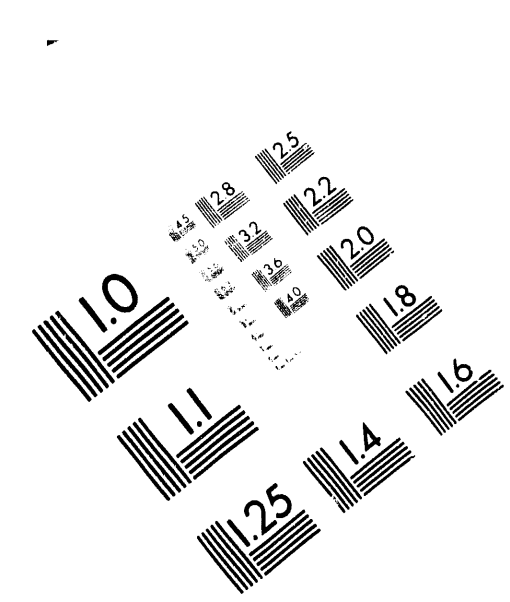

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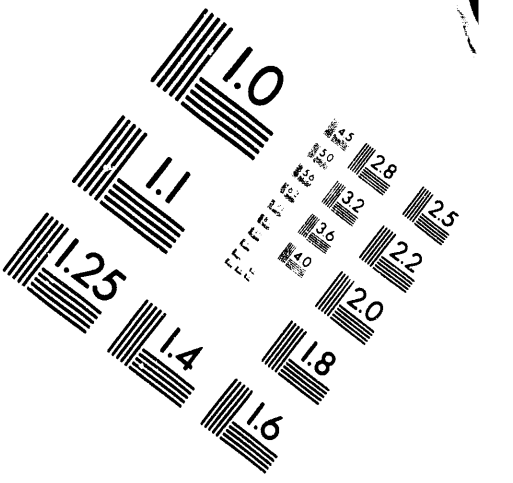

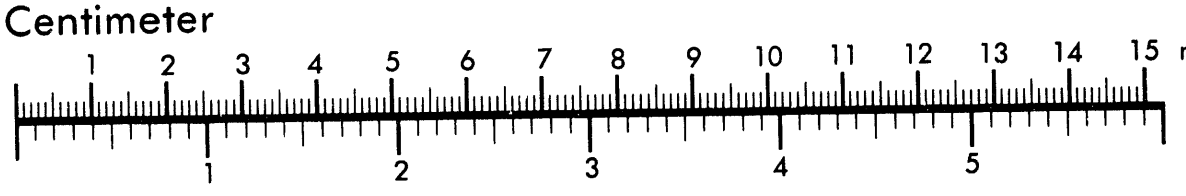

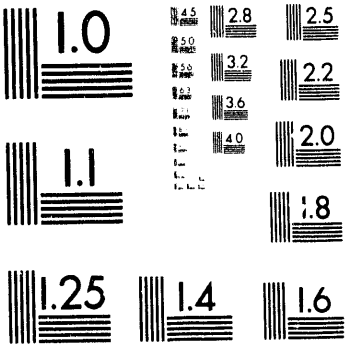

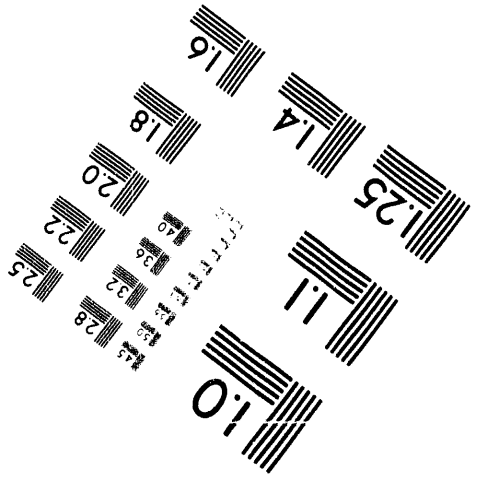

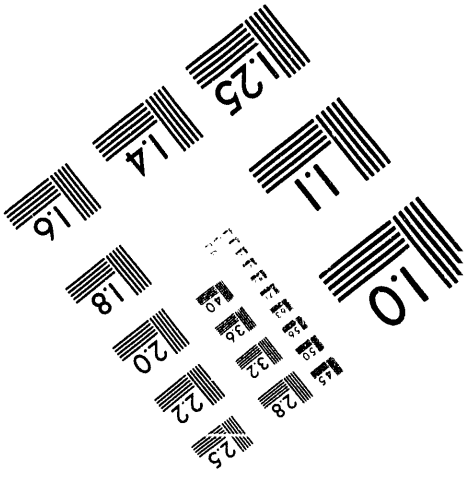

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WSRC-RP-92-995

ACUTE AND CHRONIC TOXICITY OF URANIUM COMPOUNDS TO CERIODAPHNIA-DAPHNIA DUBIA (U)

March, 1993

Westinghouse Savannah River Company
Savannah River Site
Aiken, SC 29808

NUCLEAR MATERIALS PROCESSING DIVISION
300 AREA ENVIRONMENTAL, SAFETY,& HEALTH

Savannah River Site

Prepared for the Department of Energy
Under Contract DE-AC09-89SR18035

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ACUTE AND CHRONIC TOXICITY OF URANIUM COMPOUNDS TO CERIODAPHNIA DUBIA (U)

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PREFACE

In May 1985, the South Carolina Department of Health and Environmental Control (SCDHEC) issued modifications to the Savannah River Site's (SRS) National Pollution Elimination Discharge System (NPDES) Permit (No. SC0000175). The permit modifications were implemented in order to allow the operation of the M-Area Liquid Effluent Treatment Facility (LETF), an Industrial Wastewater Treatment facility. The M-Area LETF was intended to treat the dilute process streams from the nickel plating and aluminum forming operations in M-Area. Special condition No. 28 of the permit required the Department of Energy (DOE) to develop, and after SCDHEC approval, implement a study to determine the impact of the LETF discharges on the receiving stream, Tims Branch. Pursuant to the granting of the permit modification, the DOE also agreed to conduct toxicity testing for uranium[1] (Ref. 1 included as Attachment I).

Acute toxicity tests with uranyl nitrate were conducted on bluegill sunfish and Daphnia pulex[2,21]. Uranyl nitrate was used as it was considered representative of the types of uranium discharged from the LETF. The 48 hour LC_{50} (Lethal Concentration for 50% of the exposed organisms) for Daphnia pulex was 0.22 mg total uranium/L. The 96 hour LC_{50} for bluegill sunfish was 1.67 mg total uranium/L. Using the daily average uranium concentration target limit (0.5 mg/L at the LETF discharge), the calculated concentration releasing to Tims Branch at the A-014 outfall was 0.060 mg/L. This provided safety factors of 7.3 to 55.7 for acute effects. If the instream dilution resulting from the average flow (7Q10) in Tims Branch were included, the safety factors were 4.4 to 33.4.

After review of the SRS report, SCDHEC stated in 1986 that the "the safety factor range of 4.4 to 33.4 is well below the safety factor of 100 used by SCDHEC to address chronic toxicity (long term) impact "[3] (Attachment II).

In 1988, SCDHEC reviewed the request for a permit modification to allow the treatment of supernate in the LETF storage tanks. They again expressed concern that the uranium concentration in the DETF effluent exceeded the level which could cause chronic effects on the receiving stream[4] (Attachment III).

After reviewing the SRS report on the instream biological effects of the LETF discharges, SCDHEC requested in 1989 that chronic toxicity tests be conducted due to the high instream effluent flow percentage and the variable nature of the acute toxicity test results[5] (Attachment IV).

In light of these concerns by SCDHEC, the Savannah River Site initiated a study to determine the chronic toxicity of three uranium compounds in the receiving stream environment.

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Acute and Chronic Toxicity of Three Uranium Compounds

A study to determine the acute and chronic toxicity of uranyl nitrate, hydrogen uranyl phosphate, and uranium dioxide to the organism Ceriodaphnia dubia was conducted. The testing was accomplished under sub-contracts administered by J. L. Keyes of the WSRC Environmental Protection Department (EPD) and with consultation from W. L. Specht of the Savannah River Technology Center (SRTC). The toxicity tests were conducted by two independent environmental consulting laboratories (Shealy Environmental, as a subcontractor to AnalytiKEM, Rock Hill, SC; and Normandeau Associates, New Ellenton, SC).

Part of the emphasis for this determination was based on concerns expressed by SCDHEC, which was concerned that a safety factor of 100 must be applied to the previous 1986 acute toxicity result of 0.22 mg/L for Daphnia pulex. This would have resulted in the LETF release limits being based on an instream concentration of 0.0022 mg/L uranium.

The acute and chronic toxicity results from this study with Ceriodaphnia dubia are summarized below:

	Uranium Concentration, mg/L		
Uranium Compound	**$UO_2(NO_3)_2$**	**HUO_2PO_4**	**UO_2**
Acute Toxicity (48 hr LC_{50})	0.073	0.100	0.050
Chronic Toxicity (ChV)	0.003	0.004	0.039

LETF Outfall Concentration Limits

The NPDES Permit renewal application to SCDHEC utilized the results of this study and recommended that the LETF release limit for uranium be based an instream concentration of 0.004 mg/L uranium. This is based on the fact that the uranium releases from the M-Area LETF will be in the hydrogen uranyl phosphate form, or a uranyl phosphate complex at the pH (6-10) of the Liquid Effluent Treatment Facility effluent stream, and at the pH of the receiving stream (5.5 to 7.0).

Based on the chronic toxicity of hydrogen uranyl phosphate, a lower uranium concentration limit for the Liquid Effluent Treatment Facility (LETF) outfall vs. the existing NPDES permit was recommended. The current NPDES permit "Guideline" for uranium at outfall M-004 is 0.500 mg/L average and 1.0 mg/L maximum, at a design flowrate of 60 gpm. It was recommended that the uranium concentration at the M-004 outfall be reduced to 0.28 mg/L average, and 0.56 mg/L, maximum, and to reduce the design flowrate to 30 gpm (0.038 MGD). The 0.28 mg/L concentration will provide an instream concentration of 0.004 mg/L uranium. The 0.28 mg/L concentration at M-004 is based on the combined flows from A-014, A-015, and A-011 outfalls (since 1985) of 1840 gpm (2.65 MGD) and was the flow rate which was utilized in the recent NPDES permit renewal application (1988 permit renewal).

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ACKNOWLEDGMENTS

The lead researchers for the two environmental laboratories which conducted the uranium toxicity studies were R. L Shealy, of Shealy Environmental, and K. E. Trapp and E. T. Korthals, of Normandeau Associates, Inc. Their professionalism and attention to detail brought this project to a successful conclusion.

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1.0 INTRODUCTION

Pursuant to granting an industrial wastewater construction permit for the M-Area Liquid Effluent Treatment Facility (LETF) in 1985, the South Carolina Department of Health and Environmental Control (SCDHEC) included certain conditions in the Savannah River Plant's NPDES permit modification.[1] The conditions included acute toxicity testing for uranium. Acute toxicity with uranyl nitrate was determined in 1986 with bluegill sunfish and Daphnia pulex.[2,21] The 96 hour LC_{50} for bluegill sunfish was 1.67 mg/L total uranium and the 48 hr. LC_{50} for Daphnia pulex was 0.22 mg/L total uranium (as uranyl nitrate). The dilution water was collected from an on-site stream, Upper Three Runs.

The water of SRS streams typically have a low total hardness (2-20 mg/L as $CaCO_3$) and a low pH (5-6). Heavy metals, including uranium, are much more toxic to aquatic organisms in soft versus hard water[7].

Using the 1985 average design flow rates for the M-Area LETF (0.086 MGD) and the daily maximum target concentration of uranium of 1.0 mg/L at the LETF outfall, the maximum uranium concentration at A-014 combined outfall was calculated to be 0.060 mg/L. The A-014 outfall is a combined outfall, including non-contact cooling water (0.72 MGD), groundwater air stripper effluent (0.58 MGD), and the LETF effluent. The monthly average at the A-014 outfall was therefore 0.030 mg/L, at the monthly average target uranium concentration of 0.50 mg/L at the LETF outfall. The instream acute toxicity safety factor was calculated to be 3.7 to 27.8 for Daphnia pulex and sunfish, respectively (for a 1.0 mg/L maximum effluent uranium concentration). The safety factor was calculated to be 7.3 to 55.8 respectively (for a 0.5 mg/L average effluent uranium concentration). These safety factors included no additional dilution from the initial receiving stream, Tims Branch.

SCDHEC commented on the results of the uranium acute toxicity study with a concern that "there appears to be a possibility of impact in Tim's Branch due to chronic toxicity at these concentrations".[3] The concentration in Tims Branch from a target daily maximum release of 1.0 mg/L would be approximately 0.050 mg/L, including the additional Tims Branch "7Q10" flow of 0.258 MGD. "The corresponding safety factor range of 4.4 to 33.4 is well below the safety factor of 100 used by SCDHEC to address chronic (long term) impact. The monthly average concentration of 0.030 mg/L at outfall A-014 and a safety factor range of 7.3 to 55.7 would not be significantly less instream"[3].

In addition to the acute toxicity studies conducted to support the NPDES permit modification, a Biological and Chemical study was also required on the receiving stream, Tim's Branch.[8] The primary conclusion of the study, completed in 1987, was that no adverse impact had occurred on the water chemistry, water quality, or aquatic communities or the Tim's Branch/Upper Three Runs system due to the effluent releases from the M-Area LETF.

SCDHEC approved the Tim's Branch Biological Study in 1989, and agreed that "the data collected showed very diverse communities (macroinvertebrates) and no signs of stress in the study area. There were no indications that the periphytic communities were significantly impacted due to the discharges".[5] However, they stated that "due to the high instream waste concentration (89.2%) at 7Q10 for Tim's Branch (0.258 MGD), chronic toxicity tests should be conducted to directly address chronic toxicity"[5].

In 1988, the SRS requested a modification to the M-Area Industrial Wastewater Treatment Permit to allow the treatment of the supernate in the M-Area Interim Treatment/Storage Facility (IT/SF) tanks.[9] The supernate had resulted from the separation of the concentrated slurry in the IT/SF tanks in a sludge and supernate layer. It was shown that the composition of the supernate was similar to the normal dilute feedstream to the M-Area wastewater treatment facility. Treatment of the supernate allowed a significant volume reduction of the amount of mixed (hazardous/radioactive) waste that would eventually have to stabilized and disposed.

SCDHEC again expressed its concerns with respect to toxicity due to uranium in the LETF effluent in their 1989 review of proposed supernate treatment permit modification.[4] They stated that "a safety factor of 100 with acute toxicity tests is used by SCDHEC to address chronic (long term) impact. Using this factor, the instream uranium concentration that would not cause an impact would need to be 0.0022 to 0.0167 mg/L. Using the 7Q10 of 0.258 MGD for Tim's Branch and the cooling water flow of 0.72 MGD, this would result in acceptable effluent limits of 0.027 to 0.21 mg/L (at the LETF outfall)"[4].

In view of these concerns by SCDHEC, and in order to determine the concentration of uranium that would not cause long term impact to the receiving stream, the SRS Reactor Materials Department initiated a chronic toxicity study on uranium compounds which could be released to Tim's Branch.[10] Uranyl nitrate was initially tested, and then hydrogen uranyl phosphate and uranium dioxide were added to the test matrix. Since uranium in the supernate from the IT/SF tanks is precipitated in the DETF as hydrogen uranyl phosphate, this was the compound of primary concern. Uranium dioxide was tested since it could be released from the autoclave filtration system in Building 313-M, directly to the LETF discharge.

The acute and chronic toxicity tests were conducted by two independent environmental/analytical laboratories, Normandeau Associates, of New Ellenton, SC and AnalytiKEM Inc., Cherry Hill, NJ and Rock Hill, SC. Shealy Environmental Services, Columbia, SC performed the toxicity tests as a sub-tier contractor to AnalytiKEM. The testing was conducted under Purchase Requisitions AX-843967 and AX 843930, respectively. Dr. J. L. Keyes, of the SRS Environmental, Safety, Health, and Quality Assurance Division (ESH&QA), Environmental Protection Department (EPD), was the Subcontract Technical Representative for both contracts.

2.0 EXPERIMENTAL CONDITIONS and QUALITY ASSURANCE

The acute and chronic toxicity testing on three uranium compounds was conducted by two independent environmental consulting laboratories, Shealy Environmental (as a subcontractor to AnalytiKEM Inc.) and Normandeau Associates. Both laboratories conducted toxicity tests, using Ceriodaphnia dubia as the test organisms, on uranyl nitrate and hydrogen uranyl phosphate. Only Normandeau Associates conducted the toxicity tests on uranium dioxide. The experimental conditions and laboratory Quality Assurance procedures are described in the following sections.

2.1 Acute and Chronic Toxicity Testing of Uranyl Nitrate

2.1.1 *Initial Acute and Chronic Toxicity Testing of Uranyl Nitrate*

The test conditions and results are shown in detail in Attachment V, "Test Report No. A16747, Revision II", AnalytiKEM Inc., Cherry Hill, NJ 08003 (January 13, 1988).

Dilution water for the toxicity tests was collected July 14, 1988, from Upper Three Runs . Creek at the north side of a bridge on Road 2-1 on the Savannah River Plant site. The water was filtered with a glass fiber filter and acclimation of the Ceriodaphnia cultures to the creek water started on July 14, 1988. Ceriodaphnia for the definitive acute and chronic tests were cultured in the creek water for approximately three weeks before being used in the toxicity tests. 100% dilution water was used for the control.

2.1.1.1 Acute Toxicity Test Methods (Shealy Environmental)

Acute bioassay test methods conformed to those described in Reference 11. All organisms used in the toxicity tests were from Shealy Environmental Services, Inc. in-house cultures which were obtained from the US EPA Newtown Laboratory April 20, 1987 (Lab ID No.87-27. Ceriodaphnia from in-house cultures were identified and preserved monthly. A standard toxicant test with the EPA reference toxicant cadmium chloride (Lab ID. No. 88-964) was performed on Ceriodaphnia cultured in water from Upper Three Runs Creek in conjunction with the acute and chronic tests. The results of this test (LC_{50} = 0.09 mg/L cadmium chloride) demonstrated that the condition of the culture was within the acceptable range for test organisms (0.056-0.19 mg/L). Test solutions and the controls were prepared in 100 ml quantities in all-glass test chambers. All concentrations and the control were tested in duplicate with ten Ceriodaphnia dubia neonates (2-24 hours old) each. The test solutions were renewed after 24 hours.

A 100 mg/L uranyl nitrate stock solution was prepared on August 4, 1988, using reagent grade uranyl nitrate, by rapidly weighing 0.0101 grams of the chemical onto a tared weighing paper in a balance containing desiccant. All uranyl nitrate test concentrations were prepared fresh daily from the 100 mg/L stock solution by dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to ± 1%) except for the 1 mg/L concentration which was made up using a 1 ml Class A volumetric pipet. The uranyl nitrate stock solution was stored at 4°C during testing. Samples of all test solutions were preserved with 0.15% metals grade nitric acid and shipped with ice packs via Federal Express to AnalytiKEM, Inc. for verification.

The 48-hour acute toxicity test was conducted August 10-12, 1988, with the following solutions:

uranyl nitrate concentration	theoretical uranium concentration
1.0 mg/L $UO_2(NO_3)_2 \cdot 6H_2O$	0.47 mg/L
0.56 mg/L	0.27 mg/L
0.32 mg/L	0.15 mg/L
0.18 mg/L	0.085 mg/L
0.10 mg/L	0.047 mg/L

Dissolved oxygen, water temperature, pH, conductivity, alkalinity and total hardness measurements were made in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers. The test organisms were placed singly in the test vessels each containing 100 ml of solution. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia. Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding.

2.1.1.2 Chronic Toxicity Bioassay Methods (Shealy Environmental)

Test methods conformed to those described in Reference 12. The 7 day chronic toxicity bioassay was performed as eight treatments exposing 10 female test organisms each. The first treatment was the control (100% filtered Upper Three Runs water). The uranyl nitrate solutions were 0.0032 mg/L, 0.0056 mg/L, 0.010 mg/L, 0.018 mg/L, 0.032 mg/L, 0.056 mg/L and 0.10 mg/L . All test solutions were prepared from the same 100 mg/L stock solution as the acute test dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to ± 1%). The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

Dissolved oxygen, water temperature, pH, conductivity, total hardness and alkalinity measurements were made in conjunction with the tests. Temperature was maintained at 25°C (± 1° C) in all test chambers during the test.

The test organisms were placed singly in test vessels each containing 15 ml of solution. The organisms were between 20 and 24 hours old at the start of the test. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at the rate of approximately 1,000,000 cells per ml. test solution per day. Selenastrum cultures were obtained from Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged Erlenmeyer flasks and maintained under bright fluorescent lighting for 6 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and order of feeding was established based on random number tables.

The uranyl nitrate solution concentrations were.

0.0032, 0.0056, 0.010, 0.018, 0.032, 0.056, and 0.100 mg/L (theoretical uranyl nitrate).

The percent uranium recovery (total measured/total theoretical) was lower than anticipated (15 to 60% recovery) at low uranium concentrations (Attachment V, Table 8).

The laboratory was therefore requested to provide documentation showing acceptable (i.e., greater than 80%) analytical recovery for uranium in the range of 50-1 parts per billion (ppb) as total uranium, in order to provide confidence in subsequent uranium testing[13]. The instrument detection limit (IDL) study was performed using Inductively Coupled Plasma - Atomic Emission Spectrophotometry (ICP), using a 200 ppb standard prepared by Inorganic Venture, Inc. The IDL was determined to be 30.6 µg/L (3 X the standard deviation on 7 analyses on the 200 ppb standard). The % recovery was demonstrated to be 87 to 101 % from 1 to 50 µg/L (ppb) theoretical uranium concentration samples. The diluted solutions were concentrated 100 to 1000 fold to allow the detection on the ICP instrument at the 1 to 50 µg/L uranium concentrations. The IDL study results are given in Attachment VI.

2.1.2 *Repeat Acute and Chronic Toxicity Testing of Uranyl Nitrate*

At the conclusion of the initial acute and chronic toxicity testing on uranyl nitrate, it was decided to:
1) Repeat the test work on uranyl nitrate to confirm the initial results,
2) Add additional uranium compounds to the test matrix, which were more representative of the chemical form in which the uranium is released to the surface streams, and
3) Add a separate analytical laboratory to perform independent confirmation toxicity tests.

The "Scope of Work" for the repeat uranyl nitrate testing and the hydrogen uranyl phosphate and uranium dioxide testing is given in Attachment VII.

2.1.2.1 Acute Toxicity Test Methods (Shealy Environmental)

The test conditions and results for the repeat tests by Shealy Environmental are shown in detail in Attachment VIII, "Test Report No. A17852 (Part I) , Acute and Chronic Toxicity of Uranyl Nitrate to Ceriodaphnia Dubia." AnalytiKEM Inc., Cherry Hill, NJ 08003 (April 11, 1989).

The 48-hour acute toxicity test was conducted January 25-27, 1989. The repeat acute test conditions were the same as the initial test, with the following exceptions:

- A plankton net (37 mm) was used to filter Upper Three Runs dilution water, rather than a glass fiber filter.
- Only Upper Three Runs water less than 96 hours old was used for the control and dilution toxicity tests.
- 100 ml vs. 250 ml beakers were used as the test vessels
- The volume of test solution was 50 ml vs. 100 ml previously
- A new 100 mg/L uranyl nitrate stock solution was prepared (January 25, 1989)

The same uranyl nitrate concentrations were used in the repeat test as in the initial acute test:

uranyl nitrate concentration	theoretical uranium concentration
1.0 mg/L $UO_2(NO_3)_2 \cdot 6H_2O$	0.47 mg/L U
0.56 mg/L	0.27 mg/L U
0.32 mg/L	0.15 mg/L U
0.18 mg/L	0.085 mg/L U
0.10 mg/L	0.047 mg/L U

2.1.2.2 Chronic Toxicity Bioassay Methods (Shealy Environmental)

The chronic test conditions in the repeat test were the same as the initial test, except for the following:

- Filtration of the U3R water samples with 37 mm plankton net
- Uranium stock solution (103 mg/L uranyl nitrate, prepared 2/9/89)
- The uranyl nitrate solution concentrations were the same as the initial test: 0.0032, 0.0056 , 0.010, 0.018, 0.032, 0.056, and 0.100 mg/L as uranyl nitrate .

2.1.3 *Confirmation of Acute and Chronic Toxicity Testing on Uranyl Nitrate*

Normandeau Associates, Inc., Southeast (NAI-SE) was the second laboratory requested to confirm the acute and chronic toxicity testing results for uranyl nitrate, and to perform additional tests on hydrogen uranyl phosphate and uranium dioxide. The NAI-SE laboratory and toxicity testing methods for all three uranium compounds are described in detail in Appendix IX: K. E. Trapp and E. T. Korthals to John Pickett and J. L. Keyes, "Acute and Chronic Toxicity of Three Uranium Compounds to Ceriodaphnia dubia", Report No. NA-SR-98, Normandeau Associates, Southeast, Aiken, SC 29802 (June, 1989).

2.1.3.1 Laboratory Methods and Quality Assurance (Normandeau Associates)

The guidelines and recommendations listed in Peltier and Weber[11] and Horning and Weber[12] were followed for handling organisms, cleaning test equipment, and conducting all toxicity testing.

Ceriodaphnia dubia used in Normandeau Associates Inc., South East (NAI-SE) toxicity tests were originally obtained from cultures maintained by the US EPA Environmental Research Laboratory in Duluth, MN. These animals were cultured by the NAI-SE aquatic toxicology laboratory in water collected from Upper Three Runs Creek. Water was collected at the Road 2-1 bridge on the SRS and filtered through a plankton net prior to use. Typical water quality values for this creek are listed in Appendix IX, Table 1-1.

All-glass (1.5 L) culture dishes served as culture chambers for a "brood" stock. The dishes were thoroughly cleaned prior to use and were covered while in use to prevent the entry of dust and other contaminants. Cultures were kept in an incubator (Lab-Line Instruments, Inc., Melrose Park, IL), and temperatures maintained at 25 $\pm$ 2°C. Water temperature was monitored continuously.

Wide-spectrum fluorescent bulbs (Color Rendering Index $\geq$ 90) were used to provide a 16L:8D photo period. Light intensity measured at the surface of the culture dishes did not exceed 800 lux.

Brood-stock C. dubia (30 organisms/culture dish) were fed every other day on a diet consisting of a mixture of algae (Selenastrum capr[illegible]utum), and YCT (yeast, cerophyll, fermented trout chow). Approximately 1 x 10^8 cells/ml of [illegible]e and 7 ml of YCT were added to each culture dish. A modified version of Bold's Basi[illegible]edia was used to maintain uni-algal cultures of S. capricornutum.

All culture dishes were examined at least three times per week, and quality assurance records were maintained for each dish. Records included: date the culture was started, source of culture material, reproductive progress, presence of ephippia, and other information on the condition of the culture deemed pertinent by the observer. The animals in these dishes served as the source of neonate ($\leq$ 24 hr old) daphnids used in both acute and chronic toxicity tests. The first broods were discarded; only neonate daphnids obtained from broods other than a first brood were used in the toxicity tests.

Water from Upper Three Runs served as the control and diluent for both the acute and chronic toxicity tests conducted on $UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2. New samples of water were collected once every 72 hr. Water was not filtered prior to use in the acute toxicity tests, but was filtered through a plankton net for use in the chronic tests. Filtration will remove potential predators from the diluent and is recommended by Horning and Weber[12].

Glassware Preparation All glassware was cleaned before and after use. It was soaked for 24 h in a 5% Contrad solution, rinsed with tap water, allowed to air-dry, and rinsed with pesticide-free acetone. The glassware was again air-dried and then soaked for 24 hr in 2% HNO_3. De-ionized water was used in the final rinses (5 times with de-ionized water) of the glassware. All borosilicate beakers used in the toxicity tests were maintained separately

from other laboratory glassware and were used only for toxicity tests. Just prior to use, these beakers were rinsed with dilution water.

Preparation of Stock Solutions Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The concentration of total uranium was confirmed analytically before each stock solution was used in a test. With the exception of $HUO_2PO_4 \cdot 4H_2O$, these solutions were prepared in sufficient quantities so that the same stock solution was used for both the acute and chronic toxicity tests.

Concentrations of total uranium (dissolved plus bound uranium) were determined using either inductively coupled plasma emission spectroscopy (EPA method 200.7[14]) or fluorometry (Method 711-B; APHA 1985[15]).

Uranyl Nitrate Reagent grade uranyl nitrate (Mallinckrodt Lot #8640 KCAP) was used to prepare the stock solution of this compound. The stock solution was prepared by adding $UO_2(NO_3)_2 \cdot 6H_2O$ to Upper Three Runs Creek water. The stock solution was measured to determine the concentration of total uranium in the "as made" stock solution of uranyl nitrate. The concentration of total uranium in this stock solution equaled 43.2 mg/L. This stock solution was used to prepare all $UO_2(NO_3)_2 \cdot 6H_2O$ solutions used in the range-finding, acute, and chronic tests.

Quality Assurance Quality assurance procedures commonly followed in the NAI-SE Aquatic Toxicology Division included the following:

1. Instruments were routinely calibrated and standardized according to manufacturers' instructions. Control charts were maintained for all measured parameters.

2. Wet chemistry methods used in determining hardness and alkalinity were standardized according to US EPA methods.

3. Records were maintained of the age, productivity, quality of food, and feeding regime of all organisms maintained by NAI-SE.

4. Reference toxicity tests were performed on a routine basis (at least monthly) to determine the acceptability and sensitivity of test organisms. Reference toxicant control charts were maintained for all test organisms cultured by NAI-SE. Results of reference tests indicated that the animals used in these tests responded in an appropriate manner.

5. In order to measure the precision with which the technician prepared these test solutions, a surrogate metal was used to prepare solutions in a manner identical to that used during the toxicity tests. Manganese was chosen as a surrogate metal for uranium because of its low analytical cost and because it is routinely used as a standard to check the ICP instrument. A HACH manganese standard (1000 mg/L) was diluted with deionized water using volumetric glassware and calibrated pipettes. A subsample of each prepared manganese solution was analyzed to determine the concentration of total manganese. Results of the manganese analyses are summarized in Attachment IX, Table 2-3. These results demonstrated that the technician responsible for preparing the solutions used in all toxicity tests conducted on the three uranium compounds prepared the manganese solutions such that the percent recovery of manganese ranged from 91.8 to 97.4%.

2.1.3.2 Acute Toxicity Test Methods (Normandeau Associates)

All test solutions for the acute static renewal toxicity tests were prepared daily. Test solutions were prepared by diluting the chemical stock solutions. Aliquots of chemical stock solutions were transferred to 500-ml volumetric flasks with calibrated volumetric pipettes or pipetters. The contents of the flasks were then adjusted to 500 ml with Upper Three Runs Creek water. Separate volumetric flasks were used to prepare tests solution of each uranium compound. Test solutions were prepared from the lowest to the highest nominal concentration of total uranium using the same volumetric flask. The volumetric flasks were then cleaned (as described above) each day before use.

C. dubia were exposed to the following dilution series for uranyl nitrate:

0 (control), 0.051, 0.127, 0.190, 0.254, 0.381 mg/L total uranium (nominal values)*

* Nominal concentrations based on dilution of measured stock solutions

Borosilicate beakers (250-ml) served as test chambers for the acute static renewal toxicity tests. Two beakers were used per test concentration, with 10 individuals per beaker. A large-bore, fire-polished, glass pipette was used to randomly transfer 10 neonate ($\leq$ 24 h old) daphnids to each test chamber. When 10 individuals had been isolated, excess water was removed and 100 ml of test solution was slowly and gently poured into the beaker. Following the addition of solution, the daphnids were observed to verify they had not been damaged during transfer.

The test temperature for the C. dubia acute static renewal toxicity tests was 25 $\pm$ 2°C. The tests were conducted in a temperature-controlled, Fisher model 307 incubator. Test organisms were exposed to a 16L:8D photo period. Specific conductance, dissolved oxygen concentration, $CaCO_3$ hardness, total alkalinity, and pH of the control and highest test concentrations were recorded at the beginning of each test and at 24 h intervals. The dissolved oxygen concentration, pH, temperature, and conductivity of intermediate test concentrations were measured and recorded at test initiation and at 24 h. Total alkalinity was determined by potentiometric titration[15], while the CDTA (cyclohexanediaminetetraacetic acid) titrimetric method[15] was used to measure $CaCO_3$ hardness. Dissolved- oxygen concentrations were measured with a YSI Model 58 DO meter (Yellow Springs Instrument Co., Yellow Springs, OH), and a YSI Model 33 conductivity meter was used to measure the conductivity of each test solution. The pH values were determined with an Orion 399A pH meter.

Death or immobilization of the C. dubia were used as the indicators of acute toxicity. The criterion used to establish lethality was cessation of all visible signs of mobility (e.g., no movement of second antennae, thoracic legs, or post abdomen). Immobilization was defined as the inability of the animals to move in the water column .

The 48 h LC_{50} values were determined by using either binomial probability or the Trimmed Spearman-Karber procedure.

2.1.3.3 Chronic Toxicity Test Methods (Normandeau Associates)

Organisms used in these tests were $\leq$ 24 hr old, and all organisms used in a given test were born within 4 hr of one another. All test solutions for the three chronic toxicity tests were prepared daily. Test solutions of uranyl nitrate and hydrogen uranyl phosphate were prepared in 500-ml volumetric flasks by diluting each chemical stock solution. Uranium dioxide test solutions were prepared in a 1000-ml volumetric flask by diluting a secondary stock solution. The secondary stock was prepared from the original stock solution and used throughout the chronic toxicity test. To prepare chronic test solutions, aliquots of chemical stock solutions were transferred to volumetric flasks with calibrated pipettes or pipetters. Each flask was brought to volume with Upper Three Runs water. A

separate volumetric flask was used to prepare test solutions for each uranium compound throughout each test. Test solutions were prepared from the lowest to the highest nominal concentration of total uranium and the volumetric flasks cleaned each day between use.

Testing was performed in 20-ml glass scintillation vials containing 15 ml of test solution. All test vials were placed in an incubator maintained at 25 ± 1°C. Temperature was monitored continuously. Test organisms were exposed to a 16L:8D photo period. Twenty individuals were exposed to each test concentration and to the control.

The following dilutions were used in the seven-day static renewal life cycle tests conducted on uranyl nitrate:

0 (Control), 0.002, 0.008, 0.023, 0.046, 0.076 mg/L total uranium (nominal)*

* Nominal concentrations based on dilution of measured stock solutions

Large-bore, fire-polished, disposable glass pipettes were used to transfer organisms. Test organisms were moved to fresh test solution every 24 h, and all young produced during a test were preserved with Lugol's solution for later enumeration. Following transfer, the organisms were observed to verify they had not been damaged.

Specific conductance, dissolved-oxygen concentration (DO), $CaCO_3$ hardness, total alkalinity, and pH were recorded for the new and old control solutions as well as the highest concentrations of test solutions. Only conductivity, DO, pH, and temperature of old, new and intermediate concentrations of test solutions were measured. The same methods used to monitor water quality parameters during the acute static renewal toxicity tests were also used during all C. dubia life cycle tests.

C. dubia were fed during each test by adding an aliquot of algal suspension/YCT mixture (0.033 ml/ml) to each vial. YCT was added to increase the protein content of the diet. The other nutritional requirements of these organisms (e.g., vitamins, dietary lipids, minerals) were provided by the algal portion of the diet.

Death or immobilization of the organisms was used as an indicator of acute toxicity[11]. The criterion used to establish lethality was cessation of all visible signs of mobility (e.g., no movement of second antennae, thoracic legs, or post abdomen). Immobilization was defined as the inability of the animals to move in the water column. On Day 7, adult survival was determined, and a count was made of the total number of young produced per test organism. During any seven day period, C. dubia individuals typically produce three broods of offspring. A test was deemed acceptable if control mortality was $\leq 20\%$ [12] and if the average number of young produced per control individual was ≥ 15.[16] Chronic toxicity was determined to have occurred if statistical analyses determined that significant differences existed between the control and test organisms.

Chronic toxicity test data were analyzed using Fisher's Exact test, the Chi-Square test, Bartlett's test, one-way analysis of variance (ANOVA), and Dunnett's Multiple Comparison.

2.2 Acute and Chronic Toxicity Testing on Hydrogen Uranyl Phosphate

2.2.1 *Initial Acute and Chronic Toxicity Testing on Hydrogen Uranyl Phosphate*

2.2.1.1 Acute Toxicity Laboratory Methods and Procedures (Normandeau Associates)

The laboratory methods and procedures for the acute toxicity tests for hydrogen uranyl phosphate were the same as described previously for uranyl nitrate (Sections 2.1.3.1 & 2.1.3.2).

Preparation of Stock Solutions Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The concentration of total uranium was confirmed analytically before each stock solution was used in a test. Insufficient quantities of hydrogen uranyl phosphate stock solution were initially prepared and another stock solution had to be made prior to initiation of the chronic toxicity test.

Hydrogen Uranyl Phosphate Reagent grade uranyl nitrate (Mallinckrodt Lot #8640 KCAP) and phosphoric acid were used to prepare hydrogen uranyl phosphate. The uranyl nitrate was mixed with phosphoric acid (1:1; moles uranium to moles phosphate). This mixture was neutralized to pH 6-7 with 1.0 N NaOH and stirred for 15 min. The resulting precipitate ($HUO_2PO_4 \cdot 4H_2O$) was filtered through Whatman #4 filter paper, washed three times with de-ionized water, transferred to a watch glass and dried at 105° C for ~16 hr.

Stock solutions of hydrogen uranyl phosphate were prepared by mixing 1 g of compound with 1 L of Upper Three Runs water for approximately 1 hr. The resultant suspension was filtered through a glass fiber filter (Whatman GF/C) and the filtrate used as a stock solution to prepare all toxicity test solutions. The stock solutions were measured to determine the concentration of total uranium in the "as made" stock solutions of hydrogen uranyl phosphate. Measured concentrations of uranium in the stock solutions equaled 1.22 and 3.8 mg/L total uranium. The first stock solution (1.2 mg/L total U) was used in the range-finding and definitive acute toxicity tests. The second stock solution (3.8 mg/L total U) was used in the chronic toxicity test.

C. dubia were exposed to the following dilution series for the acute toxicity tests on hydrogen uranyl phosphate:

0 (control), 0.040, 0.060, 0.080, 0.100, and 0.120 mg/L, total uranium (nominal values)*

* Nominal concentrations based on dilution of measured stock solutions.

2.2.1.2 Chronic Toxicity Laboratory Methods and Procedures (Normandeau Associates)

The laboratory methods and procedures for the chronic toxicity tests for hydrogen uranyl phosphate were the same as described previously for uranyl nitrate.(Section 2.1.3.3).

The following dilutions were used in the seven-day static renewal life cycle tests on hydrogen uranyl phosphate:

0 (Control), 0.006, 0.02, 0.06, 0.12, 0.20 mg/L total uranium*

* Nominal concentrations based on dilution of measured stock solutions

2.2.1.3 Acute Toxicity and Laboratory Methods and Procedures (Shealy Environmental)

The Shealy Environmental laboratory and toxicity testing methods for the hydrogen uranyl phosphate tests are described in detail in Attachment X, "Test Report No. A17852 (Part II), Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia". AnalytiKEM Inc., Cherry Hill, NJ 08003 (July, 1989).

The laboratory and acute toxicity testing procedures were the same as a previously described for Shealy Environmental (Sections 2.1.1.1), with the following exceptions:

Dilution water for the toxicity tests was collected January 23 through February 16, 1989 from Upper Three Runs Creek at the north side of a bridge on Road 2-1 on the Savannah River Site. The water was filtered with a plankton net, and only water less than 96 hr old was used for the toxicity tests. Ceriodaphnia for the acute and chronic tests had been cultured in the creek water since October 25, 1988. 100% dilution water was used for the control.

A standard toxicant test with the EPA reference toxicant cadmium chloride (Lab. ID. No. 88-964) was performed on Ceriodaphnia cultured in water from Upper Three Runs in conjunction with the acute and chronic tests. The results of this test (LC_{50} = 0.08-0.17 mg/L cadmium chloride) demonstrated that the condition of the culture was within the acceptable range for test organisms (0.059 - 0.199 mg/L).

The hydrogen uranyl phosphate (HUP) was prepared as recommended in Attachment VII, by mixing uranyl nitrate and phosphoric acid at a 1 mole uranium to 1 mole phosphate ratio, and then neutralizing to pH 6-7 with sodium hydroxide. The precipitate was stirred for 15 minutes, and then filtered through #40 Whatman filter paper. The compound was rinsed three times with deionized water and dried overnight at 105°C.

A 104 mg/L HUP stock solution was prepared on 2/17/89 for the acute test, by weighing 0.0104 gm HUP into 100 ml water. The solution was stirred for 5 minutes, allowed to settle, and the aliquots for the test concentrations were drawn off of the top of the stock solution using Class A volumetric pipettes. Samples of all test solutions were preserved with 0.15% metals grade nitric acid and shipped with ice packs via Federal Express to AnalytiKEM, Inc. for verification.

The 48-hour acute toxicity test was conducted February 17-19, 1989, with the following hydrogen uranyl phosphate concentrations:

0.32, 0.56, 1.0, 1.8, and 3.2 mg/L (as theoretical hydrogen uranyl phosphate).

2.2.1.4 Chronic Toxicity Bioassay Methods (Shealy Environmental)

The 7-day chronic toxicity assay was performed February 9-16, 1989, as seven treatments exposing 10 female test organisms each. The laboratory chronic toxicity testing procedures were the same as a previously described for Shealy Environmental (Section 2.1.1.2). The first treatment was the control (100% filtered Upper Three Runs water). The hydrogen uranyl phosphate solution concentrations were 0.056 mg/L, 0.10 mg/L, 0.18 mg/L, 0.32 mg/L, 0.56 mg/L, and 1.0 mg/L (as hydrogen uranyl phosphate). All test solutions were prepared from stock hydrogen uranyl phosphate solutions prepared daily by dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to $\pm$ 1%). The HUP stock solutions were prepared in the same manner as the acute test with each new solution being stirred for 5 minutes and the precipitate being allowed to settle for 30 minutes. The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

2.2.2 *Repeat Acute and Chronic Toxicity Testing of Hydrogen Uranyl Phosphate*

2.2.2.1 Laboratory Methods and Quality Assurance (Normandeau Associates)

The laboratory methods and Quality Assurance procedures used by Normandeau Associates for the repeat HUP testing were the same as the initial tests, with the following exceptions:

Water from Upper Three Runs served as the water for the culture of the Ceriodaphnia dubia and as control and dilutant for both the repeat acute and chronic toxicity tests conducted on $HUO_2PO_4 \cdot 4H_2O$. Typical water quality values for this creek are listed in Attachment XII, Table 1-1. Water for all toxicity tests was collected from the Road 2-1 bridge located on SRS. Water was collected the day each test was initiated and was used within 72 hr of collection. New samples of water were collected once every 72 hr. Water was not filtered prior to use in the acute toxicity tests, but was filtered through a plankton net for use in the chronic tests.

Glassware Preparation All glassware was cleaned before and after use. Glassware was first rinsed with pesticide-free acetone, then with methanol followed by methylene chloride. It was soaked for 24 hr in a 5% Contrad solution and rinsed with deionized water. It was air-dryed, and then soaked for 24 hr in 2% HNO_3. Deionized water was then used in the final rinses (5 times) and rinsed with pesticide-free acetone. All borosilicate beakers used in the toxicity tests were maintained separately from other laboratory glassware and were used only for toxicity tests. Just prior to use, these beakers were rinsed with dilution water.

Preparation of Stock Solutions Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The same hydrogen uranyl phosphate stock solution was used for both the acute and chronic tests. The concentration of the dissolved uranium in the stock solution was confirmed analytically before it was used in a test.

Hydrogen Uranyl Phosphate Reagent grade uranyl nitrate (Mallinckrodt Lot #8640 KCAP) and phosphoric acid were used to prepare hydrogen uranyl phosphate. The uranyl nitrate was mixed with phosphoric acid (1:1 moles uranium to moles phosphate). This mixture was neutralized to pH 6-7 with 1.0 N NaOH and stirred for 15 min. The resulting precipitate ($HUO_2PO_4 \cdot 4H_2O$) was filtered through Whatman #4 filter paper, washed three times with deionized water, transferred to a watch glass and dried at 105° C for approximately 16 hr.

A saturated stock solution of hydrogen uranyl phosphate were prepared by mixing 0.4 gm of compound with 19 L of Upper three Runs water for approximately 16 hr. The resultant suspension was filtered through a 0.45 µm polycarbonate filter (Millipore Corp., Bedford, MA.). The filtrate was analyzed to determine the amount of dissolved uranium. The measured concentration of uranium in the stock solution equaled 0.26 ± 0.02 mg/L uranium. The filtrate used as a stock solution to prepare all toxicity test solutions.

Quality Assurance The Quality Assurance procedures used by Normandeau Associates were same as previously described (Sec. 2.1.3.1)

2.2.2.2 Acute Toxicity Test Methods (Normandeau Associates)

The acute toxicity procedures used for the repeat HUP testing were the same as the initial tests (Section 2.1.3.2), with the following exceptions:

The 48 hr static tests were performed with and without food added to the test solutions. The unfed acute test was conducted June 29 - July 1, 1989. The fed acute test was conducted July 11 - July 13, 1989.

All test solutions for the acute static renewal toxicity tests were prepared daily. Test solutions were prepared by diluting the HUP stock solution. The undiluted stock solution served as the highest test concentration.

C. dubia were exposed to the following dilution series of hydrogen uranyl phosphate in both acute toxicity tests (fed and un-fed):

0 (control), 0.10, 0.13, 0.16, 0.20, 0.23 and 0.26 mg/L uranium (nominal U concentration).

Graduated cylinders and a variable pipetter were used to transfer aliquots of chemical stock solution to 500 ml volumetric flasks. The flask contents were then adjusted to 500 ml with U3R water. Test solutions were prepared from the lowest to the highest nominal concentration if dissolved uranium using the same volumetric flask.

In the acute toxicity test with fed organisms, a mixture of algal suspension and yeast - trout chow -cerophyll was added at a final concentration of 0.033 ml/ml test solution. Organisms were fed at test initiation and after 24 hr.

The 48 h LC_{50} values were determined by the Trimmed Spearman-Karber procedure.

2.2.2.3 Chronic Toxicity Test Methods (Normandeau Associates)

The chronic toxicity procedures used for the repeat HUP testing were the same as the initial tests (Section 2.1.3.3), with the following exceptions:

Testing was performed in 20-ml cups (Solo Corp.) containing 15 ml of test solution.

The following dilutions were used in the seven-day static renewal life cycle tests conducted hydrogen uranyl phosphate:

0 (Control), 0.0002, 0.0006, 0.002, 0.006, 0.020, 0.060, 0.120, and 0.200 mg/L dissolved uranium*.

* Nominal concentrations based on dilution of measured stock solutions

2.2.2.4 Acute Toxicity Test Methods (Shealy Environmental)

The Shealy Environmental laboratory and toxicity testing methods for the repeat hydrogen uranyl phosphate tests are described in detail in Attachment XIII, "Test Report No. 80084 Final, Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia." AnalytiKEM Inc., Rock Hill, SC 29730 (January 5, 1990).

The laboratory methods and procedures used by Shealy Environmental for the repeat hydrogen uranyl phosphate acute toxicity testing were the same as the initial HUP tests, with the following exceptions:

Dilution water for the toxicity tests was collected August 9, 1989 from Upper Three Runs at the north side of a bridge on Road 2-1 on the Savannah River Plant site. Only one batch of water was used for the acute and chronic toxicity tests due to the appearance of sporadic toxicity in the U3R water.

Standard toxicant test with the EPA reference toxicant cadmium chloride (Lab. ID. No. 88-964) are performed twice monthly on Ceriodaphnia cultured in water from Upper Three Runs Creek in conjunction with the acute and chronic tests. The results of this test demonstrated that the condition of the culture was within the acceptable range for test organisms (Central tendency = 0.138 ppm, Upper Limit = 0.25 ppm, Lower Limit = 0.03 ppm).

The hydrogen uranyl phosphate (HUP) was prepared by Shealy as recommended in Attachment XI, by mixing uranyl nitrate and phosphoric acid at a 1 mole uranium to 1 moles phosphate ratio, and then neutralizing to pH 6-7 with sodium hydroxide. The precipitate was stirred for 15 minutes, and then filtered through #40 Whatman filter paper. The compound was rinsed three times with deionized water and dried overnight at 105°C. Shealy sent the dried compound to AnalytiKEM, Inc. for preparation of a uranium stock solution.

A 1.05 mg/L HUP stock solution was prepared AnalytiKEM, Inc. by filtering a 2000 mg/L solution of HUP through a 0.45 µm filter, and then returned to Shealy. The toxicity test concentrations were prepared by dosing the dilution water with the appropriate aliquot of the uranium stock solution using Class A volumetric filters. After all testing was completed by Shealy, the uranium stock solution was returned to AnalytiKEM, Inc., for verification of the concentration. The uranium content was verified on September 29, 1989 to be 1.00 mg/L uranium.

