	T6399	-
Filtration at pH 10	9/20/96	T6399	metals
EDTA Chelation	9/20/96	T6399	metals; surfactants
Sublation	9/20/96	T6399	surfactants

Methodology for the individual treatments is discussed below by treatment. All treatments also were conducted on dilution water controls (Upper Three Runs Creek).

2.2.1 Filtration at pH 10

A 300 mL aliquot of effluent at 100% concentration was adjusted to pH 10.0 and filtered with a 0.45 μ membrane filter. After filtration the pH was readjusted to the initial pH, at 7.48.

2.2.2 EDTA Chelation Test

A 15 g/L stock solution of disodium EDTA was prepared. This stock solution was added to aliquots of 80% and 100% effluent to provide EDTA concentrations of 1,5,10,25, and 50 mg/L.

2.2.3 Sublation Test

An 80 mL aliquot of 100% effluent was vigorously aerated in glass vessel such that foam produced by the aeration overflowed into an outer glass vessel. The foam which overflowed was collected and labelled as the "sublate." The effluent remaining in the inner vessel after foam was removed was also collected and labelled as the "residual."

2.3 *Toxicity Testing Methods*

Acute toxicity tests were set with all untreated and treated effluent samples. The baseline test and filtration at pH 10 test were comprised of two replicates of five neonate test organisms at each test concentration. The EDTA Chelation test was comprised of one replicate of five neonate test organisms at each EDTA/effluent combination. The sublation test was comprised of one replicate of five neonate test organisms at each test concentration. A treated control (one replicate) was prepared for each treatment set, where dilution water was subjected to the same treatment as the effluent.

3.0 RESULTS

3.1 *Summary*

Upon arrival the sample was observed to be have a clear color. Residual chlorine when the sample was received was slightly elevated, at 0.10 mg/L. However, by the time the treatments and tests were conducted residual chlorine had decreased to less than 0.05 mg/L. Hardness was very low, at 10.0 mg/L, as was alkalinity (53.6 mg/L). The results are summarized as follows;

<u>Treatment</u>	<u>Start Date</u>	<u>Sample ID</u>	<u>Result</u>
Baseline	9/20/96	T6399	Sample toxic; LC ₅₀ =89%
pH 10 Filtration	9/20/96	T6399	Toxicity not reduced
EDTA Chelation	9/20/96	T6399	Toxicity removed
Sublation	9/20/96	T6399	Toxicity not reduced

3.2 *Discussion*

The September 11, 1996 sample of A-005 effluent was slightly less toxic than the sample collected in July (Figure 1). The September baseline test showed no significant acute toxicity at effluent concentrations of 80% or less. Effluent in the July sample showed moderate toxicity in the 80% dilution. It is believed that the difference in toxicity may be attributed to the presence of residual chlorine in the July test, and absence in the September test.

Filtration at pH 10 was not effective at significantly reducing effluent toxicity. This may be because effluent concentrations of copper in A-005 effluent are quite low prior to treatment, and the treatment is not effective in this concentration range.

The EDTA chelation test showed that toxicity could be completely removed by EDTA in the absence of residual chlorine. This result is consistent with copper as a toxicant. The optimal EDTA concentration was 1-5 mg/L.

The sublation test did not provide any evidence of the presence of surfactants. Typically, if surfactants are present the residual solution should be substantially less toxic than the subluted portion. In this test, however, both solutions were equally toxic.

Follow-up treatments/testing conducted in September 1996 provided results consistent with the conclusion of initial Phase I treatments that indicated that copper and residual chlorine both play a role in effluent toxicity. It appears that most of the toxicity is due to copper, and residual chlorine contributes only a small portion of the toxicity.

Figure 1
WSRC A-005 Effluent
Phase 1 TIE Follow-Up Testing Results
September 1996

BASELINE & pH 10 FILTRATION TESTING

Effluent Concentration	Baseline Mortality		pH 10 Filtration Mortality
	July 96	Sept 96	July 96
0%	0%	0%	0%
20%	0%	0%	0%
40%	0%	0%	0%
60%	0%	0%	0%
80%	30%	0%	10%
100%	90%	100%	80%
48 Hr LC50	74.8%	89.0%	90.8%
Toxic Units	1.34	1.12	1.10
Hardness (mg/L)	8	10	

Toxicity - Baseline & pH 10 Filtered

EDTA CHELATION TEST (September 1996)

EDTA Conc. (mg/L)	Effluent Concentration		
	0%	80%	100%
0	0%	0%	100%
1	0%	0%	0%
5	0%	0%	0%
10	0%	10%	20%
25	40%	100%	100%
50	100%	100%	100%
Initial pH (Control)	6.16		6.49
Initial pH (5 mg/L)	6.28		7.94