The 48-hour acute toxicity test was conducted August 17-19, 1989, with the following hydrogen uranyl phosphate concentrations:

0.10, 0.15, 0.20, 0.25, and 0.30 mg/L as theoretical uranium.

2.2.2.5 Chronic Toxicity Bioassay Methods (Shealy Environmental)

The laboratory methods and procedures used by Shealy Environmental for the repeat HUP chronic toxicity testing were the same as the initial tests, with the following exceptions:

The 7-day chronic toxicity assay was performed September 2-9, 1989, as six treatments exposing 10 female test organisms each. The first treatment was the control (100% filtered Upper Three Runs water). The hydrogen uranyl phosphate solution concentrations were 0.020 mg/L, 0.035 mg/L, 0.050 mg/L, 0.065 mg/L, and 0.080 mg/L, as theoretical uranium.

2.3 Acute and Chronic Toxicity Testing on Uranium Dioxide

2.3.1 *Acute Toxicity Laboratory Methods and Procedures (Normandeau Associates)*

The laboratory methods and procedures for the acute toxicity tests for uranium dioxide by Normandeau Associates were the same as described previously for uranyl nitrate (Sections 2.1.3.1 & 2.1.3.2).

Preparation of Stock Solutions Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The concentration of total uranium was confirmed analytically before each stock solution was used in a test. These solutions were prepared in sufficient quantities so that the same stock solution was used for both the acute and chronic toxicity tests.

Uranium Dioxide Uranium dioxide (UO_2) used in this study was provided by the Department of Energy, Savannah River Office. The sample consisted of a liquid overlying a layer of solid material that had settled on the bottom of the container. The liquid portion was decanted and filtered through a glass fiber filter (Whatman GF/C). The fine particulates remaining in the filtrate were allowed to settle. A pipette was used to transfer the solution without re-suspension of the particulate material. This solution served as the UO_2 stock solution for the acute and chronic toxicity tests. The stock solution was measured to determine the concentration of total uranium in the "as made" stock solutions of uranium dioxide. Measured concentration of uranium in this solution equaled 114 mg/L total uranium.

C. dubia were exposed to the following dilution series for uranium dioxide for the acute toxicity tests:

0 (control), 0.01, 0.04, 0.07, 0.10, 0.13 mg/L total uranium (nominal values)*

* Nominal concentrations based on dilution of measured stock solutions.

2.3.2 *Chronic Toxicity Testing Methods (Normandeau Associates)*

The chronic toxicity procedures used for the uranium dioxide testing were the same as the chronic tests on uranyl nitrate (Section 2.1.3.3), with the following exceptions:

The following dilutions were used in the seven-day static renewal life cycle tests conducted on uranium dioxide:

0 (Control), 0.0015, 0.005, 0.015, 0.03,, 0.05 mg/L total uranium*

* Nominal U concentrations based on dilution of measured stock solutions.

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3.0 TOXICITY TEST RESULTS

3.1 Acute and Chronic Toxicity Test Results on Uranyl Nitrate

3.1.1 *Initial Acute and Chronic Toxicity Tests on Uranyl Nitrate.*

3.1.1.1 Initial Acute Toxicity Test Results (Shealy Environmental)

The results of the initial Shealy 48-hour acute toxicity bioassay are given in Table 1. The acute toxicity was determined vs. the concentration of total uranium recovered from the analyzed uranyl nitrate solutions. Actual recovered and theoretical uranium concentrations were essentially the same at the concentration ranges used in the acute tests. Mortality occurred in the 0.081 mg/L (100% mortality), 0.140 mg/L (100% mortality), 0.290 mg/L (100% mortality) and 0.490 mg/L (100% mortality) recovered uranium concentrations. No mortality occurred in the control or the 0.044 mg/L uranium concentration. These data were used to determine a 48 -hour LC_{50} (median lethal concentration) value with the Binomial Method.[11] This calculation resulted in a 48-hour LC_{50} value of 0.060 mg/L uranium with 95% confidence limits of 0.044 and 0.081 mg/L.

Water chemistry data taken in conjunction with the acute bioassay are given in Attachment V, (Table 5). All parameters monitored were within acceptable limits for bioassay purposes.

3.1.1.2 Initial Chronic Toxicity Bioassay (Shealy Environmental)

The results of the 7-day chronic toxicity test conducted August 5-12, 1988, are given in Table 2. Mortality occurred in the 0.0015 mg/L (10% mortality), 0.0047 mg/L (20% mortality) and 0.0085 mg/L (10% mortality) theoretical uranium concentrations. No mortality occurred in the control. Reproduction in the control averaged 32.9 offspring per female. One male was observed in the 0.027 mg/L and 0.047 mg/L uranium concentrations. Males were not included in calculating the reproduction data as directed by SCDHEC (Mr. Dave Graves, Biological Services Division, personal communication to Shealy Environmental).

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test and homogeneity of variances using Bartlett's Test. Log transformed data were found to be normally distributed (Chi-Square = 6.915, critical value = 13.28) with homogeneous variances (Bartlett's = 8.22; critical value = 12.59). Statistical analyses of the results using Dunnett's Multiple Comparison Procedure indicated chronic toxicity at the 0.0027 mg/L, 0.0047 mg/L, 0.0085 mg/L, 0.015 mg/L, 0.027 mg/L and 0.047 mg/L theoretical uranyl nitrate (as uranium) concentrations (actual recovered uranium concentrations <0.0013, 0.0021, 0.0014, 0.0096, 0.015 and 0.044 mg/L, respectively).

The no observed effect concentration (NOEC) was 0.0015 mg/L uranyl nitrate (as theoretical uranium) (<0.0013 mg/L actual recovered uranium) while the lowest observed effect concentration (LOEC) was 0.0027 mg/L uranyl nitrate (as uranium) (<0.0013 mg/L actual uranium). The chronic value (ChV), taken as the geometric mean of the NOEC and LOEC, was 0.0020 mg/L uranyl nitrate (as theoretical uranium).

Water chemistry data taken in conjunction with the chronic toxicity test are given in Attachment V, Table 7. All parameters monitored were within acceptable limits for bioassay purposes.

Table 1

Initial Acute Toxicity Results on Uranyl Nitrate by Shealy Environmental

Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of uranyl nitrate to Ceriodaphnia dubia. August 10 - 12, 1988. Concentrations in theoretical and actual recovered uranium. Ten test organisms per replicate.

			Number Affected After		%
		Replicate	24 Hours	48 Hours	Mortality
Control		A	0	0	0
		B	0	0	
Theoretical U	Recovered U				
mg/L					
0.047	0.044	A	0	0	0
		B	0	0	
0.085	0.081	A	0	10	100
		B	0	10	
0.15	0.14	A	10	10	100
		B	10	10	
0.27	0.29	A	10	10	100
		B	10	10	
0.47	0.49	A	10	10	100%
		B	10	10	

LC_{50} = 0.060 mg/L U, based on recovered (measured uranium).

Table 2

Initial Chronic Toxicity Results on Uranyl Nitrate by Shealy Environmental

Average reproduction of Ceriodaphnia dubia in the uranyl nitrate solutions was as follows:

		Offspring per female	% Mortality
Control		= 32.9	0
Theoretical U	Recovered U		
mg/L			
0.0015	<0.0013	= 30.3	10
0.0027	<0.0013	= 23.5	0
0.0047	0.0021	= 21.2	20
0.0085	0.0014	= 16.6	10
0.015	0.0096	= 17.5	0
0.027	0.015	= 18.3	0
0.047	0.044	= 15.7	0

NOEC = 0.0015 mg/L U, LOEC = 0.0027 mg/L, ChV = 0.0020 mg/l, theoretical uranium

3.1.2 *Repeat Acute and Chronic Toxicity Testing of Uranyl Nitrate*

3.1.2.1 Repeat Acute Toxicity Test (Shealy Environmental)

The results of the second Shealy 48-hour acute toxicity bioassay are given in Table 3. The acute toxicity was determined vs. the concentration of total uranium recovered from the analyzed uranyl nitrate solutions. Theoretical and recovered uranium concentrations were essentially the same at the concentration ranges used in the acute tests. Mortality occurred in the 0.051 mg/L (15% mortality), 0.088 mg/L (45% mortality), 0.160 mg/L (95% mortality) and 0.270 mg/L (95% mortality) and 0.500 mg/L (100% mortality) recovered uranium concentrations. No mortality occurred in the control. These data were used to determine a 48 -hour LC_{50} (median lethal concentration) value with the Probit Method.[11] This calculation resulted in a 48-hour LC_{50} value of 0.089 mg/L uranium with 95% confidence limits of 0.072 and 0.107 mg/L.

Water chemistry data taken in conjunction with the acute bioassay are given in Attachment VIII, Table 4. All parameters monitored were within acceptable limits for bioassay purposes.

3.1.2.2 Repeat Chronic Toxicity Bioassay (Shealy Environmental)

The 7-day chronic toxicity test was conducted February 9-16, 1989, as seven treatments exposing 10 test organisms each. The results are given in Table 4. Mortality of the adult females occurred in the 0.0015 mg/L (10% mortality), 0.0027 mg/L (10% mortality), 0.0047 mg/L (10% mortality), 0.0085 mg/L (10% mortality) and 0.015 mg/L (20% mortality) 0.0015 mg/L (10% mortality), 0.0047 mg/L (20% mortality) theoretical uranium concentrations. No mortality occurred in the control or the 0.027 theoretical uranium concentration. Reproduction in the control averaged 19.5 offspring per female. One male was observed in the 0.0027 mg/L uranium concentration. Males were not included in calculating the reproduction data as specified by SCDHEC.

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test. Log transformed data were found to be not normally distributed (Chi-Square = 20.91, critical value = 13.28) with homogeneous variances (Bartlett's = 8.22; critical value = 12.59). Statistical analyses of the results using Wilcoxon Rank Sum Test indicated chronic toxicity at the 0.0047 mg/L, 0.0085 mg/L, 0.015 mg/L, and 0.027 mg/L as theoretical uranium concentrations (recovered uranium concentrations 0.0025, 0.0039, 0.0081, 0.016, and 0.036 mg/L, respectively).

The no observed effect concentration (NOEC) was 0.0027 mg/L as theoretical uranium, while the lowest observed effect concentration (LOEC) was 0.0047 mg/L as theoretical uranium. The chronic value (ChV), taken as the geometric mean of the NOEC and LOEC, was 0.0036 mg/L as theoretical uranium.

Water chemistry data taken in conjunction with the chronic toxicity test are given in Attachment VIII, Table 6. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 14 and 15. For those days pH's of less than 6 were recorded for all concentrations and the control.

Table 3

Repeat Acute Toxicity Results on Uranyl Nitrate by Shealy Environmental

Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of Uranyl nitrate to Ceriodaphnia dubia, January 25-27, 1989. Concentrations in uranyl nitrate, theoretical uranium and actual recovered uranium. Ten test organisms per replicate.

Test Concentration			Replicate	Number Affected After 24 Hours	48 Hours	% Mortality
Control			A	0	0	0
			B	0	0	
$UO_2(NO_3)_2 \cdot 6H_2O$	Theoretical U mg/L	Actual Recovered U				
0.10	0.047	0.051	A	0	1	15
			B	0	2	
0.18	0.085	0.088	A	0	4	45
			B	0	5	
0.32	0.15	0.160	A	6	9	95
			B	8	10	
0.56	0.27	0.270	A	5	10	95
			B	8	9	
1.0	0.47	0.500	A	10	10	100
			B	10	10	

LC_{50} = 0.089 mg/L uranium (as recovered/measured uranium)

Table 4

Repeat Chronic Toxicity Results on Uranyl Nitrate by Shealy Environmental

Average reproduction in the uranyl nitrate solutions was as follows:

			Offspring per female	% Mortality
Control			= 19.5	0
$UO_2(NO_3)_2 \cdot 6H_2O$	Theoretical U mg/L	Recovered U		
0.0032	0.0015	0.00033	=19.9	10
0.0056	0.0027	0.0025	= 17.7	10
0.010	0.0047	0.0039	= 9.0	10
0.018	0.0085	0.0081	= 10.7	10
0.032	0.015	0.016	= 8.3	20
0.056	0.027	0.036	= 6.9	0

NOEC = 0.0027 mg/L, LOEC = 0.0047 mg/L, ChV = 0.0036 mg/L (as theoretical uranium)

3.1.3 *Confirmation Acute and Chronic Toxicity Testing on Uranyl Nitrate*

3.1.3.1 Confirmation Acute Toxicity Test on Uranyl Nitrate (Normandeau Associates)

The results of the 48-hour static renewal acute toxicity test conducted November 21-23, 1988 on uranyl nitrate are given in Table 5. The acute toxicity was determined vs. the nominal (theoretical) concentration of total uranium in the uranyl nitrate solutions. Theoretical and actual recovered uranium concentrations were essentially the same at the concentration ranges used in the acute tests. Significant mortality occurred in the four highest test concentrations in the initial 24 hr.. At 48 hours, partial mortality (15%) was observed at the lowest concentration (0.051 mg/L) and complete mortality was observed at all other test concentrations (0.127, 0.190, 0.254, and 0.381 mg/L) nominal uranium concentrations. Five % mortality occurred in the control. These data were used to determine a 48 -hour LC_{50} (median lethal concentration) value using a Binomial probability method of calculation.

This resulted in a 48-hour LC_{50} value of 0.071 mg/L uranium with 95% confidence limits of 0.051 and 0.127 mg/L, nominal (theoretical) uranium.

Water chemistry data taken in conjunction with the acute bioassay are given in Attachment IX to this report. (Appendix 2, Table 1)

3.1.3.2 Confirmation Chronic Toxicity Test on Uranyl Nitrate (Normandeau Associates)

The 7-day chronic toxicity test was conducted December 9-16, 1988. The results are given in Table 6. Mortality of the adult females occurred in the 0.002 mg/L (35% mortality), 0.008 mg/L (10% mortality), 0.023 mg/L (5% mortality), 0.046 mg/L (5% mortality) and 0.076 mg/L (10 mortality) theoretical uranium concentrations. Five % mortality occurred in the control. Reproduction in the control averaged 15.5 offspring per female.

Mortality was relatively high among the original females exposed to the 0.002 mg/L nominal uranium concentration. Although it is not known why the mortality was so high at this test concentration, it is believed that this response was unrelated to the uranium exposure. The results of the exposure to the 0.002 mg/L uranium were not considered in further analysis of these test results.

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test and Bartlett's Test. The results were found to be normally distributed and the variances were homogenous (Chi-Square = 9.16, critical value = 13.28: Bartlett's = 2.36; critical value = 9.49). Statistical analyses of the results using Dunnett's multiple comparison test indicated that exposure of C. dubia to concentrations equal to or greater than 0.008 mg/L total uranium resulted in a significant difference in production of young compared to the control.

It was recommended that:
No Observed Effect Concentration (NOEC) was <0.008 mg/L and,
Lowest Observed Effect Concentration (LOEC) was 0.008 mg/L as theoretical uranium.

The theoretical/nominal (as prepared) uranium concentrations were utilized for the determination of the NOEC and LOEC.

Water chemistry data taken in conjunction with the chronic toxicity test are given in Attachment IX, Appendix 3, Tables 1 and 3 to this report. The statistical analyses are detailed in Attachment IX.

Table 5

Confirmation Acute Toxicity Results on Uranyl Nitrate by Normandeau Associates

Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of Uranyl nitrate to Ceriodaphnia dubia. November 21-23, 1988. Twenty test organisms were exposed to each concentration, with two tests per concentration. (10 organisms per replicate)

Test Concentration		Number Affected After 24 Hours	48 Hours	% Mortality
Control		0	1	5
	mg/L			
Theoretical U	Recovered U			
0.051	0.042	0	3	15
0.127	0.117	19	20	100
0.190	0.219	20	--	100
0.254	0.325	20	--	100
0.381	0.322	20	--	100

LC_{50} = 0.071 mg/l U (as theoretical uranium)

Table 6

Confirmation Chronic Toxicity Results on Uranyl Nitrate by Normandeau Associates

Static life cycle test, performed on Ceriodaphnia dubia, December 9-16, 1988. 20 females per test concentration. Average reproduction in the uranyl nitrate solutions was as follows:

		Offspring per female	% Mortality
Control		15.5	5
mg/L			
Nominal U	Measured U		
0.002	0.002	9.70	35
0.008	0.007	11.40	10
0.023	0.020	12.55	5
0.046	0.039	8.65	5
0.076	0.064	5.05	10

NOEC = <0.008 mg/L U, LOEC = 0.008 mg/L U (as theoretical uranium).

3.2 Acute and Chronic Toxicity Testing of Hydrogen Uranyl Phosphate

3.2.1 *Initial Acute and Chronic Test Results on Hydrogen Uranyl Phosphate*

3.2.1.1 Acute Toxicity Test Results on Hydrogen Uranyl Phosphate (Normandeau Associates)

The acute toxicity of $HUO_2PO_4 \cdot 4H_2O$ to C. dubia was determined. The results of initial and final basic water chemistry analyses performed on all solutions used in this acute test are listed in Attachment IX, Appendix 2, Table 2.

No test organism mortality was observed in any of the test concentrations of hydrogen uranyl phosphate after 24 hr of exposure (Table 7). Partial mortality was observed at all test concentrations 48 hr following test initiation. A 48 h LC_{50} of 0.110 mg/L nominal uranium was calculated from these data, using a Trimmed Spearman-Karber calculation. (95% confidence limits = 0.10 to 0.12 mg/L nominal uranium).

3.2.1.2 Chronic Toxicity Test Results on Hydrogen Uranyl Phosphate (Normandeau Associates)

Test results are given in Table 8. The concentration of total uranium was measured daily in the next to the highest test solution of hydrogen uranyl phosphate (0.120 mg/L nominal uranium concentration) used in the seven-day chronic test resulted in a measured average of 0.119 mg/L, with a standard deviation of 0.020. The daily uranium analyses are given in Attachment IX, Table 3-2. The results of all initial and final basic water chemistry analyses performed on all $HUO_2PO_4 \cdot 4H_2O$ solutions are listed in Attachment IX, Appendix 3, Tables 3 and 4.

Some mortality was observed among C. dubia exposed to all test concentrations of $HUO_2PO_4 \cdot 4H_2O$ used in the chronic test (Table 8). Both brood and young production were reduced among test organisms as compared to the control individuals. These data suggested that exposure to all of the concentrations of total uranium in the form of $HUO_2PO_4 \cdot 4H_2O$ adversely affected C. dubia.

Fisher's Exact test was used to analyze adult survival data. The results of this test indicated no significant difference existed in the percent survival among C. dubia exposed to any of the solutions used in this chronic test. Offspring production by individuals exposed to all test concentrations of $HUO_2PO_4 \cdot 4H_2O$ was included in all other analyses performed on this data.

The results of the Chi-Square Goodness of Fit and Bartlett's Test indicated that data were normally distributed and that the variances were homogeneous. Parametric procedures were used to perform all other analyses.

A one-way analysis of variance (ANOVA) was conducted to determine if significant differences existed in the offspring produced by C. dubia exposed to test concentrations of hydrogen uranyl phosphate. Results of this test indicated that reproduction among the various treatment groups differed significantly. Dunnett's multiple comparison test indicated that exposure of C. dubia to all test concentrations $\geq$ to 0.006 mg/L total uranium resulted in a significant reduction in production of young when compared to the control. This seven-day life cycle test determined that the NOEC for $HUO_2PO_4 \cdot 4H_2O$ expressed as total uranium equals some value < 0.006 mg/L and the LOEC equals 0.006 mg/L . The Minimum Significant Difference (MSD) test determined that a 28.5% reduction in the mean number of offspring from the control production (i.e. mean offspring production of $\leq$ 11.01) could be detected.

Table 7

Initial Acute Toxicity Testing Conducted on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on hydrogen uranyl phosphate, November 21 - 23, 1988. Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate). Test vessels = 250-ml glass beakers containing 100 ml/beaker.

Nominal Concentration (mg/L total U)[a]	Measured Concentration (mg/L total U) (11-21-88)	(11-22-88)	Total Mortalities 24h	48h	% Mortality at 48 hr
Control			0	0	0
0.040			0	3	15
0.060			0	1	5
0.080	0.064	0.061	0	4	20
0.100			0	5	25
0.120	0.131	0.117	0	14	70

[a]Nominal concentrations based on measured stock solutions.

LC_{50} = 0.110 mg/L U (nominal uranium)

Table 8

Initial Chronic Toxicity Testing Conducted on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of a static renewal life cycle test for Ceriodaphnia dubia exposed to hydrogen uranyl phosphate, December 13 - 20 1988.

Nominal* Concentration (mg/L total U)	Mean Measured Concentrations (mg/L total U)	X (SD)[a] of young per female	X broods[b] per female	% Mortality of original females
Control		15.40 (7.79)	2.3	10
0.006		9.45 (6.12)	2.1	20
0.020		9.10 (6.17)	1.7	30
0.060		8.60 (5.37)	1.8	20
0.120	0.119	9.80 (5.49)	2.1	5
0.200		8.25 (5.16)	1.8	15

*Nominal concentrations based on dilution of measured stock solutions.

[a]Mean value based on number of young produced by 20 original females.
[b]Mean value based on surviving original females.
(SD = Standard Deviation)

NOEC = < 0.006 mg/L total U LOEC = 0.006 mg/L total U ChV = NA

3.2.1.3 Acute Toxicity Test Results on Hydrogen Uranyl Phosphate (Shealy Environmental)

The results of the initial Shealy 48-hour acute toxicity bioassay for hydrogen uranyl phosphate are given in Table 9. The acute toxicity was determined vs. the concentration of theoretical uranium concentrations. The recovered uranium concentrations results were sporadic, and ranged from 2 to 10 times lower than the theoretical uranium concentration. Mortality occurred in the 0.54 mg/L (20% mortality), 0.97 mg/L (100% mortality), and 1.72 mg/L (100% mortality) theoretical uranium concentrations. No mortality occurred in the 0.17 mg/L or 0.30 mg/L theoretical uranium concentrations and the control. These data were used to determine a 48-hour LC50 (median lethal concentration) value with the Binomial method.[11] This calculation resulted in a 48-hour LC50 value of 0.65 mg/L theoretical uranium concentration with 95% confidence limits of 0.54 and 0.197 mg/L. Extrapolating from the theoretical uranium concentrations to the actual recovered uranium concentrations results in a 48-hr LC_{50} of ~0.070 mg/L uranium.

The recovered uranium values vs. the theoretical values indicated that the technique of weighing a known amount of hydrogen uranyl phosphate did not result in the expected amount of soluble HUP in solution. As discussed below (Section 3.2.2.3), the acute toxicity test was therefore repeated with HUP, starting with a known stock solution of predetermined uranium concentration.

Water chemistry data taken in conjunction with the acute bioassay are given in Attachment X, Table 5. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 17 for the 1.0, 1.8, and 3.2 mg/L HUP concentrations, which were <6.0.

3.2.1.4 Chronic Toxicity Test Results on Hydrogen Uranyl Phosphate (Shealy Environmental)

The initial 7-day chronic toxicity test with HUP was conducted February 9-16, 1989, as seven treatments exposing 10 test organisms each. The results are given in Table 10. Mortality of the adult females occurred in the control (10% mortality) 0.030 mg/L (10% mortality), 0.054 mg/L (10% mortality), 0.17 mg/L (10% mortality), 0.30 mg/L (20% mortality) and 0.54 mg/L (10% mortality) theoretical uranium concentrations. No mortality occurred in the 0.097 theoretical uranium concentration. Reproduction in the control averaged 17.0 offspring per female.

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test. and for homogeneous variances using Bartlett's test. Non-transformed data were found to be normally distributed (Chi-Square = 3.22, critical value = 12.59), with homogeneous variances (Bartlett's test p value = 0.445,p=0.01). Statistical analyses of the results using Dunnett's multiple comparison Procedure indicated chronic toxicity at the 0.30 mg/L and 0.54 mg/L as theoretical uranium concentrations (recovered uranium concentrations 0.043 and 0.063 mg/L, respectively).

The no observed effect concentration (NOEC) was 0.17 mg/L as theoretical uranium (0.050 recovered uranium), while the lowest observed effect concentration (LOEC) was 0.30 mg/L as theoretical uranium (0.043 recovered uranium). The chronic value (ChV) was estimated to be approximately 0.050 mg/L as recovered uranium.

The theoretical vs. the actual recovered uranium concentrations for the chronic toxicity tests indicated that the procedure for preparing the test solutions from the precipitated HUP resulted in much lower than expected concentrations of dissolved uranium in solution, as had been observed for the acute toxicity tests. The chronic toxicity tests for hydrogen uranyl phosphate were repeated (Section 3.2.2.4).

Water chemistry data taken in conjunction with the chronic toxicity test are given in Attachment X, Table 7. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 14 and 15. For these days pH's of less than 6 were recorded for all concentrations and the control. Note, a similar variance was recorded for the pH's of the uranyl nitrate toxicity tests being conducted on the same days (Section 3.1.2.2).

Table 9

Initial Acute Toxicity Results on Hydrogen Uranyl Phosphate by Shealy Environmental

Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia. February 17-19, 1989. Concentrations in hydrogen uranyl phosphate, theoretical uranium, and actual recovered uranium. Ten test organisms per replicate.

Test Concentration			Replicate	Number Affected After 24 Hours	Number Affected After 48 Hours	% Mortality
Control			A	0	0	0
			B	0	0	
$HUO_2PO_4 \cdot H_2O$	Theoretical U mg/L	Actual Recovered U				
0.32	0.17	0.036	A	0	0	0
			B	0	0	
0.56	0.30	0.190	A	0	0	0
			B	0	0	
1.0	0.54	0.059	A	2	2	20
			B	0	2	
1.8	0.97	0.099	A	10	-	100
			B	8	10	
3.2	1.72	0.170	A	10	-	100
			B	10	-	

LC_{50} = ~0.070 mg/L U (as recovered uranium)

Table 10

Initial Chronic Toxicity Results on Hydrogen Uranyl Phosphate by Shealy Environmental

Average reproduction of Ceriodaphnia dubia in the hydrogen uranyl phosphate solutions was as follows:

			Offspring per female	% Mortality
Control			= 17.0	10
$HUO_2PO_4 \cdot H_2O$	Theoretical U	Actual Recovered U*		
	mg/L			
0.056	0.030	0.028	= 12.5	10
0.10	0.054	0.021	= 13.1	10
0.18	0.097	0.037	= 13.8	0
0.32	0.17	0.050	= 13.3	10
0.56	0.30	0.043	= 9.0	20
1.0	0.54	0.063	= 7.0	10

*Average of 2 determinations

Calculated values:

NOEC = 0.17 mg/L U as theoretical uranium (0.050 recovered uranium),
LOEC = 0.30 mg/L as theoretical uranium (0.043 recovered uranium)
ChV estimated to be approximately 0.050 mg/L as recovered uranium.

The NOEC and LOEC were concluded to be < 0.021 mg/L U, based on the relatively high statistical variance of the control reproducibility (25.8%), which resulted in a "low" critical value of 12.59.

3.2.2 *Repeat Acute and Chronic Toxicity Testing on Hydrogen Uranyl Phosphate*

3.2.2.1 Acute Toxicity Test Results on Hydrogen Uranyl Phosphate by Normandeau Associates

Acute Toxicity Test - Unfed Organisms Results of the repeat acute toxicity test with unfed test organisms are summarized in Table 11. Partial test organism mortality was observed in the control, 0.16, 0.23, and 0.26 mg/L dissolved uranium nominal test concentrations after 24 h of exposure. Complete mortality was observed in the 0.16, 0.20, 0.23, and 0.26 mg/L dissolved uranium (nominal) concentrations at test termination. Control mortality at test termination equaled 5% . Based on these test results, the 48 hr LC_{50} for unfed test organisms equaled 0.12 mg/L nominal uranium concentration (95% confidence limits = 0.11 to 0.13 mg/L dissolved uranium. The 48 hr LC_{50} for unfed test organisms equaled 0.10 mg/L measured uranium concentration (95% confidence limits = 0.09 to 0.11 mg/L dissolved uranium.

The results of basic water chemistry analyses performed on all solutions used in this acute toxicity test are listed in Attachment XII, Appendix 2, Tables 1 through 4.

Acute Toxicity Test - Fed Organisms A 48 hr static renewal acute toxicity test was conducted to determine the effect of food (algae/YTC) on the toxicity of hydrogen uranyl phosphate to C. dubia. Results of this test are summarized in Table 12. No test organism mortality was observed in any test concentration after 24 h of exposure. At test termination partial mortality (i.e., 5%) was observed in the 0.26 mg/L nominal uranium exposure. Based on these test results, the nominal 48 h LC_{50} equaled > 0.26 mg/L dissolved uranium. The nominal and measured uranium concentrations were the same in this test.

The results of basic water chemistry analyses performed on all solutions used in the repeat acute toxicity tests are listed in Attachment XII, Appendix 2, Tables 5 through 8.

Table 11

Repeat Acute Toxicity Test with Unfed Organisms on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate June 29 -July 1, 1989. Test organisms were not fed during the test.

Nominal Concentration (mg/L total U)	Measured Concentrations (mg/L) (6-29-89)	Measured Concentrations (mg/L) (6-30-89)	Total Mortalities[a] 24hr	48hr	% Mortality at 48 hr
Control	<0.01	- - -	1	1	5
0.10	0.08	0.10	0	2	10
0.13	0.10	0.10	0	13	65
0.16	0.12	0.11	1	20	100
0.20	0.12	0.16	0	20	100
0.23	0.20	0.20	1[b]	10[b]	100
0.26	0.22	0.22	2	20	100

48 hr LC_{50} = 0.10 mg/L U (measured uranium)

Table 12.

Repeat Acute Toxicity Test with Fed Organisms on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate, July 11-13, 1989. Test organisms were fed during the test.

Nominal Concentration (mg/L total U)	Measured Concentrations (mg/L) (7-11-89)	Measured Concentrations (mg/L) (7-12-89)	Total Mortalities[a] 24h	48h	% Mortality at 48 hr
Control	- - -	- - -	0	0	0
0.10	- - -	- - -	0	0	0
0.13	- - -	- - -	0	0	0
0.16	- - -	- - -	0	0	0
0.20	- - -	- - -	0	0	0
0.23	- - -	- - -	0	0	0
0.26	0.24	0.28	0	1	5

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate).

[b]One replicate of the 0.23 mg/L test concentration was lost on 30 June 1989 due to a broken beaker.

LC_{50} = > 0.26 mg/L nominal uranium concentration

3.2.2.2 Repeat Chronic Toxicity Test Results on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of the repeat seven-day C. dubia static renewal chronic toxicity test on HUP by Normandeau Associates are summarized in Table 13. Partial mortality was observed by test termination among C. dubia exposed to 0.06, 0.12, and 0.20 mg/L dissolved uranium. Both brood and mean young production were substantially reduced among organisms exposed to test concentrations $\geq$ 0.060 mg/L dissolved uranium compared to the control individuals.

The results of Fisher's Exact test indicated a significant difference existed in the percent survival between the control group and C. dubia exposed to 0.20 mg/L dissolved uranium.

The results of the Chi-Square Goodness of Fit and Bartlett's test indicated that data were normally distributed and that the variances were homogeneous. Therefore, parametric procedures were used to perform all other analyses.

Results of the ANOVA test indicated that reproduction among the various treatment groups differed significantly. Dunnett's multiple comparison test indicated that exposure of C. dubia to 0.0002, 0.0006, 0.020, 0.060, and 0.120 mg/L dissolved uranium test concentrations resulted in a significant reduction in production of young when compared to reproduction of the control group.

A review of this data indicated that the response of the test organisms deviated from the concentration-response pattern typically associated with chronic toxicity tests. It is not possible to determine if the reduced reproduction observed in the 0.0002 mg/L dissolved uranium test concentration is truly the result of exposure to hydrogen uranyl phosphate or an aberrant response. However, based on a strict interpretation of the statistical results, the nominal NOEC and LOEC for hydrogen uranyl phosphate equaled < 0.0002 and 0.0002 mg/L dissolved uranium, respectively. If the response observed at 0.0002 mg/L is atypical, then the NOEC equaled 0.002 mg/L dissolved uranium and the LOEC equaled 0.006 mg/L dissolved uranium.

Based on all information, it was concluded that the NOEC and LOEC for hydrogen uranyl phosphate be reported as 0.002 and 0.006 mg/L dissolved uranium, respectively .

The minimum significant difference (MSD) test determined that a 15.3 to 15.4% reduction in the mean number of offspring from the control production (i.e., mean offspring reduction of $\leq$ 4.5 to 4.6 vs. control could be detected among these data). The critical value was therefore 29.7 - 4.5 = 25.2.

The results of all initial and final basic water chemistry analyses performed on all test solutions are listed in Attachment XII, Appendix 3, Tables 1 and 2.

Table 13

Repeat Chronic Toxicity Test Conducted on Hydrogen Uranyl Phosphate by Normandeau Associates

Results of a seven-day static renewal chronic toxicity test for Ceriodaphnia dubia exposed to hydrogen uranyl phosphate, July 8 - 15, 1989.

Nominal [a,b] Concentration (mg/L)	Measured Concentrations (7-8-89) (mg/L)[b]	X (SD) of young per female[c]	X broods per female[d]	% Mortality of original females
Control		29.65 (6.34)	3.4	0
0.0002	<0.002	22.10 (6.28)	2.9	0
0.0006	<0.002	27.68 (3.53)	3.0	0
0.002	0.002	26.05 (3.97)	3.0	0
0.006	0.004	23.90 (4.96)	2.9	0
0.020	0.030	25.00 (7.41)	2.9	0
0.060	0.040	18.35 (7.42)	2.6	5
0.120	0.140	13.00 (7.02)	2.3	10
0.200	0.200	9.00 (4.22)	1.8	70

[a]Nominal concentrations extrapolated from the measured concentration of dissolved uranium in the stock solution.
[b]mg/L as dissolved uranium.
[c]Mean value based on number of young produced by 20 original females. One organism was lost due to mechanical injury in the 0.0006 and 0.006 mg/L test concentrations. Mean values reflect only 19 individuals in those two concentrations. One male was present in the 0.12 mg/L test concentration. The male was not included in statistical analysis of young production.
[d]Mean value based on surviving original females.
SD = Standard Deviation

NOEC = 0.002 mg/L nominal uranium concentration

LOEC = 0.006 mg/L nominal uranium concentration

ChV = 0.004 mg/L

3.2.2.3 Repeat Acute Test Results on Hydrogen Uranyl Phosphate by Shealy Environmental

The results of the second Shealy 48-hour acute toxicity bioassay for hydrogen uranyl phosphate conducted by Shealy Environmental are given in Table 14. The acute toxicity was determined vs. the concentration of theoretical uranium concentrations. A separate dilution series was analyzed to compare theoretical (by dilution) vs. measured uranium concentrations. The results agreed very closely, with a % recovery of 101 % (Attachment XIII, Supplemental Test Report). Mortality occurred in the 0.15 mg/L (30% mortality), 0.20 mg/L (55% mortality), 0.25 mg/L (70% mortality) and 0.30 mg/L (100% mortality) theoretical uranium concentrations. No mortality occurred in the 0.10 mg/L uranium solution or the control. These data were used to determine a 48-hour LC_{50} (median lethal concentration) value with the Probit Method.[11] This calculation resulted in a 48-hour LC_{50} value of 0.190 mg/L theoretical uranium concentration with 95% confidence limits of 0.170 and 0.210 mg/L.

Water chemistry data taken in conjunction with the acute bioassay are given in Attachment XIII, Table 5. All parameters monitored were within acceptable limits for bioassay purposes.

3.2.1.4 Chronic Toxicity Test Results on Hydrogen Uranyl Phosphate (Shealy Environmental)

The repeat 7-day chronic toxicity test by Shealy on HUP was conducted on September 2-9, 1989, as five treatments exposing 10 test organisms each. The results are given in Table 15. Mortality of the adult females occurred in the control (10% mortality) 0.020 mg/L (10% mortality), 0.035 mg/L (20% mortality), 0.050 mg/L (30% mortality), 0.065 mg/L (40% mortality) and 0.080 mg/L (70% mortality) theoretical uranium concentrations. Reproduction in the control averaged 17.4 offspring per female.

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test. and for homogeneous variances using Bartlett's test. Log transformed data were found to be normally distributed, with homogeneous variances (Bartlett's test p value = [illegible], p=0.01). Statistical analyses of the results using Dunnett's multiple comparison procedure indicated chronic toxicity at the 0.065 mg/L uranium concentration.

The no observed effect concentration (NOEC) was 0.050 mg/L as theoretical uranium, while the lowest observed effect concentration (LOEC) was 0.065 mg/L as theoretical uranium. The chronic value (ChV), taken as the geometric mean of the NOEC and the LOEC, was 0.057 mg/L as theoretical uranium.

Water chemistry data taken in conjunction with the chronic toxicity test are given in Attachment XIII, Table 7. All parameters monitored were within acceptable limits for bioassay purposes.

Table 14

Repeat Acute Toxicity Results on Hydrogen Uranyl Phosphate by Shealy Environmental

Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia. August 17-19, 1989. Ten test organisms per replicate.

Test Concentration	Replicate	Number Affected After 24 Hours	Number Affected After 48 Hours	% Affected
Control	A	0	0	0
	B	0	0	
Theoretical U, mg/L				
0.10	A	0	0	0
	B	0	0	
0.15	A	0	2	30
	B	0	4	
0.20	A	0	4	55
	B	0	7	
0.25	A	2	8	70
	B	4	6	
0.30	A	3	10	100
	B	5	10	

LC_{50} = 0.190 mg/L U (theoretical uranium concentration)

Table 15

Repeat Chronic Toxicity Results on Hydrogen Uranyl Phosphate by Shealy Environmental

Average reproduction of Ceriodaphnia dubia in the hydrogen uranyl phosphate solutions was as follows:

	Offspring per female	% Mortality
Control	= 17.4	10
Theoretical U, mg/L		
0.020	= 14.4	10
0.035	= 13.3	20
0.050	= 14.9	30
0.065	= 10.8	40
0.080	= 6.9	70

Calculated

NOEC = 0.050 mg/L U, LOEC = 0.065 mg/L U, ChV = 0.057 mg/L U (theoretical uranium)

The high statistical variance of the control reproduction resulted in a difference of 35% from the control that was "significantly" different. The "critical" value was 17.4 -6.1 = 11.3. It was concluded that the NOEC and LOEC were <0.0020 mg/L U.

3.3 Acute and Chronic Toxicity Test Results on Uranium Dioxide

3.3.1 *Acute Toxicity Test Results on Uranium Dioxide (Normandeau Associates)*

The acute toxicity of UO_2 to C. dubia was determined by Normandeau Associates in a 48 hr. acute static toxicity test (Table 16). The results of initial and final basic water chemistry analyses performed on all solutions used in this acute test are listed in Attachment IX, Appendix 2, Table 3.

Exposure to test concentrations $\geq$ 0.100 mg total uranium resulted in complete mortality to test organisms 48 hr following test initiation. The results of this test were used to estimate a 48 hr LC_{50} of 0.050 mg/L nominal uranium concentration (95% confidence intervals = 0.04 - 0.06 mg/L nominal uranium concentration, based on a Trimmed Spearman-Karber calculation.

3.3.2 *Chronic Toxicity Test Results on Uranium Dioxide (Normandeau Associates)*

The percent survival was high among C. dubia exposed to all test concentrations of UO_2 ($\geq$ 85% - Table 17) The mean number of young and broods produced by organisms exposed to concentrations of total uranium < 0.050 mg/L were similar. However, exposure to 0.050 mg/L total uranium resulted in a reduction in both brood size and offspring production per test organism as compared to the other test concentrations. These data indicate that exposure to increasing concentrations of uranium as UO_2 reduced C. dubia reproductive success.

The results of Fisher's Exact test indicated no significant difference existed in the percent of survival among C. dubia exposed to any of the concentrations used in this chronic test. Offspring production by individuals exposed to all concentrations of UO_2 were included in further statistical tests.

The results of the Chi-Square Goodness of Fit test and Bartlett's test indicated that data were normally distributed and that the variances were homogeneous. All further analyses were performed using parametric methods.

The results of the one-way ANOVA indicated that reproduction among the various treatment groups differed significantly. Dunnett's multiple comparison test determined that exposure of C. dubia to concentrations equal to 0.050 mg/L total uranium resulted in a significant reduction in production of young when compared to the control. Based on these observations, it was concluded that the NOEC for total uranium in the form of uranium dioxide equals 0.030 mg/L and the LOEC equals 0.050 mg/L. The ChV equals 0.040 mg/L uranium in the form of uranium dioxide.

The results of the MSD test performed on this set of data determined that a 26.1% reduction in the mean number of offspring from the control production (i.e. mean offspring production of $\leq$ 11.43) could be detected.

The results of all initial and final basic water chemistry analyses performed on all solutions of UO_2 used in this chronic toxicity test are listed in Attachment IX, Appendix 3, Tables 5 and 6. The daily concentrations of total uranium measured in each of the test solutions used in the test are listed in Attachment IX, Table 3-2.

Table 16
Acute Toxicity Test Conducted on Uranium Dioxide by Normandeau Associates

Results of a Ceriodaphnia dubia 48 hr. static renewal acute toxicity test conducted on uranium dioxide, December 2-4, 1988.

Nominal Concentration (mg/L total U)[b]	Measured Concentration (mg/L total U) (12-02-88)	Measured Concentration (mg/L total U) (12-03-88)	Total Mortalities[a] 24h	48h	% Mortality at 48 h
Control			0	0	0
0.010			0	0	0
0.040			0	4	20
0.070	0.060	0.068	1	14	70
0.100			3	20	100
0.130	0.120	0.182	11	20	100

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate). Test vessels = 250-ml glass beakers containing 100 ml/beaker.
[b]Nominal concentrations based on measured stock solutions.

LC_{50} = 0.050 mg/L U (nominal uranium concentration)

Table 17
Chronic Toxicity Test Conducted on Uranium Dioxide by Normandeau Associates

Results of a static renewal life cycle test for Ceriodaphnia dubia exposed to uranium dioxide, performed December 9-16, 1988.

Nominal* Concentration	Mean Measured Concentrations (mg/L total U)	X(SD)** of young per female[a]	X broods per female[b]	% Mortality of original females
Control		15.20 (4.99)	2.3	5
0.0015		11.25 (5.74)	1.9	15
0.005		11.70 (5.61)	2.4	5
0.015		13.60 (6.70)	2.2	5
0.030		11.45 (4.80)	2.4	5
0.050	0.035	7.90 (4.91)	1.9	15

*Nominal concentrations based on measured stock solutions.
**SD = Standard Deviation
[a]Mean value based on number of young produced by 20 original females.
[b]Mean value based on surviving original females.

NOEC = 0.030 mg/L U, LOEC = 0.050 mg/L U (nominal uranium)

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4.0 DISCUSSION

The initial acute and chronic toxicity testing of uranium nitrate using Ceriodaphnia dubia was conducted by Shealy Environmental. The acute and chronic toxicity of uranyl nitrate to Ceriodaphnia dubia was greater than anticipated, based on the previous tests conducted in 1986 with Daphnia pulex.[2,22] Shealy was asked to repeat the toxicity test for uranyl nitrate, and a second environmental laboratory (Normandeau Associates) was contracted to perform an independent confirmation study.

Additional uranium compounds (hydrogen uranyl phosphate and uranium dioxide) were added to the test matrix, as the uranium would be present in these forms in the LETF effluents, rather than as uranyl nitrate. The uranyl ion (UO_2^{++}) will exist as the diphosphate complex, $UO_2(HPO_4)_2^{=}$ in the surface waters of the SRS streams, if sufficient phosphate is present[18]. Since the LETF effluent has typically a 100 fold molar excess of phosphate to uranium, more than sufficient phosphate is present to complex the uranium.

The initial toxicity tests by Shealy Environmental on hydrogen uranyl phosphate (HUP) were conducted using dilutions of a stock solution, prepared from a weighed amount of HUP. However, the % recovery of uranium in the actual dilution series solutions of HUP was significantly different from the theoretical uranium concentrations (based on calculated dilution). Therefore, the acute and chronic toxicity of HUP was re-determined (at both laboratories) using dilution of a stock solution of a known dissolved uranium concentration.

The toxicity of uranium dioxide was determined only by Normandeau Associates.

4.1 Acute and Chronic Toxicity of Uranyl Nitrate to Ceriodaphnia dubia

4.1.1 *Acute Toxicity*

The acute toxicity of uranyl nitrate was determined on Daphnia pulex in 1986[2,21], by Environmental and Chemical Sciences, Inc. (ECS) laboratory (the precursor to Normandeau Associates). The acute toxicity of uranyl nitrate was then determined using Ceriodaphnia dubia in 1989. The previous and current results are summarized below:

ACUTE TOXICITY RESULTS ON URANYL NITRATE

		LC50 (mg/L)
Organism	Laboratory	
Daphnia pulex	ECS	0.220
Ceriodaphnia dubia	Shealy	0.060
	Shealy (repeat)	0.089
	Normandeau	0.071
	Average (Ceriodaphnia dubia)	0.073

The initial acute toxicity test with Ceriodaphnia dubia indicated that those organisms were much more sensitive to uranyl nitrate than Daphnia pulex. The repeat acute toxicity tests with uranyl nitrate conducted by Shealy, and the independent testing by Normandeau, confirmed the initial results with Ceriodaphnia dubia. The results of the acute toxicity results for uranyl nitrate with Ceriodaphnia dubia are sufficiently similar to use an average of the three tests.

4.1.2 *Chronic Toxicity*

The chronic toxicity of uranyl nitrate was determined using Ceriodaphnia dubia in 1989 by two independent laboratories. The results are summarized below:

CHRONIC TOXICITY RESULTS ON URANYL NITRATE (Ceriodaphnia dubia)

Laboratory	NOEC*	LOEC**	ChV***
	mg/L total uranium		
Shealy (initial)	0.0015	0.0027	0.002
Shealy (repeat)	0.0027	0.0047	0.004
Normandeau	<0.008	0.008	<0.008
Average			0.003

*NOEC = No Observable Effect Concentration
**LOEC = Lowest Observable Effect Concentration
***ChV= Geometric mean of NOEC and LOEC

The chronic toxicity results by Shealy were very similar for both the initial and repeat uranyl nitrate tests. The Normandeau result was confounded by the fact that the toxicity response at the lowest test concentration tested (0.002 mg/L uranium) had much higher than expected mortality of the original females, such that the reproductive response was suspect. It could only be concluded that the NOEC was less than 0.008 mg/L uranium. Therefore, it was concluded that the average of the two ChV values (0.003 mg/L) determined by Shealy should be used as the ChV for uranyl nitrate to Ceriodaphnia dubia in the very soft waters of the Savannah River Site.

4.2 Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia

4.2.1 *Acute Toxicity*

The acute toxicity of hydrogen uranyl nitrate was determined using Ceriodaphnia dubia by two independent laboratories. The results are summarized below:

ACUTE TOXICITY RESULTS OF HYDROGEN URANYL PHOSPHATE TO CERIODAPHNIA DUBIA

Laboratory	48 Hour LC_{50} (mg/L dissolved uranium)
Shealy	0.070 (measured uranium)
Shealy (repeat)	0.190 (nominal uranium)*
Normandeau	0.110 (nominal uranium concentration)**
Normandeau(repeat)	0.100 (measured uranium)
Normandeau(repeat)	>0.260 - "fed during test" (measured uranium)
Average	0.120 ("fed" results not included)

* nominal concentration based on stock solution dilution, but a test concentration series gave excellent agreement between nominal and measured uranium concentrations
** nominal concentration based on stock solution dilution.

The initial acute toxicity test with hydrogen uranyl phosphate (HUP) by Shealy gave very different results for the nominal uranium concentrations (prepared by dilution of a 104 mg/L HUP stock solution) vs. the measured uranium concentrations. The HUP uranium stock solution was prepared by Shealy by adding a weighed amount of HUP to distilled water and allowing it to settle (as directed by WSRC). The nominal concentrations of the test solutions were based on dilution of the stock solution's theoretical HUP concentration. However, measured uranium concentrations indicated that the dissolved uranium in the test solutions was approximately 10X lower than assumed, based on dilution of the stock solution. It was concluded that only about 10% of the HUP in the initial Shealy stock solution was soluble.

The repeat acute toxicity test series was conducted by Shealy using a HUP stock solution prepared by using filtration after addition of a weighed amount of HUP to distilled water. The uranium concentration in the filtered stock solution was then determined to be 1.05 mg/L uranium, and the test solutions were prepared by serial dilution based on the measured uranium concentration.

The initial stock solution of HUP was prepared by Normandeau using filtration after addition of a weighed amount of HUP to distilled water. The uranium concentration in the initial filtered stock solution was then determined (1.2 and 3.8 mg/L), and the test solutions were prepared by serial dilution based on the measured uranium concentration in the stock solutions. The nominal uranium concentrations in the test solutions agreed closely in two cross checks of "nominal" vs. measured concentrations in the initial acute toxicity tests, and agreed within 10% on the repeat acute toxicity series by Normandeau. The HUP stock solution for the repeat series by Normandeau was prepared similarly to the initial test series, with a measured uranium concentration of 0.26 mg/L.

The acute toxicity results for the two hydrogen uranyl phosphate tests conducted by Normandeau agreed very closely with one another (0.100 vs. 0.110 mg/L U). The Shealy results were significantly different (0.070 vs. 0.190 mg/L U), but the average of the two Shealy results (0.130 mg/L U) is close to the Normandeau average (0.105 mg/L U). It was concluded that the "best" number for acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia in the soft waters of the SRS streams was an LC_{50} = 0.120 mg/L uranium.

Additional testing by Normandeau Associates indicates that this may be a very conservative acute LC_{50} concentration in the "real world". It was hypothesized that the uranyl phosphate anion $UO_2(PO_4)_2^{=}$ or the UO_2^{++} cation could be absorbed by algae or fungal material in a real stream environment. This would remove the toxicant, and result in much less sensitivity to in-stream organisms. This hypothesis was tested by adding food during the 48 hour acute toxicity tests with Ceriodaphnia dubia . A portion of the food settled to the bottom of the test container, potentially removing the uranium from solution. The acute toxicity responses indicated that this situation did occur, as no acute toxicity was noted up to a concentration of 0.26 mg/L uranium.

The SC Department of Health and Environmental Control (SCDHEC) uses as a "rule of thumb" that chronic toxicity can occur at 1/100 of the acute toxicity concentration. If this were the case for hydrogen uranyl phosphate, then the chronic toxicity would be in the range of 0.001 mg/L uranium. As discussed below, chronic toxicity for HUP was determined to be 0.004 mg/L uranium. Since the organisms are fed during the chronic toxicity tests, it is concluded that chronic test conditions (fed) are more representative of "instream" conditions than acute toxicity test conditions (unfed). It is also possible that a ratio of acute to chronic toxicity of 100 is not appropriate at the very low concentrations of uranium that cause toxicity affects in the very soft waters. Under these conditions, a ratio of acute to chronic toxicity of 10 to 20, that was demonstrated by this study for both uranyl nitrate and hydrogen uranyl phosphate, is more correct.

4.2.2 *Chronic Toxicity*

The chronic toxicity of hydrogen uranyl phosphate was determined by two independent laboratories. The results are summarized below:

CHRONIC TOXICITY RESULTS OF HYDROGEN URANYL PHOSPHATE
(Ceriodaphnia dubia)

Laboratory	NOEC*	LOEC**	ChV***
	mg/L total uranium		
Shealy (initial)	<0.021	<0.021	<0.021
Shealy (repeat)	<0.020	<0.020	<0.020
Normandeau (initial)	<0.006	0.006	<0.006
Normandeau (repeat)	0.002	0.006	0.004

*NOEC = No Observable Effect Concentration
**LOEC = Lowest Observable Effect Concentration
***ChV = Geometric mean of NOEC and LOEC

It can be seen from the summary table that the initial and repeat Normandeau chronic toxicity results agreed very closely. The initial chronic toxicity test conducted by Normandeau used a concentration of 0.006 mg/L uranium as the lowest test concentration. The repeat series included uranium concentrations of 0.006, 0.002, 0.0006, and 0.0002 mg/L, in order to bracket the suspect toxicity concentration range.

A statistical analysis of the repeat Normandeau data using Dunnett's multiple comparison test indicated that exposure of C. dubia to concentrations of 0.0002, 0.006, 0.020, 0.060, and 0.120 mg/L uranium resulted in significant reduction in production of young vs. the reproduction in the control (Table 13). The control had a reproduction mean of 29.6 young per female. Based on this data set, a 15.3% reduction in C. dubia young could be detected. That is, mean offspring production $\leq$ 25.2 would be significantly different from the control. The result for the 0.0002 concentration is apparently anomalous, as the next two higher concentrations tested (0.0006 and 0.002) were not statistically different from control. Therefore, it was concluded that the Normandeau results indicted that the Lowest Observable Effect Concentration was 0.006 mg/L uranium, and the No Observable Effect Concentration was 0.002 mg/L uranium.

The initial Shealy chronic toxicity results indicated that the LOEC and NOEC for uranium were 0.050 and 0.043 mg/L recovered (measured) uranium, respectively (Table 10). The recovered uranium values for this test series were quite variable, and did not correlate with the nominal uranium concentrations. This was due to the dilution of the stock solution to provide the test solutions, as discussed previously. In addition, the statistical analyses of the reproductive data determined that a 25.8% reduction in the mean number of offspring vs. the control production could be detected (Minimum Significant Difference; MSD). The critical value would therefore be 17.0 (control) - 4.4 = 1[illegible] The two lowest concentrations tested (as measured uranium) were 0.021 and 0.028 mg/L, [illegible] resulted in 13.1 and 12.5 neonates/female. If the variance of the control reproductive test [illegible]n slightly smaller, the minimum significant difference would have been less, the critical re[illegible]ve value would have been higher, and all of the test concentrations would have been significant[illegible]fferent from control. Therefore it is concluded that the initial chronic toxicity tests conducted [illegible] Shealy on hydrogen uranyl phosphate resulted in a NOEC and LOEC of <0.021 mg/L uranium.

In the repeat chronic toxicity tests by Shealy, a series of uranium concentrations from 0.020 to 0.080 mg/L uranium was utilized. The statistical analyses of the reproductive data determined that the minimum significant difference that could be detected was a 35% reduction in the mean number of offspring vs. the control production (Dunnett's Multiple Comparison Procedure). The critical value would therefore be 17.4 (control) - 6.09 = 11.3. Therefore, the lowest concentration at which an effect could be statistically detected was 0.065 mg/L, and the no effect concentration was 0.050 mg/L uranium (Table 15). However, if the statistical variance of the control had been less, and the minimum detectable difference had been on the order of 15%, then the critical value would have been approximately 14.8, and all concentrations tested would have been different from control. It is concluded that the repeat Shealy chronic toxicity tests on hydrogen uranyl phosphate resulted in NOEC and LOEC values of <0.020 mg/L uranium.

The results of the repeat chronic toxicity results by Normandeau and Shealy are shown in Figure 1. The results are plotted as a dose relationship, showing uranium concentration vs. average no. of offspring per female.

It can be seen that the 0.0006 and 0.002 mg/L uranium concentrations (in the Normandeau test series) were not statistically different from the control. I.e..; the test reproduction was greater than 25.2 neonates/female. The LOEC was 0.006 mg/L. The Minimum Significant Difference (MSD) of 15% is indicated in the figure.

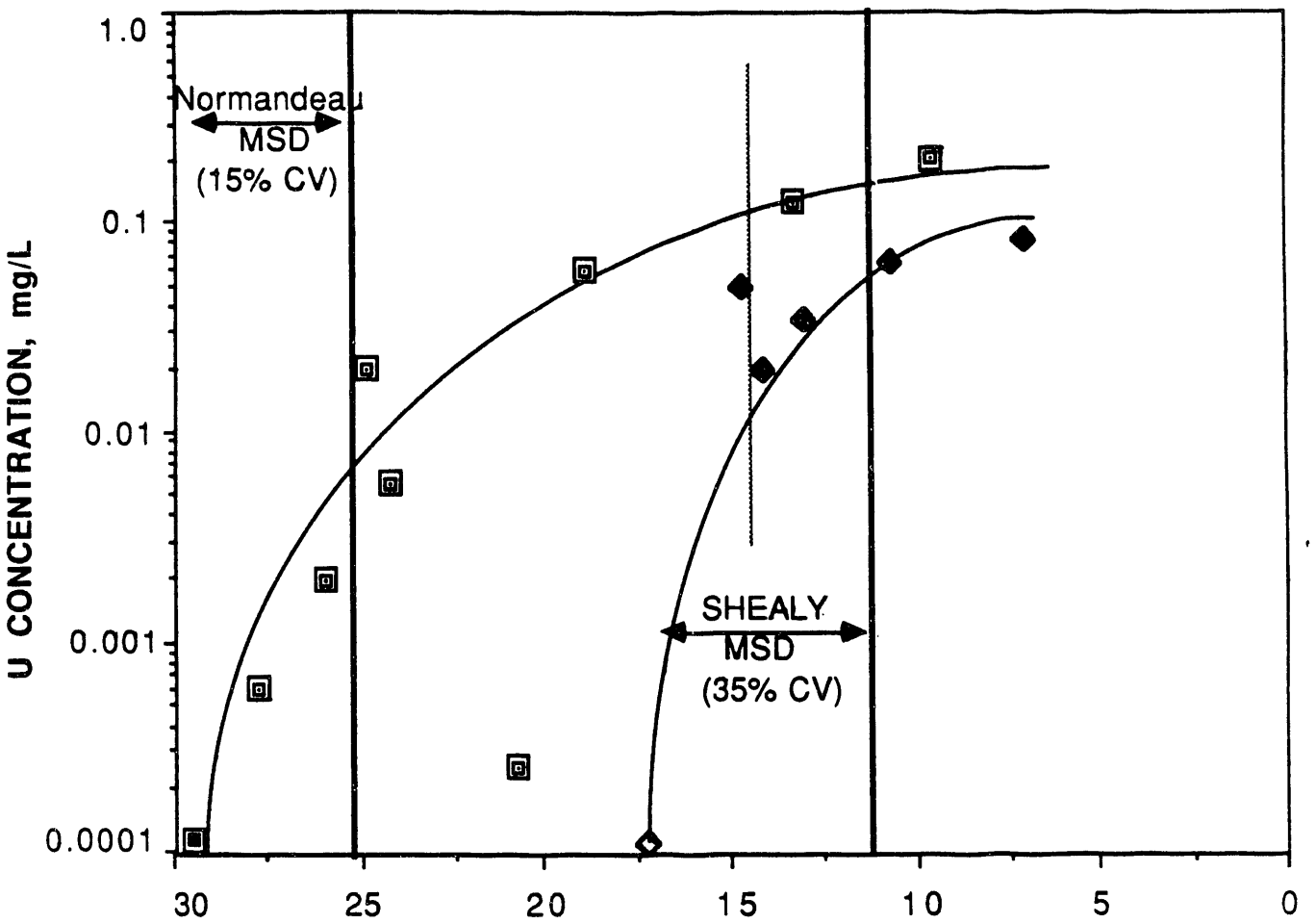
In the case of the Shealy test series, the MSD was 35%. Therefore, the test concentrations of 0.020, 0.035, and 0.050 had reproductive results which were not statistically different from the control (17.4 offspring/female). However, if the MSD had been 15%, instead of 35%, the critical value would be 14.8, and all test concentrations would have been different.

It is apparent from this type of analysis that the statistical variance of the control reproduction is critical to the determination of whether the test concentrations in a chronic toxicity test are "actually" different from the control. There is no minimum control reproductive variance established in the EPA protocols for conducting chronic toxicity tests, but as demonstrated above, a "high" variance can affect the conclusions of the test. It is the authors' recommendation that a dose vs. reproductive rate plot be developed for those situations where the organism is very sensitive to the toxicant, as is the case with C. dubia to uranium.

Figure 1

CHRONIC TOXICITY of HYDROGEN URANYL PHOSPHATE

Ceriodaphnia dubia



KEY

- Normandeau Asssoc'
- (Control)
- Shealy Environ'
- (Control)

MSD = MINIMUM SIGNIFICANT DIFFERANCE

4.3 Acute and Chronic Toxicity of Uranium Dioxide to Ceriodaphnia dubia

4.3.1 *Acute Toxicity*

The acute toxicity of uranium dioxide was determined using Ceriodaphnia dubia by Normandeau Associates. The results are summarized below:

ACUTE TOXICITY RESULTS OF URANIUM DIOXIDE (Ceriodaphnia dubia)
by Normandeau Associates

48 Hour LC_{50} (mg/L dissolved uranium)
0.050 (nominal uranium concentration)*

* nominal concentration based on stock solution dilution.

The acute toxicity LC_{50} of 0.050 mg/L uranium was surprising due to highly insoluble nature of the uranium dioxide. It was hypothesized that uranium dioxide would be less toxic than uranyl nitrate or hydrogen uranyl phosphate (HUP) due to the fact that the dissolved uranium would be present as U^{+4} complexes[18], which could have different physiological toxicity affects. However, the expectation for uranium dioxide was borne out with respect to chronic toxicity, which was chronically toxic at much higher concentrations than uranyl nitrate or HUP.

4.3.2 *Chronic Toxicity*

The chronic toxicity of uranium dioxide was determined using Ceriodaphnia dubia by Normandeau laboratory. The results are summarized below:

CHRONIC TOXICITY RESULTS OF URANIUM DIOXIDE (Ceriodaphnia dubia)
by Normandeau Associates

NOEC*	LOEC**	ChV***
mg/L total uranium		
<0.030	0.050	0.040

*NOEC = No Observable Effect Concentration
**LOEC = Lowest Observable Effect Concentration
***ChV = Geometric mean of NOEC and LOEC

The chronic toxicity results indicate that the oxidation state of uranium in the environment can affect the chronic toxicity to the instream organisms. In a reducing environment, which is commonly found at the bottom of many stagnant fresh water ponds and estuaries (due to the decay of settled vegetation), it is quite possible that the +6 state of uranium (UO^{2++}) could be reduced to the +4 state, with a attendant reduction in chronic toxicity due to uranium.

4.4 Comparison to Previous Uranium Toxicity Results

Although there have been few studies concerning the toxicity of uranium to aquatic organisms, previous results do exist to compare with the results of this study.

4.4.1 *Acute Uranium Toxicity*

4.4.1.1 Fish

Tarzwell and Henderson[19] exposed fathead minnows (Pimephales promelas) to uranyl nitrate, uranyl sulfate, and uranyl acetate and determined the TLM in hard and soft waters (1960). (TLM is a measurement which is essentially equivalent to an LC_{50}). They found that the minnows were much less sensitive to uranyl concentration in hard water than in soft water. Davies[20] obtained 96 hr LC_{50}'s for uranium to brook and rainbow trout (1980). The water hardness and alkalinity levels were slightly greater than those used by Tarzwell. Poston[7] reported an LC_{50} for fathead minnows (P. promelas) in Columbia river water (1984). Trapp[2&21] reported acute toxicity results similar to the Tarzwell results, using bluegill (Lepomis macrochirus), in the very soft water of Upper Three Runs, a Savannah River Site stream (1986). Bywater[22] reported the acute toxicity of uranium on a number of northern Australian freshwater fishes in extremely soft receiving waters (1991). The acute toxicity results for the effects of uranyl ion on fish are summarized below:

Study	Date	Fish	Hardness*	U Compound	96 Hr LC_{50} mg/L U**
Tarzwell	1960	Fathead minnow (P. promelas)	400	uranyl sulfate	119
			20	uranyl sulfate	2.6
			20	uranyl nitrate	2.7
			20	uranyl acetate	3.2
Davies	1980	Rainbow trout (S. gairdneri)	31	NA	6.2
		Brook trout (S. fontinalis)	31	NA	8.0
Poston	1984	Fathead minnow (P. promelas)	70	uranyl nitrate	16.7
Trapp	1986	Bluegill (L. macrochirus)	3	uranyl nitrate	1.67
Bywater	1991	Reticulated Perchlet (A. macleayi)	3#	uranyl sulfate	0.80
		Purple spotted gudgeon (M. mogurnda)	3#	uranyl sulfate	1.5

** Hardness expressed as mg/L $CaCO_3$
** LC_{50} expressed as mg/L total uranium
# Total alkalinity as mg/L
NA Not Available

4.4.1.2 Waterfleas (Cladocerans/Daphnids)

Previous acute toxicity testing with uranium compounds was conducted by Poston in 1984[7] using Daphnia magna in moderately hard water (hardness = 70). The 48 hr. LC_{50} was 6.0 mg/L total uranium. Testing with higher hardness waters gave similar results to those with fish, in that the Daphnids were much less sensitive to uranium at higher hardness. Poston reported a regression equation relating hardness and LC_{50} of: $LC_{50} = -159.8 + 39.3 \ln CaCO_3$ mg/L hardness. The correlation coefficient for that data set exceeded 0.9.

Trapp[2&21] reported an LC_{50} of 0.22 mg/L total uranium, using Daphnia pulex, in the very soft waters of Upper Three Runs (hardness = 3.0 mg/L $CaCO_3$). The acute toxicity results again reflect the influence of water hardness, with the daphnids being much more sensitive to uranium in very soft water.

Bywater[22] reported a 24 hour LC_{50} of 1.3 mg/L uranium for the cladoceran Moinodaphnia macleayi in the extremely soft waters of a northern Australian stream (1991).

The results reported in the current study, using Ceriodaphnia dubia in a very soft water, are in agreement with the previous results with D. pulex, although it appears that C. dubia is more sensitive to uranyl compounds than D. pulex. The acute toxicity results for cladocerans and daphnids are summarized below:

Summary of Acute Toxicity Results for Uranium

Study	Date	Cladoceran	Hardness*	U Compound	48 Hr LC_{50} mg/L U**
Poston	1984	Daphnia magna	195	uranyl nitrate	52***
			130	uranyl nitrate	37***
			70	uranyl nitrate	6
Trapp	1986	Daphnia pulex	3	uranyl nitrate	0.220
Bywater	1991	M. macleayi	3#	uranyl sulfate	1.3##
Shealy (V)	1989	Ceriodaphnia dubia	3	uranyl nitrate	0.060
Shealy (VIII)	1989	Ceriodaphnia dubia	3	uranyl nitrate	0.089
Normad' (IX)	1989	Ceriodaphnia dubia	3	uranyl nitrate	0.071
Shealy (X)	1990	Ceriodaphnia dubia	3	uranyl phosphate	0.070
Shealy (XIII)	1990	Ceriodaphnia dubia	3	uranyl phosphate	0.190
Normad'(IX)	1989	Ceriodaphnia dubia	3	uranyl phosphate	0.110
Normad'(XII)	1990	Ceriodaphnia dubia	3	uranyl phosphate	0.100

* Hardness expressed as mg/L $CaCO_3$
** LC_{50} Expressed as mg/L total uranium
*** Average of two tests
# Total alkalinity as mg/L
24 hour LC_{50}

4.4.2 *Chronic Uranium Toxicity*

4.4.2.1 Waterfleas (Daphnids)

Poston[22] reported a chronic toxicity effect (reproduction) for D. magma (in soft water, hardness = 70) of 0.52 mg/l total uranium. The reproductive effect was at a concentration approximately one tenth that of the acute LC_{50} effect under similar conditions.

The results of this study indicate chronic toxicity effects to C. dubia at very low uranium concentrations, using the very soft water of Upper Three Runs creek as the control and dilution water. This is attributed to a combination of the use of C. dubia and the effect of the very soft water. The chronic toxicity results for uranyl nitrate and uranyl phosphate are summarized below:

Summary of Chronic Toxicity Results of Uranium on Daphnids

Study	Date	Daphnid	Hardness*	U Compound	ChV mg/L U**
Poston	1984	Daphnia magma	70	uranyl nitrate	0.52
Shealy (V)#	1989	Ceriodaphnia dubia	3	uranyl nitrate	0.002
Shealy (VIII)	1989	Ceriodaphnia dubia	3	uranyl nitrate	0.004
Normad'(IX)	1989	Ceriodaphnia dubia	3	uranyl nitrate	<0.008
Shealy (X)	1990	Ceriodaphnia dubia	3	uranyl phosphate	<0.021
Shealy (XIII)	1990	Ceriodaphnia dubia	3	uranyl phosphate	<0.020
Normad'(IX)	1989	Ceriodaphnia dubia	3	uranyl phosphate	<0.006
Normad'(XII)	1990	Ceriodaphnia dubia	3	uranyl phosphate	0.004

4.5 NPDES Permit Renewal Proposal

The chronic toxicity results reported in this study were utilized to calculate the proposed uranium release concentration from the M-Area Liquid Effluent Treatment Facility (LETF). The average discharge rate from the LETF is 0.038 million gallons per day (MGD). This effluent is diluted to approximately 2.65 MGD by non-contact cooling waters (A-014), effluent from a groundwater clean-up air-stripper (M-005), effluent from the A-Area sanitary treatment facility (A-015) and overflows from the A-Area powerhouse (A-011 outfall). The dilution factor is therefore 69.8. None of the dilution streams contain detectable uranium, so the use of them to calculate a dilution factor for uranium is appropriate. Dilution from the receiving stream, Tims Branch, was not included - as it is a ephemeral stream. The "7Q10" for Tims Branch has been determined to be zero, in years with very low rainfall amounts. In these years the flow in the stream is composed entirely of flows from the operational facilities in the A and M areas of the Savannah River Site.

The chronic toxicity of uranyl phosphate (0.004 mg/L total uranium) was selected as the appropriate compound of concern. Uranyl ions will be complexed with phosphate anions in the pH environment (5.5 to 7.0) of the receiving stream[18]. The concentration of uranium proposed in the NPDES permit renewal application for the DETF effluent was therefore 0.28 mg/L (0.004 mg/L x 69.8 dilution factor).

This proposed DETF concentration is believed to be conservative, as the hardness in the area of the receiving stream (Tims Branch) to which the process effluents initially discharge is significantly higher (~20 mg/L as $CaCO_3$) than the hardness of the Upper Three Runs water used for the chronic toxicity tests[23]. However, the hardness in Tims Branch is reduced to a level of approximately 6 mg/L just prior to its confluence with Upper Three Runs[24]. Therefore, in order to protect the entire reach of the initial receiving stream and the subsequent stream, the chronic toxicity level of 0.004 mg/l total uranium as uranyl phosphate is believed to be appropriate.

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5.0 REFERENCES

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3. A. Coffey to S. R. Wright. *Re: NPDES Permit #SC0000175 (Condition #28) Uranium Acute Toxicity Study Savannah River Plant., M-Area,* South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (October 16, 1986).

4. N. Weatherup to S. R. Wright. *Re: DETF-IST/M Area - DOE/Savannah River Plant,* South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (June 17, 1988).

5. C. C. Montgomery to S. R. Wright. *Re: S.R.P M-Area, Biological and Chemical Assessment of M-Area Process Discharge to Tim's Branch - June 1985 to Dec. 1986.* South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (March 15, 1989).

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ATTACHMENTS

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II. A. Coffey to S. R. Wright. Re: NPDES Permit #SC0000175 (Condition #28) Uranium Acute Toxicity Study Savannah River Plant., M-Area, South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (October 16, 1986).

III. N. Weatherup to S. R. Wright. Re: DETF-IST/M Area - DOE/Savannah River Plant, South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (June 17, 1988).

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ACUTE AND CHRONIC TOXICITY OF URANIUM COMPOUNDS TO CERIODAPHNIA DUBIA (U)

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Records Administration, 773-52A *

*With Attachments

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ATTACHMENT I

J. Bart Ruiter to R. P. Whitfield, Re: NPDES Modifications Dated may 23, 1985 - Savannah River Plant, South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (May 22, 1985).

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May 22, 1985

R.P. Whitfield
Department of Energy
Savannah River Operations Office
P.O. Box A
Aiken, S.C. 29802

Re: NPDES Modifications Dated May 23, 1985
Savannah River Plant

Dear Mr. Whitfield:

In the latest NPDES modification effective May 23, 1985, there was a few target dates which Savannah River Plant (SRP) must meet and an agreement concerning the monitoring of uranium. This letter will clarify the dates of the special conditions and the agreement on uranium.

The specific conditions which have deadlines are items 28, 30, and 31.

(A) Special condition 28 - Biological Study Plan for outfall A-014 on the aquatic communities in the receiving stream.

1. Submit plan to DHEC for review and approval by September 23, 1985.

2. Implementation of plan by January 23, 1986.

(B) Special condition 30 - Submit a completed 2-C Form for outfalls M-005, M-004, and A-014 four months after discharging from outfalls.

(C) Special condition 31 - Assessment of ground water contamination resulting from 300-M area operations and necessary cleanup activities of this area to be submitted by December 31, 1985.

The South Carolina Department of Health and Environmental Control (SCDHEC) feels confident that uranium can be regulated by the Department based on water quality, however; the Department of Energy (DOE) feels that this parameter is under their jurisdiction by the Atomic Energy Act. In an attempt to prevent delays in the liquid effluent treatment facility operations which will eliminate wastewater discharge to the M-Area seepage basin, the Department agreed not to place a uranium limit on the permit at this time, but to record results in the discharge monitoring reports and to follow the procedure listed in your letter of February 15, 1985, item 1.

The Liquid Effluent Treatment Facilities (LETF) will be operated so as to achieve the target limits for uranium of 1.0 mg/l daily maximum and 0.5 mg/l monthly average. If the target limits are not met, SRP will notify the South Carolina Department of Health & Environmental Control (SCDHEC) in writing. This written notification will include (1) the reasons for the excursion, (2) remedial actions necessary and (3) a request for SCDHEC concurrence with the chosen course of action.

Also, DOE has agreed to do toxicity testing for uranium as described below.

SCDHEC-approved acute toxicity tests with bluegill sunfish and daphnids will be conducted to support the Outfall M-004 target limits for uranium. The tests will be 48-hour (daphnids) and 96-hour (bluegill sunfish) static acute tests following Environmental Protection Agency and American Society for Testing & Materials standards protocols. The chemical species of uranium present in the LETF effluent will be used in these tests. Dilution water for the studies will be from the receiving stream, Tims Branch. Test results will be transmitted to SCDHEC within 120 days of SCDHEC concurrence of this program. The uranium toxicity studies will be conducted by Environmental and Chemical Sciences, Inc. of Aiken, South Carolina. Their SCDHEC biological certification number is #02102.

Since no schedule was presented for the toxicity testing, this office will offer the following schedule:

A. Submit to DHEC for review and approval a toxicity study plan by August 23, 1985.

B. Complete study by November 23, 1985.

C. Submit report on findings by February 23, 1986.

Please note although the Department is in overview roll at this time, if we are not satisfied with DOE's responses or decide to go forward with the issue at a later date, we reserve the right to do so.

The NPDES permit modification for outfall M-004 submitted September 25, 1985, stated the following concerning total toxic organics (TTO):

"A solvent management plan and certification statement will be submitted to SCDHEC in lieu of monitoring total toxic organics at M-004 outfall of the LETF."

As of this date this office has not received the solvent management plan. It should be noted that this plan must be submitted to DHEC for review an approval prior to discharging from outfall M-004. Therefore, we are requesting the immediate submittal of the plan in order for this office to have an adequate time for review.

In Jim Joy's letter of April, 1985 he requested a schedule for the submittal and completion dates for when DOE feels the plans can be implemented for the 50% mixing zone monitoring plan (special conditions #16) and the biological monitoring plan (special conditions #17). This office would like that these schedules be submitted to DHEC by June 7, 1985 to insure that this office will have sufficient time to review and approve these plans.

If you should have any questions please call.

Sincerely,

Bart Ruiter

J. Bart Ruiter, Engineer
Industrial & Agricultural Wastewater Division
Bureau of Water Pollution Control

JBR/jf

cc: Kin Hill, Lower Savannah District

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ATTACHMENT II

A. Coffey to S. R. Wright. Re: NPDES Permit #SC0000175 (Condition #28) Uranium Acute Toxicity Study Savannah River Plt., M-Area, South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (October 16, 1986).

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South Carolina Department of Health and Environmental Control

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Euta M. Colvin, M.D.

October 16,1986

Mr. S.R. Wright, Acting Director
Environmental Division
U.S.DOE/Savannah River Plant
P.O. Box A
Aiken, S.C. 29802

Re: NPDES Permit #SC0000175 (Condition #28) Uranium Acute Toxicity Study Savannah River Plt., M-Area

Dear Mr. Wright:

This Office has reviewed the memo and attached report from C.V. Muska, June 30, 1986, in support of Outfall M-004 NPDES uranium target limits. Static toxicity tests were conducted February 5-14, 1986, according to accepted methods, using Daphnia pulex and bluegill sunfish (Lepomis macrochirus) as test organisms. Results were reported appropriately.

As stated in the memo, safety factors calculated from a maximum uranium concentration at the A-014 Outfall, of 0.06 mg/l, and a LC_{50} range of 0.22-1.67mg/l, result in a safety factor range of 3.7-27.8. Also, it is suggested that, "receiving stream dilution is adequate to protect the aquatic organisms...".

The 7Q10 of Tim's Branch is 0.258 MGD (0.977 million liters per day). This level of dilution would result in a maximum concentration of 0.05 mg/l in Tim's Branch at the discharge. The corresponding safety factor range of 4.4-33.4 is well below the safety factor of 100 used by SCDHEC to address chronic (long term) impact. The monthly average uranium concentration of 0.03 mg/l and safety range of 7.3-55.7 would not be significantly less instream. In addition, static toxicity tests generally result in higher LC_{50} values than do flow through tests, making organisms appear less sensitive.

In view of the safety factor ranges for the maximum and average uranium instream (target) concentrations at 7Q10, there appears to be a possibility of impact in Tim's Branch due to chronic toxicity at these concentrations.

In accordance with your July 30, 1986 memo, please submit the instream water chemistry/biological study report by May 23, 1987.

If I may be of any assistance, please feel free to call me at 734-5252.

Sincerely,

Alan Coffey

Alan Coffey, Engineer
Industrial & Agricultural Wastewater
Division

AC/jf

cc: Kin Hill
Gary Hoover
Jack Roberts

ATTACHMENT III

N. Weatherup to S. R. Wright. Re: DETF-IST/M Area - DOE/Savannah River Plant, South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (June 17, 1988).

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South Carolina Department of Health and Environmental Control

2600 Bull Street
Columbia, S.C. 29201

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Michael D. Jarrett

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Toney Graham, Jr. M.D.

June 17, 1988

C.R. Wright, Director
Environmental Division
Department of Energy
Savannah River Operations Office
P.O. Box A
Aiken, S.C. 29802

Re: DETF-IST/M Area
DOE/Savannah River Plant

Dear Mr. Wright:

Per your May 3, 1988 letter, this Office has reviewed the engineering report, plans and specifications for the system to decant and transfer supernatant from the interim storage tanks to the dilute effluent treatment facility at the Savannah River Plant. Our concerns are as follows:

1. Table 2-1 of the report shows that the IST No. 8 supernatant has a higher uranium concentration than the existing DETF influent. A review of the DMR Data from October 1986, shows that effluent uranium concentration ranged as follows:

   A. Daily Maximum: .047 to 1.19 mg/l
   B. Daily Average: .034 to .301 mg/l

   The treatability data shows that the proposed mixture can be treated to 0.03 mg/l. In light of the past performance, please address the capability of the existing equipment to treat this influent with a higher uranium concentration to meet the 0.5 mg/l effluent level. Note, this level may be too high based on discussions in Item 2.

2. Through previous correspondence and discussions, this Office has expressed its concern with the toxicity due to uranium in the effluent. Biological testing was required with the results showing a 96-Hr. LC_{50} = 1.67 mg/l for blue gill fish and a 48-Hr. LC_{50} = 0.22 mg/l for flathead minnows. The biological testing was performed in solutions prepared from water obtained from upper Three Runs Creek and various concentrations of uranyl nitrate.

   A safety factor of 100 with acute toxicity tests is used by SCDHEC to address chronic (long term) impact. Using this factor, the instream uranium concentration that would not cause an impact would need to be 0.0022 mg/l to 0.0167 mg/l. Using the 7Q10 of 0.258 for Tims Branch and the cooling water flow OF 0.72 MGD, this would result in acceptable effluent limits of 0.027 to 0.21 mg/l.

A review of the DMR data from October 1986 is provided in Item 1. The effluent levels are all above 0.027 mg/l and many exceed the 0.21 mg/l level.

This Office, therefore, continues to be concerned with the possible impact of the M004 Discharge on the receiving water. Based on these discussions, and the addition of a wastewater stream with a higher concentration of uranium to the DETF, this Office proposes to modify the NPDES Permit to require seven day chronic toxicity testing for the discharge from M004. This testing will demonstrate the impact of the actual effluent levels and forms of uranium as well as the additive impact of all the constituents in the wastestream.

Attached please find the proposed Part III modification. Based on the results of this testing, the target limits for uranium may be modified and/or limits may be placed in the Permit.

3. The treatability study states that a composite sample of IST No. 8 supernatant was mixed with ten samples of M-Area dilute wastewater. The treatability study was then performed (page 2-8). Average concentrations for the mixed samples and treatability results were provided. Please provide the separate analysis for the 10 samples and the treatability results for the ten samples.

4. Tables 2-3, 2-4, 2-5 and 2-6 note that tanks 1-6 where sampled in November, 1986 and analyzed December, 1986 through October, 1987 and that tank 8 was sampled in January, 1987 and analyzed November, 1987 through January, 1988. Standard methods specifies the maximum recommended storage time for metals is six months. For nitrates and phosphates the maximum recommended storage time is 48 hours. Was this testing performed by a South Carolina certified laboratory? Please address the effect on the analytical results and treatability results.

5. A discussion of treatment for IST No. 4 is discussed on page 2-11. It is stated that the addition of Sodium Hydroxide will raise the pH which will result in a reduction of Uranium and sludge in the tank. A treatability study should be provided to demonstrate this. In addition, this Office has concerns regarding dissolving the sludge and then treating the supernatant through the DETF. It appears that this will only be transferring the sludge from tank #4 to the DETF for disposal.

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6. The treatability study was performed only on tank #8. The concentration of the other tank's supernatant vary considerably from this. As an example, Uranium varies from 12 mg/l in tank #8 to 180 mg/l in tank #4 and 63 mg/l in tank #3. Aluminum varies from 2100 mg/l in tank #8 to 6300 mg/l in tank #2 and 4400 mg/l in tank #5. Please address how this will be accounted for. It appears additional treatability studies are needed.

7. The treatability study for tank #8 states that a 30:1 dilution of the IST supernatant is needed. Will a greater dilution be needed for the tanks with higher concentrations? This Office has concerns that dilution is needed to achieve the limitations. As discussed previously, we do not feel dilution is a solution to the treatment of wastewater.

8. The proposed project addresses the treatment of the supernatant but not the ultimate disposal of the sludges associated with the Liquid Effluent Treatment Facility (LETF). While this project will be provided additional time, this Office feels that SRP should address ultimate disposal of the sludge at this time. A schedule for accomplishing this goal should also be submitted.

9. In the Engineering Report, page 2-6, the connection of the IST supernatant discharge through an existing unused header, to the DETF Equalization Tanks is discussed. Please provide plans and specifications for the existing unused header and the connection.

10. In the Design Criteria and Calculations Equipment Specifications, Section 5.1.4, the flow control instrumentation is discussed as being identical to the existing waste acid and waste caustic instrumentation for additions to the equalization tanks, which SCDHEC approved in the original LETF construction permit. Please submit specifications for this instrumentation.

Please review our concerns at your earliest convenience. In addition, send comments and/or approval of the proposed Draft modification as soon as possible. If you have questions, please contact me or Sue Schweikart at 734-5300.

Sincerely,

Nancy Weatherup

Nancy Weatherup, P.E.
Industrial and Agricultural
Wastewater Division

NW/jf
cc: Kim Cauthen, Lower Savannah
David Wilson, BSHWM

35. A completed NPDES Form 2C containing actual influent and effluent data from the F/H Effluent Treatment Facility shall be submitted to SCDHEC within four (4) months after the ETF first begins to discharge.

36. The Permittee shall develop and, after SCDHEC's approval, implement a biological study on a quarterly basis to determine the impact of Outfall H-016 on the aquatic communities in the receiving stream. The biological study program will consist of a one-year baseline study prior to discharge and a three-year post startup study. The biological study plan shall include an assessment of the instream macroinvertebrate community. Also, the biological study plan shall determine if bioaccumulation of mercury is occurring in the aquatic organisms in the receiving stream. The Permittee shall submit the biological study plan to SCDHEC for approval no later than four (4) months after the Outfall H-016 permit modification becomes effective. After submittal and review of the biological study final report (final report will contain both base line and post startup studies, SCDHEC has the authority to request mitigative action and/or continued studies of a nature and frequency to be agreed on with the Permittee.

37. The Permittee shall develop and maintain at the permitted facility a complete Operations and Maintenance Manual for the waste treatment plants. The manual shall be available for on-site review during normal working hours. The manual shall contain operation and maintenance instructions for all equipment and appurtenances associated with the waste treatment plant. The manual shall contain a general description of the treatment process(es), operating characteristics that will produce maximum treatment efficiency and corrective action to be taken should operating dificulties be encountered.

38. Where applicable, the definition for "daily average" shall be taken to be equivalent to the definition for "monthly average" provided in Part I., C.8.a. of the NPDES Permit boiler plate.

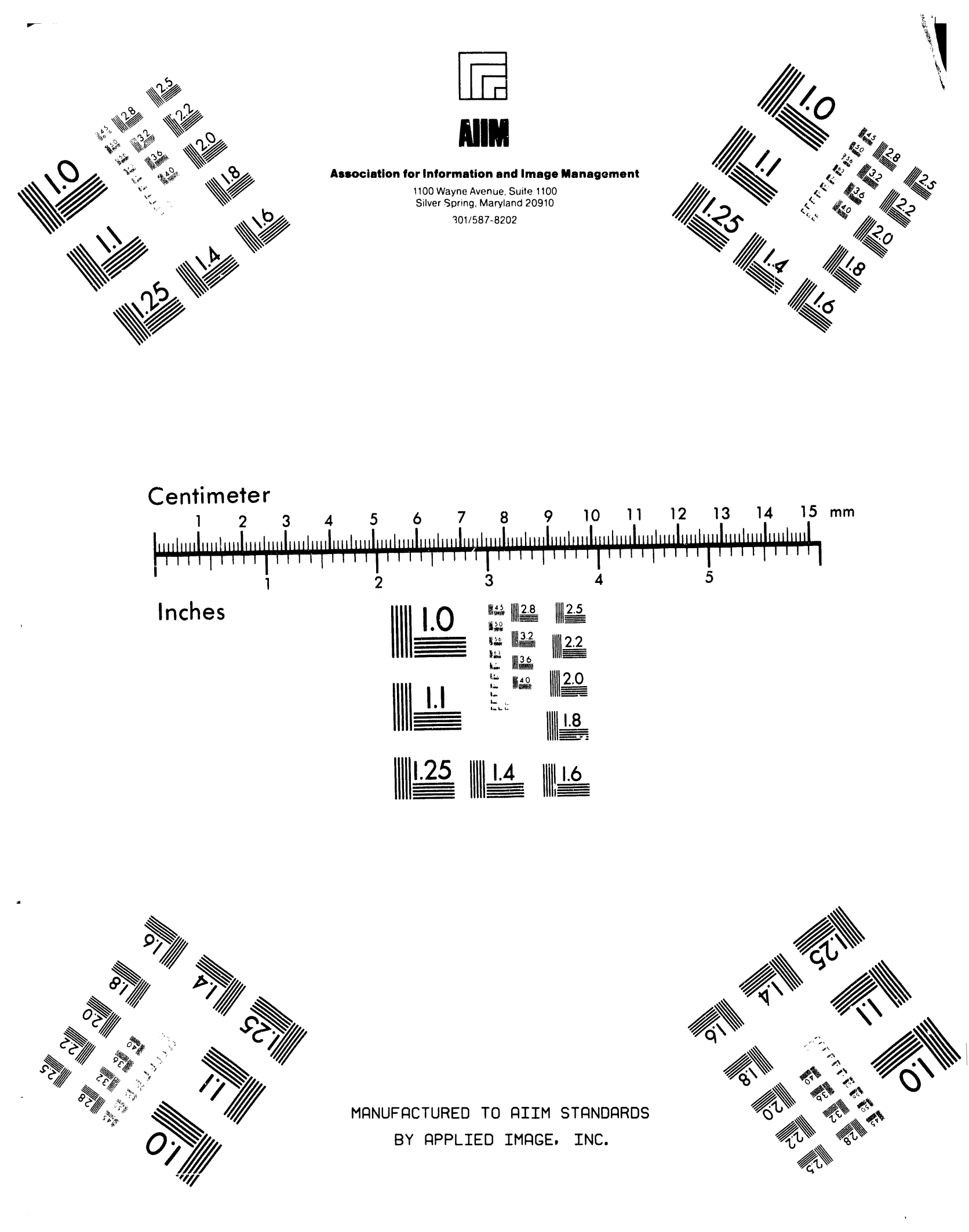
39. For Outfall M004:

A. On a monthly basis, a 7-day chronic renewal toxicity test shall be conducted using a control and the instream waste concentration (IWC) of 8%. The test shall be conducted using Ceriodaphnia dubia as the test organism and in accordance with "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organism" (EPA/600/4-85/014). Testing shall begin October 1, 1988 and results shall be submitted to the Department within fifteen days of completion of each monthly test. Twelve consecutive acceptable months of toxicity results may result in quarterly testing in lieu of monthly tests.

B. Reopener Clause - toxicity

If any monthly test results indicate a sigrificant difference in Ceriodaphnia dubia reproduction or survival between the control and instream waste concentration at the 95% confidence level (p = 0.05), a toxicity reduction plan shall be submitted within 60 days of the Department notification. Upon Department approval, this shall become a part of this permit. This permit may be modified or revoked and reissued to incorporate toxicity limitations and monitoring requirements in the event toxicity testing or other studies conducted on the effluent or receiving stream indicate that detrimental effects may be expected in the receiving stream as a result of this discharge.

DRAFT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ATTACHMENT IV

C. C. Montgomery to S. R. Wright. Re: S.R.P M-Area, Biological and Chemical Assessment of M-Area Process Discharge to Tim's Branch - June 1985 to Dec. 1986. South Carolina Department of Health and Environmental Control (SCDHEC), Columbia, SC, 29201 (March 15, 1989).

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South Carolina Department of Health and Environmental Control

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Oren L. Brady, Jr.
Moses H. Clarkson, Jr
Euta M. Colvin, M.D.
Henry S. Jordan, M.D

March 15, 1989

S. R. Wright, Director
Environmental Division
Savannah River Operations Office
P.O. Box A
Aiken, South Carolina 29802

Re: S.R.P. M - Area
Biological and Chemical Assessment of M-Area Process Discharge to Tim's Branch - June 1985 - Dec. 1986.

Dear Mr. Wright:

SCDHEC Staff have reviewed the above report, submitted as required by Part III, Item #28, of the NPDES Permit. Below is a list of their observations and comments.

1. Chemistry

   A. Sediment TKN values below the discharge are very elevated relative to statewide sediment data.

   B. The N:P ratio suggests the potential for algal blooms at certain times of the year.

2. Macroinvertebrates

   A. The studies conducted were very thorough. The data collected showed very diverse communities and no signs of stress in the study area.

   B. Taxa richness improved downstream of the discharge due to higher dissolved oxygen levels and higher flow rates.

   C. Total biomass and densities were similar at all locations.

3. Periphyton

   A. There were no indications that the periphytic communities were significantly impacted due to the discharges.

4. Toxicity

   A. Acute toxicity tests were conducted with NCC+PAS, NCC+PAS+LETF, and Tim's Branch water.

   B. There appeared to be no real decline of toxicity during the year of testing but rather indications that both effluent combinations were variable in nature.

   C. Acute toxicity in Tim's Branch water was low throughout the year, but no toxicity should be expected. Any toxicity should be unacceptable instream.

   D. Due to the high instream waste concentration (89.2%) at 7Q10 (0.258 mgd), chronic toxicity tests should be conducted to directly address chronic toxicity. The high IWC and the variable nature of acute toxicity indicate a real potential for chronic toxicity.

Questions concerning these comments should be directed to Mr. Butch Younginer, Manager of Water Quality Monitoring Section, at 734-5401.

Sincerely,

Cathy C. Montgomery

Cathy C. Montgomery
Environmental Quality Manager
Water Quality Assessment and
Enforcement Division

CCM\jh
cc: Steve Thomas
Kim Cauthen
Butch Younginer

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ATTACHMENT V

Test Report No. A16747, Revision II, Acute and Chronic Toxicity of Uranyl Nitrate to Ceriodaphnia Dubia, Task Order Contract AX843930, Task I". AnalytiKEM Inc., Cherry Hill, NJ 08003 (January, 1989).

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Rcvd. E+E 1/16/89

AnalytiKEM An American NuKEM Company

AnalytiKEM Inc.
28 Springdale Road
Cherry Hill. NJ 08003
609/751-1122
215/923-2068

A16747) 1117

TEST REPORT NO. ~~A17747~~, Revision II

January 13, 1989

Prepared for:

E.I. DuPont de Nemours & Company
Atomic Energy Division
Savannah River Plant
Aiken, SC 29808-0001

Attention: John L. Keyes

Date of Sample Receipt: July 29, 1988

Sampled by: Client

Sample Quantity: Fourteen (14)

NJ Certification No. NJ 04012

NY Certification No. NY 10815

SC Certification No. SC 94004

NC Certification No. NC 258

Reviewed & Approved by: Patricia de Andino for

Name: Michael Shmookler, Ph.D.

Title: Technical Director

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ACUTE AND CHRONIC TOXICITY OF URANYL NITRATE TO CERIODAPHNIA DUBIA

Report To

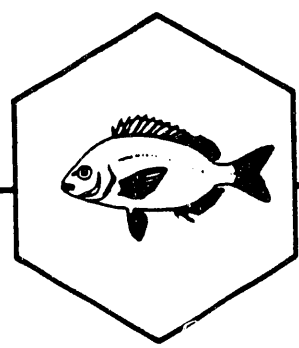
Savannah River Plant

Aiken, South Carolina

Revised January 1989

SHEALY ENVIRONMENTAL SERVICES, INC.

BIOLOGISTS, TOXICOLOGISTS & CHEMISTS



ACUTE AND CHRONIC TOXICITY OF URANYL NITRATE TO CERIODAPHNIA DUBIA

Report To

Savannah River Plant

Aiken, South Carolina

Revised January 1989

Submitted By

SHEALY ENVIRONMENTAL SERVICES, INC.
Columbia, South Carolina
(803) 254-9915

SCDHEC Laboratory Certification No. 26103

Richard L. Shealy, President

I. INTRODUCTION

Acute and chronic toxicity tests were conducted August 5 - 12, 1988, for the Savannah River Plant to assess the acute and chronic toxicity of uranyl nitrate to Ceriodaphnia dubia.

II. METHODS

Dilution water for the toxicity tests was collected July 14, 1988, from Upper Three Runs Creek at the northside of a bridge on Road 2-1 on the Savannah River Plant site. The water was filtered with a glass fiber filter and acclimation of the Ceriodaphnia cultures to the creek water started on July 14, 1988. Ceriodaphnia for the definitive acute and chronic tests were cultured in the creek water for approximately three weeks before being used in the toxicity tests.

A total of four range-finding tests were conducted July 19 - August 5, 1988, with concentrations ranging from 0.0018mg/l - 1.0 mg/l reagent grade uranyl nitrate (0.00085 - 0.4740 mg/l theoretical Uranium) (Table 1). These tests were used to determine test concentrations for the definitive acute and chronic tests.

A. Acute Toxicity Test.

Test methods conformed to those described in USEPA (1985a; see Table 2). The 48-hour acute toxicity test was conducted August 10 - 12, 1988, with the following uranyl nitrate concentrations: 1.0 mg/l (0.490mg/l actual recovered uranium), 0.56 mg/l (0.290mg/l uranium), 0.32 mg/l (0.140mg/l uranium), 0.18 mg/l (0.081mg/l uranium) and 0.10 mg/l (0.044mg/l uranium). For the control, 100% dilution water was

Table 1: Summary of results of range-finding tests with Uranyl nitrate conducted July 19 - August 5, 1988. Concentrations reported in theoretical uranium.

Test Date/Type of Test		Test Concentrations	Results
July 19-20, 1988	Chronic	0, .0125, .1185, .2370 and .3555mg/l	Excessive mortality Concentrations too high
July 25-27, 1988	Chronic	0, .0047, .0085, .0152, .0265 .0474, .0711 and .0948mg/l	Excessive mortality Concentrations too high
July 28-29, 1988	Acute	*0, .0047, .0085, .0152 .0265, .0474 and .0853mg/l	Sporatic mortality Solutions remade
July 29-Aug. 3	Chronic	0, .00085, .0015, .0027 .0047, .0085 and .0152mg/l	No effect - Determined that fresh solutions should be used for each rewewal and that higher conc's needed

*Concentration range repeated from previous test because of sporatic mortality and low percentage of recovery of uranyl nitrate from previously analyzed samples.

Table 2: Summary of test conditions for the acute toxicity bioassay with Ceriodaphnia dubia.