EDTA Addition vs Toxicity

SUBLATION TEST (September 1996)

Effluent Concentration	Mortality at 48 Hours		
	Untreated	Residual	Sublate
0%	0%	0%	0%
100%	100%	100%	0%

Toxicity - Sublation Test

APPENDIX F-3

A-005, PHASE III RESULTS

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TOXICITY IDENTIFICATION EVALUATION

PHASE III

Spiking Tests

Westinghouse Savannah River Company

Sample Identification:A-005

September 1996

1.0 INTRODUCTION

A-005 effluent was identified as acutely toxic to *Ceriodaphnia dubia* in June 1996. A Phase I TIE initiated in July 1996 and completed in September 1996 indicated that copper was the main toxicant in A-005 effluent, with residual chlorine also playing a role in toxicity. No other metals were detected at levels considered to be potentially toxic. In order to confirm copper as the toxicant, simulated A-005 was prepared and copper was spiked into the simulated effluent. This report presents the results of these investigations, initiated on October 10, 1996 and identified as Phase III.

2.0 METHODS

Baseline testing conducted on September 20-22, 1996 indicated acute toxicity only in undiluted effluent. Based upon this information simulated effluent was prepared as a formulation of 60% effluent and 40% moderately hard synthetic freshwater (MHSF) diluted to a hardness of 8 mg/L (the hardness of the effluent). The diluted MHSF was prepared by adding 360 mL of ultra-pure Type I water to 40 mL of MHSF at a hardness of 80 mg/L. Copper was spiked into this simulated effluent.

An acute definitive toxicity test was set with five copper concentrations and a control. Copper was spiked as copper sulfate.

3.0 RESULTS

Copper was spiked at a range of 2 - 20 µg/L into the simulated effluent. However, the simulated effluent at 60% dilution had a baseline level of copper of 10 µg/L. The nominal series of copper concentrations was 2, 5, 10, 15, and 20 µg/L. Adding the baseline level of copper provided a series of copper concentrations of 7, 10, 15, 20, and 25 µg/L. The results are summarized as follows;

48 Hr Mortality By Dilution

<u>Cu Concentration</u>	<u>Cu Only</u>
0 µg/L	80%
7 µg/L	90%
10 µg/L	100%
15 µg/L	100%
20 µg/L	100%
25 µg/L	100%

It can be observed that acute toxicity was present in the effluent without any amount of spiked copper. This made it difficult to determine whether toxicity in spiked dilutions was due to copper or toxicity of the simulated effluent. It is not clear why the non-spiked simulated effluent was toxic, because the baseline test suggested toxicity should not be present. Data generated at ETT Environmental in association with water effect ratio testing indicates an LC_{50} of approximately 9.0 µg/L for total copper in soft diluted mineral water (DMW - hardness = 50 mg/L). However, A-005 effluent is much softer than soft diluted mineral water. Based upon the EPA water criterion formula for copper at a hardness of 8 mg/L the CMC (maximum concentration to avoid acute toxicity) is $e^{(0.9422 \ln(\text{hardness}) - 1.464)}$ is 1.6 µg/L. This is a conservative estimate of the range at which copper might be acutely toxic in A-005 effluent.

It should be noted that hardness itself, if too low, can result in toxicity. In order to determine if the soft DMW contributed to toxicity apart from the influence of copper, a series of DMW dilutions was prepared in which hardness was varied. The results indicated that 35% mortality occurred in DMW with a hardness of 2 mg/L, 95% mortality in ultra-pure water with a hardness of <0.1 mg/L, and no significant mortality in DMW with a hardness of 5 mg/L and higher. This suggests that water with a hardness of 8 mg/L should not have been significantly toxic due only to low hardness.

On November 6, 1996 a final series of tests was conducted in which copper was spiked into reconstituted water with a hardness of 5 mg/L. Spiked copper concentrations were 0, 1, 2, 5, and 20 µg/L. Results showed complete mortality of test organisms at copper concentrations of 2 µg/L and higher. Minimal mortality was noted at copper concentrations of 1 µg/L and less. This indicates that in A-005 effluent, the toxic copper level can be expected to be between 2 µg/L (the value in reconstituted water of similar hardness) and 10 µg/L (the simulated effluent concentration in which 100% mortality was noted).

4.0 CONCLUSION

Copper was present at toxic levels in the A-005 effluent. Copper levels must be reduced by as much as 90% to eliminate copper acute toxicity, or alternatively, hardness must be increased to moderately hard levels (80-100 µg/L) to eliminate copper toxicity.