1. Temperature:	25 ± 1 C
2. Light intensity:	Ambient laboratory levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	250 ml beakers
5. Volume of test solution:	100 ml
6. Age of test organisms	2-24 hour old neonates
7. No. animals per test vessel:	10
8. No. replicate test vessels per concentration:	2
9. Total no. organisms per concentration:	20
10. Feeding regime:	No feeding required
11. Aeration:	None, unless D.O. falls below 40% saturation, at which time gentle single-bubble aeration started
12. Dilution water:	Upper Three Runs Creek Water at the Savannah River Plant Road 2-1
13. Test duration:	48 hours
14. Effect measured:	Mortality - no movement of appendages on gentle prodding

used. All organisms used in the toxicity tests were from SHEALY ENVIRONMENTAL SERVICES, INC. in-house cultures which were obtained from the USEPA Newtown Laboratory April 20, 1987, Lab I.D. No. 87-271. Ceriodaphnia from in-house cultures are identified and preserved monthly. A standard toxicant test with the EPA reference toxicant cadmium chloride (Lab. I.D. No. 88-964) was performed on Ceriodaphnia cultured in water from Upper Three Runs Creek in conjunction with the acute and chronic tests. The results of this test (LC50=0.09 mg/l cadmium chloride) demonstrated that the condition of the culture was within the acceptable range for test organisms (0.056-0.198mg/l). Test solutions and the controls were prepared in 100 ml quantities in all-glass test chambers. All concentrations and the control were tested in duplicate with ten Ceriodaphnia dubia neonates (2-24 hours old) each. The test solutions were renewed after 24-hours. A 100 mg/l uranyl nitrate stock solution was prepared on August 4, 1988, using reagent grade uranyl nitrate by rapidly weighing 0.0101 grams of the chemical onto a tared weighing paper in a balance containing desiccant. All uranyl nitrate test concentrations were prepared fresh daily from the 100mg/l stock solution by dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to ± 1%) except for the 1mg/l concentration which was made up using a 1 ml Class A volumetric pipet. The uranyl nitrate stock solution was stored at 4°C during testing. Samples of all test solutions were preserved with 0.15% metals grade nitric acid and shipped with ice packs via Federal Express to ANALYTIKEM, INC. for verification.

Dissolved oxygen, water temperature, pH, conductivity, alkalinity and total hardness measurements were made in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers. The test organisms were placed singly in the test vessels each containing 100 ml of solution. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia. Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding.

B. Chronic Toxicity Bioassay

Test methods conformed to those described in USEPA (1985b; see Table 3). The 7-day chronic toxicity bioassay was performed as eight treatments exposing 10 female test organisms each. The first treatment was the control (100% filtered Upper Three Runs Creek Water). The uranyl nitrate solutions were 0.0032mg/l, 0.0056mg/l, 0.01mg/l, 0.018mg/l, 0.032mg/l , 0.056mg/l and 0.10mg/l (actual recovered uranium values of <0.0013mg/l, <0.0013mg/l, 0.0021mg/l, 0.0014mg/l, 0.0096mg/l, 0.015mg/l and 0.044mg/l, respectively). All test solutions were prepared from the same 100mg/l stock solution as the acute test dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to ± 1%). The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

Table 3: Summary of test conditions for chronic toxicity bioassay with Ceriodaphnia dubia.

1. Temperature:	25 ± 1°C
2. Light intensity:	Ambient laboratory levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	1 ounce SOLO plastic disposable cups
5. Volume of test solution:	15 ml
6. Age of test organisms	2-24 hour neonates and all released within the same four hour period
7. No. animals per test vessel:	1
8. No. replicate test vessels per concentration:	10
9. Total no. organisms per concentration:	10
10. Feeding regime:	Selenastrum capricornutum at the rate of 1-2,000,000 cells per ml. test soln. per day
11. Aeration:	None
12. Dilution water:	Upper Three Runs Creek Water at the Savannah River Plant Road 2-1
13. Test duration:	7 days
14. Effect measured:	Mortality - no movement of appendages on gentle prodding and number of offspring produced

Dissolved oxygen, water temperature, pH, conductivity, total hardness and alkalinity measurements were made in conjunction with the tests. Temperature was maintained at 25° C (± 1° C) in all test chambers during the test.

The test organisms were placed singly in test vessels each containing 15 ml of solution. The organisms were between 20 and 24 hours old at the start of the test. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at a rate of approximately 1,000,000 cells per ml. test solution per day. Selenastrum cultures were obtained from Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged erlenmeyer flasks and maintained under bright fluorescent lighting for 6 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and order of feeding was established based on random number tables.

III. RESULTS

A. Acute Toxicity Bioassay

The results of the 48-hour acute toxicity bioassay are given in Table 4. All results are reported as test concentrations of total uranium recovered from the analyzed uranyl nitrate solutions. Mortality occurred in the 0.081 (100% mortality), 0.140 (100% mortality),

Table 4. Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of Uranyl nitrate to Ceriodaphnia dubia, August 10 - 12, 1988. Concentrations in theoretical and actual recovered uranium. Ten test organisms per replicate.

Test Concentration		Replicate	Number Affected After 24 Hours	48 Hours	% Affected
Control		A	0	0	0%
		B	0	0	
Theoretical U	Actual Recovered U				
0.047mg/l	0.044mg/l	A	0	0	0%
		B	0	0	
0.085mg/l	0.081mg/l	A	0	10	100%
		B	0	10	
0.15mg/l	0.14mg/l	A	10	10	100%
		B	10	10	
0.27mg/l	0.29mg/l	A	10	10	100%
		B	10	10	
0.47mg/l	0.49mg/l	A	10	10	100%
		B	10	10	

0.290mg/l (100% mortality) and 0.490mg/l (100% mortality) recovered uranium concentrations. No mortality occurred in the control or the 0.044 mg/l uranium concentration. These data were used to determine a 48-hour LC50 (median lethal concentration) value with the Binomial Method (EPA, 1985a). This calculation resulted in a 48-hour LC50 value of 0.060mg/l uranium with 95% confidence limits of 0.044 and 0.081 mg/l.

Water chemistry data taken in conjuction with the acute bioassay are given in Table 5. All parameters monitored were within acceptable limits for bioassay purposes.

B. Chronic Toxicity Bioassay

The results of the 7-day chronic toxicity test conducted August 5 - 12, 1988, are given in Table 6. Mortality occurred in the <0.0013mg/l (10% mortality), 0.0021mg/l (20% mortality) and 0.0014mg/l (10% mortality) recovered uranium concentrations. No mortality occurred in the control. Reproduction in the control averaged 32.9 offspring per female. One male was observed in the 0.015mg/l and 0.044mg/l uranium concentrations. Males were not included in calculating the reproduction data as specified by SCDHEC (Mr. Dave Graves, Biological Services Division, personal communication).

Table 5. Water quality data recorded in conjunction with the 48-hour static renewal bioassay to determine the acute toxicity of Uranyl nitrate to Ceriodaphnia dubia, August 10 - 12, 1988.

Exposure Period	Parameter	Test Concentrations					
		Control	0.10mg/l	0.18mg/l	0.32mg/l	0.56mg/l	1.0mg/l
0 Hours	D.O. (mg/l)	7.8	7.8	7.8	7.8	7.8	7.8
	Temp. (^{o}C)	26.0	26.0	26.0	25.8	25.8	25.8
	pH (SU)	7.6	7.1	7.7	7.1	7.1	7.0
	Cond. (umhos/cm)	21	22	23	23	23	22
	Tot. Hard. (mg/l)	6.1	-	-	-	-	-
	Tot. Alk. (mg/l)	1.1	-	-	-	-	-
(Before Renewal)							
24 Hours	D.O. (mg/l)	7.7	7.7	7.7	7.7	7.7	7.7
	Temp. (^{o}C)	26.0	26.0	26.0	25.8	25.8	25.8
(After Renewal)							
24 Hours	D.O. (mg/l)	8.1	8.1	8.1	-	-	-
	Temp. (^{o}C)	26.0	26.0	26.0	-	-	-
	pH (SU)	7.31	6.87	6.87	-	-	-
48 Hours	D.O. (mg/l)	8.0	8.0	8.0	-	-	-
	Temp. (^{o}C)	26.0	25.8	25.8	-	-	-
	pH (SU)	7.76	7.04	7.06	-	-	-

Table 6. Survival and reproduction of Ceriodaphnia dubia exposed to solutions of uranyl nitrate August 5 - 12, 1988. Concentrations in theoretical and actual recovered uranium.

X = Death 0 = Live - No Reproduction # = Reproduction

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Control	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	2	0	3	2	0	3	0	3	3	2
	4	8	0	3	1	3	9	0	7	0	0
	5	0	12	11	11	8	10	10	2	8	0
	6	14	15	1	12	15	12	15	12	11	14
	7	12	12	17	0	3	16	11	13	2	1
	TOTAL	36	39	35	26	29	50	36	37	24	17

Mean Number of Young/Female = 32.9 (S.D.= ±9) Survival = 100%

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.0015mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
<0.0013mg/l	3	0	2	0	1	0	0	2	0	2	0
	4	1	6	2	0	5	0	0	0	0	0
	5	7	0	7	12	11	7	9	10	11	11
	6	9	11	10	10	12	10	8	12	11	12
	7	14	12	X/12	0	15	14	9	5	2	9
	TOTAL	31	31	X/31	23	43	31	28	27	26	32

Mean Number of Young/Female = 30.3 (S.D.= ±5) Survival = 90%

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.0027mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
<0.0013mg/l	3	0	0	0	0	0	3	0	0	4	0
	4	6	5	0	2	5	0	1	7	0	0
	5	7	12	7	7	12	8	5	13	5	10
	6	11	11	2	9	9	15	12	0	7	7
	7	6	0	13	0	0	0	1	12	1	0
	TOTAL	30	28	22	18	26	26	19	32	17	17

Mean Number of Young/Female = 23.5 (S.D.= ±6) Survival = 100%

Table 6. (Continued)

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.0047 mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
0.0021	3	0	0	0	0	0	0	0	0	0	0
	4	2	3	3	0	0	5	1	3	0	1
	5	9	7	7	9	10	11	8	6	6	10
	6	10	12	X/0	X/2	12	7	16	11	5	10
	7	4	0	-	-	1	17	2	0	2	0
	TOTAL	25	22	X/10	X/11	23	40	27	20	13	21

Mean Number of Young/Female = 21.2 (S.D.= ±9) Survival = 80%

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.0085mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
0.0014mg/l	3	0	0	0	0	0	X/0	0	0	0	0
	4	0	3	4	0	0	-	5	1	0	0
	5	0	9	9	7	8	-	7	9	8	11
	6	0	13	5	4	0	-	0	12	0	13
	7	12	0	14	0	0	-	0	0	12	0
	TOTAL	12	25	32	11	8	X/0	12	22	20	24

Mean Number of Young/Female = 16.6 (S.D.= ±10) Survival = 90%

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.015mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
0.0096mg/l	3	0	0	0	0	0	0	0	0	0	0
	4	3	0	0	4	8	0	3	0	0	0
	5	4	5	8	10	0	0	2	4	10	8
	6	6	5	13	10	10	12	4	5	8	11
	7	16	0	2	0	2	0	1	0	0	1
	TOTAL	29	10	23	24	20	12	10	9	18	20

Mean Number of Young/Female = 17.5 (S.D.= ±7) Survival = 100%

Table 6. (Continued)

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.027mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
0.015mg/l	3	0	0	0	0	0	0	0	0	0	0
	4	3	1	0	1	3	0	3	0	0	0
	5	7	8	7	9	9	8	8	7	5	0
	6	5	11	12	5	8	11	10	9	11	0
	7	0	1	0	1	1	0	1	0	0	0
	TOTAL	15	21	19	16	21	19	22	16	16	0*

Mean Number of Young/Female = 18.3 (S.D.= ±3) Survival = 100%

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Theoretical U											
0.047mg/l	1	0	0	0	0	0	0	0	0	0	0
Recovered U	2	0	0	0	0	0	0	0	0	0	0
0.044mg/l	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	5	4	4	3	4	4	3	4	5	0	0
	6	1	9	8	7	10	10	9	7	8	0
	7	5	6	8	4	0	5	2	5	6	0
	TOTAL	10	19	19	15	14	18	15	17	14	0*

Mean Number of Young/Female = 15.7 (S.D.= ±3) Survival = 100%

* Male - Not included in reproduction data.

Average reproduction in the uranyl nitrate solutions was as follows:

Control		= 32.9 offspring per female
Theoretical U	Recovered U	
0.0015mg/l	<0.0013mg/l	= 30.1 offspring per female
0.0027mg/l	<0.0013mg/l	= 23.5 offspring per female
0.0047mg/l	0.0021mg/l	= 21.2 offspring per female
0.0085mg/l	0.0014mg/l	= 16.6 offspring per female
0.015mg/l	0.0096mg/l	= 17.5 offspring per female
0.027mg/l	0.015mg/l	= 18.3 offspring per female
0.047mg/l	0.044mg/l	= 15.7 offspring per female

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test and homogeneity of variances using Bartlett's Test. Log transformed data were found to be normally distributed (Chi-Square = 6.915, critical value = 13.28) with homogeneous variances (Bartlett's = 8.22; critical value = 12.59). Statistical analyses of the results using Dunnett's Multiple Comparison Procedure indicated chronic toxicity at the 0.0027mg/l, 0.0047mg/l, 0.0085mg/l, 0.015mg/l, 0.027mg/l and 0.047mg/l theorectial uranyl nitrate (as uranium) concentrations (actual recovered uranium concentrations <0.0013, 0.0021, 0.0014, 0.0096, 0.015 and 0.044mg/l, respectively). The no observed effect concentration (NOEC) was 0.0015mg/l uranyl nitrate (as uranium) (<0.0013mg/l actual recovered uranium) while the lowest observed effect concentration (LOEC) was 0.0027mg/l uranyl nitrate (as uranium) (<0.0013mg/l actual uranium). The chronic value (ChV), taken as the geometric mean of the NOEC and LOEC, was

0.0020mg/l uranyl nitrate (as uranium).

Note: The theoretical (as prepared) uranium concentrations were utilized for the determination of the NOEC, LOEC, and chronic value (ChV), due to the analytical detection level limit (<0.0013mg/l U).

Water chemistry data taken in conjunction with the chronic toxicity test are given in Table 7. All parameters monitored were within acceptable limits for bioassay purposes.

Table 7. Water chemistry data recorded in conjunction with the seven day toxicity bioassay to assess the chronic toxicity of Uranyl nitrate to Ceriodaphnia dubia, August 5 - 12, 1988.

Date	Treatment	D.O. (mg/l)	Temp. (°C)	pH (SU)	Cond. (umhos/cm)	Hard. (mg/l)	Alk. (mg/l)
8/5	Control	8.0	24.5	7.0	18	6.1	1.1
	0.0032 mg/l	8.0	24.5	7.0	21	-	-
	0.0056 mg/l	8.0	24.5	7.1	21	-	-
	0.010 mg/l	8.0	24.5	7.0	21	-	-
	0.018 mg/l	8.0	24.5	7.0	21	-	-
	0.032 mg/l	8.0	24.5	6.9	21	-	-
	0.056 mg/l	8.0	24.5	6.9	21	-	-
	0.10 mg/l	8.0	24.5	7.4	22	-	-
8/6	(Before Renewal)						
	Control	7.8	-	-	-	-	-
	0.0032 mg/l	7.8	-	-	-	-	-
	0.0056 mg/l	7.8	-	-	-	-	-
	0.010 mg/l	7.9	-	-	-	-	-
	0.018 mg/l	7.9	-	-	-	-	-
	0.032 mg/l	8.0	-	-	-	-	-
	0.056 mg/l	8.0	-	-	-	-	-
	0.10 mg/l	8.0	-	-	-	-	-
8/6	(After Renewal)						
	Control	8.0	26.0	7.5	-	-	-
	0.0032 mg/l	8.0	26.0	7.3	-	-	-
	0.0056 mg/l	8.0	26.0	7.2	-	-	-
	0.010 mg/l	8.0	26.0	7.4	-	-	-
	0.018 mg/l	8.0	26.0	7.3	-	-	-
	0.032 mg/l	8.0	26.0	7.4	-	-	-
	0.056 mg/l	8.0	26.0	7.3	-	-	-
	0.01 mg/l	8.0	26.0	7.3	-	-	-
8/7	(Before Renewal)						
	Control	7.8	-	-	-	-	-
	0.0032 mg/l	7.8	-	-	-	-	-
	0.0056 mg/l	7.9	-	-	-	-	-
	0.010 mg/l	7.9	-	-	-	-	-
	0.018 mg/l	7.8	-	-	-	-	-
	0.032 mg/l	7.8	-	-	-	-	-
	0.056 mg/l	7.8	-	-	-	-	-
	0.01 mg/l	7.8	-	-	-	-	-

Table 7. Continued:

Date	Treatment	D.O. (mg/l)	Temp. (°C)	pH (SU)	Cond. (umhos/cm)	Hard. (mg/l)	Alk. (mg/l)
8/7	(After Renewal)						
	Control	7.9	26.0	6.7	-	-	-
	0.0032 mg/l	7.9	26.0	6.7	-	-	-
	0.0056 mg/l	7.9	26.0	7.1	-	-	-
	0.010 mg/l	7.8	26.0	7.0	-	-	-
	0.018 mg/l	7.8	26.0	7.1	-	-	-
	0.032 mg/l	7.8	26.0	7.1	-	-	-
	0.056 mg/l	7.9	26.0	7.1	-	-	-
	0.01 mg/l	7.5	26.0	7.2	-	-	-
8/8	(Before Renewal)						
	Control	7.7	-	-	-	-	-
	0.0032 mg/l	7.6	-	-	-	-	-
	0.0056 mg/l	7.2	-	-	-	-	-
	0.010 mg/l	7.2	-	-	-	-	-
	0.018 mg/l	6.8	-	-	-	-	-
	0.032 mg/l	7.0	-	-	-	-	-
	0.056 mg/l	7.0	-	-	-	-	-
	0.01 mg/l	7.5	-	-	-	-	-
8/8	(After Renewal)						
	Control	7.7	26.0	7.5	-	-	-
	0.0032 mg/l	7.7	26.0	7.3	-	-	-
	0.0056 mg/l	7.8	26.0	7.1	-	-	-
	0.010 mg/l	7.5	26.0	7.1	-	-	-
	0.018 mg/l	7.5	26.0	7.1	-	-	-
	0.032 mg/l	7.5	26.0	7.1	-	-	-
	0.056 mg/l	7.5	26.0	7.0	-	-	-
	0.10 mg/l	7.5	26.0	7.1	-	-	-
8/9	(Before Renewal)						
	Control	7.7	-	-	-	-	-
	0.0032 mg/l	7.7	-	-	-	-	-
	0.0056 mg/l	7.6	-	-	-	-	-
	0.010 mg/l	7.6	-	-	-	-	-
	0.018 mg/l	7.5	-	-	-	-	-
	0.032 mg/l	7.6	-	-	-	-	-
	0.056 mg/l	7.6	-	-	-	-	-
	0.10 mg/l	7.4	-	-	-	-	-

Table 7. Continued:

Date	Treatment	D.O. (mg/l)	Temp. (oC)	pH (SU)	Cond. (umhos/cm)	Hard. (mg/l)	Alk. (mg/l)
8/9	(After Renewal)						
	Control	7.7	26.0	7.1	-	-	-
	0.0032 mg/l	7.7	26.0	7.0	-	-	-
	0.0056 mg/l	7.7	26.0	7.0	-	-	-
	0.010 mg/l	7.7	26.0	7.0	-	-	-
	0.018 mg/l	7.7	26.0	7.0	-	-	-
	0.032 mg/l	7.7	26.0	7.0	-	-	-
	0.056 mg/l	7.7	26.0	7.0	-	-	-
	0.10 mg/l	7.5	26.0	7.0	-	-	-
8/10	(Before Renewal)						
	Control	7.3	-	-	-	-	-
	0.0032 mg/l	7.3	-	-	-	-	-
	0.0056 mg/l	7.2	-	-	-	-	-
	0.010 mg/l	7.4	-	-	-	-	-
	0.018 mg/l	7.3	-	-	-	-	-
	0.032 mg/l	7.3	-	-	-	-	-
	0.056 mg/l	7.3	-	-	-	-	-
	0.01 mg/l	7.4	-	-	-	-	-
8/10	(After Renewal)						
	Control	7.4	26.0	7.6	-	-	-
	0.0032 mg/l	7.3	26.0	7.0	-	-	-
	0.0056 mg/l	7.3	26.0	7.0	-	-	-
	0.010 mg/l	7.4	26.0	7.0	-	-	-
	0.018 mg/l	7.5	26.0	7.0	-	-	-
	0.032 mg/l	7.4	26.0	7.0	-	-	-
	0.056 mg/l	7.5	26.0	7.0	-	-	-
	0.10 mg/l	7.5	26.0	7.0	-	-	-
8/11	(Before Renewal)						
	Control	7.5	-	-	-	-	-
	0.0032 mg/l	7.4	-	-	-	-	-
	0.0056 mg/l	7.4	-	-	-	-	-
	0.010 mg/l	7.5	-	-	-	-	-
	0.018 mg/l	7.6	-	-	-	-	-
	0.032 mg/l	7.5	-	-	-	-	-
	0.056 mg/l	7.5	-	-	-	-	-
	0.10 mg/l	7.5	-	-	-	-	-

Table 7. Continued:

Date	Treatment	D.O. (mg/l)	Temp. (°C)	pH (SU)	Cond. (umhos/cm)	Hard. (mg/l)	Alk. (mg/l)
8/11	(After Renewal)						
	Control	7.5	25.5	6.8	-	-	-
	0.0032 mg/l	7.5	25.5	6.9	-	-	-
	0.0056 mg/l	7.5	25.5	6.8	-	-	-
	0.010 mg/l	7.5	25.5	6.8	-	-	-
	0.018 mg/l	7.5	25.5	6.9	-	-	-
	0.032 mg/l	7.5	25.5	6.9	-	-	-
	0.056 mg/l	7.5	25.5	6.9	-	-	-
	0.10 mg/l	7.5	25.5	6.8	-	-	-
8/12	(Final)						
	Control	7.5	25.5	-	-	-	-
	0.0032 mg/l	7.4	25.5	-	-	-	-
	0.0056 mg/l	7.5	25.5	-	-	-	-
	0.010 mg/l	7.5	25.5	-	-	-	-
	0.018 mg/l	7.4	25.5	-	-	-	-
	0.032 mg/l	7.4	25.5	-	-	-	-
	0.056 mg/l	7.5	25.5	-	-	-	-
	0.10 mg/l	7.5	25.5	-	-	-	-

IV. REFERENCES

USEPA. 1985a. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA 600/4-85/013. 216pp.

USEPA. 1985b. Short-Term Methods For Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-85/014. 162pp.

V. ANALYSIS OF TEST SOLUTIONS

Two methods for the preparation of the test solutions used in this study were investigated. These are:

1. Standard serial dilution method

2. Dosing with microliter syringe

Analysis of solutions, prepared using Method 1, for uranyl nitrate concentration (as total uranium) produced highly variable, non-reproducible results. Actual analyte recoveries range from 0 to greater than 200% of the theoretical concentration.

Analysis of solutions prepared using Method 2 produced more consistent and acceptable data. Problems were encountered, however, at uranyl nitrate concentrations below 0.1 mg/L. These results are presented in Table 8.

Table 8. Results of analysis of test solutions for uranyl nitrate hexahydrate $UO_2(NO_3)_2 \cdot 6H_2O$ concentration.

Theoretical Concentration of $UO_2(NO_3)_2 \cdot 6H_2O$, mg/L	Theoretical Concentration of Uranium (Derived from Weight % Calculation)	Uranium, total (Observed) mg/l	Percent Uranium Recovery
0.0032	.0015	<.0013*	N/A
0.0056	.0027	<.0013*	N/A
0.010	.0047	.0021	45
0.018	.0085	.0014	16
0.032	.015	.0096	64
0.056	.027	.015	56
0.100	.047	.044	94
0.180	.085	.081	95
0.320	.15	.140	93
0.560	.270	.290	107
1.00	.470	.490	104

Notes:

MW $UO_2(NO_3)_2 \cdot 6H_2O$ = 502.13
Atomic Weight Uranium = 238.03

(All concentrations in Table 6 are calculated as total Uranium.)

* Detection limit of 0.0013

Note: Low level detection limits were achieved by sample preconcentration.

See Table 1 & 2 which were submitted to DuPont on October 24, 1988.

VI. METHODOLOGY

Metals

Aqueous

Sample Preparation Methods

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW846, Third Edition, USEPA, November 1986.

- Method 3010: Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by Flame Atomic Absorption Spectroscopy or Inductively Coupled Plasma Spectroscopy.

Sample Analysis Methods

Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, USEPA, March 1983.

- Method 200.7: Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes.

VII. QUALITY CONTROL DATA

Matrix Spike/Matrix Spike Duplicate Recovery Data

Constituent	Sample Spiked	Amount of Spike	Recovery MS	MSD	RPD
Uranium	DI Water	.10	116	124	7
Units		(mg/l)	(%)	(%)	(%)

Note: Deionized water was spiked with uranium to give a 0.100 mg/l final concentration.

Definition of Terms

MS - Matrix Spike
MSD - Matrix Spike Duplicate
RPD - Relative Percent Difference

ATTACHMENT VI

W. A. Fithian to J. L. Keyes. *References: Contract AX 843390 Task 1,2.* AnalytiKEM Inc., Cherry Hill, NJ 08003 (October, 1988).

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AnalytiKEM An American NuKEM Company

AnalytiKEM Inc.
28 Springdale Road
Cherry Hill, NJ 08003
609/751-1122
215/923-2068

John Does this meet our needs? JLK 10/27

October 24, 1988

E.I. du Pont de Nemours & Company, Inc.
Atomic Energy Division
Savannah River Plant
Aiken, South Carolina 29808-0001

Attention: John L. Keyes

References: Contact AX 843930 Task 1, 2

Dear Mr. Keyes:

In response to your letter of September 21, 1988, AnalytiKEM is pleased to document the Uranium results requested. Instrument detection limit (IDL) and spike recovery studies were undertaken, and the results are as follows:

1) The IDL study was performed by ICP. A 200 ppb Uranium standard was run seven times and the three sigma recovery limits were calculated. The data is presented in Table 1.

2) The spike recovery study was performed by ICP as well. Five standards were run at the following levels: 1 ppb, 5 ppb, 10 ppb, 25 ppb and 50 ppb. Results are presented in Table 2. The standards were concentrated by factors (described in Table 2) to enable their detection by ICP.

I trust that this data satisfies your needs. If there are any questions, please do not hesitate to call.

Very truly yours,

AnalytiKEM, Inc.

William A. Fithian
Laboratory Manager

WAF/eml

cc: P. de Andino
J. Shearard
J. McLaughlin

Table 1

Uranium Detection Limit Study

Uranium Standard Recoveries

Actual Values:	201
	194
	19[illegible]
	204
	222
	214
	216
	206
	219

σ = 10.2 ug/l

3 X σ = INSTRUMENT DETECTION LIMIT = 30.6 ug/l

Note: Study was conducted at 385.958 nm

Table 2

Uranium Standard Recovery Study

STANDARD AMOUNT (ug/l)	CONCENTRATION FACTOR	CONCENTRATION (ug/l) RECOVERED IN CONCENTRATE	CALCULATED CONCENTRATION	PERCENT RECOVERY
1.0	1000/10	87	0.87 ug/l	87
5.0	1000/10	504	5.04 ug/l	101
10	100/10	96	9.6 ug/l	96
25	100/10	248	24.8 ug/l	99
50	100/10	459	45.9 ug/l	92

Note: Stock Uranium Standard Solution was prepared by Inorganic Venture, Inc. in 2% nitric.

- Methodology

Metals

Aqueous

Sample Analysis Methods

Methods for Chemical Analysis of Wate and Wastes, EPA 600/4-79-020, USEPA, March 1983.

- Method 200.7: Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes.

ATTACHMENT VII

J. B. Pickett to J.L. Keyes. Inter-Office Memorandum, *Scope of Work for M-Area Effluent Toxicity Tests*. E. I. Du Pont de Nemours & Co., Savannah River Plant, Aiken, SC 29808 (September 30, 1988).

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OSR 3-4A

DUPONT

INTER-OFFICE MEMORANDUM

SAVANNAH RIVER PLANT

2/9/30/88 JMB

TO: J. L. KEYES, 703-A
ENVIRONMENT & ENERGY DEPARTMENT

FROM: J. B. PICKETT, 320-4M
RAW MATERIALS ENGINEERING & TECHNOLGY DEPARTMENT

SCOPE OF WORK FOR M-AREA EFFLUENT TOXICITY TESTS

A scope of work for the toxicity testing to be done by Analytikem is attached. Please transmit the scope to them.

JBP:smr
Att

CC: P. C. MAHONEY, 730-M
W. L. SPECHT, 773-42A
H. L. MARTIN, 730-M

PROPOSED SCOPE OF WORK FOR
M-AREA TOXICITY TESTING BY ANALYTIKEM (AX-843930)
TASK ORDER NO. _____

1. Chemical Testing

- Test the following three chemicals for acute & chronic toxicity.
  - Acute toxicity = 48 hr LC_{50}
  - Chronic toxicity = 7 day LOEC(Lowest Observed Effect Concentration) and NOCE(No Observed Effect Concentration)

  - Uranyl Nitrate - procure commercially
  - Hydrogen Uranyl Phosphate - prepare (procedure attached)
  - Uranium Dioxide - supplied by Du Pont

- Weighed amounts of each chemical in a known volume of water to be used. Also, each test solution (ie, each of the diluted toxicity solutions) is to be analyzed for total uranium concentration, with an analytical detection limit of 0.001 mg/L (as U).

- - Ceriodaphnia dubia to be used
  - Upper Three Runs Creek water as control(collected at SRP Road F or upstream of Road F.
  - Serial dilutions

- Chemical concentration (gms solid/liter of water) for both acute toxicity LC_{50} and the Chronic Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) is to be determined.

- Timing: Initial results on $UO_2(NO_3)_2$ to be completed by 8/31/88. Results on HUO_2PO_4 within three weeks of receiving the preparation details and on UO_2 within three weeks of receiving material from M-Area. M-Area plans to ship the UO_2 by 9/2/88.

2. Effluent Testing

- The following effluents are to be tested for acute and chronic toxicity.

  - M-005 (Air stripper effluent) plus non-contact cooling water; to be collected in sewer line downstream of M-005 and cooling water mixing point.

  - M-004 (LETF Effluent); to be collected when LETF operating.

  - A-014 Composite; to be collected when air stripper and LETF are operating, plus non-contact cooling water.

  - M-004 and simulated supernatant (H. L. Martin to prepare)

- A-014 composite and simulated supernatant (H. L. Martin to prepare)

- Tims Branch water above A-014 discharge (immediately below Beaver Dam; which is upstream of the A-014 discharge and the Tims Branch mixing zone).

- Du Pont will prepare shipping orders for UO_2 and composite samples (J. B. Pickett)

- Composite collection will probably be by Dupont personnel.

- The composite samples will be collected over a 24 Hr. period.

- Timing (TBD) Toxicity results to be transmitted within 3 weeks of receipt of samples.

3. Toxicity Testing Details

All toxicity tests will be conducted using EPA approved methods (Peltier and Weber,1985; Horning and Weber; 1985), except that the acute tests will be conducted at 25°C rather than 20°C.

ATTACHMENT

Preparation of $HUO_2PO_4 \cdot 4H_2O$ (mw = 438 gms/mole)

1) Mix uranyl nitrate and phosphoric acid on a 1 mole U to 1 mole PO_4 ratio.

2) Neutralize to pH 6-7 with NaOH.

3) Stir 15 minutes, and filter precipitate.

4) Rinse three times with D.I. water.

5) Dry ppt at 105° C overnight.

Resulting compound should be: $HUO_2PO_4 \cdot 4H_2O$ (Hydrogen uranyl phosphate, or hydrogen autenite)

ATTACHMENT VIII

Test Report No. A17852 (Part I), Acute and Chronic Toxicity of Uranyl Nitrate to Ceriodaphnia dubia. AnalytiKEM Inc., Cherry Hill, NJ 08003 (July, 1989).

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Rcd 8/10/89 gmp
Edited 10/13/89 gmp *

AnalytiKEM An American NuKEM Company

AnalytiKEM Inc.
28 Springdale Road
Cherry Hill, NJ 08003
609/751-1122
215/923-2068

TEST REPORT NO. A17852 (Part I)

ACUTE AND CHRONIC TOXICITY OF URANYL NITRATE TO CERIODAPHNIA DUBIA

April 11, 1989
July 13, 1989 (Revised) gmp

Prepared for:

Westinghouse Savannah River Company
Savannah River Site
P.O. Box 616
Aiken, SC 29802

Attention: John L. Keyes

NJ Certification No. NJ 04012

NY Certification No. NY 10815

SC Certification No. SC 94004

NC Certification No. NC 258

Reviewed & Approved by: Michael Shmookler

Name: Michael Shmookler, Ph.D.

Title: Technical Director

Accepted as is, did not request revised report gmp
minor corrections, editorial change and, cated in
final report. gmp 10/13/89.

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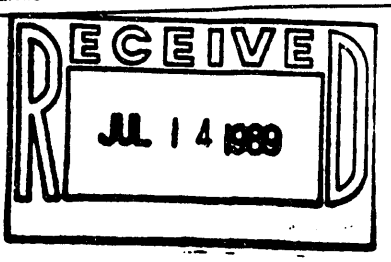
SHEALY ENVIRONMENTAL SERVICES, INC.
BIOLOGISTS, TOXICOLOGISTS & CHEMISTS

400 GRAYMONT AVENUE
COLUMBIA, SOUTH CAROLINA 29205

(803) 254-9915

July 13, 1989



Mr. Joseph P. McLaughlin, Manager
ANALYTIKEM, INC.
454 Anderson Road, BTC 532
Rock Hill, SC 29730

Dear Mr. McLaughlin:

Enclosed please find the revised reports on the Ceriodaphnia chronic toxicity tests for Hydrogen uranyl phosphate, uranyl nitrate and the M-Area effluents. As you will see from the reports we have made as many of the requested revisions as possible. Additionally, we have the following comments concerning technical concerns/questions which were addressed by Dr. John Pickett, Dr. Winona Specht and Mr. John Keyes:

1. All water samples have been filtered through a 37 um plankton net. The EPA protocol from EPA/600/4-85/014 is 30 um. The new EPA Bulletin (EPA/600/4-89/001), however; specifies to use a 60 um plankton net. Please advise as to which bore size is to be used in future toxicity tests. (Response to Item Number 1 of letter dated May 24, 1989).

2. For all future test samples and effluents will be aerated vigorously for 5 minutes when necessary to eliminate problem of supersaturation when samples are warmed to 25°C. (Response to Item Number 2 of letter dated May 24, 1989).

3. There is no EPA criteria for acceptable coefficients of variation, however; as requested we will maintain coefficients of variation for the control groups below 35% in all future tests. This may require that some tests be repeated at SAVANNAH RIVER SITE's expense. (Response to Item Number 5 of letter dated May 24, 1989).

4. Concerning the question of pH decline overtime in the hydrogen uranyl phosphate and uranyl nitrate tests we are enclosing calibration records for the pH readings for review. Please note the same dilution water was used for both tests. (Response to Item Number 6 of letter dated May 24, 1989).

5. The chronic values for the hydrogen uranyl phosphate test was an typographical error which was corrected in the revised report. The chronic value for the uranyl nitrate is correct as 0.0031 ppm and not 0.0037 ppm as Dr. Specht indicated. The value was derived as follows:

$$\text{Antilog}\left[\frac{\text{Log (NOEC)} + \text{Log (LOEC)}}{2}\right] =$$

$$\text{Antilog}\left[(0.0025 + 0.0039)/2)\right] = 0.0031$$

(Response to Item Number 7 of letter dated May 24, 1989).

Please call if you have any questions.

Sincerely,

Richard L. Shealy
President

for uranyl nitrate 2/9-2/16/89
H/HP

METER CALIBRATIONS

Parameter / Calibration	2/9 Initial	2/10 Day 1	2/11 Day 2	2/12 Day 3	2/13 Day 4	2/14 Day 5	2/15 Day 6	2/16 Day 7
pH Meter #	-	3	3	3	3	3	3	3
Buffer Temp.	18°C	19°C	18°C	18.5°C	18°	20°	20°	22°
Buffer 7	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.01
Buffer 10	-	-	-	-	-	-	-	-
Buffer 4	4=4.00	4=3.99	4=4.00	4=4.00	4=4.01	4=4.00	4=4.00	4=4.00
Slope (Diff mV)	151 ✓	151 ✓	163 ✓	169 ✓	174 ✓		172 ✓	151 ✓
Final Ck.	7=7.05	7=7.03	7=7.06	7=7.04	7=7.05	7=7.05	7=7.06	7=7.05
D.O. Meter #	2	2	2	2	2	2	2	2
Redline Ck.	✓	✓	✓	✓	✓	✓	✓	✓
Temperature	18.5°C	18.5	19°C	18	18°	21	22	22.5
Initial	9.50	9.50	9.30	9.40	9.30	9.20	8.40	8.90
Chart	9.35	9.35	9.26	9.45	9.45	8.90	8.72	8.64
Reset	9.35	9.35	9.26	9.45	9.46	8.90	8.72	8.64
Final Ck.	9.20	9.10	9.00	9.20	9.40	8.80	8.60	8.50

ACUTE AND CHRONIC TOXICITY OF URANYL NITRATE TO CERIODAPHNIA DUBIA

Report To

WESTINGHOUSE SAVANNAH RIVER COMPANY

Savannah River Site

Aiken, South Carolina

For; Report No A17852 (Part I)

Revised July 1989

Submitted By:

SHEALY ENVIRONMENTAL SERVICES, INC.
Columbia, South Carolina
(803) 254-9915

SCDHEC Laboratory Certification No. 26103

Richard L. Shealy, President

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I. INTRODUCTION

Acute and chronic toxicity tests were conducted January 25 - February 16, 1989, for the Savannah River Site to assess the acute and chronic toxicity of uranyl nitrate to Ceriodaphnia dubia.

II. METHODS

Dilution water for the toxicity tests was collected January 23 (Lab I.D. No. 89-0129), February 9, 1989 (Lab I.D. No. 89-0364), and February 13, 1989 (Lab I.D. No. 89-0371) from Upper Three Runs Creek at the northside of a bridge on Road 2-1 on the Savannah River Plant site by Mr. Jeff Bullard and shipped iced to the laboratory via Federal Express. The water was filtered with a plankton net (37 mm) and only water less than 96 hours old was used for the toxicity tests. Ceriodaphnia for the acute and chronic tests had been cultured in creek water since October 25, 1988.

A. Acute Toxicity Test

Test methods conformed to those described in USEPA (1985a; see Table 1). The 48-hour acute toxicity test was conducted January 25 - 27, 1989, with the following concentrations of uranyl nitrate: 1.0 mg/l (0.500 mg/l actual recovered uranium), 0.56 mg/l (0.270 mg/l recovered uranium), 0.32 mg/l (0.160 mg/l recovered uranium), 0.18 mg/l (0.088 mg/l recovered uranium) and 0.10 mg/l (0.051 mg/l recovered uranium). For the control, 100% dilution water was used.

All organisms used in the toxicity tests were from SHEALY ENVIRONMENTAL SERVICES, INC.'s in-house cultures which were obtained

Table 1: Summary of test conditions for the acute toxicity bioassay with *Ceriodaphnia dubia*.

1. Temperature:	25 ± 1°C
2. Light intensity:	Ambient laboratory levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	100 ml beakers
5. Volume of test solution:	50 ml
6. Age of test organisms:	2-24 hour old neonates
7. No. animals per test vessel:	10
8. No. replicate test vessels per per concentration:	2
9. Total no. organisms per concentration:	20
10. Feeding regime:	No feeding required
11. Aeration:	None, unless D.O. falls below 40% saturation, at which time gentle single-bubble aeration started.
12. Dilution water:	Upper Three Runs Creek Water at the Savannah River Plant Road 2-1
13. Test duration:	48 hours
14. Effect measured:	Mortality - no movement of appendages on gentle prodding

from the USEPA Newton Laboratory April 20, 1987, Lab I.D. No. 87-271. Ceriodaphnia from in-house cultures are identified and preserved monthly. Standard toxicant tests with the EPA reference toxicant cadmium chloride and laboratory reagent grade cadmium chloride are performed monthly on Ceriodaphnia cultured in water from Upper Three Runs Creek and in conjunction with the chronic toxicity tests. The results of these tests (LC50's = 0.08 - 0.17 mg/l cadmium chloride) demonstrated that the condition of the cultures were within the acceptable range for the test organisms (0.059 - 0.199 mg/l cadmium chloride). Test solutions and the controls were prepared in 50 ml quantities in all-glass test chambers. All concentrations and the control were tested in duplicate with ten Ceriodaphnia dubia neonates (2-24 hours old) each. The test solutions were renewed after 24-hours. A 100 mg/l uranyl nitrate stock solution was prepared on January 25, 1989, using reagent grade uranyl nitrate by rapidly weighing 0.0100 grams of the chemical onto a tared weighing paper in a balance containing desiccant. All uranyl nitrate test concentrations for the acute test were prepared from the 100 mg/l stock solution using Class A volumetric pipets. The uranyl nitrate stock solution was stored at or below 4°C during testing. Samples of all test solutions for the acute and chronic tests were preserved with 0.15% metals grade nitric acid and shipped with ice packs via Federal Express to ANALYTIKEM, INC. for verification.

Dissolved oxygen, water temperature, pH, conductivity, alkalinity and total hardness measurements were made in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers. The

test organisms were placed singly in the test vessels each containing 50 ml of solution. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia. Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding.

B. Chronic Toxicity Bioassay

Test methods conformed to those described in USEPA (1985b; see Table 2). The 7-day chronic toxicity bioassay was performed February 9 - 16, 1989, as seven treatments exposing 10 test organisms each. The first treatment was the control (100% filtered Upper Three Runs Creek Water). The uranyl nitrate solutions were 0.0032 mg/l, 0.0056 mg/l, 0.01 mg/l, 0.018 mg/l, 0.032 mg/l and 0.056 mg/l, as shown in Table 3. Actual recovered uranium values were 0.00033 mg/l and 0.0025 mg/l, 0.0039 mg/l, 0.0081 mg/l, 0.016 mg/l and 0.036 mg/l, respectively). All test solutions were prepared from a 103 mg/l stock uranyl nitrate solution prepared February 9, 1989, by dosing the dilution water with the appropriate aliquot using Hamilton microliter syringes (accuracy and reproducibility to ± 1%). The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

Table 2: Summary of test conditions for the chronic toxicity bioassay with *Ceriodaphnia dubia*.

1. Temperature:	25 ± 1°C
2. Light intensity:	Ambient laboratory levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	1 ounce SOLO plastic disposable cups
5. Volume of test solution:	15 ml
6. Age of test organisms:	2-24 hour neonates and all released within the same four hour period
7. No. animals per test vessel:	1
8. No. replicate test vessels per per concentration:	10
9. Total no. organisms per concentration:	10
10. Feeding regime:	*Selenastrum capricornutum* at the rate of 1-2,000,000 cells per ml test soln. per day
11. Aeration:	None
12. Dilution water:	Upper Three Runs Creek Water at the Savannah River Site Road 2-1
13. Test duration:	7 days
14. Effect measured:	Mortality - no movement of appendages on gentle prodding and number of offspring produced

Dissolved oxygen, water temperature, pH, and conductivity measurements were made daily in conjunction with the test. Temperature was maintained at 25° ± 1°C in all test chambers during the test.

The test organisms were placed singly in the test vessels each containing 15 ml of solution. The organisms were between 19 and 23 hours old at the start of the test. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at a rate of approximately 1,000,000 cells per ml. test solution per day. Selenastrum cultures were obtained from Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged Erlenmeyer flasks and maintained under bright fluorescent lighting for 6 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and order of feeding was established based on random number tables.

III. RESULTS

A. Acute Toxicity Bioassay

The results of the 48-hour acute toxicity bioassay are given in Table 3. All results are reported as test concentrations of total uranium recovered from the analyzed uranyl nitrate solutions. Mortality occurred in the 0.051 mg/l (15% mortality), 0.088 mg/l (45% mortality), 0.160 mg/l (95% mortality), 0.270 mg/l (95% mortality),

Table 3. Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of uranyl nitrate to Ceriodapnia dubia, January 25 - 27, 1989. Concentrations in theoretical and actual recovered uranium. Ten test organisms per replicate.

Test Concentration			Replicate	Number Dead After 24 Hours	Number Dead After 48 Hours	% Mortality
Control			A	0	0	0%
			B	0	0	
$UO_2(NO_3)_2 \cdot 6H_2O$	Theoretical U	Actual Recovered U				
0.10 mg/l	0.047 mg/l	0.051 mg/l	A	0	1	15%
			B	0	2	
0.18 mg/l	0.085 mg/l	0.088 mg/l	A	0	4	45%
			B	0	5	
0.32 mg/l	0.15 mg/l	0.160 mg/l	A	6	9	95%
			B	8	10	
0.56 mg/l	0.27 mg/l	0.270 mg/l	A	5	10	95%
			B	8	9	
1.0 mg/l	0.47 mg/l	0.500 mg/l	A	10	10	100%
			B	10	10	

and 0.500 mg/l (100% mortality) recovered uranium concentrations. No mortality occurred in the control. These data were used to determine a 48-hour LC50 (median lethal concentration) value with the Probit Method (EPA, 1985a). This calculation resulted in a 48-hour LC50 value of 0.089 mg/l recovered uranium with 95% confidence limits of 0.072 and 0.107 mg/l (See Appendix A).

Water chemistry data taken in conjunction with the acute bioassay are given in Table 4. All parameters monitored were within acceptable limits for bioassay purposes.

B. Chronic Toxicity Bioassay

The results of the 7-day chronic toxicity test are given in Table 5. Mortality of the adult females occurred in the 0.00033 mg/l (10% mortality), 0.0025 mg/l (10% mortality), 0.0039 (10% mortality), 0.0081 mg/l (10% mortality), and 0.016 mg/l (20% mortality) recovered uranium concentations. No mortality occurred in the control or the 0.036 mg/l actual uranium concentration. Reproduction in the control averaged 20 offspring per female. One male was observed in the 0.0025 mg/l uranium concentration. Males were not included in calculating the reproduction data as specified by Mr. Dave Graves (SCDHEC, Biological Services Division, personal communication).

Table 4. Water quality data recorded in conjunction with the 48-hour static renewal bioassay to determine the acute toxicity of Uranyl nitrate to Ceriodaphnia dubia, January 25 - 27, 1989.

Exposure Period	Parameter	Control	Test Concentrations (Actual Uranium) 0.051mg/l	0.088mg/l	0.160mg/l	0.270mg/l	0.500mg/l
0 Hours	D.O. (mg/l)	9.20	9.20	9.20	9.20	9.20	9.20
	Temp. (°C)	24.5	24.5	24.5	24.5	24.5	24.5
	pH (SU)	6.30	6.24	6.19	6.19	6.17	6.17
	Cond. (umhos/cm)	18	18	18	18	18	18
	Tot. Hard. (mg/l)	3.9	-	-	-	-	3.9
	Tot. Alk. (mg/l)	3.5	-	-	-	-	3.5
(Before Renewal) 24 Hours	D.O. (mg/l)	7.60	7.60	7.60	7.60	7.60	7.80
	Temp. (°C)	26.0	26.0	26.0	25.8	25.8	25.8
	pH (SU)	6.39	6.38	6.35	6.33	6.38	6.35
(After Renewal) 24 Hours	D.O. (mg/l)	8.00	8.00	8.00	7.90	8.00	-
	Temp. (°C)	25.5	25.5	25.5	25.5	25.5	-
	pH (SU)	6.48	6.39	6.40	6.39	6.32	-
	Tot. Hard. (mg/l)	3.9	-	-	-	3.9	-
	Tot. Alk. (mg/l)	3.5	-	-	-	3.5	-
48 Hours	D.O. (mg/l)	7.30	7.20	7.40	7.30	7.30	-
	Temp. (°C)	24.8	24.8	24.8	24.8	24.8	-
	pH (SU)	6.55	6.55	6.53	6.59	6.79	-

Table 5: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site.

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Control	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	11	0	3	2	2	7	0	4	2	4
	5	6	0	6	9	8	5	2	6	4	2
	6	6	3	10	0	11	0	4	7	10	6
	7	4	3	11	8	11	4	0	0	5	9
	TOTAL	27	6	30	19	32	16	6	17	21	21
	ADULT	L	L	L	L	L	L	L	L	L	L

$\bar{X}$= 19.5 S.D. = 8.89

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.0032) mg/l uranyl nitrate Recovered Uranium 0.00033 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	4	2	3	5	3	2	3	0	2	9
	5	6	3	4	4	10	5	4	6	5	2
	6	10	10	9	7	3	10	4	9	2	6
	7	0	0	5	10	5	5	8	D/0	5	9
	TOTAL	20	15	21	26	21	22	19	15	14	26
	ADULT	L	L	L	L	L	L	L	D	L	L

$\bar{X}$= 19.9 S.D. = 4.28

Table 5: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site. (Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.0056) mg/l uranyl nitrate Recovered Uranium 0.0025 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	4	4	4	4	2	4	2	2	0	4
	5	0	4	3	6	11	3	3	3	0	8
	6	12	5	10	D/9	0	4	10	9	0	0
	7	3	10	0	-	4	0	1	1	0	10
	TOTAL	19	23	17	19	17	11	16	15	0	22
	ADULT	L	L	L	D	L	L	L	L	L*	L

$\bar{X}$= 17.7 S.D. = 3.64

*Male not included in reproduction data.

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.010) mg/l uranyl nitrate Recovered Uranium 0.0039 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	3	3	0	D/0	0	0	2	3	4
	5	0	0	3	2	-	5	6	2	3	0
	6	0	7	4	10	-	5	4	3	3	5
	7	0	0	0	1	-	0	0	8	0	4
	TOTAL	0	10	10	13	0	10	10	15	9	13
	ADULT	L	L	L	L	D	L	L	L	L	L

$\bar{X}$= 9.0 S.D. = 5.10

Table 5: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site. (Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.018) mg/l uranyl nitrate Recovered Uranium 0.0081 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	5	0	3	2	2	3	2	0	2	2
	5	3	0	8	0	4	3	4	6	4	5
	6	3	0	0	D/0	6	8	6	6	10	5
	7	0	0	0	-	0	0	1	4	0	0
	TOTAL	11	0	11	2	12	14	13	16	16	12
	ADULT	L	L	L	D	L	L	L	L	L	L

$\bar{X}$= 10.7 S.D. = 5.43

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.032) mg/l uranyl nitrate Recovered Uranium 0.016 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	3	3	2	D/0	4	2	1	1	4	3
	5	0	5	6	-	0	2	2	4	3	4
	6	3	5	7	-	D/0	3	4	0	3	5
	7	0	2	0	-	-	2	0	0	0	0
	TOTAL	6	15	15	0	4	9	7	5	10	12
	ADULT	L	L	L	D	D	L	L	L	L	L

$\bar{X}$= 8.3 S.D. = 4.85

Table 5: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site.
(Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Dilution (0.056) mg/l uranyl nitrate Recovered Uranium 0.0036 mg/l	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	2	0	3	0	0	7	2	0	0	3
	5	0	0	4	0	0	0	0	3	4	0
	6	2	6	7	0	4	3	2	5	5	0
	7	1	2	0	0	2	0	0	0	2	0
	TOTAL	5	8	14	0	6	10	4	8	11	3
	ADULT	L	L	L	L	L	L	L	L	L	L

$\bar{X}$= 6.9 S.D. = 4.15

Average reproduction and mortality of the adult females in the uranyl nitrate solutions was as follows:

			Offspring per Female	% Mortality
	Control		= 19.5	0%
$UO_2(NO_3)_2.6H_2O$	Theoretical U	Recovered U		
0.0032	0.0015 mg/l	0.00033 mg/l	= 19.9	10%
0.0056	0.0027 mg/l	0.0025 mg/l	= 17.7	10%
0.010	0.0047 mg/l	0.0039 mg/l	= 9.0	10%
0.018	0.0085 mg/l	0.0081 mg/l	= 10.7	10%
0.032	0.015 mg/l	0.016 mg/l	= 8.3	20%
0.056	0.027 mg/l	0.036 mg/l	= 6.9	0%

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test. Log transformed data were found to be not normally distributed (Chi-Square = 20.91, critical value = 12.59). Statistical analyses of the results using the Wilcoxon Rank Sum Test indicated chronic toxicity at the 0.0039 mg/l, 0.0081 mg/l, 0.016 mg/l, and 0.036 mg/l recovered uranium concentrations. The no observed effect concentration (NOEC) was 0.0025 mg/l actual recovered uranium (0.0056 mg/l uranyl nitrate) while the lowest observed effect concentration (LOEC) was 0.0039 actual recovered uranium (0.010 mg/l uranyl nitrate). The chronic value (ChV), taken as the geometric mean of the NOEC and LOEC, was 0.0031 mg/l recovered uranium (0.0075 mg/l uranyl nitrate).

0.0036 Theoretical Uranium

Water chemistry data taken in conjunction with the chronic toxicity test are given in Table 6. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 14 and 15. For these days pH's of less than 6 standards units were recorded for all concentrations and the control.

Table 6. WATER CHEMISTRY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site.

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
Control (0%)	Temp. (deg. °C)	25.5		25.0		25.0		24.5		25.0		24.8		24.5	25.0	
	D.O. (ppm)	9.00	8.30	8.50	8.20	8.70	8.00	8.30	7.90	8.20	7.30	9.00	7.00	8.90	7.30	
	pH (SU)	6.35		6.24		6.17		6.20		6.15		5.86		5.89		
	Alk.(ppm CaCO3)	3.0		3.0		3.0		3.0		3.0		3.0		3.0		
	Hard.(ppm CaCO3)	3.8		3.8		3.8		3.8		3.8		3.8		3.8		
	Cond.(umhos/cm)	20		20		20		20		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
0.00033 mg/l Recovered Uranium	Temp. (deg.°C)	25.5		25.0		24.5		24.5		24.0		25.5		24.5	25.0	
	D.O. (ppm)	9.00	8.30	8.50	8.20	8.70	8.10	8.30	7.90	8.20	7.50	9.20	7.60	9.10	7.70	
	pH (SU)	6.35		6.25		6.15		6.19		6.15		5.88		5.90		
	Cond.(umhos/cm)	19		19		19		19		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
0.0025 mg/l Recovered Uranium	Temp. (deg.°C)	25.5		25.0		24.0		24.5		24.5		25.0		24.0	25.0	
	D.O. (ppm)	8.95	8.30	8.50	8.20	8.75	8.00	8.30	7.90	8.35	7.45	9.00	7.50	9.00	7.90	
	pH (SU)	6.32		6.30		6.14		6.15		6.10		5.88		5.88		
	Cond.(umhos/cm)	19		19		20		20		19		19		19		

Table 6. WATER CHEMISTRY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site. (Continued)

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg.°C)	25.5		25.0		24.0		25.0		24.0		25.0		24.5	25.0	
0.0039																
mg/l	D.O. (ppm)	9.00	8.40	8.50	8.25	8.70	8.00	8.30	7.90	8.40	7.60	8.95	7.70	9.35	7.90	
Recovered																
Uranium	pH (SU)	6.28		6.18		6.10		6.14		6.06		5.86		5.87		
	Cond.(umhos/cm)	19		19		20		20		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg.°C)	25.5		24.0		24.0		25.0		25.0		25.0		24.5	25.0	
0.0081																
mg/l	D.O. (ppm)	9.00	8.40	8.50	8.20	8.80	8.00	8.30	7.60	8.20	7.40	8.95	7.90	9.20	7.70	
Recovered																
Uranium	pH (SU)	6.25		6.15		6.10		6.10		6.05		5.86		5.85		
	Cond.(umhos/cm)	19		19		20		20		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg.°C)	25.5		24.0		25.0		24.5		26.0		24.0		24.0	25.0	
0.016																
mg/l	D.O. (ppm)	8.90	8.30	8.50	8.20	8.70	7.90	8.20	7.70	8.15	7.00	9.20	8.00	9.25	7.80	
Recovered																
Uranium	pH (SU)	6.24		6.10		6.06		6.05		6.01		5.87		5.89		
	Cond.(umhos/cm)	19		19		20		20		19		19		19		

Table 6. WATER CHEMISTRY DATA for the Ceriodaphnia Chronic Toxicity Test conducted with uranyl nitrate for the Savannah River Site. (Continued)

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg.°C)	25.5		24.0		25.0		24.0		26.0		25.0		24.5	25.0	
0.036	D.O. (ppm)	8.95	8.20	8.50	8.20	8.70	8.00	8.10	7.60	8.20	7.30	8.95	7.50	9.20	7.80	
mg/l Recovered Uranium	pH (SU)	6.24		6.10		6.05		6.04		6.01		5.85		5.86		
	Cond.(umhos/cm)	20		20		25		25		23		23		23		
	Alk.(ppm CaCO3)	3.0		3.0		3.0		3.0		3.0		3.0		3.0		
	Hard.(ppm CaCO3)	3.8		3.8		3.8		3.8		3.8		3.8		3.8		

APPENDIX A

Probit Analyses of Acute Data

(Concentration in Actual Recovered Uranium)

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

Probit Analysis of Uranyl Nitrate Acute Toxicity Test

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.0510	20	3	0.1500	0.1500	0.1486
0.0880	20	9	0.4500	0.4500	0.4961
0.1600	20	19	0.9500	0.9500	0.8690
0.2700	20	19	0.9500	0.9500	0.9827
0.5000	20	20	1.0000	1.0000	0.9995

Chi - Square Heterogeneity = 2.587

Mu = -1.053247
Sigma = 0.229407

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	9.591172	0.836020	(7.952574,	11.229771)
Slope	4.359067	0.802859	(2.785463,	5.932670)

Theoretical Spontaneous Response Rate = 0.0000

Probit Analysis of Uranyl Nitrate Acute Toxicity Test

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0259	0.0119	0.0380
EC 5.00	0.0371	0.0207	0.0500
EC10.00	0.0450	0.0276	0.0582
EC15.00	0.0512	0.0335	0.0646
EC50.00	0.0885	0.0715	0.1068
EC85.00	0.1529	0.1244	0.2162
EC90.00	0.1741	0.1387	0.2611
EC95.00	0.2109	0.1620	0.3476
EC99.00	0.3023	0.2144	0.6009

Probit Analysis of Uranyl Nitrate Acute Toxicity Test

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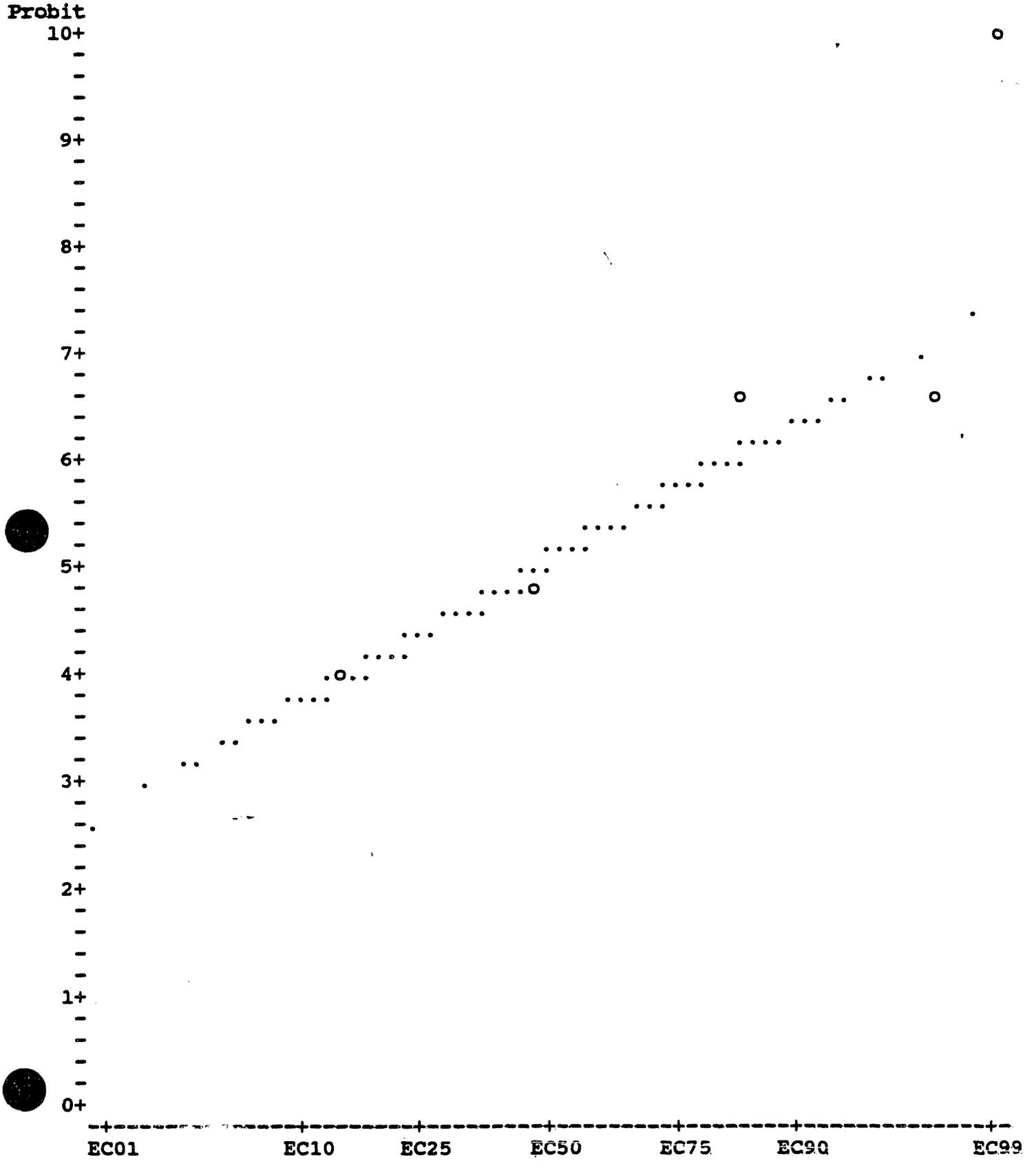

APPENDIX B

Wilcoxon's Rank Sums

Wilcoxon's Rank Sums

Uranyl Nitrate Conc. (mg/l)	Recovered U Conc. (mg/l)	Rank Sum	No. of Replicates	Critical Rank Sum
0.0032	0.00033	101.5	10	73
0.0056	0.0025	79.5	9	60
0.010	0.0039	71	10	73
0.018	0.0081	72	10	73
0.032	0.016	66	10	73
0.056	0.036	66	10	73

IV. METHODOLOGY

Metals

Aqueous

Sample Preparation Methods

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW846, Third Edition, USEPA, November 1986.

- Method 3010: Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by Flame Atomic Absorption Spectroscopy or Inductively Coupled Plasma Spectroscopy.

Sample Analysis Methods

Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, USEPA, March 1983.

- Method 200.7: Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes.

V. Analysis of Test Solutions

Results of analysis of test solutions of uranyl nitrate hexahydrate $UO_2(NO_3)_2 \cdot 6H_2O$ concentration (MW = 502.13)

Theoretical Concentration Total Uranium, mg/L	Observed Concentration Total Uranium, mg/L	Percent Recovery
0.0015	0.00033	22
0.0026	0.0025	96
0.0047	0.0039	83
0.0085	0.0081	95
0.015	0.016	106
0.026	0.036	140
0.047	0.051	109
0.085	0.088	104
0.150	0.160	106
0.263	0.270	103
0.470	0.500	106
51.0	48.0	94

VI. QUALITY CONTROL DATA

Matrix Spike/Matrix Spike Duplicate Recovery Data

Constituent	Sample Spiked	Amount of Spike	Recovery Matrix Spike
Uranium	DI Water	10	102
Uranium	DI Water	10	105
Uranium	DI Water	100	93
Units		(ug)	(%)

ATTACHMENT IX

K. E. Trapp and E. T. Korthals to John Pickett and J. L. Keyes. *Acute and Chronic Toxicity of Three Uranium Compounds to Ceriodaphnia dubia.* Report No. NA-SR-98, Normandeau Associates, Southeast, Aiken, SC 29802 (June, 1989).

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Acute and Chronic Toxicity of Three Uranium Compounds to *Ceriodaphnia dubia*

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E.T. Korthals

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June 1989

This report NAI-SR-98, was prepared for
Westinghouse Savannah River Company
Savannah River Site
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Aiken, South Carolina 29802

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June 1989

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TABLES

EXECUTIVE SUMMARY

Studies were conducted to assess the acute and chronic toxicities of three uranium compounds discharged from the M-Area Effluent Treatment Facility (ETF) into Tim's Branch, a tributary of Upper Three Runs Creek. The ETF facility is designed to process effluent discharged from the Fuel and Target Fabrication Facility (M-Area) of the Savannah River Plant. The waterflea, Ceriodaphnia dubia served as the test organism.

C. dubia static renewal toxicity tests were conducted to assess the acute toxicity of $UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2. Test results indicated that based on nominal concentrations of total U, UO_2 was the most acutely toxic of the three compounds (48 h LC50 = 0.05 mg/L total U; 95% confidence limits = 0.04 - 0.06 mg/L total U). The C. dubia 48 h LC50 for $UO_2(NO_3)_2 \cdot 6H_2O$ was 0.07 mg/L total U (95% confidence limits = 0.05 - 0.13 mg/L total U; nominal concentrations) while the LC50 for $HUO_2PO_4 \cdot 4H_2O$ equaled 0.11 mg/L total U (95% confidence limits = 0.10 - 0.12 mg/L total U; nominal concentrations).

C. dubia seven-day static renewal toxicity tests were conducted to determine the chronic toxicity of each of the three uranium compounds. A conservative interpretation of the chronic toxicity test results determined that the NOEC for uranyl nitrate in Upper Three Runs Creek water was <0.008 mg/L total U while the

LOEC equaled 0.008 mg/L total U (nominal concentrations). The NOEC and LOEC for $HUO_2PO_4 \cdot 4H_2O$ equaled <0.006 and 0.006 mg/L total U, respectively (nominal concentrations). The results of the C. dubia chronic toxicity test conducted on uranium dioxide determined that the NOEC equaled 0.03 mg/L total U and that the LOEC equaled 0.05 mg/L total U (nominal concentrations).

1.0 INTRODUCTION

Low concentrations of three different uranium compounds [$UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2] are currently being discharged from various outfalls located in M-Area on the Savannah River Plant (SRP). Discharge from these outfalls enters Tim's Branch, a tributary of Upper Three Runs Creek. Tim's Branch flows into this creek near Road C on SRP. Upper Three Runs Creek is a blackwater creek that runs through SRP property and discharges into the Savannah River. The water in Upper Three Runs Creek is generally acidic and demonstrates little or no buffering capacity (see Table 1-1). E. I. du Pont de Nemours & Co. requested that Normandeau Southeast (NAI-SE, SC DHEC Laboratory Identification Number 02101) determine the toxicity of each of the three uranium compounds to the waterflea, Ceriodaphnia dubia. Information generated from these tests will help determine if the three uranium compounds are having an effect on the aquatic biota of Tim's Branch or Upper Three Runs Creek.

This series of toxicity tests was designed to assess both the acute and chronic toxicity of the three uranium compounds. The toxicity tests were conducted in three stages. The first stage consisted of a series of range-finding tests in which C. dubia were exposed to a wide range of concentrations of each of the three uranium compounds. This phase was exploratory and its

Table 1-1. Results of basic water-chemistry analyses conducted on water samples collected from Upper Three Runs Creek and the Road 2-1 bridge on the Savannah River Plant. January 1986 - January 1988.

	pH	Total Hardness ($CaCO_3$ mg/L)	Total Alkalinity[a] ($CaCO_3$ mg/L)	Conductivity (mS/cm)
1986				
Jan. 14	5.50	2.5	1.0	0.020
Feb. 5	6.33	2.6	3.8	0.009
Mar. 5	5.90	4.5	1.0	0.010
Apr. 29	5.30	1.5	< DL	0.018
May 16	4.82	4.0	0.5	0.015
June 17	5.30	2.5	0.5	0.010
Aug. 13	5.53	3.5	2.0	0.015
Oct. 27	6.95	3.0	< DL	0.010
Dec. 9	5.30	5.0	< DL	0.012
1987				
Apr. 6	5.80	5.5	2.0	0.020
July 17	5.40	3.5	1.5	0.018
Sept. 16	5.15	2.5	< DL	0.012
Oct. 15	5.90	6.0	2.5	0.020
Dec. 9	5.90	5.0	1.5	0.020
1988				
Jan. 11	5.50	6.0	1.5	0.022

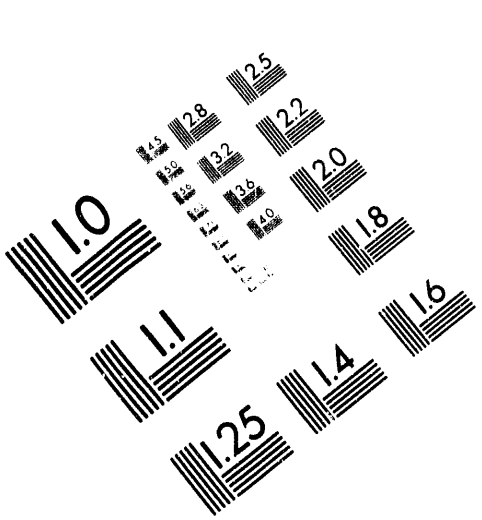
[a]Detection Limit = 0.1 mg/L.

purpose was to reduce the number of concentrations tested in the second phase by approximating concentrations of each compound that would produce 50% mortality among the test organisms. Once these initial tests were completed, definitive acute static renewal 48 h toxicity tests were conducted. This represented the second phase of testing and was designed to establish the concentration of each uranium compound that was lethal to 50% of the test organisms (lethal concentration, or LC50) within 48 h. The 95% confidence limits for each LC50 were also calculated. The 95% confidence limits of an LC50 provide some indication of the range of concentrations over which a similar acute response might be observed.

When the acute toxicities of $UO_2(NO_3)_2 \cdot 6H_2O$, UO_2 and $HUO_2PO_4 \cdot 4H_2O$ to C. dubia had been determined, the third stage of testing was initiated. Seven-day C. dubia static renewal life cycle tests were performed to identify those concentrations of each uranium compound that could have sublethal, long-term (chronic), adverse effects on aquatic organisms. C. dubia were exposed to a range of concentrations of each of the three compounds for seven days. The LC50 values determined for each compound in the acute toxicity tests were used to help establish the concentrations used in each chronic test. During any seven-day period, C. dubia individuals typically produce three broods of offspring. Test organism survival and offspring production served as criteria for determining the chronic toxicity of each uranium compound.

Statistical analyses of the C. dubia life cycle test results were used to identify a no-observable-effect concentration (NOEC), which was the highest concentration of toxicant [e.g., $UO_2(NO_3)_2 \cdot 6H_2O$, UO_2, or $HUO_2PO_4 \cdot 4H_2O$] that produced no statistically significant reduction in the survival or reproduction of test organisms when compared to control organism survival and reproduction. A lowest-observed-effect concentration (LOEC) was also identified. The LOEC represents that concentration of toxicant which produces a statistically significant adverse effect on test organism survival and reproduction (Horning and Weber 1985).

The results of the acute and seven-day chronic toxicity tests will be used to help determine a threshold or safe discharge concentration for each uranium compound so that these compounds can be released from M-Area outfalls without disrupting the normal propagation of fish and other aquatic life inhabiting Tim's Branch or Upper Three Runs Creek.



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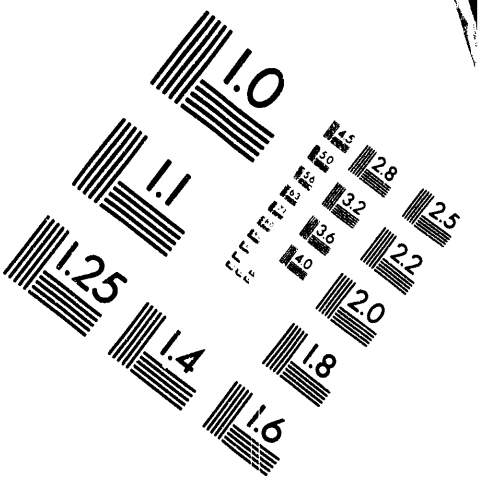

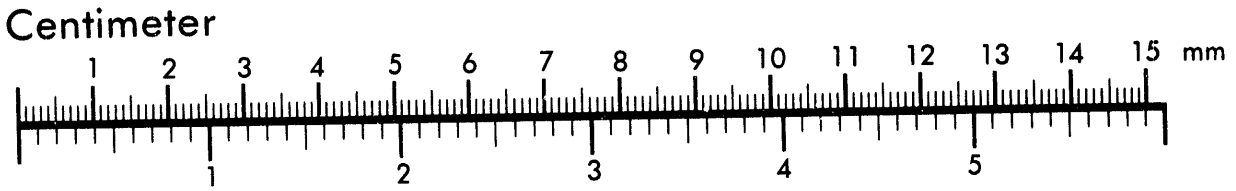

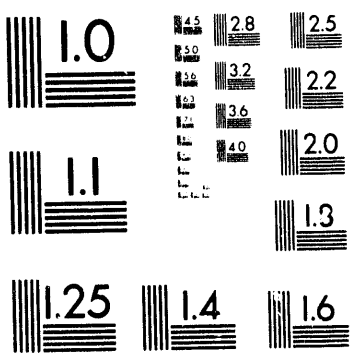

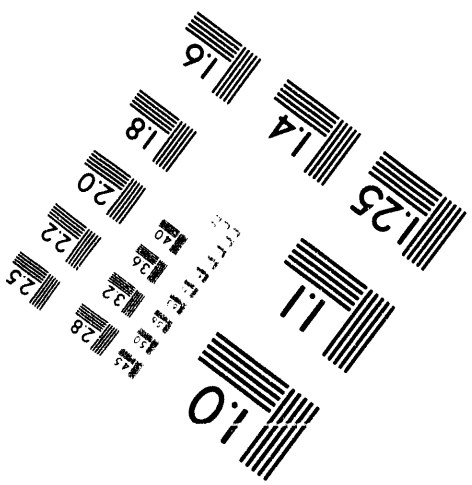

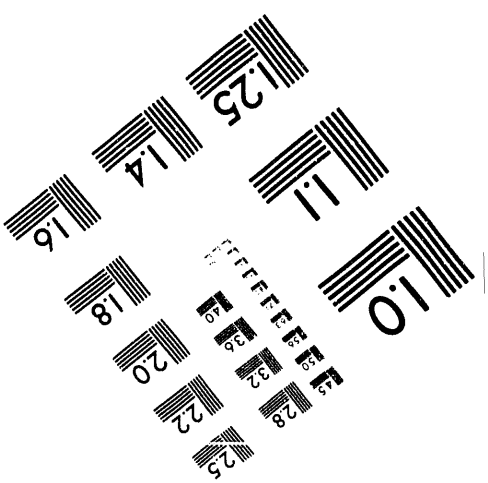

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2.0 METHODS AND MATERIALS

2.1 PREPARATION OF STOCK SOLUTIONS

Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The concentration of total uranium was confirmed analytically before each stock solution was used in a test. With the exception of $HUO_2PO_4 \cdot 4H_2O$, these solutions were prepared in sufficient quantities so that the same stock solution was used for both the acute and chronic toxicity tests. Insufficient quantities of hydrogen uranyl phosphate stock were initially prepared and another stock solution had to be made prior to initiation of the chronic toxicity test.

Concentrations of total uranium (dissolved plus bound uranium) were determined using either inductively coupled plasma emission spectroscopy (EPA method 200.7; EPA 1983) or fluorometry (Method 711-B; APHA 1985).

2.1.1 Uranyl Nitrate

Reagent grade uranyl nitrate (Mallinckrodt Lot #8640 KCAP) was used to prepare the stock solution of this compound. The stock solution was prepared by adding $UO_2(NO_3)_2 \cdot 6H_2O$ to Upper Three Runs Creek water. The stock solution was measured to determine the concentration of total uranium in the "as made" stock solution of uranyl nitrate. The concentration of total

uranium in this stock solution equaled 43.2 mg/L. This stock solution was used to prepare all $UO_2(NO_3)_2 \cdot 6H_2O$ solutions used in the range-finding, acute, and chronic tests.

2.1.2 Hydrogen Uranyl Phosphate

Reagent grade uranyl nitrate (Mallinkcrodt Lot #8640 KCAP) and phosphoric acid were used to prepare hydrogen uranyl phosphate. The uranyl nitrate was mixed with phosphoric acid (1:1; moles uranium to moles phosphate). This mixture was neutralized to pH 6-7 with 1.0 N NaOH and stirred for 15 min. The resulting precipitate ($HUO_2PO_4 \cdot 4H_2O$) was filtered through Whatman #4 filter paper, washed three times with deionized water, transferred to a watch glass and dried at 105° C for approximately 16 h.

Stock solutions of hydrogen uranyl phosphate were prepared by mixing 1 g of compound with 1 L of Upper Three Runs Creek water for approximately 1 h. The resultant suspension was filtered through a glass fiber filter (Whatman GF/C) and the filtrate used as a stock solution to prepare all toxicity test solutions. The stock solutions were measured to determine the concentration of total uranium in the "as made" stock solutions of hydrogen uranyl phosphate. Measured concentrations of uranium in the stock solutions equaled 1.2 and 3.8 mg/L total uranium. The first stock solution (1.2 mg/L total U) was used

in the range-finding and definitive acute toxicity tests. The second stock solution (3.8 mg/L total U) was used in the chronic toxicity test.

2.1.3 Uranium Dioxide

Uranium dioxide (UO_2) used in this study was received from E.I. du Pont de Nemours & Company, Savannah River Laboratory. The sample consisted of a liquid overlying a layer of solid material that had settled on the bottom of the container. The liquid portion was decanted and filtered through a glass fiber filter (Whatman GF/C). The fine particulates remaining in the filtrate were allowed to settle. A pipette was used to transfer the solution without resuspension of the particulate material. This solution served as the UO_2 stock solution for the acute and chronic toxicity tests. The stock solution was measured to determine the concentration of total uranium in the "as made" stock solutions of uranium dioxide. Measured concentration of uranium in this solution equaled 114 mg/L total uranium.

2.2 LABORATORY PROTOCOL

The guidelines and recommendations listed in Horning and Weber (1985) and Peltier and Weber (1985) were followed for handling organisms, cleaning test equipment, and conducting al

toxicity tests. Laboratory procedures are listed in detail, and deviations from methodology given in Horning and Weber (1985) or Peltier and Weber (1985) are noted. The waterflea, Ceriodaphnia dubia, served as the test organism.

2.2.1 Culture Methods

Ceriodaphnia dubia used in NAI-SE toxicity tests were originally obtained from cultures maintained by the US EPA Environmental Research Laboratory in Duluth, MN. These animals are now cultured by the NAI-SE aquatic toxicology laboratory in water collected from Upper Three Runs Creek (Aiken County, SC). Water is collected at the Road 2-1 bridge on the SRP and is filtered through a plankton net prior to use. Typical water quality values for this creek are listed in Table 1-1.

All-glass (1.5 L) culture dishes serve as culture chambers for a "brood" stock. The dishes are thoroughly cleaned prior to use and are covered while in use to prevent the entry of dust and other contaminants. Cultures are kept in an incubator (Lab-Line Instruments, Inc., Melrose Park, IL), and temperatures are maintained at 25 $\pm$ 2^{o}C. Water temperature is monitored continuously.

Wide-spectrum fluorescent bulbs (Color Rendering Index $\geq$ 90) are used to provide a 16L:8D photoperiod. Light intensity measured at

the surface of the culture dishes does not exceed 800 lux.

Brood-stock C. dubia (30 organisms/culture dish) are fed every other day on a diet consisting of a mixture of algae. (Selenastrum capricornutum), and YCT (yeast, cerophyll, fermented trout chow). Approximately 1 x 10^8 cells/mL of algae and 7 mL of YCT were added to each culture dish. A modified version of Bold's Basic Media (Appendix 1) is used to maintain uni-algal cultures of S. capricornutum.

All culture dishes are examined at least three times per week, and quality assurance records are maintained for each dish. Records include date the culture was started, source of culture material, reproductive progress, presence of ephippia, and other information on the condition of the culture deemed pertinent by the observer. The animals in these dishes serve as the source of neonate (≤ 24 h old) daphnids used in both acute and chronic toxicity tests. The first broods are discarded; only neonate daphnids obtained from broods other than a first brood are used in the toxicity tests.

2.2.2 Test Procedures

2.2.2.1 Collection of Water

Water from Upper Three Runs Creek served as the control and

diluent for both the acute and chronic toxicity tests conducted on $UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2. Water for all toxicity tests was collected from the Road 2-1 bridge located on SRP. Water was collected the day each test was initiated and was used within 72 h of collection. New samples of water were collected once every 72 h. Water was not filtered prior to use in the acute toxicity tests, but was filtered through a plankton net for use in the chronic tests. Filtration will remove potential predators from the diluent and is recommended by Horning and Weber (1985).

2.2.2.2 Ceriodaphnia dubia Acute Static Renewal Toxicity Tests

Several range-finding tests were performed to determine the concentrations of $UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2 to be used in subsequent definitive tests. Each range-finding test consisted of a control group and groups of at least five neonate daphnids, each of which was exposed approximately 48 h to one of at least four test concentrations. Based on the results of these range-finding tests, definitive tests of 48 h duration were initiated to establish the LC50 of each of the three uranium compounds.

All test solutions for the three definitive acute static renewal toxicity tests were prepared daily. Test solutions were prepared by diluting the previously described chemical stock

solutions. Aliquants of chemical stock solutions were transferred to 500-mL volumetric flasks with calibrated volumetric pipettes or pipetters. The contents of the flasks were then adjusted to 500 mL with Upper Three Runs Creek water. Separate volumetric flasks were used to prepare tests solution of each uranium compound. Test solutions were prepared from the lowest to the highest nominal concentration of total uranium using the same volumetric flask. The volumetric flasks were then cleaned (as described in Section 2.3) each day before use. Test concentrations used in each definitive acute toxicity test were based on information supplied by range-finding tests. Every attempt was made to prepare a typical geometric dilution series; however, the availability of pipettes capable of delivering the required volumes of stock solution restricted the choice of uranium concentrations used in each test. Because of this restriction, test concentrations spanned the necessary concentration range but deviated somewhat from a "typical" dilution series.

C. dubia were exposed to the following dilution series for each of the three compounds:

Uranyl Nitrate: 0 (control), 0.051, 0.127, 0.190, 0.254, 0.381 mg/L total uranium (nominal values).

Hydrogen Uranyl Phosphate: 0 (control), 0.04, 0.06, 0.08, 0.10, 0.12 mg/L total uranium (nominal values).

Uranium Dioxide: 0 (control), 0.01, 0.04, 0.07, 0.10, 0.13 mg/L total uranium (nominal values).

Test conditions are summarized in Table 2-1. Borosilicate beakers (250-mL) served as test chambers for the acute static renewal toxicity tests. Two beakers were used per test concentration, with 10 individuals per beaker. A large-bore, fire-polished, glass pipette was used to randomly transfer 10 neonate ($\leq$ 24 h old) daphnids to each test chamber. When 10 individuals had been isolated, excess water was removed and 100 mL of test solution was slowly and gently poured into the beaker. Following the addition of solution, the daphnids were observed to verify they had not been damaged during transfer.

The test temperature for the C. dubia acute static renewal toxicity tests was 25 $\pm$ 2°C. The tests were conducted in a temperature-controlled, Fisher model 307 incubator. Test organisms were exposed to a 16L:8D photoperiod. Specific conductance, dissolved oxygen concentration, $CaCO_3$ hardness, total alkalinity, and pH of the control and highest test concentrations were recorded at the beginning of each test and at 24 h intervals. The dissolved oxygen concentration, pH, temperature, and conductivity of intermediate test concentrations were measured and recorded at test initiation and at 24 h. Total alkalinity was determined by potentiometric titration (APHA 1985), while the CDTA (cyclohexanediaminetetraacetic acid)

Table 2-1. SUMMARY OF TEST CONDITIONS: Ceriodaphnia dubia[a] 48 h static renewal acute toxicity test

1. Test Temperature	25 ± 2° C .
2. Light quality	Ambient illumination
3. Light intensity	ambient laboratory levels
4. Photoperiod	16L:8D
5. Test vessel size/type	250-mL borosilicate glass beakers
6. Number of organisms per vessel	10
7. Number of replicates	2 per concentration
8. Age of organisms	≤ 24 h
9. Total number of organisms per concentration	20
10. Aeration	None, unless DO is ≤ 40% saturation
11. Diluent	Upper Three Runs Creek water
12. Test Duration	48 h
13. Effect Measured	Mortality (LC50 ± 95% confidence limits)
14. Chemical Parameters Measured on diluent and highest test concentration	DO, °C, pH, conductivity, hardness alkalinity, (daily on new and old solutions)
15. Chemical Parameters Measured on intermediate test concentrations	DO, °C, pH, conductivity (daily on new and old solutions)

[a]Adapted from Peltier and Weber 1985.

titrimetric method (APHA 1985) was used to measure $CaCO_3$ hardness. Dissolved-oxygen concentrations were measured with a YSI Model 58 DO meter (Yellow Springs Instrument Co., Yellow Springs, OH), and a YSI Model 33 conductivity meter was used to measure the conductivity of each test solution. The pH values were determined with an Orion 399A pH meter.

Death or immobilization of the C. dubia were used as the indicators of acute toxicity. The criterion used to establish lethality was cessation of all visible signs of mobility (e.g., no movement of second antennae, thoracic legs, or postabdomen). Immobilization was defined as the inability of the animals to move in the water column (ASTM 1984).

2.2.2.3 Ceriodaphnia dubia Chronic Toxicity Tests

Organisms used in these tests were $\leq$ 24 h old, and all organisms used in a given test were born within 4 h of one another. All test solutions for the three chronic toxicity tests were prepared daily. Test solutions of uranyl nitrate and hydrogen uranyl phosphate were prepared in 500-mL volumetric flasks by diluting each chemical stock solution. Uranium dioxide test solutions were prepared in a 1000-mL volumetric flask by diluting a secondary stock solution. The secondary stock was prepared from the original stock solution and used throughout the chronic toxicity test. To prepare chronic test solutions, aliquants of chemical stock solutions were transferred to

volumetric flasks with calibrated pipettes or pipetters. Each flask was brought to volume with Upper Three Runs Creek water. A separate volumetric flask was used to prepare test solutions for each uranium compound throughout each test. Test solutions were prepared from the lowest to the highest nominal concentration of total uranium and the volumetric flasks cleaned each day between use.

Test conditions are summarized in Table 2-2. Testing was performed in 20-mL glass scintillation vials containing 15 mL of test solution. All test vials were placed in an incubator maintained at 25 ± 1°C. Temperature was monitored continuously. Test organisms were exposed to a 16L:8D photoperiod. Twenty individuals were exposed to each test concentration and to the control. The following dilutions were used in the seven-day static renewal life cycle tests conducted on each of the three uranium compounds:

Uranyl Nitrate: 0 (Control), 0.002, 0.008, 0.023, 0.046, 0.076 mg/L total uranium (nominal)

Hydrogen Uranyl Phosphate: 0 (Control), 0.006, 0.02, 0.06, 0.12, 0.20 mg/L total uranium (nominal)

Uranium Dioxide: 0 (Control), 0.0015, 0.005, 0.015, 0.03, 0.05 mg/L total uranium (nominal)

Table 2-2 SUMMARY OF TEST CONDITIONS: *Ceriodaphnia dubia*[a]
7 d chronic static renewal toxicity test

1. Test Temperature	25 ± 1° C
2. Light quality	Ambient illumination
3. Light intensity	ambient laboratory levels
4. Photoperiod	16L:8D
5. Test vessel size/type	20-mL borosilicate glass scintillation vials
6. Number of organisms per vessel	1
7. Number of replicates	20 per concentration
8. Age of organisms	≤ 24 h
9. Total number of organisms per concentration	20
10. Aeration	None, unless DO is ≤ 40% saturation
11. Diluent	Upper Three Runs Creek water
12. Test Duration	7 d
13. Effect Measured	Mortality, reduced young production (NOEC and LOEC)
14. Chemical Parameters Measured on diluent and highest test concentration	DO, °C, alkalinity, hardness, pH, conductivity (daily on new solutions)
15. Chemical Parameters Measured on intermediate test concentrations	DO, °C, pH, conductivity (daily on old and new solutions)

[a]Adapted from Horning and Weber 1985.

Large-bore, fire-polished, disposable glass pipettes were used to transfer organisms. Test organisms were moved to fresh test solution every 24 h, and all young produced during a test were preserved with Lugol's solution (APHA 1985) for later enumeration. Following transfer, the organisms were observed to verify they had not been damaged.

Specific conductance, dissolved-oxygen concentration (DO), $CaCO_3$ hardness, total alkalinity, and pH were recorded for the new and old control solutions as well as the highest concentrations of test solutions. Only conductivity, DO, pH, and temperature of old, new and intermediate concentrations of test solutions were measured. The same methods used to monitor water quality parameters during the acute static renewal toxicity tests were also used during all C. dubia life cycle tests.

C. dubia were fed during each test by adding an aliquot of algal suspension/YCT mixture (0.033 mL/mL) to each vial. YCT was added to increase the protein content of the diet. The other nutritional requirements of these organisms (e.g., vitamins, dietary lipids, minerals) were provided by the algal portion of the diet.

Death or immobilization of the organisms was used as an indicator of acute toxicity (Peltier and Weber 1985). The criterion used to establish lethality was cessation of all visible signs of mobility (e.g., no movement of second antennae,

thoracic legs, or postabdomen). Immobilization was defined as the inability of the animals to move in the water column (ASTM 1984). On Day 7, adult survival was determined, and a count was made of the total number of young produced per test organism. During any seven day period, C. dubia individuals typically produce three broods of offspring. A test was deemed acceptable if control mortality was $\leq$ 20% (Horning and Weber 1985) and if the average number of young produced per control individual was $\geq$ 15. (SC DHEC 1988). Chronic toxicity was determined to have occurred if statistical analyses determined that significant differences existed between the control and test organisms.

2.3 GLASSWARE PREPARATION

All glassware was cleaned before and after use. It was soaked for 24 h in a 5% Contrad solution, rinsed with tap water, allowed to air-dry, and rinsed with pesticide-free acetone. The glassware was again air-dried and then soaked for 24 h in 2% HNO_3. Deionized water was used in the final rinses (5 times with deionized water) of the glassware. All borosilicate beakers used in the toxicity tests were maintained separately from other laboratory glassware and were used only for toxicity tests. Just prior to use, these beakers were rinsed with dilution water.

2.4 DATA ANALYSIS

The 48 h LC50 values were determined by using either binomial probability (Stephan et al. 1978) or the Trimmed Spearman-Karber procedure (Hamilton et al. 1977, 1978). Chronic toxicity test data were analyzed using Fisher's Exact test, the Chi-Square test, Bartlett's test, one-way analysis of variance (ANOVA), and Dunnett's Multiple Comparison test (Sokal and Rohlf 1981, Zar 1984).

2.5 QUALITY ASSURANCE

Quality assurance procedures commonly followed in the NAI-SE Aquatic Toxicology Division include the following:

1. Instruments are routinely calibrated and standardized according to manufacturers' instructions. Control charts are maintained for all measured parameters.
2. Wet chemistry methods used in determining hardness and alkalinity are standardized according to US EPA methods.
3. Records are maintained of the age, productivity, quality of food, and feeding regime of all organisms maintained by NAI-SE.
4. Reference toxicity tests are performed on a routine basis (at least monthly) to determine the acceptability and sensitivity of test organisms. Reference toxicant control charts are maintained for all test organisms

cultured by NAI-SE. Results of reference tests indicated that the animals used in these tests responded in an appropriate manner.

5. In order to measure the precision with which the technician prepared these test solutions, a surrogate metal was used to prepare solutions in a manner identical to that used during the toxicity tests. Manganese was chosen as a surrogate metal for uranium because of its low analytical cost and because it is routinely used as a standard to check the ICP instrument. A HACH manganese standard (1000 mg/L) was diluted with deionized water using volumetric glassware and calibrated pipets. A subsample of each prepared manganese solution was analysed to determine the concentration of total manganese. Results of the manganese analyses are summarized in Table 2-3. These results demonstrated that the technician responsible for preparing the solutions used in all toxicity tests conducted on the three uranium compounds prepared the manganese solutions such that the percent recovery of manganese ranged from 91.8 to 97.4%.

Table 2-3. Chemical analysis of manganese test dilutions.

Nominal concentrations of manganese reflect the same dilution factor as that used in chronic toxicity tests conducted on uranyl nitrate, hydrogen uranyl phosphate, uranium dioxide.

	Nominal (mgMn/L)	Measured (mgMn/L)	% Recovery
Test #1	1.58	1.56	98.7
	5.26	5.18	98.5
	15.79	15.7	99.4
	31.58	29.9	94.7
	52.63	50.1	95.2
			$\bar{x} \pm sd = 97.3 \pm 2.2$
Test #2	0.015	0.013	86.7
	0.05	0.044	88.0
	0.15	0.140	93.3
	0.3	0.278	95.7
	0.5	0.476	95.2
			$\bar{x} \pm sd = 91.8 \pm 4.2$
Test #3	0.05	0.046	92.0
	0.18	0.169	93.9
	0.53	0.503	94.9
	1.06	1.01	95.3
	1.76	1.69	96.0
			$\bar{x} \pm sd = 94.4 \pm 1.6$

3.0 RESULTS

3.1 URANIUM ANALYSES

3.1.1 Acute Toxicity Test Solutions

The concentration of total uranium concentrations in each of the three stock solutions was measured before the acute toxicity tests were initiated. Total uranium concentrations of the stock solutions were determined by using inductively coupled plasma-atomic emmission spectrophotometry (EPA 200.7, EPA 1983; detection limit = 0.1 mg/L).

The stock solutions were used to prepare each test solution. The test solutions were prepared daily (See Section 2.2.2.2). The concentration of total uranium in the highest and the intermediate dilution for each uranium compound was analytically verified. Concentrations of total uranium measured in the test solutions used in the static renewal acute toxicity tests are summarized in Table 3.1. Two methods were employed to analyse these samples; ICP (EPA 200.7, EPA 1983 detection limit = 0.1 mg/L) or fluorometric technique (SM 711B, APHA 1985; detection limit = 0.02 mg/L). The method selected for each analysis was based on the detection limit.

Table 3-1. Uranium Concentrations for Acute Toxicity Tests

Results of chemical analyses performed on the highest and intermediate concentrations of uranium compounds used in three Ceriodaphnia dubia 48 h static renewal acute toxicity tests. Upper Three Runs Creek water served as the control and diluent for these tests.

Measured Stock Solution Concentrations (mg/L total U)	Nominal* Concentrations (mg/L total U)	Measured Concentrations Date		
		Nov 21	Nov 22	Mean
URANYL NITRATE				
43.0	0.051	0.047	0.038	0.043
	0.127	0.143	0.090	0.117
	0.190	0.219	NA	----
	0.254	0.325	NA	----
	0.381	0.322	NA	----
HYDROGEN URANYL PHOSPHATE				
1.2	0.08	0.064	0.061	0.062
	0.12	0.131	0.117	0.124
		Dec 2	Dec 3	Mean
URANIUM DIOXIDE				
114.0	0.070	0.060	0.068	0.064
	0.130	0.120	0.182	0.151

NA = Not applicable; complete test organism mortality occurred within 24 h of test initiation and these test solutions were not renewed.

*Nominal concentrations based on dilution of measured stock solutions.

For purposes of clarity, all concentrations of uranium in the text are expressed in terms of the nominal concentrations of total uranium. Toxicity endpoints (e. g. LC50 values and NOECs) are converted to measured concentrations of total uranium in Section 4.0 of this text.

3.1.2 Chronic Toxicity Test Solutions

With the exception of hydrogen uranyl phosphate, the concentration of total uranium in stock solutions was not determined again prior to performing the chronic toxicity tests. A new stock of hydrogen uranyl phosphate had to be prepared to conduct the chronic toxicity test. The concentration of total uranium in this new stock solution was determined by the same method previously noted. (See Table 3.2)

Test concentrations were prepared daily using the stock solutions to prepare the appropriate test solutions (See Section 2.2.2.3). Total uranium concentrations were measured daily in the highest test solution used in each of the three chronic tests. Results of uranium analyses for the three static renewal chronic toxicity tests are summarized in Table 3.2. The total uranium concentrations of these solutions was determined by the fluorometric technique (SM 711B, APHA 1985).

Table 3-2. Uranium Concentrations for Chronic Toxicity Tests

Results of chemical analyses performed on the highest test concentration of uranium compounds in three Ceriodaphnia dubia static renewal chronic toxicity tests. New test solutions prepared daily. Water from Upper Three Runs Creek served as the control and diluent for these tests.

Measured Stock Solution Concentrations (mg/L total U)	Nominal* Concentrations (mg/L total U)	Measured Concentrations								
		DATE								
		Dec 9	Dec 10	Dec 11	Dec 12	Dec 13	Dec 14	Dec 15	Mean	std.
URANYL NITRATE										
43.0	0.002		0.002							
	0.008		0.007							
	0.023		0.020							
	0.046		0.039							
	0.076	0.085	0.068	0.043	0.025	0.048	0.053	0.096	0.060	0.025
URANIUM DIOXIDE										
114.0	0.05	0.033	0.044	0.027	0.065	0.033	0.027	0.018	0.035	0.015
		DATE								
		Dec 13	Dec 14	Dec 15	Dec 16	Dec 17	Dec 18	Dec 19		
HYDROGEN URANYL PHOSPHATE										
3.8	0.12	0.124	0.104	0.133	0.129	0.098	0.097	0.150	0.119	0.020

*Nominal concentrations based on dilution of measured stock solutions.

For purposes of clarity, all concentrations of uranium in the text are expressed in terms of the nominal concentrations of total uranium. Toxicity endpoints (e. g. LC50 values and NOECs) are converted to measured concentrations of total uranium in Section 4.0 of this text.

3.2 48 H ACUTE TOXICITY TESTS (CERIODAPHNIA DUBIA)

3.2.1 Uranyl Nitrate

A 48 h acute static toxicity test was conducted to determine the LC50 of $UO_2(NO_3)_2$ to C. dubia. The results of initial and final basic water chemistry analyses performed on all solutions used in this acute test are listed in Table 1 in Appendix 2.

Significant mortality ($\geq$ 95% mortality) was observed at the four highest test concentrations 24 h following test initiation (Table 3-3). At 48 h, partial mortality was observed at the lowest concentrations tested; complete mortality was observed at all other test concentrations (Table 3-3). These test results were used to estimate a 48 h LC50 of 0.07 mg/L total uranium (95% confidence intervals = 0.05 to 0.127 mg/L total uranium; Table 3-3).

Table 3-3. Acute Toxicity Test Conducted on Uranyl Nitrate

Results of a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on uranyl nitrate. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent for this test. 21 - 23 November 1988.

Nominal Concentration (mg/L total U)[b]	Measured Concentrations (mg/L total U) (11-21-88)	Measured Concentrations (mg/L total U) (11-22-88)	Total Mortalities[a] 24h	48h	% Mortality at 48 h
Control			0	1	5
0.051	0.047	0.038	0	3	15
0.127	0.143	0.090	19	20	100
0.190	0.219		20	--	100
0.254	0.325		20	--	100
0.381	0.322		20	--	100

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate). Test vessels = 250-mL glass beakers containing 100 mL/beaker.

[b]Nominal concentrations based on measured stock solutions.

48 h LC50 = 0.071 mg/L
95% Confidence Limits =
0.051 - 0.127 mg/L

Method of Calculation =
Binominal Probability

3.2.2 Hydrogen Uranyl Phosphate

The acute toxicity of $HUO_2PO_4 \cdot 4H_2O$ to C. dubia was also determined. The results of initial and final basic water chemistry analyses performed on all solutions used in this acute test are listed in Table 2 in Appendix 2.

No test organism mortality was observed in any of the test concentrations of hydrogen uranyl phosphate after 24 h of exposure (Table 3-4). Partial mortality was observed at all test concentrations 48 h following test initiation and a 48 h LC50 of 0.11 mg/L total uranium was calculated from these data (95% confidence limits = 0.10 to 0.12 mg/L total uranium; Table 3-4).

3.2.3 Uranium Dioxide

The toxicity of UO_2 to C. dubia was determined in a 48 h acute static toxicity test. The results of initial and final basic water chemistry analyses performed on all solutions used in this acute test are listed in Table 3 in Appendix 2.

Exposure to test concentrations $\geq$ 0.10 mg total uranium resulted in complete mortality to test organisms 48 h following test initiation (Table 3-5). The results of this

Table 3-4. Acute Toxicity Test Conducted on Hydrogen Uranyl Phosphate

Results of a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent for this test. 21 - 23 November 1988.

Nominal Concentration (mg/L total U)[b]	Measured Concentrations (mg/L total U) (11-21-88)	Measured Concentrations (mg/L total U) (11-22-88)	Total Mortalities[a] 24h	48h	% Mortality at 48 h
Control			0	0	0
0.04			0	3	15
0.06			0	1	5
0.08	0.064	0.061	0	4	20
0.10			0	5	25
0.12	0.131	0.117	0	14	70

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate). Test vessels = 250-mL glass beakers containing 100 mL/beaker.

[b]Nominal concentrations based on measured stock solutions.

48 h LC50 = 0.11 mg/L
95% Confidence Limits =
0.10 - 0.12 mg/L

Method of Calculation =
Trimmed Spearman-Karber

Table 3-5. Acute Toxicity Test Conducted on Uranium Dioxide

Results of a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on uranium dioxide. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent for this test. 2 - 4 December 1988.

Nominal Concentration (mg/L total U)[b]	Measured Concentrations (mg/L total U) (12-2-88)	Measured Concentrations (mg/L total U) (12-3-88)	Total Mortalities[a] 24h	48h	% Mortality at 48 h
Control			0	0	0
0.01			0	0	0
0.04			0	4	20
0.07	0.060	0.068	1	14	70
0.10			3	20	100
0.13	0.120	0.182	11	20	100

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate). Test vessels = 250-mL glass beakers containing 100 mL/beaker.

[b]Nominal concentrations based on measured stock solutions.

48 h LC50 = 0.050 mg/L
95% Confidence Limits =
0.04 - 0.06 mg/L

Method of Calculation =
Trimmed Spearman-Karber

test were used to estimate a 48 h LC50 of 0.050 mg/L total uranium (95% confidence intervals = 0.04 - 0.06 mg/L total uranium; Table 3-5).

3.3 CHRONIC SEVEN-DAY STATIC RENEWAL LIFE CYCLE TESTS (CERIODAPHNIA DUBIA)

The purpose of the chronic toxicity tests was to determine concentrations of $UO_2(NO_3)_2 \cdot 6H_2O$, $HUO_2PO_4 \cdot 4H_2O$, and UO_2 that would adversely affect the test organisms, either by reducing adult C. dubia survival or by reducing their reproductive capacity.

3.3.1 Uranyl Nitrate

The results of initial and final basic water chemistry analyses performed on all solutions of $UO_2(NO_3)_2 \cdot 6H_2O$ used in this chronic test are listed in Appendix 3 (Tables 1 and 2). The concentration of total uranium in the highest test solution of uranyl nitrate used in the seven-day chronic was measured daily. These values are listed in Table 3-2.

By the end of the test, mortality was relatively high among the original females C. dubia exposed to 0.002 mg/L total uranium (35 % mortality; Table 3-6). Few deaths were observed among organisms exposed to the other test

Table 3-6. Chronic Toxicity Test Conducted on Uranyl Nitrate

Results of a static renewal life cycle test for Ceriodaphnia dubia exposed to uranyl nitrate. Work was performed for E. I. du Pont de Nemours & Company, Aiken, SC. Water collected from Upper Three Runs Creek served as the control and diluent. 9 - 16 December 1988.

Nominal* concentration (mg/L)	Mean Measured Concentrations (mg/L total U)	Measured concentrations Dec 10 (mg/L total U)	$\bar{X}$ (SD) of young per female[a]	$\bar{X}$ broods per female[b]	% Mortality of original females
Control			15.5 (5.22)	2.3	5
0.002		0.002	9.70 (8.45)	1.6	35
0.008		0.007	11.40 (5.80)	2.2	10
0.023		0.020	12.55 (5.14)	2.2	5
0.046		0.039	8.65 (5.92)	1.8	5
0.076	0.060	0.068	5.05 (8.45)	1.4	10

[a]Mean value based on number of young produced by 20 original females.
[b]Mean value based on surviving original females.
*Nominal concentrations based on measured stock solutions.

NOEC = 0.023 mg/L total U

LOEC = 0.046 mg/L total U

msd = 11.54

concentrations (Table 3-6). Although it is not known why mortality was so high among C. dubia exposed to 0.002 mg/L total uranium, it is believed that this response was unrelated to exposure to the uranium. The mean number of broods ranged in size from 1.4 to 2.3 per individual (Table 3 - 6). Mean reproduction (number of young per female) among control individuals was higher (15.5 young) than mean reproduction of individuals exposed to all test concentrations of uranyl nitrate (Table 3-6). These data suggested that exposure to increasing concentrations of total uranium adversely affected C. dubia reproduction.

The adult survival data were analyzed using Fisher's Exact test (Sokal and Rohlf 1981) to determine if the proportion of "successes" (i.e., percent survival) in the control group was the same as that in each test concentration. If statistically significant differences were detected in the percent survival of adults between the control and any test group, then such groups represented two different populations and could not be compared in further statistical analyses. The results of this test (Table 3-7) indicated that the percent survival among C. dubia exposed to 0.002 mg/ L total uranium differed significantly from the control. Based on these results, the survival NOEC equals < 0.002 mg/L total uranium and the LOEC = 0.002 mg/L. However, as discussed above, these results are believed to be anomalous and unrelated to the effects of exposure to the

Table 3-7. "Fisher's Exact" Test Procedure for Adult Survival Rate for Uranyl Nitrate

Results of Fisher's Exact test conducted on the percent survival of Ceriodaphnia dubia exposed to a control and five concentrations of uranyl nitrate. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December 1988.

H_o: The proportion of C. dubia survival in the control group is the same as that of C. dubia exposed to each of five concentrations of uranyl nitrate.

H_a: The proportion of C. dubia survival in the control group is not equal to that of C. dubia exposed to each of five concentrations of uranyl nitrate.

Comparison	Critical value	b value	Significantly Different ?
Control vs. 0.002	14	13	Y
Control vs. 0.008	14	18	N
Control vs. 0.023	14	19	N
Control vs. 0.046	14	19	N
Control vs. 0.076	14	18	N

test solution. Based on this observation, the results of exposure to 0.002 mg/L were not considered in further analyses of these test results.

Additional statistical analyses were conducted to determine if exposure to concentrations ≥ 0.008 mg/L total uranium had a significant effect on C. dubia offspring production. Tests were conducted to determine if the number of young produced by C. dubia exposed to the control and to the test concentrations of ≥ 0.008 mg/L total uranium were normally distributed and if the variances for this data set were homogeneous (equal). These conditions must exist to correctly perform parametric tests such as analysis of variance (ANOVA) and Dunnett's multiple comparison test. The results of the Chi-Square Goodness of Fit and Bartlett's Test (Table 3-8) demonstrated that data were normally distributed and that the variances were homogenous. Therefore, parametric tests were used to perform all further analyses.

A one-way analysis of variance (ANOVA) was conducted to determine if significant differences existed in the offspring produced by C. dubia exposed to the control and the test concentrations ≥ 0.008 mg/L total uranium. Results of this test (Table 3-9 A) indicated that reproduction among the various treatment groups differed significantly. A multiple comparison test (Dunnett's multiple comparison test) was

Table 3-8. Chi-Square Statistical Analysis - Uranyl Nitrate

Results of a Chi-Square Goodness of Fit test and Bartlett's test conducted on the number of young produced by Ceriodaphnia dubia exposed to uranyl nitrate. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December, 1988.

Chi-Square Goodness of Fit test:

calculated $\chi^2 = 9.16$

χ^2 critical value (0.01,4) = 13.277

The data are normally distributed.

Bartlett's test:

calculated B = 2.36

χ^2 critical value 0.05 [4,19] = 9.49

The variances are homogeneous.

Table 3-9. Analysis of Variance and Dunnett's Multiple Comparison Test - Uranyl Nitrate

Results of a one-way analysis of variance (Table A) and one-tailed Dunnett's comparison test (Table B) for the number of young produced by Ceriodaphnia dubia exposed to uranyl nitrate. Water from Upper Three Runs Creek was used as the control and diluent. A test to determine the minimum significant difference detectable among these data was conducted (Table C). All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December, 1988.

Table A. One way analysis of variance (ANOVA)

Ho: $\mu 1 = \mu 2 = \mu 3 = \mu 4$

Ha: The mean numbers of young produced by C. dubia exposed to both the control and four concentrations of uranyl nitrate are not equal.

Source	d.f.	S.S.	MS	F	Critical F
concentration	4	1213.36	303.34	10.79	2.53
error	95	2671.00	28.12		
total	99	3884.36			

Table B. Dunnett's multiple comparison test

Ho: $\mu c = \mu a$

Ha: $\mu c \neq \mu a$

Comparison	$(\bar{X}c - \bar{X}a)$	SE	$\lvert q' \rvert$	p	$q'_{0.05(1),119,p}$
control vs. 0.008	3.85	1.68	2.29	2	1.66*
control vs. 0.023	2.70	1.68	1.61	3	1.93*
control vs. 0.046	6.60	1.68	3.93	4	2.08*
control vs. 0.076	10.20	1.68	6.07	5	2.18*

NOEC = <0.008 mg/L total uranium
LOEC = 0.008 mg/L total uranium
*Number of offspring were significantly different (at 95% probability) from control.

performed to identify those treatment groups whose offspring production differed significantly from that of the control. This test (Table 3-9 B) indicated that exposure of C. dubia to concentrations equal to 0.008 mg/L total uranium and ≥ 0.09 mg/L total uranium resulted in a significant reduction in production of young when compared to the control. According to this statistical procedure, only exposure to 0.023 mg/L total uranium failed to significantly reduce C. dubia reproduction (Table 3-9 B).

A review of this data indicated that the response by the test organisms deviated from the concentration-response pattern typically associated with chronic toxicity tests. It is not possible to determine if the reduced reproduction observed at the 0.008 mg/L test concentration is truly the result of exposure to uranyl nitrate or an aberrant response. Based on strict interpretation of the statistical results, the NOEC for uranyl nitrate equals a concentration < 0.008 mg/L total uranium while the LOEC = 0.008 mg/L (Table 3-9 B). If the response observed at 0.008 mg/L is atypical, then the NOEC would equal 0.023 mg/L and 0.046 mg/L total uranium would equal the LOEC. These conjectures cannot be supported without additional data. It is therefore recommended that the most conservative determination of NOEC and LOEC (<0.008 and 0.008 mg/L, respectively) be reported for this compound.

A minimum significant difference test (MSD test; Horning and Weber 1985) was conducted to determine how great a difference had to exist in the mean number of young produced by two groups before a significant difference could be detected. The results of this test (Table 3-9 C) indicated that a 24.3% reduction in the mean number of offspring from the control production (i.e., mean offspring production of 11.54 or less) could be detected.

3.3.2 Hydrogen Uranyl Phosphate

The daily concentrations of total uranium measured in the next to the highest test solution of hydrogen uranyl phosphate (0.120 mg/L) used in the seven-day chronic test are listed in Table 3-2. The results of all initial and final basic water chemistry analyses performed on all $HUO_2PO_4 \cdot 4H_2O$ solutions are listed in Appendix 3 (Tables 3 and 4).

Some mortality was observed among C. dubia exposed to all test concentrations of $HUO_2PO_4 \cdot 4H_2O$ used in the chronic test (Table 3-10). Both brood and young production were reduced among test organisms as compared to the control individuals (Table 3-10). These data suggested that exposure to all of the concentrations of total uranium in the form of $HUO_2PO_4 \cdot 4H_2O$ adversely affected C. dubia.

Table 3-9. Analysis of Variance and Dunnett's Multiple Comparison Test - Uranyl Nitrate (continued)

Table C. The minimum significant difference (MSD) detectable among these data

MSD = 3.71

Control mean = 15.25 young; 15.25 - 3.71 = 11.54

For this data set, a 24.3% (3.71 young) reduction in C. dubia production of young could be detected. That is, mean offspring production $\leq$ 11.54 would be significantly different from offspring production of the control group.

Table 3-10. Chronic Toxicity Test Conducted on Hydrogen Uranyl Phosphate.

Results of a static renewal life cycle test for Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. Work was performed for E. I. du Pont de Nemours & Company, Aiken, SC. Water collected from Upper Three Runs Creek served as the control and diluent. 13 - 20 December 1988.

Nomianl* Concentration (mg/L total U)	Mean Measured Concentrations (mg/L total U)	$\bar{X}$ (SD) of young per female[a]	$\bar{X}$ broods per female[b]	% Mortality of original females
Control		15.40 (7.79)	2.3	10
0.006		9.45 (6.22)	2.1	20
0.02		9.10 (6.07)	1.7	30
0.06		8.60 (5.37)	1.8	20
0.12	0.119	9.80 (5.49)	2.1	5
0.20		8.25 (5.16)	1.8	15

[a]Mean value based on number of young produced by 20 original females.
[b]Mean value based on surviving original females.
*Nominal concentrations based on measured stock solutions.

NOEC = < 0.006 mg/L total U LOEC = 0.006 mg/L total U

MSD = 28.5% reduction
= 15.4 - 4.4 = 11.0

Fisher's Exact test was used to analyse adult survival data. The results of this test (Table 3-11) indicated no significant difference existed in the percent survival among C. dubia exposed to any of the solutions used in this chronic test. Offspring production by individuals exposed to all test concentrations of $HUO_2PO_4 \cdot 4H_2O$ was included in all other analyses performed on this data.

The results of the Chi-Square Goodness of Fit and Bartlett's Test (Table 3-12) indicated that data were normally distributed and that the variances were homogeneous. Parametric procedures were used to perform all other analyses.

A one-way analysis of variance (ANOVA) was conducted to determine if significant differences existed in the offspring produced by C. dubia exposed to test concentrations of hydrogen uranyl phosphate. Results of this test (Table 3-13 A) indicated that reproduction among the various treatment groups differed significantly. Dunnett's multiple comparison test (Table 3-13 B) indicated that exposure of C. dubia to all test concentrations $\geq$ to 0.006 mg/L total uranium resulted in a significant reduction in production of young when compared to the control. This seven-day life cycle test determined that the NOEC for $HUO_2PO_4 \cdot 4H_2O$ expressed as total uranium equals some value < 0.006 mg/L and the LOEC

Table 3-11. "Fisher's Exact" Test Procedure for Adult Survival Rate for Hydrogen Uranyl Nitrate

Results of Fisher's Exact test conducted on the percent survival of Ceriodaphnia dubia exposed to a control and five concentrations of hydrogen uranyl phosphate. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 13 to 20 December, 1988.

H_o: The proportion of C. dubia survival in the control group is the same as that of C. dubia exposed to each of five concentrations of hydrogen uranyl phosphate.

H_a: The proportion of C. dubia survival in the control group is not equal to that of C. dubia exposed to each of five concentrations of hydrogen uranyl phosphate.

Comparison	Critical value	b value	Significantly Different ?
Control vs. 0.006	12	16	N
Control vs. 0.02	12	14	N
Control vs. 0.06	12	16	N
Control vs. 0.12	12	17	N
Control vs. 0.2	12	17	N

Table 3-12. Chi-Square Goodness of Fit and Bartlett's Test - Hydrogen Uranyl Phosphate

Results of a Chi-Square Goodness of Fit test and a Bartlett's test conducted on the number of young produced by Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 13 to 20 December, 1988.

Chi-Square Goodness of Fit test:

calculated $\chi^2 = 0.20$

χ^2 critical value (0.01,4) = 13.277

The data are normally distributed.

Bartlett's test:

calculated B = 4.53

χ^2 critical value 0.05 [5,19] = 11.07

The variances are homogeneous.

Table 3-13. Analysis of Variance and Dunnett's Comparison Test - Hydrogen Uranyl Phosphate

Results of a one-way analysis of variance (Table A) and one-tailed Dunnett's comparison test (Table B) for the number of young produced by Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. Water from Upper Three Runs Creek was used as the control and diluent. A test to determine the minimum significant difference detectable among these data was conducted (Table C). All concentrations are expressed as nominal concentrations of total uranium. 13 to 20 December, 1988.

Table A. One-way analysis of variance (ANOVA)

Ho: $\mu 1 = \mu 2 = \mu 3 = \mu 4 = \mu 5$

Ha: The mean numbers of young produced by C. dubia exposed to both the control and five concentrations of hydrogen uranyl phosphate are not equal.

Source	d.f.	S.S.	MS	F	Critical F
concentration	5	705.50	141.10	3.82	2.37
error	114	4213.30	36.96		
total	119	4918.80			

Table B. One-Tailed Dunnett's Comparison Test

Ho: $\mu c = \mu a$

Ha: $\mu c \neq \mu a$

Comparison	$(\bar{X}c - \bar{X}a)$	SE	$\lvert q' \rvert$	p	$q'_{0.05(1),\ 119\ p}$
control vs. 0.006	5.95	1.92	3.10	2	1.66*
control vs. 0.02	6.30	1.92	3.28	3	1.93*
control vs. 0.06	6.80	1.92	3.54	4	2.08*
control vs. 0.12	5.60	1.92	2.92	5	2.18*
control vs. 0.2	7.15	1.92	3.72	6	2.26*

NOEC = < 0.006 mg/L total uranium
LOEC = 0.006 mg/L

equals 0.006 mg/L (Table 3-13 B). The MSD test determined that a 28.5% reduction in the mean number of offspring from the control production (i.e. mean offspring production of $\leq$ 11.01) could be detected (Table 3-13C).

3.3.3. Uranium Dioxide

The results of all initial and final basic water chemistry analyses performed on all solutions of UO_2 used in this chronic toxicity test are listed in Appendix 3 (Tables 5 and 6). The daily concentrations of total uranium measured in each of the test solutions used in the test are listed in Table 3-2.

The percent survival was high among C. dubia exposed to all test concentrations of UO_2 ($\geq$ 85%; Table 3-14). The mean number of young and broods produced by organisms exposed to concentrations of total uranium < 0.050 mg/L were similar. However, exposure to 0.050 mg/L total uranium resulted in a reduction in both brood size and offspring production per test organism as compared to production by the control and organisms exposed to the other test concentration (Table 3-14). These data indicate that exposure to increasing concentrations of uranium as UO_2 reduced C. dubia reproductive success.

Table 3-13. Analysis of Variance and Dunnett's Comparison Test - Hydrogen Uranyl Phosphate (continued)

Table C. The minimum significant difference (MSD) detectable among these data

MSD = 4.39

Control mean = 15.4 young; 15.4 - 4.39 = 11.01

For this data set, a 28.5% (4.39 young) reduction in C. dubia production of young could be detected. That is, mean offspring production ≤ 11.01 would be significantly different from offspring production of the control group.

Table 3-14. Chronic Toxicity Test Conducted on Uranium Dioxide

Results of a static renewal life cycle test for Ceriodaphnia dubia exposed to uranium dioxide. Work was performed for E. I. du Pont de Nemours & Company, Aiken, SC. Water collected from Upper Three Runs Creek served as the control and diluent. 9 - 16 December 1988.

Nominal* Concentration (mg/L total U)	Mean Measured Concentrations (mg/L total U)	$\bar{X}$ (SD) of young per female[a]	$\bar{X}$ broods per female[b]	% Mortality of original females
Control		15.20 (4.99)	2.3	5
0.0015		11.25 (5.74)	1.9	15
0.005		11.70 (5.61)	2.4	5
0.015		13.60 (6.70)	2.2	5
0.030		11.45 (4.80)	2.4	5
0.050	0.035	7.90 (4.91)	1.9	15

[a]Mean value based on number of young produced by 20 original females.
[b]Mean value based on surviving original females.
*Nominal concentrations based on measured stock solutions.

NOEC = 0.03 mg/L total U

LOEC = 0.050 mg/L total U

The results of Fisher's Exact test (Table 3-15) indicated no significant difference existed in the percent survival among C. dubia exposed to any of the concentrations used in this chronic test. Offspring production by individuals exposed to all concentrations of UO_2 were included in further statistical tests.

The results of the Chi-Square Goodness of Fit test and Bartlett's test indicated that data were normally distributed and that the variances were homogeneous (Table 3-16). All further analyses were performed using parametric methods.

The results of the one-way ANOVA indicated that reproduction among the various treatment groups differed significantly (Table 3-17 A). Dunnett's multiple comparison test determined that exposure of C. dubia to concentrations equal to 0.050 mg/L total uranium resulted in a significant reduction in production of young when compared to the control. Based on these observations, it was concluded that the NOEC for total uranium in the form of uranium dioxide equals 0.030 mg/L and the LOEC equals 0.050 mg/L (Table 3-17 B).

The results of the MSD test performed on this set of data (Table 3-17 C) determined that a 26.1% reduction in the mean number of offspring from the control production (i.e. mean

offspring production of ≤ 11.43) could be detected.

Table 3-15. "Fisher's Exact" Test Procedure for Adult Survival Rate for Uranium Dioxide.

Results of Fisher's Exact test conducted on the percent survival of Ceriodaphnia dubia exposed to a control and five concentrations of uranium dioxide. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December, 1988.

H_o: The proportion of C. dubia survival in the control group is the same as that of C. dubia exposed to each of five concentrations of uranium dioxide.

H_a: The proportion of C. dubia survival in the control group is not equal to that of C. dubia exposed to each of five concentrations of uranium dioxide.

Comparison	Critical value	b value	Significantly Different ?
Control vs. 0.0015	14	17	N
Control vs. 0.005	14	19	N
Control vs. 0.015	14	19	N
Control vs. 0.030	14	19	N
Control vs. 0.050	14	17	N

All concentrations are expressed as nominal concentrations of total uranium. 13 to 20 December, 1988.

Table 3-16. Chi-Square Goodness of Fit and Bartlett's Test - Uranium Dioxide

Results of a Chi-Square Goodness of Fit test and Bartlett's test conducted on the number of young produced by Ceriodaphnia dubia exposed to uranium dioxide. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December, 1988.

Chi-Square Goodness of Fit test:

calculated $\chi^2 = 2.24$

χ^2 critical value (0.01,4) = 13.277

The data are normally distributed.

Bartlett's test:

calculated B = 3.16

χ^2 critical value 0.05 [5,19] = 11.07

The variances are homogeneous.

Table 3-17. Analysis of Variance and Dunnett's Comparison Test - Uranium Dioxide

Results of a one-way analysis of variance (Table A) and one-tailed Dunnett's comparison test (Table B) for the number of young produced by Ceriodaphnia dubia exposed to uranium dioxide. Water from Upper Three Runs Creek was used as the control and diluent. A test to determine the minimum significant difference detectable among these data was conducted (Table C). All concentrations are expressed as nominal concentrations of total uranium. 9 to 16 December, 1988.

Table A. One-way analysis of variance (ANOVA)

Ho: $\mu 1 = \mu 2 = \mu 3 = \mu 4 = \mu 5$

Ha: The mean numbers of young produced by C. dubia exposed to both the control and five concentrations of uranium dioxide.

Source	d.f.	S.S.	MS	F	Critical F
concentration	5	608.60	121.72	4.03	2.37
error	114	3444.70	30.22		
total	119	4053.30			

Table B. One-Tailed Dunnett's Comparison Test

Ho: $\mu c = \mu a$

Ha: $\mu c \neq \mu a$

Comparison	$(\bar{X}c - \bar{X}a)$	SE	$\lvert q' \rvert$	p	$q'_{0.05(1)119, p}$
control vs. 0.0015	3.95	1.74	2.27	2	1.66*
control vs. 0.005	3.50	1.74	2.01	3	1.93*
control vs. 0.015	1.60	1.74	0.92	4	2.08
control vs. 0.030	3.75	1.74	2.16	5	2.18*
control vs. 0.050	7.30	1.74	4.20	6	2.26*

NOEC = 0.03 mg/L total uranium
LOEC = 0.05 mg/L total uranium

Table 3-17. Analysis of Variance and Dunnett's Comparison Test - Uranium Dioxide (continued)

Table C. The minimum significant difference (MSD) detectable among these data

MSD = 3.97

Control mean = 15.4 young; 15.4 - 3.97 = 11.43

For this data set, a 26.1% (3.97 young) reduction in C. dubia production of young could be detected. That is, mean offspring production $\leq$ 11.43 would be significantly different from offspring production of the control group.

4.0 DISCUSSION AND SUMMARY

Laboratory tests were conducted to determine the acute and chronic toxicities of three uranium compounds. These compounds are present in an effluent discharged from the M-Area Dilute Effluent Treatment Facility (DETF) into Tim's Branch, a tributary of Upper Three Runs Creek. The waterflea, Ceriodaphnia dubia was used in all toxicity tests. These organisms were reared in water collected from Upper Three Runs Creek; water from the creek also served as the control and diluent for both the acute and chronic tests.

The concentration of total uranium was determined in all stock solutions prior to testing. The nominal concentration of uranium in each toxicity test solution was determined by controlled dilutions of the stock solution. Additional analyses were performed to determine the actual concentration of uranium in the various solutions used in the toxicity tests. With the exception of uranyl nitrate, the concentration of total uranium was determined both in the highest test solution and in an intermediate test solution used in each of the three acute toxicity tests. Uranium concentrations were measured only in the highest solution of UO_2 and the next to highest solution concentration of HUO_2PO_4 used in the chronic toxicity tests. The concentrations of uranium in the highest solution used each day of the chronic toxicity test conducted on $UO_2(NO_3)_2 \cdot 6H_2O$ was measured daily as were uranium

concentrations of all test solutions used on 10 December 1988. The results of all these uranium analyses are listed in Tables 4-1 and 4-2.

As listed in Table 4-1, the percent recovery of total uranium in test solutions of uranyl nitrate used in the acute toxicity test ranged from 70.9 to 128%. Solutions used to conduct the chronic toxicity test ranged from 32.9 to 126.3% recovery (Table 4-2). Additional uranium analyses were performed to verify the concentrations of uranium in the various uranyl nitrate test solutions. The concentrations of total uranium in all test solutions used the first day of the acute toxicity test (11-21-88; Table 4-1) were measured as were uranium concentrations in all renewal test solutions used the second day of the acute test (11-22-88; Table 4-1). Concentrations of uranium were also measured in all solutions used on Day 1 of the chronic toxicity test (12-10-88; Table 4-2). These analyses demonstrated that percent recovery was relatively consistent for test solutions prepared the same day for a given test (Tables 4-1 and 4-2).

The percent recoveries of total uranium for the test solutions of hydrogen uranyl phosphate and uranium dioxide were similar to those for uranyl nitrate (Tables 4-1 and 4-2). Percent recovery for test solutions used in the acute toxicity test of hydrogen uranyl phosphate ranged from 76.3 to 109.2% (Table 4-1) while total uranium percent recoveries

Table 4-1. Percent Recoveries for Uranium in Solutions used in Acute Toxicity Test

Nominal Concentrations (mg/L total U)	Measured Concentrations DATE Nov 21	Measured Concentrations DATE Nov 22	% Recovery DATE Nov 21	% Recovery DATE Nov 22	Mean % Recovery
URANYL NITRATE					
0.051	0.047	0.038	92.1	74.5	96.9
0.127	0.143	0.090	113	70.9	(n = 7)
0.190	0.219		115		
0.254	0.325		128		
0.381	0.322		84.5		
Nominal LC50 = 0.070 mg/L; (95% confidence limits = 0.051 - 0.127 mg/L)			"Measured" LC50 = (0.07 mg/L) x 0.969 = 0.068 mg/L (95% confidence limits = 0.049 - 0.123 mg/L)		
HYDROGEN URANYL PHOSPHATE					
0.08	0.064	0.061	80.0	76.3	90.73
0.12	0.131	0.117	109.2	97.5	(n = 4)
Nominal LC50 = 0.110 mg/L; (95% confidence intervals = 0.10 - 0.12 mg/L)			"Measured" LC50 = (0.11mg/L) x 0.9073 = 0.10 mg/L (95% confidence intervals = 0.09 - 0.11 mg/L)		
URANIUM DIOXIDE	DATE Dec 2	DATE Dec 3	DATE Dec 2	DATE Dec 3	
0.070	0.060	0.068	85.7	97.14	104
0.130	0.120	0.182	92.3	140.0	(n = 4)
Nominal LC50 = 0.050 mg/L; (95% confidence intervals = 0.04 - 0.06 mg/L)			"Measured" LC50 = (0.05 mg/L) x 1.04 = 0.052 mg/L (95% confidence intervals = 0.042 - 0.062 mg/L)		

Table 4-2. Percent Recoveries for Uranium in Solutions used in Chronic Toxicity Tests.

Nominal Concentrations (mg/L total U)	Measured Concentrations DATE Dec 9	Dec 10	Dec 11	Dec 12	Dec 13	Dec 14	Dec 15	Mean % Recovery
URANYL NITRATE								
0.002		0.002						
0.008		0.007						
0.023		0.020						
0.046		0.039						
0.076	0.085	0.068	0.043	0.025	0.048	0.053	0.096	
		% Recovery						
0.002		100						
0.008		87.5						
0.023		87.0						
0.046		84.8						
0.076	112	89.5	56.6	32.9	63.2	69.7	126.3	82.68 (n = 11)

Nominal
NOEC = 0.023 mg/L total U
LOEC = 0.046 mg/L total U

"Measured"
NOEC = (0.023) x 0.8268 = 0.019 mg/L total U
LOEC = (0.046) x 0.8268 = 0.038 mg/L total U

CONSERVATIVE CALCULATIONS:

Nominal
NOEC = <0.008 mg/L total U
LOEC = 0.008 mg/L total U

"Measured"
NOEC = (<0.008) x 82.68 = <0.006 mg/L total U
LOEC = (0.008) x 82.68 = 0.006 mg/L total U

Table 4-2 (continued). Percent Recoveries for Uranium in Solutions used in Chronic Toxicity Tests.

Nominal Concentrations (mg/L total U)	Measured Concentrations DATE Dec 9	Dec 10	Dec 11	Dec 12	Dec 13	Dec 14	Dec 15	
URANIUM DIOXIDE								
0.050	0.033	0.044	0.027	0.065	0.033	0.027	0.018	
			% Recovery					Mean % Recovery
0.050	66.0	88.8	54.0	130	66.0	54.0	36.0	70.69 (n = 7)

Nominal
NOEC = 0.03 mg/L total U
LOEC = 0.05 mg/L total U

"Measured"
NOEC = (0.03) x 0.7069 = 0.021 mg/L total U
LOEC = (0.05) x 0.7069 = 0.035 mg/L total U

HYDROGEN URANYL PHOSPHATE								
0.120	0.124	0.104	0.133	0.129	0.098	0.097	0.150	
			% Recovery					Mean % Recovery
0.120	103	86.7	111	108	81.7	80.8	125	99.5 (n = 7)

Nominal
NOEC <0.006 mg/L total U
LOEC 0.006 mg/l total U

"Measured"
NOEC = (<0.006) (0.995) = <0.006 mg/L total U
LOEC = (0.006) (0.995) = 0.006 mg/L total U

ranged from 80.8 to 125% in chronic test solutions (Table 4-2). The percent recovery for test solutions used in the acute toxicity test conducted on UO_2 ranged from 85.7 to 140% (Table 4-1). Test solutions used in the chronic toxicity test were somewhat more variable, with percent recoveries of total uranium ranging from 36 to 130% (Table 4-2).

Using nominal concentrations, the C. dubia 48 h static renewal acute toxicity tests demonstrated that uranium dioxide was the most toxic of the three uranium compounds (LC50 = 0.050 mg/L total U; 95% confidence limits = 0.040 - 0.060 mg/L total U). Test results also demonstrated that the acute toxicities of uranyl nitrate and hydrogen uranyl phosphate were less toxic (LC50s = 0.070 and 0.110 mg/L total U, respectively; Table 4-3). In a similar study, Trapp (1986) found that the 48 h LC50 for D. pulex with uranyl nitrate equaled 0.22 mg/L total U (95% confidence interval = 0.17 - 0.36 mg/L total U). These test results suggest that uranium is somewhat more toxic to C. dubia than to D. pulex.

Static renewal seven-day toxicity tests were performed to determine the chronic toxicity of each of the three uranium compounds to C. dubia. The results of the test performed on uranyl nitrate deviated from a typical toxicity concentration-response curve and made data interpretation difficult. However, until additional data is available to supplement the results of this test, it is recommended that

Table 4-3. Summary of Toxicity Endpoints

SUMMARY

	Acute Toxicity (mg/L total U)	
	Nominal	"Measured"*
	LC50	LC50
Uranyl Nitrate	0.070	0.068
Uranium Dioxide	0.050	0.052
Hydrogen Uranyl Phosphate	0.110	0.100

	Chronic Toxicity (mg/L total U)			
	Nominal		"Measured"*	
	NOEC	LOEC	NOEC	LOEC
Uranyl Nitrate (calculated)	0.023	0.046	0.019	0.038
Uranyl Nitrate (conservative)	<0.008	0.008	<0.006	0.006
Uranium Dioxide	0.030	0.050	0.021	0.037
Hydrogen Uranyl Phosphate	<0.006	0.006	<0.006	0.006

*Calculated based on analytical % recovery.

the more conservative assessment of the data be used to determine the NOEC and LOEC values for this compound. In this instance, the NOEC and LOEC values for uranyl nitrate equalled <0.008 and 0.008 mg/L total uranium, respectively. Chronic test results determined that the NOEC and LOEC for hydrogen uranyl phosphate in Upper Three Runs Creek water equaled <0.006 mg/L and 0.006 mg/L total U, respectively (Table 4-3). The C. dubia chronic toxicity test conducted on UO_2 indicated that the NOEC for this compound in Upper Three Runs Creek water equaled 0.030 mg/L total U and that the LOEC equaled 0.050 mg/L total U (Table 4-3).

C. dubia exposed to all test solutions of hydrogen uranyl phosphate exhibited essentially identical responses; exposure to increasing concentrations of total uranium failed to elicit corresponding reductions in test organism reproduction (Table 3-10). The chemical form of the soluble uranium in the hydrogen uranyl phosphate solutions is probably that of an anionic phosphate complex $[UO_2(HPO_4)_2^{=}]$, whereas the chemical form in a uranyl nitrate solution is the cation UO_2^{++} (Langmuir 1978). These differently charged complexes may act very differently in solution (Poston et al. 1984); for example, the anionic phosphate complex may be absorbed by the food added to the test chamber each day. The occurrence of this phenomenon could be tested by adding food to a series of hydrogen uranyl phosphate test solutions. Food would be allowed to settle for 24 h and the test solutions filtered

to remove all food particles. Uranium analyses performed on tests solutions before and after the addition of food would determine if the food had removed uranium from solution, thereby altering the concentration gradient of uranium in the test solutions of hydrogen uranyl phosphate. Such a phenomenon could account for the "flat" response observed among organisms exposed to solutions of $HUO_2PO_4 \cdot 4H_2O$ during the chronic toxicity test.

A test was conducted to determine if filtration through a 0.45 μm filter reduced the concentration of total uranium in the test solutions by removing uranium bound to suspended particles. Solutions of hydrogen uranyl phophate were prepared in the same mannner (see Section 2.2.2.3) as those used in the chronic toxicity test. A portion of each sample was filtered through a 0.45 μm filter. The concentration of total uranium was measured in filtered and unfiltered solutions. These measurements demonstrated that the uranium was present as a filterable compound in the hydrogen uranyl phosphate solutions (Table 4-4).

In summary, the results of both the acute and chronic toxicity tests conducted on the three uranium compounds demonstrated that low concentrations of these compounds adversely affect the organism C. dubia.

Table 4-4. Concentrations of total uranium measured in filtered (0.45 μm filter) and unfiltered solutions of hydrogen uranyl phosphate.

Nominal Concentrations (mg/L total U)	Unfiltered (mg/L total U)	Filtered (mg/L total U)
0.050	0.041	0.0044*
0.100	0.040	0.0029*
0.200	0.129	0.026

*Samples were concentrated prior to analyses.

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APPENDIX 1

Composition of Bold's Modified Media

Appendix 1.

Composition of Modified Bold's Media[a]

Major Components

Na
K
Ca
Mg
NO_3
PO_4
Cl
SO_4

Minor components

H_3BO_3
EDTA
Fe (II)
Zn (II)
Mn (II)
Cu (II)
Mo (VI)
Co (II)

Vitamins

Thiamine Hydrochloride
D-Pantothenic Acid, Calcium
Biotin
Cyanocobalamin (B_{12})

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[a]Arthur L. Buikema, Jr., pers. comm.

APPENDIX 2

Water Chemistry Data for 48 h Acute Toxicity Tests

Table 1. Summary of basic water chemistry for a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on uranyl nitrate. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 21 - 25 November 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.51 ± 0.54 (6.85-8.17) n = 4	23.8 ± 1.0 (23.0-25.0) n = 4	6.41 ± 0.36 (6.20-6.95) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	2.1 ± 0.2 (2.0-2.5) n = 4	3.75 ± 1.50 (3.00-6.00) n = 4
0.051	7.49 ± 0.36 (7.09-7.96) n = 4	23.6 ± 0.7 (23.0-24.6) n = 4	6.24 ± 0.14 (6.15-6.45) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	---	---
0.127	7.52 ± 0.45 (7.01-8.10) n = 4	23.6 ± 0.5 (23.2-24.4) n = 4	6.29 ± 0.18 (6.15-6.55) n = 4	0.018 ± 0.003 (0.015-0.020) n = 4	1.2 (0.5-2.0) n = 2	3.0 --- n = 2
0.190	7.25 (7.08-7.42) n = 2	24.2 (24.0-24.3) n = 2	6.32 (6.15-6.50) n = 2	0.020 (0.015-0.020) n = 2	---	---
0.254	7.24 (7.03-7.45) n = 2	24.0 (23.3-24.1) n = 2	6.32 (6.15-6.50) n = 2	0.018 (0.015-0.020) n = 2	---	---
0.381	7.30 (7.13-7.47) n = 2	24.0 (23.7-24.3) n = 2	6.38 (6.20-6.55) n = 2	0.018 --- n = 2	2.0 --- n = 2	4.0 (3.00-5.00) n = 2

[a] mg/L total uranium (nominal concentrations)

[b] mg/L as $CaCO_3$

Table 2. Summary of basic water chemistry for a Ceriodaphnia dubia ~~48 h~~ static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 21 - 23 November 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.65 ± 0.60 (6.85-8.17) n = 4	23.8 ± 0.9 (23.0-25.0) n = 4	6.36 ± 0.41 (6.00-6.95) n = 4	0.015 ± 0.004 (0.010-0.020) n = 4	2.1 ± 0 --- n = 4	3.75 ± 1.50 (3.00-6.00) n = 4
0.04	7.53 ± 0.28 (7.26-7.85) n = 4	24.0 ± 0.6 (23.1-24.6) n = 4	6.34 ± 0.26 (6.10-6.70) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	---	---
0.06	7.42 ± 0.49 (6.79-7.97) n = 4	24.1 ± 1.0 (23.0-25.4) n = 4	6.50 ± 0.37 (6.10-7.00) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	---	---
0.08	7.47 ± 0.36 (7.08-7.94) n = 4	24.4 ± 0.8 (23.9-25.5) n = 4	6.43 ± 0.19 (6.25-6.70) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	---	---
0.10	7.46 ± 0.45 (7.98-8.02) n = 4	24.1 ± 0.4 (23.7-24.6) n = 4	6.38 ± 0.17 (6.20-6.60) n = 4	0.015 ± 0 --- n = 4	---	---
0.12	7.38 ± 0.31 (7.03-7.79) n = 4	24.0 ± 0.7 (23.5-24.9) n = 4	6.38 ± 0.19 (6.20-6.65) n = 4	0.016 ± 0.002 (0.015-0.020) n = 4	2.2 ± 0.5 (2.0-3.0) n = 4	3.4 ± 1.0 (2.0-4.0) n = 4

[a] mg/L total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

Table 3. Summary of basic water chemistry for a Ceriodaphnia dubia 48 h static renewal acute toxicity test conducted on uranium dioxide. Work was performed for E.I. du Pont de Nemours & Company, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 2 - 4 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[a]	Hardness (mg/L)[a]
Control	7.41 ± 0.58 (6.53-7.72) n = 4	25.0 ± 0.6 (24.4-25.5) n = 4	6.29 ± 0.14 (6.15-6.45) n = 4	0.020 --- n = 4	2.2 ± 0.5 (2.0-3.0) n = 4	4.00 ± 0.82 (3.00-5.00) n = 4
0.01	7.49 ± 0.52 (6.73-7.89) n = 4	24.9 ± 0.7 (24.1-25.8) n = 4	6.05 ± 0.19 (5.80-6.20) n = 4	0.020 --- n = 4	---	---
0.04	7.39 ± 0.58 (6.54-7.81) n = 4	25.2 ± 0.4 (24.6-25.7) n = 4	6.16 ± 0.27 (5.85-6.45) n = 4	0.020 --- n = 4	---	---
0.07	7.50 ± 0.65 (6.61-8.19) n = 4	25.0 ± 0.3 (24.7-25.3) n = 4	6.20 ± 0.31 (5.80-6.55) n = 4	0.020 --- n = 4	---	---
0.10	7.70 ± 0.86 (6.63-8.73) n = 4	25.1 ± 0.4 (24.4-25.3) n = 4	6.20 ± 0.31 (5.80-6.55) n = 4	0.020 --- n = 4	---	---
0.13	7.56 ± 0.62 (6.67-8.05) n = 4	24.5 ± 0.2 (24.2-24.8) n = 4	6.38 ± 30.1 (6.90-6.60) n = 4	0.020 --- n = 4	2.6 ± 0.7 (2.0-3.4) n = 4	4.1 ± 0.2 (4.0-4.5) n = 4

[a] mg/L total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

APPENDIX 3

Water Chemistry Data for Chronic Toxicity Tests

Table 1. Summary of initial water chemistry for Ceriodaphnia dubia 7 d chronic static renewal toxicity test conducted with uranyl nitrate. Work was performed for E.I. du Pont de Nemours & Company, Savannah River Laboratory, Aiken SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 9 - 16 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.76 ± 0.44 (6.91-8.18) n = 7	24.3 ± 0.6 (24.0-25.7) n = 7	5.99 ± 0.12 (5.75-6.10) n = 7	0.015 ± 0 --- n = 7	2.0 ± 0.8 (1.0-3.0) n = 7	3.4 ± 0.4 (3.0-4.0) n = 7
0.002	7.67 ± 0.60 (6.38-8.16) n = 7	24.2 ± 0.2 (24.0-24.6) n = 7	6.04 ± 0.11 (5.90-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.008	7.52 ± 0.49 (6.43-7.84) n = 7	24.5 ± 0.6 (24.0-25.7) n = 7	6.04 ± 0.11 (5.90-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.023	7.48 ± 0.51 (6.37-7.89) n = 7	24.5 ± 0.7 (24.0-26.0) n = 7	6.03 ± 0.11 (5.90-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.046	6.47 ± 0.53 (6.38-8.16) n = 7	24.4 ± 0.6 (24.0-25.8) n = 7	6.03 ± 0.13 (5.85-6.05) n = 7	0.015 ± 0 --- n = 7	---	---
0.076	7.50 ± 0.65 (6.17-8.28) n = 7	24.3 ± 0.5 (24.0-25.3) n = 7	6.07 ± 0.11 (5.85-6.20) n = 7	0.015 ± 0 --- n = 7	1.7 ± 0.5 (2.0-1.0) n = 7	3.7 ± 0.5 (4.0-3.0) n = 7

[a] mg/L as total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

Table 2. Summary of basic water chemistry for Ceriodaphnia dubia 7 d chronic static renewal toxicity test conducted with uranyl nitrate; 24 h readings. Work was performed for E. I. du Pont de Nemours Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 9 - 16 December 1988.

Concentration (ppm)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[a]	Hardness (mg/L)[a]
Control	7.47 ± 0.16 (7.21-7.73) n = 7	24.4 ± 0.3 (24.1-24.9) n = 7	6.19 ± 0.20 (5.95-6.50) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	2.1 ± 1.2 (0-4.0) n = 7	3.5 ± 0.5 (3.0-4.0) n = 7
0.002	7.32 ± 0.30 (7.00-7.78) n = 7	24.2 ± 0.2 (24.0-24.5) n = 7	6.15 ± 0.15 (6.00-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.008	7.35 ± 0.32 (6.74-7.64) n = 7	24.0 ± 0.4 (23.2-24.5) n = 7	6.19 ± 0.09 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.023	7.42 ± 0.30 (7.03-7.77) n = 7	24.2 ± 0.2 (24.0-24.6) n = 7	6.21 ± 0.07 (6.10-6.30) n = 7	0.016 ± 0.002 (0.015-0.020)	---	---
0.046	7.34 ± 0.36 (6.92-7.80) n = 7	24.1 ± 0.2 (24.0-24.5) n = 7	6.19 ± 0.09 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.076	7.53 ± 0.33 (7.31-7.80) n = 7	24.1 ± 0.1 (24.0-24.2) n = 7	6.19 ± 0.10 (6.05-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	1.8 ± 0.7 (1.0-3.0) n = 7	3.7 ± 0.5 (3.0-4.0) n = 7

[a]mg/L as total uranium (nominal concentrations).

Table 3. Summary of initial water chemistry for Ceriodaphnie dubia 7 d chronic static renewal toxicity test conducted with hydrogen uranyl phosphate. Work was performed for E.I. du Pond de Nemours & Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 13 - 20 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity[b] (mg/L)	Hardness[b] (mg/L)
Control	7.66 ± 0.40 (6.91-8.18) n = 7	24.2 ± 0.3 (24.0-24.7) n = 7	6.04 ± 0.09 (5.95-6.20) n = 7	0.015 ± 0 --- n = 7	2.4 ± 1.0 (1.0-4.0) n = 7	3.4 ± 0.5 (3.0-4.0) n = 7
0.006	7.40 ± 0.40 (6.53-7.74) n = 7	24.5 ± 0.6 (24.0-25.5) n = 7	6.06 ± 0.11 (5.90-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.02	7.30 ± 0.41 (6.67-7.93) n = 7	24.6 ± 0.7 (24.0-25.9) n = 7	6.08 ± 0.10 (5.95-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.06	7.38 ± 0.41 (6.64-7.88) n = 7	24.6 ± 0.7 (24.0-25.6) n = 7	6.08 ± 0.11 (5.95-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.12	7.40 ± 0.39 (6.64-7.74) n = 7	24.7 ± 0.7 (24.0-25.8) n = 7	6.11 ± 0.09 (6.00-6.20) n = 7	0.015 ± 0 --- n = 7	---	---
0.20	7.54 ± 0.39 (6.71-7.94) n = 7	24.4 ± 0.5 (24.0-25.3) n = 7	6.11 ± 0.11 (5.95-6.25) n = 7	0.015 ± 0 --- n = 7	1.9 ± 0.7 (1.0-3.0) n = 7	4.0 ± 0 --- n = 7

[a] mg/L as total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

Table 4. Summary of basic water chemistry for Ceriodaphnia dubia 7 d chronic static renewal toxicity test conducted with hydrogen uranyl phosphate; 24 h readings. Work was performed for E.I. du Pont de Nemours & Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 13 - 20 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.53 ± 0.18 (7.21-7.73) n = 7	24.3 ± 0.3 (24.1-24.9) n = 7	6.11 ± 0.10 (5.95-6.20) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	2.4 ± 0.5 (2.0-3.0) n = 7	3.1 ± 0.4 (3.0-4.0) n = 7
0.006	7.38 ± 0.26 (6.93-7.66) n = 7	24.3 ± 0.3 (24.0-24.8) n = 7	6.19 ± 0.02 (6.15-6.20) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.02	7.28 ± 0.26 (6.83-7.55) n = 7	24.3 ± 0.3 (24.1-24.9) n = 7	6.24 ± 0.06 (6.15-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.06	7.30 ± 0.26 (6.97-7.64) n = 7	24.5 ± 0.5 (24.0-25.3) n = 7	6.26 ± 0.07 (6.15-6.35) n = 7	0.016 ± 0.002 (0.015-0.020)	---	---
0.12	7.24 ± 0.20 (7.02-7.48) n = 7	24.5 ± 0.5 (24.0-25.1) n = 7	6.30 ± 0.06 (6.20-6.40) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.20	7.38 ± 0.41 (6.69-7.71) n = 7	24.2 ± 0.3 (24.0-24.7) n = 7	6.29 ± 0.09 (6.15-6.45) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	2.3 ± 0.8 (2.0-4.0) n = 7	4.0 ± 0 --- n = 7

[a] mg/L as total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

Table 5. Summary of initial basic water chemistry for Ceriodaphnia dubia 7 d chronic static renewal toxicity test conducted with uranium dioxide. Work was performed for E.I. du Pont de Nemours & Company, Savannah River Laboratory, Aiken SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 9 - 16 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.76 ± 0.44 (6.91-8.18) n = 7	24.3 ± 0.6 (24.0-25.7) n = 7	6.14 ± 0.38 (5.75-6.95) n = 7	0.015 ± 0 --- n = 7	2.0 ± 0.8 (1.0-3.0) n = 7	3.4 ± 0.4 (3.0-4.0) n = 7
0.0015	7.71 ± 0.54 (6.82-8.56) n = 7	24.4 ± 0.6 (24.0-25.6) n = 7	5.96 ± 0.07 (5.85-6.05) n = 7	0.015 ± 0 --- n = 7	---	---
0.005	7.64 ± 0.63 (6.52-8.65) n = 7	24.6 ± 0.7 (24.0-26.0) n = 7	5.99 ± 0.14 (5.75-6.15) n = 7	0.015 ± 0 --- n = 7	---	---
0.015	7.54 ± 0.53 (6.71-8.49) n = 7	24.4 ± 0.4 (24.1-25.3) n = 7	6.01 ± 0.12 (5.80-6.15) n = 7	0.015 ± 0 --- n = 7	---	---
0.03	7.38 ± 0.57 (6.71-8.49) n = 7	24.3 ± 0.4 (24.1-25.3) n = 7	6.02 ± 0.14 (5.80-6.15) n = 7	0.015 ± 0 --- n = 7	---	---
0.05	7.46 ± 0.56 (6.71-8.49) n = 7	24.4 ± 0.6 (24.1-25.3) n = 7	6.05 ± 0.12 (5.806.15) n = 7	0.015 ± 0 --- n = 7	2.0 ± 0.6 (1.0-3.0) n = 7	4.0 ± 0 --- n = 7

[a] mg/L total uranium (nominal concentrations)
[b] mg/L as $CaCO_3$

Table 6. Summary of basic water chemistry for Ceriodaphnia dubia 7 d chronic static renewal toxicity test conducted with uranium dioxide; 24 h readings. Work was performed for E.I. du Pont de Nemours & Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 9 - 16 December 1988.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.47 ± 0.16 (7.21-7.73) n = 7	24.4 ± 0.3 (24.1-24.9) n = 7	6.19 ± 0.20 (5.95-6.50) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	2.0 ± 1.0 (1.0-4.0) n = 7	3.5 ± 0.5 (3.0-4.0) n = 7)
0.0015	7.32 ± 0.34 (6.82-7.71) n = 7	24.2 ± 0.2 (24.0-24.5) n = 7	6.13 ± 0.11 (6.00-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.005	7.38 ± 0.37 (6.81-7.79) n = 7	24.3 ± 0.2 (24.0-24.6) n = 7	6.21 ± 0.10 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.015	7.31 ± 0.28 (6.97-7.68) n = 7	24.2 ± 0.2 (24.0-24.7) n = 7	6.22 ± 0.10 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020)	---	---
0.03	7.31 ± 0.30 (6.97-7.68) n = 7	24.2 ± 0.1 (24.0-24.7) n = 7	6.21 ± 0.11 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020 n = 7	---	---
0.05	7.34 ± 0.32 (6.97-7.68) n = 7	24.1 ± 0.2 (24.0-24.7) n = 7	6.20 ± 0.12 (6.10-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	2.3 ± 1.0 (1.0-4.0) n = 7	---

[a] mg/L total uranium (nominal concentrations

[b] mg/L as $CaCO_3$

ATTACHMENT X

Test Report No. A17852 (Part II), Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia. AnalytiKEM Inc., Cherry Hill, NJ 08003 (July, 1989).

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Rpt'; 8/10/89 JPP
Edited 10/16/89 JPP+

AnalytiKEM An American NuKEM Company

AnalytiKEM Inc.
28 Springdale Road
Cherry Hill, NJ 08003
609/751-1122
215/923-2068

TEST REPORT NO. A17852 (Part II)

ACUTE AND CHRONIC TOXICITY OF HYDROGEN URANYL PHOSPHATE TO CERIODAPHNIA DUBIA

April 20, 1989
July 13, 1989 (Revised)

Prepared for:

Westinghouse Savannah River Company
Savannah River Site
P.O. Box 616
Aiken, SC 29802

Attention: John L. Keyes

NJ Certification No. NJ 04012

NY Certification No. NY 10815

SC Certification No. SC 94004

NC Certification No. NC 258

Reviewed & Approved by: Michael Shmookler

Name: Michael Shmookler, Ph.D.

Title: Technical Director

Accepted as is, did not request revised report
minor corrections and editorial changes JPP
made to final (July, '89) report. JPP

AnalytiKEM

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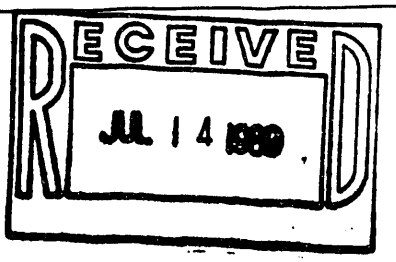
SHEALY ENVIRONMENTAL SERVICES, INC.
BIOLOGISTS, TOXICOLOGISTS & CHEMISTS

400 GRAYMONT AVENUE
COLUMBIA, SOUTH CAROLINA 29205

(803) 254-9915

July 13, 1989



Mr. Joseph P. McLaughlin, Manager
ANALYTIKEM, INC.
454 Anderson Road, BTC 532
Rock Hill, SC 29730

Dear Mr. McLaughlin:

Enclosed please find the revised reports on the Ceriodaphnia chronic toxicity tests for Hydrogen uranyl phosphate, uranyl nitrate and the M-Area effluents. As you will see from the reports we have made as many of the requested revisions as possible. Additionally, we have the following comments concerning technical concerns/questions which were addressed by Dr. John Pickett, Dr. Winona Specht and Mr. John Keyes:

1. All water samples have been filtered through a 37 um plankton net. The EPA protocol from EPA/600/4-85/014 is 30 um. The new EPA Bulletin (EPA/600/4-89/001), however; specifies to use a 60 um plankton net. Please advise as to which bore size is to be used in future toxicity tests. (Response to Item Number 1 of letter dated May 24, 1989).

2. For all future test samples and effluents will be aerated vigorously for 5 minutes when necessary to eliminate problem of supersaturation when samples are warmed to 25°C. (Response to Item Number 2 of letter dated May 24, 1989).

3. There is no EPA criteria for acceptable coefficients of variation, however; as requested we will maintain coefficients of variation for the control groups below 35% in all future tests. This may require that some tests be repeated at SAVANNAH RIVER SITE's expense. (Response to Item Number 5 of letter dated May 24, 1989).

4. Concerning the question of pH decline overtime in the hydrogen uranyl phosphate and uranyl nitrate tests we are enclosing calibration records for the pH readings for review. Please note the same dilution water was used for both tests. (Response to Item Number 6 of letter dated May 24, 1989).

5. The chronic values for the hydrogen uranyl phosphate test was an typographical error which was corrected in the revised report. The chronic value for the uranyl nitrate is correct as 0.0031 ppm and not 0.0037 ppm as Dr. Specht indicated. The value was derived as follows:

$$\text{Antilog}\left[\frac{\text{Log (NOEC)} + \text{Log (LOEC)}}{2}\right] =$$

$$\text{Antilog}\left[(0.0025 + 0.0039)/2)\right] = 0.0031$$

(Response to Item Number 7 of letter dated May 24, 1989).

Please call if you have any questions.

Sincerely,

Richard L. Shealy
President

for urea/nitrate 2/9-2/16/89
H/HP

METER CALIBRATIONS

Parameter Calibration	2/9 Initial	2/10 Day 1	2/11 Day 2	2/12 Day 3	2/13 Day 4	2/14 Day 5	2/15 Day 6	2/16 Day 7
pH Meter #	3	3	3	3	3	3	3	3
Buffer Temp.	18°C	19°C	18°C	18.5°C	18°	20°	22°	22°
Buffer 7	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.00	7=7.01
Buffer 10	-	-	-	-	-	-	-	-
Buffer 4	4=4.00	4=3.99	4=4.00	4=4.00	4=4.01	4=4.00	4=4.00	4=4.00
Slope (Difference)	151 ✓	151 ✓	163 ✓	169 ✓	174 ✓		172 ✓	151 ✓
Final Ck.	7=7.05	7=7.03	7=7.06	7=7.04	7=7.05	7=7.05	7=7.06	7=7.05
D.O. Meter #	2	2	2	2	2	2	2	2
Redline Ck.	✓	✓	✓	✓	✓	✓	✓	✓
Temperature	18.5°C	18.5	19°C	18	18°	21	22	22.5
Initial	9.50	9.50	9.30	9.40	9.30	9.20	8.40	8.90
Chart	9.35	9.35	9.26	9.45	9.45	8.90	8.72	8.64
Reset	9.35	9.35	9.26	9.45	9.46	8.90	8.72	8.64
Final Ck.	9.20	9.10	9.00	9.20	9.40	8.80	8.60	8.50

ACUTE AND CHRONIC TOXICITY OF HYDROGEN URANYL PHOSPHATE TO CERIODAPHNIA DUBIA

Report To

WESTINGHOUSE SAVANNAH RIVER COMPANY

Savannah River Site

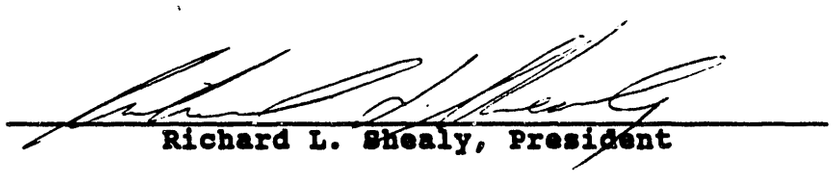
Aiken, South Carolina

Revised July 1989

Submitted By:

SHEALY ENVIRONMENTAL SERVICES, INC.
Columbia, South Carolina
(803) 254-9915

SCDHEC Laboratory Certification No. 26103



Richard L. Shealy, President

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I. INTRODUCTION

Acute and chronic toxicity tests were conducted January 27 - February 19, 1989, for the Savannah River Site to assess the acute and chronic toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia.

II. METHODS

Dilution water for the toxicity tests was collected January 23 (Lab I.D. No. 89-0129), January 27, 1989 (Lab I.D. 89-0158), February 9, 1989 (Lab I.D. No. 89-0364), February 13, 1989 (Lab I.D. No. 89-0371), and February 16, 1989 (Lab I.D. 89-0394) from Upper Three Runs Creek at the northside of a bridge on Road 2-1 on the Savannah River Site by Mr. Jeff Bullard and shipped iced to the laboratory via Federal Express. The water was filtered with a plankton net (37 um mesh) and only water less than 96 hours old was used for the toxicity tests. Ceriodaphnia for the acute and chronic tests had been cultured in water from Upper Three Runs Creek since October 25, 1988.

A. Range-Finding Tests

Range-finding tests were conducted January 27 - 29 and January 30 - February 1, 1989, with concentrations ranging from 0.32 mg/l to 10 mg/l hydrogen uranyl phosphate (0.21 mg/l - 6.5 mg/l theoretical uranium) (Table 1.) These tests were used to determine test concentrations for the definitive acute and chronic tests.

B. Acute Toxicity Test

Test methods conformed to those described in USEPA (1985a; see Table 2). The 48-hour acute toxicity test was conducted February 17 - 19,

Table 1: Summary of results of range-finding tests with hydrogen uranyl phosphate conducted January 27 - February 1, 1989.

Test Date	Test Concentrations	Results
January 27 - 29, 1989	0, 1, 1.8, 3.2, 5.6 & 10 mg/l H.U.P. ~~(0, .65, 1.2, 2.1, 3.6 & 6.5 mg/l~~ theoretical uranium) 0, .54, .97, 1.7, 3.0, & 5.4 mg/L *	High mortality in all test concentrations after 24 hours except the 1 ppm concentration. Lower concentrations needed.
January 31 - February 1, 1989	0, .32, .56, 1.0, 1.8, & 3.2 mg/l H.U.P. ~~(0, .21, .36, .65, 1.8 & 2.1 mg/l~~ * theoretical uranium) 0, .17, .30, .54, .97, 1.7 mg/L	35% mortality in 1.8 mg/l concentration. 90% mortality in 3.2 ppm concentration. Same test concentrations used in the definitive acute test conducted February 17 - 19, 1989.

Should used wrong conversion (0.65)
should have used 0.54
10/16/89

Table 2: Summary of test conditions for the acute toxicity bioassay with Ceriodaphnia dubia.

1.	Temperature:	25 ± 1°C
2.	Light intensity:	Ambient laboratory levels
3.	Photoperiod:	16 h light/8 h dark
4.	Size of test vessel:	100 ml beakers
5.	Volume of test solution:	50 ml
6.	Age of test organisms:	2-24 hour neonates
7.	No. animals per test vessel:	10
8.	No. replicate test vessels per per concentration:	2
9.	Total no. organisms per concentration:	20
10.	Feeding regime:	No feeding required.
11.	Aeration:	None, unless D.O. falls below 40% saturation, at which time gentle single-bubble aeration started.
12.	Dilution water:	Upper Three Runs Creek Water at the Savannah River Site Road 2-1
13.	Test duration:	48 hours
14.	Effect measured:	Mortality - no movement of appendages on gentle prodding

1989, with the following hydrogen uranyl phosphate concentrations: 3.2 mg/l (0.170 mg/l actual recovered uranium), 1.8 mg/l (0.099 mg/l recovered uranium), 1.0 mg/l (0.059 mg/l recovered uranium), 0.56 mg/l (0.190 mg/l recovered uranium) and 0.32 mg/l (0.036 mg/l recovered uranium). For the control, 100% dilution water was used.

All organisms used in the toxicity tests were from SHEALY ENVIRONMENTAL SERVICES, INC.'s in-house cultures with original stock culture obtained from the USEPA Newton Laboratory April 20, 1987, Lab I.D. No. 87-271. Ceriodaphnia from in-house cultures are identified and preserved monthly. Standard toxicant tests with the EPA reference toxicant cadmium chloride and laboratory reagent grade cadmium chloride are performed monthly on Ceriodaphnia cultured in water from Upper Three Runs Creek and in conjunction with the chronic toxicity tests. The results of these tests (LC50's = 0.08 - 0.17 mg/l cadmium chloride) demonstrated that the condition of the cultures were within the acceptable range (0.059 - 0.199 mg/l cadmium chloride) for the test organisms . Test solutions and the controls were prepared in 50 ml quantities in all-glass test chambers. All concentrations and the control were tested in duplicate with ten Ceriodaphnia dubia neonates (2-24 hours old) each. The test solutions were renewed after 24-hours. The hydrogen uranyl phosphate compound was prepared using a procedure provided by Dr. John Pickett by mixing uranyl nitrate and phosphoric acid on a 1 mole U to 1 mole PO_4 ratio and neutralized to a pH of 6 - 7 standard units with sodium hydroxide. The compound was stirred for 15 minutes and the precipitate filtered through a #40 Whatman filter paper. The compound was then rinsed three times with

deionized water and dried overnight at 105°C. A 104 mg/l hydrogen uranium phosphate stock solution was prepared on February 17, 1989 for the acute test. The hydrogen uranyl phosphate solution was prepared by rapidly weighing 0.0104 grams of the chemical onto a tared weighing paper in a balance containing desiccant. Another stock solution (106 ppm) was prepared in the same manner on February 18, 1989 for the renewal. Both hydrogen uranyl phosphate stock solutions were stirred with a magnetic stirrer for five minutes at a constant setting and the precipitate allowed to settle for 30 minutes. Aliquots of the hydrogen uranyl stock solution were drawn off the top of the solution after the precipitate was allowed to settle. The test concentrations were prepared by dosing the dilution water with the appropriate aliquot of the hydrogen uranyl stock solution using Class A volumetric pipets. Samples of hydrogen uranyl phosphate solutions were preserved with 10% metals grade nitric acid and shipped with ice packs to ANALYTIKEM, INC. via Federal Express for analyses.

Dissolved oxygen, water temperature, pH, conductivity, total alkalinity and hardness measurements were made in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers.

The test organisms were placed singly in the test vessels each containing 50 ml of solution. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia.

Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding.

C. Chronic Toxicity Bioassay

Test methods conformed to those described in USEPA (1985b; see Table 3). The 7-day chronic toxicity bioassay was performed February 9 - 16, 1989, as seven treatments exposing 10 test organisms each. The first treatment was the control (100% filtered Upper Three Runs Creek Water). The hydrogen uranyl phosphate solutions were 0.056 mg/l, 0.10 mg/l, 0.18 mg/l, 0.32 mg/l, 0.56 mg/l and 1.0 mg/l, (actual recovered uranium values of 0.028 mg/l and 0.021 mg/l, 0.037 mg/l, 0.050 mg/l, 0.043 mg/l and 0.063 mg/l, respectively). All test solutions were prepared from stock hydrogen uranyl phosphate solutions prepared daily by dosing the dilution water with the appropriate aliquot using Class A volumetric pipets and Hamilton microliter syringes (accuracy and reproducibility ± 1%). The hydrogen uranyl phosphate solutions were prepared in the same manner as for the acute test with each new solution being stirred by a magnetic stirrer for 5 minutes on a constant setting and the precipitate allowed to settle for 30 minutes. Samples of all test concentrations from each day were preserved with 10% metals grade nitric acid and shipped with ice packs to ANALYTIKEM, INC. for analysis. The values given for actual recovered uranium represent averaged values from analyzed samples of the hydrogen uranyl phosphate solutions from two days. The test organisms were exposed to each treatment in individual test chambers. Test solutions were renewed daily.

Table 3: Summary of test conditions for the chronic toxicity bioassay with Ceriodaphnia dubia.

1. Temperature:	25 ± 1°C
2. Light intensity:	Ambient laboratory levels
3. Photoperiod:	16 h light/8 h dark
4. Size of test vessel:	1 ounce SOLO plastic disposable cups
5. Volume of test solution:	15 ml
6. Age of test organisms:	2-24 hour neonates and all released within the same four hour period
7. No. animals per test vessel:	1
8. No. replicate test vessels per per concentration:	10
9. Total no. organisms per concentration:	10
10. Feeding regime:	Selenastrum capricornutum at the rate of 1-2,000,000 cells per ml test soln. per day
11. Aeration:	None
12. Dilution water:	Upper Three Runs Creek Water at the Savannah River Site Road 2-1
13. Test duration:	7 days
14. Effect measured:	Mortality - no movement of appendages on gentle prodding and number of offspring produced

Dissolved oxygen, water temperature, pH, and conductivity measurements were made daily in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers during the test.

The test organisms were placed singly in the test vessels each containing 15 ml of solution. The organisms were between 16 and 20 hours old at the start of the test. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at a rate of approximately 1,000,000 cells per ml. per day in each solution. Selenastrum cultures were obtained from Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged Erlenmeyer flasks and maintained under bright fluorescent lighting for 6 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and order of feeding was established based on random number tables.

III. RESULTS

A. Acute Toxicity Bioassay

The results of the 48-hour acute toxicity bioassay are given in Table 4. Mortality occurred in the 0.059 mg/l (20% mortality), 0.099 mg/l (100% mortality) and 0.170 mg/l (100% mortality) actual recovered uranium.

Table 4. Number and percentage of <u>Ceriodaphnia</u> showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to <u>Ceriodapnia dubia</u>. Concentrations in hydrogen uranyl phosphate, theoretical uranium, and actual recovered uranium. Ten test organisms per replicate.

Test Concentration			Replicate	Number Dead After 24 Hours	Number Dead After 48 Hours	% Mortality
Control			A	0	0	0%
			B	0	0	
H.U.P.*	Theoretical U	Actual Recovered U				
0.32	~~0.208~~ 0.17# mg/l	0.036 mg/l	A	0	0	0%
			B	0	0	
0.56	~~0.364~~ 0.30# mg/l	0.190 mg/l	A	0	0	0%
			B	0	0	
1.0	~~0.650~~ 0.54# mg/l	0.059 mg/l	A	2	2	20%
			B	0	2	
1.2						
1.8	~~1.170~~ 0.97# mg/l	0.099 mg/l	A	10	10	100%
			B	8	10	
3.2	~~2.080~~ 1.72# mg/l	0.170 mg/l	A	10	-	100%
			B	10	-	

*Hydrogen Uranyl Phosphate

They (Shealy) calculated 48 Hr LC50 of 1.2 mg/L HUP
This would translate to 0.070 mg/L recovered U. and to 0.65 theoretical

theoretical U values revised by JBP, 10/18/94.
Shealy used U:mg conversion; should have used 0.54 instead of 0.65 JBP

concentrations. No mortality occurred in the 0.190 mg/l, 0.036 mg/l concentrations or the control. These data were used to determine a 48-hour LC50 (median lethal concentration) value with the Binomial Method (EPA, 1985a). This calculation resulted in a 48-hour LC50 value of 1.20 mg/l hydrogen uranyl phosphate with 95% confidence limits of 1.0 and 1.8 mg/l. A 48-hour LC50 value in terms of recovered uranium could not be calculated since the recovered uranium concentrations were sporatic and not in a progressive series.

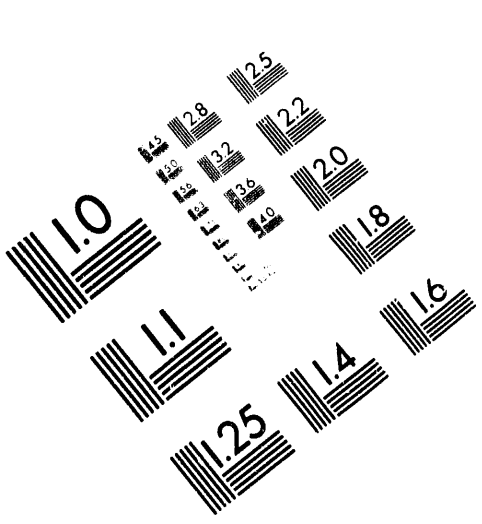
Water chemistry data taken in conjunction with the acute bioassay are given in Table 5. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 17 for the 1.0 mg/l, 1.8 mg/l and 3.2 mg/l hydrogen uranyl phosphate concentrations. For these concentrations, pHs of less than 6 S.U.s were observed.

B. Chronic Toxicity Bioassay

The results of the 7-day chronic toxicity test are given in Table 6. Mortality occurred in the control (10% mortality), 0.056 mg/l (10% mortality), 0.10 mg/l (10% mortality), 0.32 mg/l (10% mortality), 0.56 mg/l (20% mortality) and 1.0 mg/l (10% mortality) hydrogen uranyl phosphate concentrations. No mortality occurred in the 0.18 mg/l hydrogen uranyl phosphate concantration. Reproduction in the control averaged 17 offspring per female.

Table 5. Water quality data recorded in conjunction with the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia, February 17 - 19, 1989.

Exposure Period	Parameter	Test Concentrations (Actual Recovered Uranium)					
		Control	0.036mg/l	0.190mg/l	0.059mg/l	0.099mg/l	0.170mg/l
0 Hours	D.O. (mg/l)	9.50	9.10	9.10	9.20	9.35	9.35
	Temp. (°C)	24.5	24.5	24.5	24.5	24.5	24.5
	pH (SU)	6.12	6.10	6.06	5.98	5.93	5.87
	Cond. (umhos/cm)	19	18	18	18	18	18
	Tot. Hard. (mg/l)	3.8	-	-	-	-	11.5
	Tot. Alk. (mg/l)	3.0	-	-	-	-	3.0
(Before Renewal)							
24 Hours	D.O. (mg/l)	7.60	7.50	7.40	7.50	7.60	7.60
	pH (SU)	6.56	6.63	6.60	6.61	6.54	6.46
(After Renewal)							
24 Hours	D.O. (mg/l)	8.30	8.30	8.30	8.20	8.20	-
	Temp. (°C)	24.0	24.0	24.0	24.0	24.0	-
	pH (SU)	6.18	6.10	6.12	6.10	6.05	-
	Cond. (umhos/cm)	19	18	18	18	18	-
	Tot. Hard. (mg/l)	3.8	-	-	-	-	11.5
	Tot. Alk. (mg/l)	3.0	-	-	-	-	3.0
48 Hours	D.O. (mg/l)	7.60	7.60	7.60	7.65	7.45	-
	Temp. (°C)	24.8	24.8	24.8	24.8	24.8	-
	pH (SU)	6.40	6.41	6.42	6.39	6.37	-



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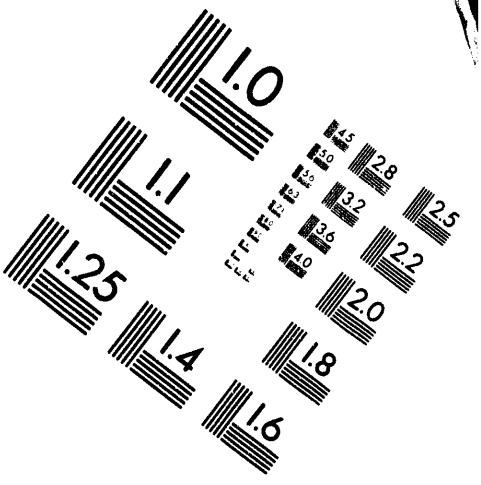

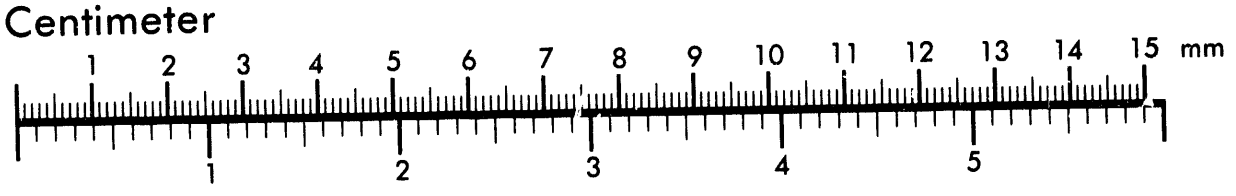

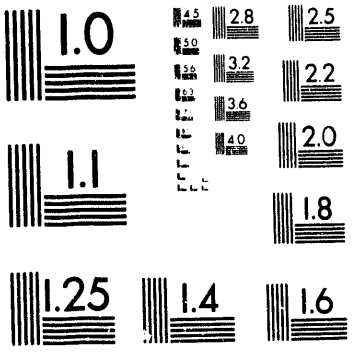

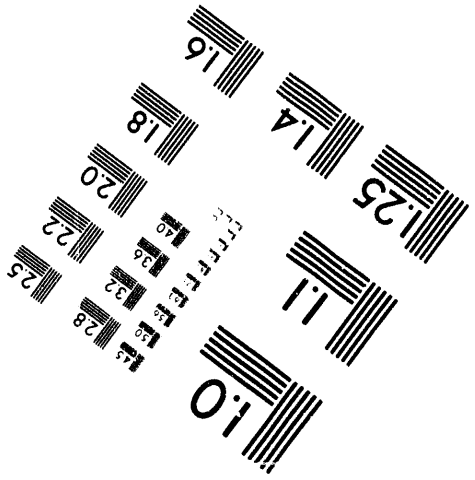

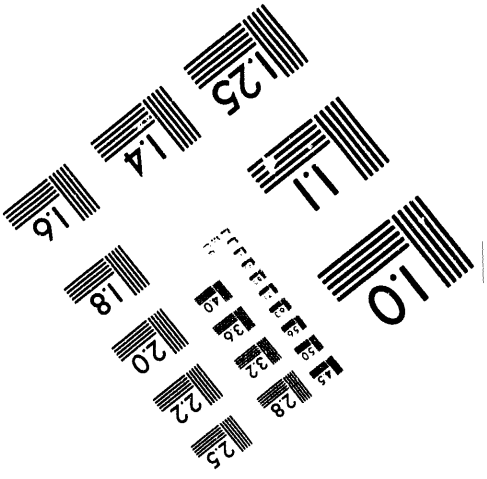

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Table 6: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site
Conducted February 9 - 16, 1989

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
Control	4	2	2	0	D/0	2	2	3	3	0	0
	5	4	3	6	-	4	4	3	7	4	0
	6	0	4	6	-	2	5	10	0	11	3
	7	5	9	10	-	16	7	9	9	2	10
	TOTAL	11	18	22	0	24	18	25	19	17	13
	ADULT	L	L	L	D	L	L	L	L	L	L

$\bar{X}$= 16.7 S.D. = 7.3

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
Dilution	2	0	0	0	0	0	0	0	0	0	0
(0.056)	3	0	0	0	0	0	0	0	0	0	0
ppm hydrogen	4	2	2	1	0	2	3	2	1	3	4
uranyl phosphate	5	0	6	0	D/0	0	4	6	0	4	0
Observed U	6	4	7	10	-	3	5	2	4	6	5
= 0.028 ppm	7	11	0	0	-	3	3	9	0	3	10
	TOTAL	17	15	11	0	8	15	19	5	16	19
	ADULT	L	L	L	D	L	L	L	L	L	L

$\bar{X}$= 12.5 S.D. = 6.4

Table 6: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site Conducted February 9 - 16, 1989 (Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
Dilution	2	0	0	0	0	0	0	0	0	0	0
(0.010)	3	0	0	0	0	0	0	0	0	0	0
ppm hydrogen	4	0	6	2	D/0	2	2	0	2	2	3
uranyl phosphate	5	0	5	3	-	2	0	10	0	0	1
Observed U	6	8	4	3	-	8	5	4	0	8	5
= 0.021 ppm	7	4	5	10	-	0	10	5	5	5	2
	TOTAL	12	20	18	0	12	17	19	7	15	11
	ADULT	L	L	L	D	L	L	L	L	L	L

$\bar{X}$= 13.1 S.D. = 6.2

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
Dilution	2	0	0	0	0	0	0	0	0	0	0
(0.18)	3	0	0	0	0	0	0	0	0	0	0
ppm hydrogen	4	2	4	2	0	2	2	0	0	2	4
uranyl phosphate	5	3	0	3	2	4	3	3	2	0	6
Observed U	6	4	3	3	6	5	4	5	3	2	5
= 0.037 ppm	7	6	2	8	8	3	5	8	4	6	4
	TOTAL	15	9	16	16	14	14	16	9	10	19
	ADULT	L	L	L	L	L	L	L	L	L	L

$\bar{X}$= 13.8 S.D. = 3.4

Table 6: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site Conducted February 9 - 16, 1989 (Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
Dilution	2	0	0	0	0	0	0	0	0	0	0
(0.32)	3	0	0	0	0	0	0	0	0	0	0
ppm hydrogen uranyl phosphate	4	3	4	2	2	1	D/1	0	2	2	0
	5	0	4	3	6	3	-	0	0	0	0
Observed U = 0.050 ppm	6	4	5	10	6	11	-	2	4	0	9
	7	4	5	6	5	0	-	12	6	5	6
	TOTAL	11	18	21	19	15	1	14	12	7	15
	ADULT	L	L	L	L	L	D	L	L	L	L

$\bar{X}$= 13.3 S.D. = 5.9

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	D/0
Dilution	3	0	0	0	0	0	0	0	0	0	-
(0.56)	4	2	0	3	0	2	2	D/0	1	2	-
ppm hydrogen uranyl phosphate	5	0	2	0	6	0	0	-	3	5	-
	6	4	2	6	6	5	2	-	4	4	-
Observed U = 0.043 ppm	7	5	2	2	0	1	11	-	2	6	-
	TOTAL	11	6	11	12	8	15	0	10	17	0
	ADULT	L	L	L	L	L	L	D	L	L	D

$\bar{X}$= 9.0 S.D. = 5.7

Table 6: REPRODUCTION/MORTALITY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site Conducted February 9 - 16, 1989 (Continued)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
	1	0	0	0	0	0	0	0	0	0	0
Dilution	2	0	0	0	0	0	0	0	0	0	0
(1.0)	3	0	0	0	0	0	D/0	0	0	0	0
ppm hydrogen uranyl phosphate	4	3	0	3	0	1	-	2	0	0	1
	5	0	2	2	0	2	-	5	0	5	6
Observed U = 0.063 ppm	6	2	3	10	4	2	-	0	2	0	5
	7	6	0	0	0	0	-	0	0	4	0
	TOTAL	11	5	15	4	5	0	7	2	9	12
	ADULT	L	L	L	L	L	D	L	L	L	L

$\bar{X}$= 7.0 S.D. = 4.7

Average reproduction in the hydrogen uranyl phosphate solutions was as follows:

Hydrogen Uranyl Phosphate	Theoretical U	Average * Recovered U		Offspring Per Female	% Mortality
Control			=	17.0	10
0.056 mg/l	0.~~036~~ .030 mg/l	0.028 mg/l	=	12.5	10
0.10 mg/l	0.~~065~~ .054 mg/l	0.021 mg/l	=	13.1	10
0.18 mg/l	0.~~117~~ .097 mg/l	0.037 mg/l	=	13.8	0
0.32 mg/l	0.~~208~~ .17 mg/l	0.050 mg/l	=	13.3	10
0.56 mg/l	0.~~364~~ .30 mg/l	0.043 mg/l	=	9.0	20
1.0 mg/l	0.650 .54 mg/l	0.063 mg/l	=	7.0	10

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test and for homogeneous variances using Bartlett's test. Non-transformed data were found to be normally distributed (Chi-Square = 3.22, critical value = 12.59) and with homogeneous variances (Bartlett's Test p value = 0.445, p = 0.01). Statistical analyses of the results using Dunnett's Multiple Comparison Procedure indicated chronic toxicity at the 0.56 mg/l and 1.0 mg/l hydrogen uranyl phosphate concentrations (actual recovered uranium concentrations of 0.043 and 0.063, respectively). The no observed effect concentration (NOEC) was 0.32 mg/l hydrogen uranyl phosphate (0.050 mg/l recovered uranium) while the lowest observed effect concentration (LOEC) was 0.56 mg/l hydrogen uranyl phosphate (0.043 mg/l recovered uranium). The chronic value (ChV), taken as the

* Average of 2 determinations (see Table IV), page 25.

[illegible] column corrected by [illegible], 10/16/[illegible].

geometric mean of the NOEC and LOEC, was 0.423 mg/l hydrogen uranyl phosphate.

Water chemistry data taken in conjunction with the chronic toxicity test are given in Table 7. All parameters monitored were within acceptable limits for bioassay purposes except for the pH readings on February 14 and 15. For these days pHs of less than 6 S.U.s were recorded for all concentrations and the control.

Recovered U values do not track HUP Nominally but the results indicate chronic toxicity somewhere 40 to 60 ppb U.

10/16/89

Table 7: WATER CHEMISTRY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site, February 9 - 16, 1989.

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
Control (0%)	Temp. (deg. C)	25.5		24.5		25.0		24.0		24.0		25.0		24.0	25.0	
	D.O. (ppm)	9.00	8.80	8.70	8.80	8.70	8.30	8.30	7.90	8.20	7.30	9.10	7.50	9.00	7.60	
	pH (SU)	6.35		6.24		6.17		6.20		6.15		5.86		5.89		
	Alk.(ppm CaCO3)	3.0		3.0		3.0		3.0		3.0		3.0		3.0		
	Hard.(ppm CaCO3)	3.8		3.8		3.8		3.8		3.8		3.8		3.8		
	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
Dilution (0.056) Observed U = 0.028 ppm	Temp. (deg. C)	25.5		24.5		25.0		25.0		26.0		25.0		24.0	25.0	
	D.O. (ppm)	9.00	8.30	8.50	8.20	8.70	8.10	8.30	7.90	8.20	7.50	9.20	7.60	9.10	7.70	
	pH (SU)	6.26		6.20		6.15		6.21		6.12		5.90		5.87		
	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
Dilution (0.10) Observed U = 0.021 ppm	Temp. (deg. C)	25.5		24.5		25.0		24.0		26.0		25.0		24.0	25.0	
	D.O. (ppm)	8.95	8.80	8.90	8.80	8.70	8.20	8.30	7.90	8.10	7.30	9.10	7.50	9.00	7.60	
	pH (SU)	6.24		6.18		6.13		6.19		6.02		5.88		5.87		
	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Table 7: WATER CHEMISTRY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site, February 9 - 16, 1989. (Continued)

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg. C)	25.5		24.5		25.0		26.0		24.5		25.0		24.0	25.0	
Dilution																
(0.18)	D.O. (ppm)	8.95	8.80	8.90	8.80	8.70	8.10	8.30	7.90	8.10	7.70	9.00	7.70	8.70	7.50	
Observed	pH (SU)	6.24		6.13		6.10		6.20		6.00		5.89		5.85		
U = 0.037																
ppm	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg. C)	25.5		25.0		25.0		26.0		24.5		24.0		24.5	25.0	
Dilution																
(0.32)	D.O. (ppm)	9.00	8.80	8.90	8.60	8.70	8.00	8.30	7.90	8.10	7.20	8.85	7.50	9.00	7.55	
Observed	pH (SU)	6.30		6.10		6.11		6.20		5.97		5.90		5.82		
U = 0.050																
ppm	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg. C)	25.5		25.0		25.0		26.0		24.5		24.0		24.5	25.0	
Dilution																
(0.56)	D.O. (ppm)	9.00	8.80	8.90	8.70	8.70	8.00	8.20	7.80	8.10	7.30	8.80	7.40	8.50	7.80	
Observed	pH (SU)	6.27		6.11		6.07		6.18		5.95		5.85		5.80		
U = 0.043																
ppm	Cond.(umhos/cm)	19		19		20		19		19		19		19		

Table 7: WATER CHEMISTRY DATA for the Ceriodaphnia chronic toxicity test conducted with hydrogen uranyl phosphate for the Savannah River Site, February 9 - 16, 1989. (Continued)

Concentration	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	Temp. (deg. C)	25.5		24.0		25.0		26.0		24.0		25.0		24.0		
Dilution																
(1.0)	D.O. (ppm)	9.05	8.80	8.90	8.65	8.70	8.10	8.30	7.80	8.20	7.00	8.50	7.50	9.00	7.50	
Observed	pH (SU)	6.26		6.09		6.08		6.15		5.97		5.90		5.85		
U = 0.063																
ppm	Cond.(umhos/cm)	19		19		20		19		19		19		19		
	Alk.(ppm CaCO3)	3.5		3.0		3.5		3.0		3.0		3.0		3.0		
	Hard.(ppm CaCO3)	3.8		3.8		3.8		3.8		3.8		3.8		3.8		

APPENDIX A

Analyses of the acute toxicity test data

with the Binomial Method

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(%)
3.2	20	20	100	9.536743E-05
1.8	20	20	100	9.536743E-05
1	20	4	20	.5908966
.56	20	0	0	9.536743E-05
.32	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT 1 AND 1.8 CAN BE USED AS STATISCALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.40501 PERCENT.

APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.200501

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

APPENDIX B

ANOVA table for the chronic toxicity test

Appendix B ANOVA table for the chronic toxicity test conducted with hydrogen uranyl phosphate February 9 - 16, 1989 (Data non-transformed)

Source	DF	SS	MS	F
Among	6	653.7	109.0	3.22
Within	63	2135.1	33.9	
Total	69	2788.8		

Dunnett's T Values

(Critical Value = 2.35)

Concentration	Calculated T
0.056 mg/l	1.70
0.10 mg/l	1.43
0.18 mg/l	1.21
0.32 mg/l	1.36
0.56 mg/l	3.02
1.0 mg/l	3.58

Chi-Square Calculated Value = 3.22 Critical Value = 12.59

Bartlett's Test p Value = 0.445 (p = 0.01)

Control = 17.0

Critical value = 12.59

$\Delta = 4.4/17.0 = 25.9\%$ for minimum significant difference. JMP

IV. Analysis of Test Solutions

Results of analysis of test solutions of uranyl phosphate $HUO_2PO_4.4H_2O$ (MW = 438)

Theoretical Concentration Total Uranium, mg/L	Observed Concentration Total Uranium, mg/L	Percent Recovery
0.17	0.036	21
0.30	0.190	63
0.54	0.059	11
0.97	0.099	10
1.72	0.170	10
0.030	0.026	87
0.054	0.031	57
0.097	0.039	40
0.17	0.055	32
0.30	0.037	12
0.54	0.057	11
0.030	0.030	100
0.054	0.011	20
0.097	0.035	36
0.17	0.044	26
0.30	0.048	16
0.54	0.068	13
55.4	48	87

V. Methodology and Quality Control Data

The methodology and quality control data are described in Sections II and IV, respectively, Part I, of this report.

ATTACHMENT XI

J. L. Keyes to J. P McLaughlin. *Scope of Work for Additional Hydrogen Uranyl Phosphate Toxicity Tests*. Westinghouse Savannah River Co., Savannah River Site, Aiken, SC 29802 (May 3, 1989).

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Westinghouse
Savannah River Company

P.O. Box 616
Aiken, SC 29802

EPS-ES-89-0046

May 9, 1989

Mr. Joseph P. McLaughlin
Contract Program Manager
AnalytiKEM, Inc.
454 S. Anderson Road, BTC 532
Rock Hill, SC 29730

Dear Mr. McLaughlin:

Contract AX #843930

Enclosed is a Scope of Work for additional Hydrogen Uranyl Phosphate acute and chronic toxicity tests. Please arrange for Shealy Environmental Services to initiate this task in a timely manner.

Questions may be directed to Dr. John Pickett at (803) 725-3838.

Yours very truly,

John L. Keyes

J. L. Keyes
Process Biologist
Environmental Protection Section

JLK:ccc
Enclosure

Westinghouse
Savannah River Company

P O Box 616
Aiker SC 29802

INTER-OFFICE MEMORANDUM

May 3, 1989

TO: J. L. KEYES, 703-15A
ENVIRONMENT & ENERGY DEPARTMENT

FROM: J. B. PICKETT, 320-4M
RAW MATERIALS ENGINEERING & TECHNOLGY DEPARTMENT

SCOPE OF WORK FOR ADDITIONAl HYDROGEN URANYL PHOSPHATE
ACUTE AND CHRONIC TOXICITY TESTS

Please transmit the attached scope of work to AnalytiKEM and to ECS/Normandeau for additional acute and chronic toxicity tests on hydrogen uranyl phosphate. I believe that the results of the initial tests were affected by small amounts of the solid compound in the stock solution, which then dissolved in the test solutions (as a function of the % dilution). I have specified a revised stock solution preparation procedure which I believe will eliminate this problem. Please call me at 5-3838 if you have any questions.

JBP:smr
Att

CC: W. L. Specht, 773-42A
C. P. Thompson, 730-M
H. L. Martin, 730-M

SCOPE OF WORK

ADDITIONAL TOXICITY TESTING ON HYDROGEN URANYL PHOSPHATE

1. Please test hydrogen uranyl phosphate for acute and chronic toxicity (LC-50, and NOEC and LOEC).

2. All toxicity tests are to be conducted using EPA approved methods (Peltier and Weber, 1985, and Horning and Weber, 1985)--except that the tests will be conducted at 25°C rather than 20°C.

3. Ceriodaphnia dubia, raised in Upper Three Run water are to be used.

   Water from Upper Three Runs, collected at Road 2-1 on the Savannah River Site is to be used for control and dilutions.

4. The preparation of the hydrogen uranyl phosphate compound, and the preparation of the subsequent stock solution is given in Attachment I.

5. The following uranium concentration ranges are suggested for the acute and chronic tests. Note: These may be modified as required--based on:

   a) the results of the acute tests and/or

   b) the stock solution concentration and available microsyringe sizes.

	Nominal Dissolved Uranium, mg/L
Acute Toxicity:	0.10, 0.080, 0.060, 0.040, and 0.020
Chronic Toxicity:	0.060, 0.048, 0.036, 0.024, 0.018, 0.012, 0.008, 0.004

6. The acute and chronic serial dilutions are to be based on the measured concentration of the uranium stock solution. The uranium stock solution is to be determined in triplicate, prior to use. Matrix spike QC analyses are to be provided when the stock solution is determined.

   The acute and chronic dilution series are to be prepared using standard microsyringe/pipet techniques, which will be called the "nominal", uranium concentrations. Duplicate samples of each of acute and chronic nominal dilution series are to be analyzed (day one of the chronic series is to be sampled and analyzed for uranium).

   The detection limit is to be 0.001 mg/L.

ATTACHMENT

Preparation of $HUO_2PO_4 \cdot 4H_2O$ (MW= 438 gm/Mole).

1) Mix uranyl nitrate and phosphoric acid on a 1 mole U to 1 mole PO_4 ratio.

2) Neutralize to pH 6-7 with NaOH.

3). Stir 15 minutes, and filter precipitate.

4) Rinse three times with D.I. water.

5) Dry ppt at 105° C overnight.

Resulting compound should be: $HUO_2PO_4 \cdot 4H_2O$ (Hydrogen uranyl phosphate, or hydrogen autenite)

Note: the "HUP" previously prepared for the initial round of tests may be utilized, if desired. Retain a 1-2 gm sample of the compound, to be used for x-ray diffraction analysis at SRS.

Preparation of HUP stock solution

1) Prepare sufficient stock solution (one lot) for all of the acute and chronic tests.

2) Weight out enough hydrogen uranyl phosphate ("HUP") to prepare ~ a 100 ppm solution. Filter the entire solution using 0.45µm filter. This should result in a filtrate with ~10 ppm of soluble "HUP". The filtrate is to be used as the stock solution for all of the toxicity tests. Note; either 0.45 µm Nucleopore® or 0.45 µm Millipore® filters are recommended.

3) The stock solution is to be maintained at 4°C during the testing.

4) The concentration of uranium in the stock solution is to be determined in triplicate, and the dilutions based on the measured value of uranium.

4) The filtrate stock solution is to be agitated or stirred vigorously prior to preparing any dilutions (or analyses) to be sure that any insoluble material is included.

ATTACHMENT XII

E. T. Korthals and K. E. Trapp to John Keyes. *Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia.* Report No. NAI-SR-106, Normandeau Associates, Southeast, Aiken, SC 29802 (December, 1989).

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Normandeau Associates, Inc.
P.O. Box 1393
Aiken, SC 29802
(803) 652-2206
(803) 652-7428 (Fax)

NORMANDEAU ASSOCIATES

RECEIVED 1/4/90

CC: J. Pickett, 730-m
W.L. Specht, 773-42A
M.D. Dukes, 703-A w/o report

2 January 1990

John Keyes, Contract Manager
Westinghouse Savannah River Comapny
Savannah River Site
P.O. Box 616
Aiken, SC 29802

Dear Mr. Keyes:

Enclosed please find the final revision for the report "Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia", NAI-SR-106 (Ref. Contract AX843967). This final revision includes comments as discussed with Dr. Pickett on 12/1/89.

Sincerely,

Eric T. Korthals
Laboratory Manager,
Aquatic Toxicology

ETK/wmb

Enclosure

ok - enclosed requested Revisions JMP 1/11/90

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NAI-SR-106

Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to *Ceriodaphnia dubia*

E.T. Korthals
K.E. Trapp

NORMANDEAU ASSOCIATES, SOUTHEAST
P.O. Box 1393
Aiken, South Carolina 29802
(803) 652-2206

Draft Report
September 1989

Final Revision
December 1989

This report NAI-SR-106, was prepared for
Westinghouse Savannah River Company
Savannah River Site
P.O. Box 616
Aiken, South Carolina 29802

John Pickett
Program Director
8
John L. Keyes
Contract Manager

by

NORMANDEAU ASSOCIATES, SOUTHEAST
P.O. Box 1393
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(803) 652-2206

Draft Report
September 1989

Final Revision
December 1989

TABLE OF CONTENTS

TABLES

EXECUTIVE SUMMARY

1. Studies were conducted to assess the acute and chronic toxicity of a uranium compound, hydrogen uranyl phosphate, discharged from the M-Area Dilute Effluent Treatment Facility (DETF) into Tim's Branch, a tributary of Upper Three Runs Creek.

2. The waterflea, Ceriodaphnia dubia, was used as the test organism in 48 h static renewal acute toxicity tests. These acute toxicity test were conducted with fed and unfed test organisms to assess the acute (short-term) toxicity of hydrogen uranyl phosphate, $HUO_2PO_4 \cdot 4H_2O$. Test results (unfed) indicated that, based on nominal concentrations of dissolved uranium, the C. dubia 48 h LC50 for hydrogen uranyl phosphate equaled 0.12 mg/L dissolved uranium (95% confidence limits = 0.11 - 0.13 mg/L). The test results also indicated that the addition of food increased the nominal 48 h LC50 to > 0.26 mg/L dissolved uranium.

3. A C. dubia seven-day static renewal chronic toxicity test was conducted to determine the chronic (long-term) toxicity of hydrogen uranyl phosphate. Exposure to nominal concentrations of hydrogen uranyl phosphate $\geq$ 0.006 mg/L dissolved uranium affected both test organism survival and reproduction. The nominal NOEC and LOEC for hydrogen uranyl phosphate equaled 0.002 and 0.006 mg/L dissolved uranium, respectively.

4. These results demonstrated that relatively low concentrations (i.e., $\leq$ 0.12 mg/L dissolved uranium) of hydrogen uranyl phosphate adversely affected the survival and reproduction of the test organism, C. dubia. Results of the acute toxicity tests also demonstrated that the addition of food to test solutions decreased the toxicity of hydrogen uranyl phosphate.

1.0 INTRODUCTION

Westinghouse Savannah River Company requested that Normandeau Southeast (NAI-SE, SC DHEC Laboratory Identification Number 02101) determine the toxicity of hydrogen uranyl phosphate to the water flea, Ceriodaphnia dubia. Information generated from these tests will help determine if the uranium compound adversely affects the aquatic biota of Upper Three Runs Creek.

Low concentrations of a uranium compound, hydrogen uranyl phosphate ($HUO_2PO_4 \cdot 4H_2O$), are currently discharged from the M-Area Dilute Effluent Treatment Facility (DETF) on the Savannah River Site (SRS). Discharge from the DETF enters Tim's Branch, a tributary of Upper Three Runs Creek. Tim's Branch flows into this creek near Road C on the SRS. Upper Three Runs Creek is a blackwater creek that runs through SRS property and discharges into the Savannah River. The water in Upper Three Runs Creek is generally acidic and demonstrates little or no buffering capacity (Table 1-1).

This series of toxicity tests was designed to assess both the acute and chronic toxicity of hydrogen uranyl phosphate. The toxicity tests were conducted in three stages. The first stage consisted of a range-finding test in which C. dubia were exposed to a wide range of hydrogen uranyl phosphate concentrations. This phase was exploratory; its purpose was to reduce the number of concentrations tested in the second phase by approximating

Table 1-1. Results of basic water chemistry analyses conducted on water samples collected from Upper Three Runs Creek and the Road 2-1 bridge on the Savannah River Site. January 1988 - August 1989.

	pH	Total Hardness ($CaCO_3$ mg/L)	Total Alkalinity[a] ($CaCO_3$ mg/L)	Conductivity (mS/cm)
1988				
Jan. 11	5.50	6.0	1.5	0.022
Apr. 7	6.10	6.5	1.0	0.020
Jun. 7	5.60	2.5	2.0	0.015
Jun. 20	6.30	3.0	3.5	0.020
Jul. 19	5.55	3.0	1.0	0.015
Aug. 8	5.80	3.5	1.5	0.020
Sept.8	5.80	3.0	2.5	0.020
Oct. 14	5.80	2.5	1.0	0.015
Nov. 3	5.95	3.0	1.0	0.015
Dec. 12	6.00	3.5	1.0	0.015
1989				
Apr. 26	6.30	4.0	3.0	0.015
May 11	5.65	3.0	1.0	0.010
June 12	5.60	3.0	1.0	0.010
July 19	6.05	3.0	1.0	0.015
Aug. 14	6.00	3.0	3.0	0.010

[a]Detection Limit = 0.1 mg/L.

concentrations of the compound that would produce 50% mortality among the test organisms.

Once this initial test was completed, a definitive 48 h static renewal acute toxicity test was conducted. This represented the second phase of testing and was designed to establish the concentration of hydrogen uranyl phosphate that was lethal to 50% of the test organisms (lethal concentration, or LC50) within 48 h. The 95% confidence limits for the LC50 were also calculated to provide some indication of the range of concentrations over which a similar acute response might be observed. The acute toxicity of hydrogen uranyl phosphate was determined with and without the addition of food to test solutions. The purpose of the acute toxicity test with fed test organisms was to assess whether the toxicity of hydrogen uranyl phosphate in the chronic toxicity test was affected by the addition of food.

When the acute toxicity of hydrogen uranyl phosphate to C. dubia had been determined, the third stage of testing was initiated. A seven-day C. dubia static renewal chronic toxicity test was performed to identify concentrations of hydrogen uranyl phosphate that could have sublethal, long-term (chronic), adverse effects on aquatic organisms. C. dubia were exposed to a range of concentrations of hydrogen uranyl phosphate for seven days. The LC50 value determined in the unfed acute toxicity test was used to establish the concentrations used in the chronic test. During a seven-day period, C. dubia individuals typically produce three

broods of offspring. Test organism survival and offspring production served as criteria for determining the chronic toxicity of hydrogen uranyl phosphate.

Statistical analyses of the C. dubia chronic toxicity test result were used to identify a no-observable-effect concentration (NOEC). The NOEC is the highest concentration of hydrogen uranyl phosphate that produces no statistically significant reduction in the survival or reproduction of test organisms when compared to control organism survival and reproduction. A lowest-observed-effect concentration (LOEC) was also identified. The LOEC represents the lowest concentration of toxicant that produces a statistically significant adverse effect on test organism survival and reproduction (Horning and Weber 1985).

The results of the acute and seven-day chronic toxicity tests will be used to help determine a threshold or safe discharge concentration for hydrogen uranyl phosphate so that the compound can be released from the DETF outfall without disrupting the aquatic community of Upper Three Runs Creek.

2.0 METHODS AND MATERIALS

2.1 PREPARATION OF STOCK SOLUTION

Unless otherwise specified, all solutions (both stock and test) were prepared using volumetric glassware and calibrated pipettes or pipetters. The same hydrogen uranyl phosphate stock solution was used for both the acute and chronic toxicity tests. The concentration of dissolved uranium in the stock solution was confirmed analytically before it was used in a test. Concentrations of dissolved uranium were determined either by fluorometry (Method 711-B, detection limit = 0.02 mg/L; APHA 1985) or by inductively coupled plasma emission spectrophotometry (EPA method 200.7, detection limit = 0.10 mg/L; EPA 1983).

Reagent grade uranyl nitrate (Mallinckrodt Lot# 8640 KCAP) and phosphoric acid were used to prepare hydrogen uranyl phosphate. The uranyl nitrate was mixed with phosphoric acid (1:1 ratio of moles uranium to moles phosphate). This mixture was neutralized to pH 6-7 with 1.0 N NaOH and stirred for 15 min. The resulting precipitate ($HUO_2PO_4 \cdot 4H_2O$) was filtered through Whatman #4 filter paper, washed three times with deionized water, transferred to a watch glass, and dried at 105^{o} C for approximately 16 h.

A saturated stock solution of hydrogen uranyl phosphate was prepared by mixing 0.4 g of the compound with 19 L of Upper

Three Runs Creek water for approximately 16 h. The resultant suspension was filtered through a 0.45 μm polycarbonate filter (Millipore Corp., Bedford, MA). The filtrate was then used as a stock solution to prepare all toxicity test solutions. The stock solution was analyzed to determine the concentration of dissolved uranium it contained. The measured concentration of uranium in the stock solution equaled 0.26 ± 0.02 mg/L dissolved uranium ($\bar{x}$ ± sd, n = 3). All nominal test concentrations were extrapolated from the concentration of dissolved uranium measured in the hydrogen uranyl phosphate stock solution.

2.2 LABORATORY PROTOCOL

The guidelines and recommendations listed in Horning and Weber (1985) and Peltier and Weber (1985) were followed for handling organisms, cleaning test equipment, and conducting all toxicity tests. Laboratory procedures are listed in detail, and deviations from methodology given in Horning and Weber (1985) or Peltier and Weber (1985) are noted. The water flea, Ceriodaphnia dubia, served as the test organism.

2.2.1 Culture Methods

Stock cultures of Ceriodaphnia dubia were originally obtained from cultures maintained by the US EPA Environmental Research Laboratory in Duluth, MN. These animals are now cultured by the NAI-SE aquatic toxicology laboratory in water collected from Upper Three Runs Creek (Aiken County, SC). Water is collected at the Road 2-1 bridge on the SRS and is filtered

through a plankton net prior to use. Typical water quality values for this creek are listed in Table 1-1.

All-glass (1.5 L) culture dishes serve as culture chambers for a "brood" stock. The dishes are thoroughly cleaned prior to use and are covered while in use to prevent the entry of dust and other contaminants. Cultures are kept in an incubator (Lab-Line Instruments, Inc., Melrose Park, IL), with the temperature maintained at 25 $\pm$ 2^{o} C. Water temperature is continously monitored. Wide-spectrum fluorescent bulbs (Color Rendering Index $\geq$ 90) are used to provide a 16L:8D photoperiod. Light intensity measured at the surface of the culture dishes did not exceed 800 lux.

Brood-stock C. dubia (30 organisms/culture dish) were fed every other day on a diet consisting of a mixture of algae (Selenastrum capricornutum) and YCT (yeast, cerophyll, fermented trout chow). Approximately 1 x 10^{8} cells/mL of algae and 5 mL of YCT were added to each culture dish. A modified version of Bold's Basic Media (Appendix 1) was used to maintain unialgal cultures of S. capricornutum.

All culture dishes were examined at least three times per week, and quality assurance records were maintained for each dish. Records include date the culture was started, source of culture material, reproductive progress, presence of ephippia, and other information on the condition of the culture deemed

pertinent by the observer. The animals in these dishes served as the source of neonate (≤ 24 h old) daphnids used in both acute and chronic toxicity tests. The first two broods were discarded; only neonate daphnids obtained from later broods were used in the toxicity tests.

2.2.2 Test Procedures

2.2.2.1 Collection of Water

Water from Upper Three Runs Creek served as the control and diluent for both the acute and chronic toxicity tests conducted on hydrogen uranyl phosphate. Water for all toxicity tests was collected from the Road 2-1 bridge located on the SRS. Water was collected prior to test initiation and was used within 72 h of collection. New samples of water were collected once every 72 h. Upper Three Runs Creek water was collected on 28 June and 11 July 1989 for the acute toxicity tests and on 7 and 11 July 1989 for the chronic toxicity test. Water was not filtered prior to use in the acute toxicity tests, but was filtered through a 30 μm plankton net prior to use in the chronic test. Filtration removes potential predators from the diluent and is recommended by Horning and Weber (1985).

2.2.2.2 48 h Ceriodaphnia dubia Static Renewal Acute Toxicity Tests

Range-Finding Test - A range-finding test was performed to determine the concentrations of hydrogen uranyl phosphate to be used in subsequent definitive tests. The range-finding test

consisted of a control group and test groups of twenty neonate daphnids, each of which was exposed for 48 h to one of seven test concentrations. The stock solution was diluted to prepare nominal dissolved uranium concentrations of 0 (control), 0.02, 0.04, 0.06, 0.08, 0.30 mg/L. Based on the results of this range-finding test, a definitive test of 48 h duration was initiated to establish the LC50 of the uranium compound.

Acute Toxicity Tests - The 48 h static renewal acute toxicity tests were performed with and without food added to the test solutions. The acute toxicity test with unfed test organisms was initated on 29 June 1989 and ended on 1 July. The acute toxicity test with fed test organisms was conducted from 11 to 13 July 1989. All test solutions for these static renewal acute toxicity tests were prepared daily by diluting the hydrogen uranyl phosphate stock solution. The undiluted stock solution served as the highest test concentration (i.e., 0.26 mg/L dissolved uranium). Test concentrations used in the acute toxicity tests were based on results of the range-finding test.

C. dubia were exposed to the following dilution series of hydrogen uranyl phosphate in both acute toxicity tests:

0 (control), 0.10, 0.13, 0.16, 0.20, 0.23, 0.26

mg/L dissolved uranium (nominal values).

Graduated cylinders and a variable pipetter were used to transfer aliquants of chemical stock solution to 500-mL volumetric flasks. The flask contents were then adjusted to 500

mL with Upper Three Runs Creek water. Test solutions were prepared from the lowest to the highest nominal concentration of dissolved uranium using the same volumetric flask. The concentration of dissolved uranium in each test solution of the acute toxicity test with unfed test organisms was analytically verified. Only the highest test concentration in the acute toxicity test with fed organisms was analyzed for dissolved uranium.

Test conditions are summarized in Table 2-1. Borosilicate glass beakers (250-mL) served as test chambers for the static renewal acute toxicity tests. Two beakers were used per test concentration, with ten individuals per beaker. A large-bore, fire-polished, glass pipette was used to randomly transfer ten neonate daphnids to each test chamber. When ten individuals had been isolated, excess water was removed and 100 mL of test solution was slowly and gently poured into the beaker. Following the addition of solution, the daphnids were observed to verify they had not been damaged during transfer.

In the acute toxicity test with fed test organisms, a mixture of algal suspension and yeast-trout chow-cerophyll (YTC) was added at a final concentration of 0.033 mL/mL test solution. This was the same final concentration of food added to the chronic toxicity test solutions. Organisms were fed at test initiation and after the 24-h renewal.

Table 2-1. SUMMARY OF TEST CONDITIONS: *Ceriodaphnia dubia*[a] 48 h static renewal acute toxicity test.

1. Test temperature	25 ± 2° C
2. Light quality	Ambient illumination
3. Light intensity	Ambient laboratory levels
4. Photoperiod	16L:8D
5. Test vessel size/type	250-mL borosilicate glass beakers
6. Number of organisms per vessel	10
7. Number of replicates	2 per concentration
8. Age of organisms	≤ 24 h
9. Total number of organisms per concentration	20
10. Aeration	None, unless DO is ≤ 40% saturation
11. Diluent	Upper Three Runs Creek water
12. Test duration	48 h
13. Effect measured	Mortality (LC50 ± 95% confidence limits)
14. Chemical parameters measured on diluent and highest test concentration	DO, °C, pH, conductivity, hardness, alkalinity (daily on new and old solutions)
15. Chemical parameters measured on intermediate test concentrations	DO, °C, pH, conductivity (daily on new and old solutions)

[a]Adapted from Peltier and Weber 1985.

The acute toxicity tests were conducted in a temperature-controlled, walk-in incubator maintained at 25 ± 2° C. Test organisms were exposed to a 16L:8D photoperiod. Specific conductance, dissolved oxygen concentration (DO), $CaCO_3$ hardness, total alkalinity, and pH of the control and highest test concentration were recorded at the beginning of each test and at 24 h intervals. Dissolved oxygen concentration, pH, temperature, and conductivity of intermediate test concentrations were measured and recorded at test initiation and at 24 h. Total alkalinity was determined by potentiometric titration (APHA 1985), while the CDTA (cyclohexanediaminetetraacetic acid) titrimetric method (APHA 1985) was used to measure $CaCO_3$ hardness. Dissolved-oxygen concentrations were measured with a YSI Model 58 DO meter (Yellow Springs Instrument Co., Yellow Springs, OH), and a YSI Model 33 conductivity meter was used to measure the conductivity of each test solution. The pH values were determined with an Orion 399A pH meter.

Immobilization and death of the C. dubia were used as the indicators of acute toxicity (Peltier and Weber 1985). The criterion used to establish lethality was cessation of all visible signs of mobility (e.g., no movement of second antennae, thoracic legs, or postabdomen). Immobilization was defined as the inability of the animals to move in the water column (ASTM 1984). A test was deemed acceptable if control organism mortality was ≤ 10% (Peltier and Weber 1985).

2.2.2.3 Seven-Day Ceriodaphnia dubia Static Renewal Chronic Toxicity Test

Organisms used in this test were ≤ 24 h old and born within a 4 h period. All test solutions for the chronic toxicity test were prepared daily. The following concentrations were used in the seven-day static renewal chronic toxicity test conducted with hydrogen uranyl phosphate:

0 (Control), 0.0002, 0.0006, 0.002, 0.006, 0.02, 0.06, 0.12, 0.20 mg/L dissolved uranium (nominal).

To prepare the chronic toxicity test solutions, aliquants of the hydrogen uranyl phosphate stock solution were transferred to a 500-mL volumetric flask with pipettes or pipetters. The volume was then adjusted to 500 mL with Upper Three Runs Creek water. Test solutions were prepared from the lowest to the highest nominal concentration of dissolved uranium. The dissolved uranium concentration in the stock solution was determined on Day 5 of the chronic toxicity test. Dissolved uranium was also measured on 8 July 1989 (Day 0) in all test solutions and daily in the highest test concentration. Test solutions with nominal concentrations ≤ 0.006 mg/L were concentrated by a factor of ten prior to analysis.

Test conditions are summarized in Table 2-2. The chronic toxicity test was initiated on 8 July 1989 and ended on 15 July. Testing was performed in 20-mL cups (Solo Corp.) containing 15 mL of test solution. Cups were assigned a randomized position in a

Table 2-2. SUMMARY OF TEST CONDITIONS: *Ceriodaphnia dubia*[a] seven-day static renewal chronic toxicity test.

1. Test temperature	25 ± 1° C
2. Light quality	Ambient illumination
3. Light intensity	Ambient laboratory levels
4. Photoperiod	16L:8D
5. Test vessel size/type	20-mL plastic cups
6. Number of organisms per vessel	1
7. Number of replicates	20 per concentration
8. Age of organisms	≤ 24 h
9. Total number of organisms per concentration	20
10. Aeration	None, unless DO is ≤ 40% saturation
11. Diluent	Upper Three Runs Creek water
12. Test duration	7 d
13. Effect measured	Mortality, reduced young production (NOEC and LOEC)
14. Chemical parameters measured on diluent and highest test concentration	DO, °C, alkalinity, hardness, pH, conductivity (daily on new solutions)
15. Chemical parameters Measured on intermediate test concentrations	DO, °C, pH, conductivity (daily on old and new solutions)

[a]Adapted from Horning and Weber 1985.

test tray which was maintained for the duration of the test. All test trays were placed in an incubator maintained at 25 ± 1° C. The incubator temperature was continuously monitored. Test organisms were exposed to a 16L:8D photoperiod. Twenty individuals were exposed to each test concentration and to the control solution.

Large-bore, fire-polished, disposable glass pipettes were used to transfer organisms. Test organisms were moved to fresh test solution every 24 h, and all young produced during a test were preserved with Lugol's solution (APHA 1985) for later enumeration. Test organism mortality and presence of young were recorded daily. Following transfer, the organisms were observed to verify they had not been damaged.

Specific conductance, DO, $CaCO_3$ hardness, total alkalinity, and pH were recorded for the new and old solutions of the control and highest test concentration. Only conductivity, DO, pH, and temperature measurements were performed on the new and old intermediate test concentrations. The same methods used to monitor water quality parameters during the static renewal acute toxicity tests were used in the chronic toxicity test.

C. dubia were fed during each test by adding an aliquot of algal suspension/YTC mixture (0.033 mL/mL) to each cup. YTC was added to increase the protein content of the diet. The other nutritional requirements of these organisms (e.g., vitamins,

dietary lipids, minerals) were provided by the algal portion of the diet.

The criterion for establishing lethality in the acute toxicity tests was also used in the chronic toxicity test. On Day 7 of the chronic toxicity test, adult survival was determined, and a count was made of the total number of young produced per test organism. A test was deemed acceptable if control mortality was $\leq$ 20% (Horning and Weber 1985) and if the average number of young produced per control individual was $\geq$ 15 (SC DHEC 1988).

Chronic toxicity was determined to have occurred if statistical analyses indicated that significant differences existed between the control and test organisms.

2.3 GLASSWARE PREPARATION

All glassware was cleaned before and after use. Glassware was first rinsed with pesticide-free acetone, then with methanol followed by methylene chloride. Following the solvent rinses, glassware was soaked for 24 h in a 5% Contrad solution and rinsed with deionized water. The glassware was air-dried, then soaked for 24 h in 2% HNO_3. Deionized water was used in the final rinses (5 times with deionized water) of the glassware. All borosilicate-glass beakers used in the toxicity tests were maintained separately from other laboratory glassware and were used only for toxicity tests. Just prior to use, these beakers were rinsed with dilution water.

2.4 DATA ANALYSIS

The 48 h LC50 values were determined by the Trimmed Spearman-Karber procedure (Hamilton et al. 1977, 1978).

Chronic toxicity test data were analyzed using Fisher's Exact test, the Chi-Square test, Bartlett's test, one-way analysis of variance (ANOVA), and Dunnett's multiple comparison test (Sokal and Rohlf 1981, Zar 1984).

Adult survival data was analyzed by Fisher's Exact Test (Sokal and Rohlf 1981) to determine if the percent survival in the control group was the same as that observed in each test concentration. If statistically significant differences were detected in the percent survival of adults between the control and any test group, then such groups represented two different populations and could not be compared further in statistical analyses.

Additional statistical analyses, Chi-Square Goodness of Fit and Bartlett's tests, were conducted to determine if the number of young produced by C. dubia exposed to the control solution and test exposures without significant mortality were normally distributed and if the variances for this data were homogeneous. These conditions must exist to perform parametric tests such as the analysis of variance (ANOVA) and Dunnett's multiple comparison test.

A one-way analysis of variance (ANOVA) was conducted to determine if significant differences in offspring production existed between C. dubia exposed to the control solution and hydrogen uranyl phosphate test solutions. If the results of this test indicated that reproduction among various treatment groups differed significantly, then Dunnett's multiple comparison test was performed to identify those test solutions in which offspring production was significantly less than that of control organisms.

Percent recoveries of dissolved uranium in test solutions were calculated based on the nominal test concentration.

2.5 QUALITY ASSURANCE

Quality assurance procedures commonly followed in the NAI-SE Aquatic Toxicology Division include the following:

1. Instruments are routinely calibrated and standardized according to manufacturers' instructions. Control charts are maintained for all measured parameters.

2. Wet chemistry methods used in determining hardness and alkalinity are standardized according to US EPA methods.

3. Records are maintained of the age, productivity, quality of food, and feeding regime of all organisms maintained by NAI-SE.

4. Reference toxicity tests are performed on a routine basis (at least twice monthly) to determine the acceptability and sensitivity of test organisms. Reference toxicant control charts are maintained for all test organisms cultured by NAI-SE. Results of reference tests indicated that the animals used in these tests responded in an appropriate manner.

3.0 RESULTS

3.1 48 H CERIODAPHNIA DUBIA STATIC RENEWAL ACUTE TOXICITY TESTS

3.1.1 Acute Toxicity Test-Unfed Organisms

Results of the acute toxicity test with unfed test organisms are summarized in Table 3-1. Partial test organism mortality was observed in the control, 0.16, 0.23, and 0.26 mg/L dissolved uranium nominal test concentrations after 24 h of exposure (Table 3-1). Complete mortality was observed in the 0.16, 0.20, 0.23, and 0.26 mg/L dissolved uranium exposures at test termination. Partial mortality was observed in the remaining test concentrations at test termination. Control mortality at test termination equaled 5% (Table 3-1). Based on these test results, the nominal 48 h LC50 for unfed test organisms equaled 0.12 mg/L dissolved uranium (95% confidence limits = 0.11 to 0.13 mg/L dissolved uranium; Table 3-1).

The results of basic water chemistry analyses performed on all solutions used in this acute toxicity test are listed in Appendix 2 (Tables 1 through 4).

3.1.2 Acute Toxicity Test-Fed Organisms

A 48 h static renewal acute toxicity test was conducted to determine the effect of food (algae/YTC) on the toxicity of hydrogen uranyl phosphate to C. dubia. Results of this

Table 3-1. Acute Toxicity Test with Unfed Organisms Conducted on Hydrogen Uranyl Phosphate.

Results of a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent for this test. 29 June - 1 July 1989.

Nominal Concentration (mg/L)[b,c]	Measured Concentrations (mg/L)[c] (6-29-89)	Measured Concentrations (mg/L)[c] (6-30-89)	Average	Total Mortalities[a] 24 h	48 h	% Mortality at 48 h	% Recovery
Control	<0.01	---		1	1	5	90
0.10	0.08	0.10	0.09	0	2	10	77
0.13	0.10	0.10	0.10	0	13	65	73
0.16	0.12	0.11	0.115	1	20	100	70
0.20	0.12	0.16	0.14	0	20	100	87
0.23	0.20	0.20	0.20	1[d]	10[d]	100	85
0.26	0.22	0.22	0.22	2	20	100	90%

[a]Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate).
Test vessels = 250-mL borosilicate glass beakers containing 100 mL/beaker.
[b]Nominal concentrations extrapolated from the measured dissolved uranium concentration in the stock solution.
[c]mg/L as dissolved uranium.
[d]One replicate of the 0.23 mg/L test concentration was lost on 30 June 1989 due to a broken beaker.

Nominal 48 h LC50 = 0.12 mg/L dissolved uranium
95% Confidence Limits = 0.11 - 0.13 mg/L

Method of Calculation = Trimmed Spearman-Karber

test are summarized in Table 3-2. No test organism mortality was observed in any test concentration after 24 h of exposure (Table 3-2). At test termination partial mortality (i.e., 5%) was observed in the 0.26 mg/L dissolved uranium exposure. Based on these test results, the nominal 48 h LC50 equaled > 0.26 mg/L dissolved uranium (Table 3-2).

The results of basic water chemistry analyses performed on all solutions used in this acute toxicity test are listed in Appendix 2 (Tables 5 through 8).

3.2 SEVEN-DAY CERIODAPHNIA DUBIA STATIC RENEWAL CHRONIC TEST

Results of the seven-day C. dubia static renewal chronic toxicity test are summarized in Table 3-3. Partial mortality was observed by test termination among C. dubia exposed to 0.06, 0.12, and 0.20 mg/L dissolved uranium (Table 3-3). Both brood and mean young production were substantially reduced among organisms exposed to test concentrations $\geq$ 0.06 mg/L dissolved uranium compared to the control individuals (Table 3-3).

The results of Fisher's Exact test (Table 3-4) indicated a significant difference existed in the percent survival between the control group and C. dubia exposed to 0.20 mg/L dissolved uranium.

Table 3-2. Acute Toxicity Test with Fed Organisms Conducted on Hydrogen Uranyl Phosphate.

Results of a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Test organisms were fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent for this test. 11 -13 July 198[illegible].

Nominal Concentration (mg/L)[b,c]	Measured Concentrations (mg/L)[c] (7-11-89)	Measured Concentrations (mg/L)[c] (7-12-89)	Total Mortality[a] 24 h	48 h	%Mortality at 48 h
Control	---	---	0	0	0
0.10	---	---	0	0	0
0.13	---	---	0	0	0
0.16	---	---	0	0	0
0.20	---	---	0	0	0
0.23	---	---	0	0	0
0.26	0.24	0.28	0	1	5

[a] Twenty organisms were exposed to each concentration. Concentrations were tested in duplicate (10 organisms/replicate).
[b] Test vessels = 250-mL borosilicate glass beakers containing 100 mL/beaker.
[b] Nominal concentrations extrapolated from the measured dissolved uranium concentration in the stock solution.
[c] mg/L as dissolved uranium.

Nominal 48 h LC50 = > 0.26 mg/L dissolved uranium

Method of Calculation = Not Applicable

Table 3-3. Chronic Toxicity Test Conducted on Hydrogen Uranyl Phosphate.

Results of a seven-day static renewal chronic toxicity test for Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Water collected from Upper Three Runs Creek served as the control and diluent. 8 - 15 July 1989.

Nominal[a] Concentration (mg/L)[b]	Measured Concentration (7-8-89) (mg/L)[b]	$\bar{X}$ (SD) of young per female[c]	$\bar{X}$ broods per female[d]	% Mortality of original females
Control	< 0.002	29.65 (6.34)	3.4	0
0.0002	< 0.002	22.10 (6.28)	2.9	0
0.0006	< 0.002	27.68 (3.53)	3.0	0
0.002	0.002	26.05 (3.97)	3.0	0
0.006	0.004	23.90 (4.96)	2.9	0
0.02	0.03	25.00 (7.41)	2.9	0
0.06	0.04	18.35 (7.42)	2.6	5
0.12	0.14	13.00 (7.02)	2.3	10
0.20	0.20	9.00 (4.22)	1.8	70

[a] Nominal concentrations extrapolated form the measured concentration of dissolved uranium in the stock solution.
[b] mg/L as dissolved uranium.
[c] Mean value based on number of young produced by 20 original females. One organism was lost due to mechanical injury in the 0.0006 and 0.006 mg/L test concentrations. Mean values reflect only 19 individuals in those two concentrations. One male was present in the 0.12 mg/L test concentration. The male was not included in statistical analysis of young production.
[d] Mean value based on surviving original females.

Nominal NOEC = 0.002 mg/L dissolved uranium

Nominal LOEC = 0.006 mg/L dissolved uranium

Table 3-4. "Fisher's Exact" Test Procedure for Adult Survival Rate for Hydrogen Uranyl Phosphate.

Results of Fisner's Exact test conducted on the percent survival of Ceriodaphnia dubia exposed to a control and eight concentrations of hydrogen uranyl phosphate in the seven day static renewal chronic toxicity test. Water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of dissolved uranium.
8 - 15 July 1989.

H_o: The proportion of C. dubia survival in the control group is the same as that of C. dubia exposed to each of eight concentrations of hydrogen uranyl phosphate.

H_a: The proportion of C. dubia survival in the control group is not equal to that of C. dubia exposed to each of eight concentrations of hydrogen uranyl phosphate.

Comparison	Critical value	b value	Significantly Different [a]
Control vs. 0.0002	15	19	N
Control vs. 0.0006	15	19	N
Control vs. 0.002	15	20	N
Control vs. 0.006	15	19	N
Control vs. 0.02	15	20	N
Control vs. 0.06	15	19	N
Control vs. 0.12	15	18	N
Control vs. 0.20	15	6	Y

[a] $P = 0.05$.

The results of the Chi-Square Goodness of Fit and Bartlett's tests (Table 3-5) indicated that data were normally distributed and that the variances were homogeneous. Therefore, parametric procedures were used to perform all other analyses.

Results of the ANOVA test (Table 3-6A) indicated that reproduction among the various treatment groups differed significantly. Dunnett's multiple comparison test (Table 3-6B) indicated that exposure of C. dubia to 0.0002, 0.006, 0.02, 0.06, and 0.12 mg/L dissolved uranium test concentrations resulted in a significant reduction in production of young when compared to reproduction of the control group.

A review of this data indicated that the response of the test organisms deviated from the concentration-response pattern typically associated with chronic toxicity tests. It is not possible to determine if the reduced reproduction observed in the 0.0002 mg/L dissolved uranium test concentration is truly the result of exposure to hydrogen uranyl phosphate or an aberrant response. However, based on strict interpretation of the statistical results, the nominal NOEC and LOEC for hydrogen uranyl phosphate equaled < 0.0002 and 0.0002 mg/L dissolved uranium, respectively. If the response observed at 0.0002 mg/L is atypical, then the nominal NOEC equaled 0.002 mg/L dissolved uranium and the nominal LOEC equaled 0.006 mg/L dissolved uranium (Table 3-

Table 3-5. Chi-Square Goodness of Fit and Bartlett's Tests for Hydrogen Uranyl Phosphate.

Results of a Chi-Square Goodness of Fit test and a Bartlett's test conducted on the number of young produced by Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. In the seven-day static renewal chronic toxicity test, water from Upper Three Runs Creek served as the control and diluent. All concentrations are expressed as nominal concentrations of dissolved uranium. 8 - 15 July 1989.

Chi-Square Goodness of Fit test:

calculated $\chi^2 = 0.678$

χ^2 critical value $_{(0.01,4)} = 13.277$

The data are normally distributed.

Bartlett's test:

calculated B = 18.40

χ^2 critical value $_{0.01\ [7,\ 18.62]} = 18.48$

The variances are homogeneous.

Table 3-6. Analysis of Variance and Dunnett's Multiple Comparison Tests for Hydrogen Uranyl Phosphate.

Results of a one-way analysis of variance (Table A) and one-tailed Dunnett's comparison test (Table B) for the number of young produced by Ceriodaphnia dubia exposed to hydrogen uranyl phosphate. In the seven-day static renewal chronic toxicity test water from Upper Three Runs Creek was used as the control and diluent. A test to determine the minimum significant difference detectable among these data was conducted (Table C). All concentrations are expressed as nominal concentrations of dissolved uranium. 8 - 15 July 1989.

Table A. One-way analysis of variance (ANOVA)

Ho: The mean numbers of young produced by C. dubia exposed to both the control and seven concentrations of hydrogen uranyl phosphate are equal.

Ha: The mean numbers of young produced by C. dubia exposed to both the control and seven concentrations of hydrogen uranyl phosphate are not equal.

Source	d.f.	S.S.	MS	F	Critical F
concentration	7	3921.57	560.22	15.30	2.09
error	149	5455.74	36.62		
total	156	9377.31			

Table 3-6 (continued). Analysis of Variance and Dunnett's Multiple Comparison Tests for Hydrogen Uranyl Phosphate.

Table B. One-Tailed Dunnett's Comparison Test

Ho: $\mu c = \mu a$

Ha: $\mu c \neq \mu a$

Comparison	$(\bar{X}c - \bar{X}a)$	SE	$\lvert q' \rvert$	p	$q'_{0.05(1),120,p}$
control vs. 0.0002	7.55	1.91	3.95	6	2.26*
control vs. 0.0006	1.97	1.94	1.02	2	1.66
control vs. 0.002	3.60	1.91	1.88	3	1.93*
control vs. 0.006	5.76	1.94	2.97	5	2.18*
control vs. 0.02	4.65	1.91	2.43	4	2.08*
control vs. 0.06	11.30	1.91	5.91	7	2.32*
control vs. 0.12	16.65	1.94	8.58	8	2.37*

Nominal NOEC = 0.002 mg/L dissolved uranium
Nominal LOEC = 0.006 mg/L dissolved uranium

*Number of offspring were significantly different from control (P = 0.05).

Table C. The minimum significant difference (MSD) detectable among these data

$MSD_{n=20} = 4.5$
$MSD_{n=19} = 4.6$

Control mean = 29.6 young; 29.6 - 4.5 = 25.1
and
29.6 - 4.6 = 25.0

For this data set, a 15.3% (4.5 young) or 15.5% (4.6 young) reduction in C. dubia production of young could be detected. That is, mean offspring production $\leq$ 25.0 or 25.1 would be significantly different from offspring production of the control group.

6B). A similar chronic toxicity test, performed 13 - 20 December 1988, determined the nominal NOEC and LOEC equaled < 0.006 and 0.006 mg/L total uranium, respectively (Trapp and Korthals 1989). Based on all information, it is recommended that the nominal NOEC and LOEC for hydrogen uranyl phosphate be reported as 0.002 and 0.006 mg/L dissolved uranium, respectively (Table 3-6B).

The minimum significant difference (MSD) test determined that a 15.3 to 15.4% reduction in the mean number of offspring from the control production (i.e., mean offspring production of $\leq$ 4.5 to 4.6) could be detected (Table 3-6C) among these data.

The results of all initial and final basic water chemistry analyses performed on all test solutions are listed in Appendix 3 (Tables 1 and 2).

3.3 URANIUM ANALYSES

In this report all concentrations of uranium are expressed in terms of the nominal concentrations of dissolved uranium. The toxicity endpoints (i.e., LC50, NOEC, and LOEC values) are converted to measured concentrations of dissolved uranium in Tables 3-7 to 3-9.

Table 3-7. Percent Recovery of Dissolved Uranium in Solutions used in an Acute Toxicity Test with Unfed Organisms Conducted with Hydrogen Uranyl Phosphate. 29 - 30 June 1989.

Nominal Concentrations (mg/L dissolved U)	Measured Concentrations[a] DATE Jun 29	Measured Concentrations[a] DATE Jun 30	% Recovery[b] DATE Jun 29	% Recovery[b] DATE Jun 30	Mean % Recovery
0.26	0.22 (0.18-0.27)	0.22 (0.20-0.25)	84.6	84.6	84.6
0.23	0.20 (0.20-0.21)	0.20 (0.16-0.25)	87.0	87.0	87.0
0.20	0.12 (0.10-0.14)	0.16 (0.12-0.19)	60.0	80.0	70.0
0.16	0.12 (0.11-0.12)	0.11 (0.08-0.14)	75.0	68.8	71.9
0.13	0.10 (0.09-0.10)	0.10 (0.09-0.11)	76.9	76.9	76.9
0.10	0.08 (0.08-0.08)	0.10 (0.10-0.11)	80.0	100.0	90.0
				Grand Mean	
				% Recovery = 80.1%	

Nominal 48 h LC50 = 0.12 mg/L dissolved uranium (95% confidence limits = 0.11 - 0.13 mg/L)

Measured 48 h LC50 = 0.10 mg/L dissolved uranium (95% confidence limits = 0.09 - 0.10 mg/L)

[a]Values in parentheses represent the range of measured dissolved uranium concentrations.

[b]Percent recovery based on the nominal concentration of dissolved uranium in test solutions.

Table 3-8. Percent Recovery of Dissolved Uranium in Solutions used in an Acute Toxicity Test with Fed Organisms Conducted with Hydrogen Uranyl Phosphate. 11 - 13 July 1989.

Nominal Concentration (mg/L dissolved U)	Measured Concentrations[a] DATE Jul 11	Measured Concentrations[a] DATE Jul 12	% Recovery[b] DATE Jul 11	% Recovery[b] DATE Jul 12	Mean % Recovery
0.26	0.24 (0.22-0.25)	0.28 (0.28-0.29)	92.3	107.7	100.0

Nominal 48 h LC50 = > 0.26 mg/L dissolved uranium

Measured 48 h LC50 = > 0.26 mg/L dissolved uranium

[a]Values in parentheses represent the range of measured dissolved uranium concentrations.

[b]Percent recovery based on the nominal concentration of dissolved uranium in the test solution.

Table 3-9. Percent Recovery for Dissolved Uranium in Solutions used in a Chronic Toxicity Test Conducted with Hydrogen Uranyl Phosphate. 8 - 15 July 1989.

Nominal Concentration (mg/L dissolved U)	Measured Concentrations[a] DATE						
	Jul 8	Jul 9	Jul 10	Jul 11	Jul 12	Jul 13	Jul 14
control	< 0.002						
0.0002	< 0.002						
0.0006	< 0.002						
0.002	0.002(0.001-0.002)						
0.006	0.004(0.004-0.004)						
0.02	0.03 (0.03-0.03)						
0.06	0.04 (0.04-0.05)						
0.12	0.14 (0.11-0.18)						
0.20	0.20 (0.18-0.23)	0.14 (0.12-0.16)	0.18 (0.17-0.20)	0.19 (0.16-0.22)	0.20 (0.18-0.21)	0.16 (0.16-0.16)	0.19 (0.17-0.21)

[a]Values in parantheses represent range of measured dissolved uranium concentrations.

Table 3-9 (cont.). Percent Recovery for Dissolved Uranium in Solutions used in a Chronic Toxicity Test Conducted on Hydrogen Uranyl Phosphate. 8 - 15 July 1989.

Nominal Concentration (mg/L dissolved U)	% Recovery[b] DATE Jul 8	Jul 9	Jul 10	Jul 11	Jul 12	Jul 13	Jul 14	Mean % Recovery
control	----							----
0.0002	----							----
0.0006	----							----
0.002	100.0							----
0.006	66.7							----
0.02	150.0							----
0.06	66.7							----
0.12	116.7							----
0.20	100.0	70.0	90.0	95.0	100.0	80.0	95.0	90.0

Grand Mean % Recovery = 94.2%

Nominal	"Measured"
NOEC = 0.002 mg/L dissolved U	NOEC = 0.002 x 0.942 = 0.002 mg/L dissolved U
LOEC = 0.006 mg/L dissolved U	LOEC = 0.006 x 0.942 = 0.006 mg/L dissolved U

CONSERVATIVE CALCULATIONS:

Nominal	"Measured"
NOEC = <0.0002 mg/L dissolved U	NOEC = <0.0002 x 0.942 = < 0.0002 mg/L dissolved U
LOEC = 0.0002 mg/L dissolved U	LOEC = 0.0002 x 0.942 = 0.0002 mg/L dissolved U

[b]Percent recovery based on the nominal dissolved uranium concentration in the test solutions.

Concentrations of dissolved uranium measured in the test solutions used in the static renewal acute toxicity tests are summarized in Tables 3-10 and 3-11. Results of uranium analyses for the static renewal chronic toxicity test are summarized in Table 3-12.

The mean percent recoveries of uranium in test solutions of the acute toxicity test with unfed test organisms ranged from 60.0 to 100.0% (Table 3-10), while in the test with fed test organisms the mean percent recovery equaled 100% (Table 3-11). Percent recoveries of dissolved uranium in all test solutions of the chronic toxicity test (Day 0) ranged from 66.7 to 150% (Table 3-12). The mean percent recovery of dissolved uranium in the highest test concentration equaled 90.0% (Table 3-12).

4.0 DISCUSSION

Results of the 48 h C. dubia static renewal acute toxicity test with unfed test organisms (nominal 48 h LC50 = 0.12 mg/L dissolved uranium) were similar to that of a study performed in November 1988 (nominal 48 h LC50 = 0.11 mg/L total uranium; Trapp and Korthals 1989) on hydrogen uranyl phosphate. Although the test results were similar, they are not directly comparable since the reported units and the physical form of hydrogen uranyl phosphate were different in the two studies. In the acute toxicity test performed in November 1988, the hydrogen uranyl phosphate in the stock solution existed as fine-particulate and dissolved compound, whereas in the present test it was only in the dissolved form (i.e., filterable through a 0.45 μm filter)

A 48 h LC50 could not be calculated for the acute toxicity test to which food was added. The results demonstrated the nominal LC50 was greater than the concentration of dissolved uranium present in the stock solution (i.e., > 0.26 mg/L dissolved uranium).

The results of the acute toxicity tests with fed and unfed test organisms were substantially different. The addition of food to test solutions decreased the toxicity of hydrogen uranyl phosphate. The chemical form of the dissolved uranium in the hydrogen uranyl phosphate test

solutions was probably that of an anionic phosphate complex [$UO_2(HPO_4)^{=}$; Langmuir 1978]. This anionic complex may have been absorbed by the food added to the test solutions. Algal mats, humic acids and other organic substances in natural waters fix uranyl ions and have been reported to remove uranium from the water column (Taylor 1983). In this toxicity test with fed test organisms, a portion of the food material settled to the bottom of the test chambers, potentially removing uranium from solution. This phenomenon may account for the difference in the results of the two acute toxicity tests performed with hydrogen uranyl phosphate. The results of these tests indicated that the addition of food may have had an affect on the toxicity of hydrogen uranyl phosphate in the chronic toxicity test.

Chronic toxicity test results indicated that the nominal NOEC and LOEC for hydrogen uranyl phosphate equaled 0.002 and 0.006 mg/L dissolved uranium, respectively. Although differing with respect to the physical form (dissolved vs. particulate) of uranium, these results were similar to that of a chronic toxicity test performed in December 1988 (nominal NOEC = <0.006 mg/L total uranium, nominal LOEC = 0.006 mg/L total uranium; Trapp and Korthals 1989).

5.0 SUMMARY

Laboratory tests were conducted to determine the acute and chronic toxicities of hydrogen uranyl phosphate. This compound is present in an effluent discharged from the M-Area Dilute Effluent Treatment Facility (DETF) into Tim's Branch, a tributary of Upper Three Runs Creek. The waterflea, Ceriodaphnia dubia, was used in all toxicity tests. These organisms were reared in water collected from Upper Three Runs Creek. This water also served as the control and diluent for both the acute and chronic tests.

The results of both the acute and chronic toxicity tests conducted on the hydrogen uranyl phosphate demonstrated that relatively low concentrations of this compound (i.e., $\leq$ 0.12 mg/L dissolved uranium) adversely affected the organism C. dubia (Table 5-1). Results of the acute toxicity tests also demonstrated that the addition of food decreased the toxicity of hydrogen uranyl phosphate (Table 5-1).

Table 5-1. Summary of Toxicity Endpoints for Hydrogen Uranyl Phosphate.

SUMMARY

Acute Toxicity (mg/L dissolved uranium) - test organisms unfed

Nominal 48 h LC50	Measured[a] 48 h LC50
0.12	0.10

Acute Toxicity (mg/L dissolved uranium) - test organisms fed

Nominal 48 h LC50	Measured[a] 48 h LC50
> 0.26	> 0.26

Chronic Toxicity (mg/L dissolved uranium)

Nominal		Measured[a]	
NOEC	LOEC	NOEC	LOEC
0.002	0.006	0.002	0.006

[a]Calculated based on measured concentration of dissolved uranium in test solutions.

6.0 LITERATURE CITED

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APPENDIX 1

Composition of Bold's Modified Media

Appendix 1.

Composition of Modified Bold's Media[a]

Major Components

Na
K
Ca
Mg
NO_3
PO_4
Cl
SO_4

Minor Components

H_3BO_3
EDTA
Fe (II)
Zn (II)
Mn (II)
Cu (II)
Mo (VI)
Co (II)

Vitamins

Thiamine Hydrochloride
D-Pantothenic Acid, Calcium
Biotin
Cyanocobalamin (B_{12})

---

[a]Arthur L. Buikema, Jr., pers. comm.

APPENDIX 2

Water Chemistry Data for 48 h Acute Toxicity Tests

Table 1. Initial basic water chemistry for a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 29 June 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	Alkalinity (mg $CaCO_3$/L)	pH	Hardness (mg $CaCO_3$/L)	Conductivity (mS/cm)
Control	7.71	24.4	2.0	5.80	3.0	0.015
0.10	7.77	24.2		5.80		0.020
0.13	7.82	24.3		5.90		0.020
0.16	7.73	24.4		5.90		0.020
0.20	7.74	24.4		5.85		0.020
0.23	7.87	24.5		5.90		0.020
0.26	7.80	24.3	2.0	5.85	2.0	0.020

[a] mg/L dissolved uranium (nominal concentrations).

Table 2. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 30 June 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)
Control	6.98	24.3	5.70	0.015
0.10	6.97	24.2	5.80	0.030
0.13	6.89	24.2	5.90	0.025
0.16	7.11	24.4	5.90	0.025
0.20	7.09	24.3	6.00	0.020
0.23	7.09	24.5	6.00	0.025
0.26	7.10	24.0	5.95	0.020

[a] mg/L dissolved uranium (nominal concentrations).

Table 3. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate; initial reading on renewal sample. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 30 June 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	Alkalinity (mg $CaCO_3$/L)	pH	Hardness (mg $CaCO_3$/L)	Conductivity (mS/cm)
Control	6.60	24.1	2.0	5.80	3.0	0.015
0.10	6.58	24.2		5.95		0.015
0.13	6.70	24.2		5.90		0.015
0.16	6.78	24.3		5.85		0.015
0.20	6.81	24.3		5.80		0.015
0.23	6.99	24.3		5.85		0.015
0.26	7.11	24.2	1.0	5.85	2.0	0.020

[a] mg/L dissolved uranium (nominal concentrations).

Table 4. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings on renewal sample. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 1 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)
Control	6.87	24.1	6.40	0.015
0.10	6.51	23.8	6.45	0.020
0.13	6.11	23.8	6.45	0.020
0.16	6.29	23.7	6.45	0.025
0.20	6.62	23.8	6.45	0.025
0.23	7.04	23.7	6.40	0.020
0.26	6.99	23.7	6.40	0.020

[a] mg/L dissolved uranium (nominal concentrations).

Table 5. Initial basic water chemistry for a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate. Test organisms were fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 11 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	Alkalinity (mg $CaCO_3$/L)	pH	Hardness (mg $CaCO_3$/L)	Conductivity (mS/cm)
Control	7.90	24.0	1.0	6.05	4.0	0.015
0.10	7.89	24.9		5.70		0.015
0.13	7.96	24.8		5.80		0.015
0.16	7.95	24.8		5.90		0.015
0.20	7.95	24.8		6.00		0.015
0.23	7.92	24.7		6.00		0.015
0.26	7.92	24.6	<0.05	6.05	2.0	0.015

[a] mg/L dissolved uranium (nominal concentrations).

Table 6. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings. Test organisms were fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 12 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)
Control	7.58	24.0	6.10	0.015
0.10	7.52	24.3	6.25	0.015
0.13	7.57	24.4	6.25	0.015
0.16	7.52	24.4	6.25	0.015
0.20	7.68	24.1	6.25	0.015
0.23	7.56	24.3	6.30	0.015
0.26	7.57	24.5	6.30	0.015

[a] mg/L dissolved uranium (nominal concentrations).

Table 7. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal acute toxicity test conducted on hydrogen uranyl phosphate; initial reading on renewal sample. Test organisms were fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 12 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	Alkalinity (mg $CaCO_3$/L)	pH	Hardness (mg $CaCO_3$/L)	Conductivity (mS/cm)
Control	7.78	24.0	1.0	5.95	4.0	0.015
0.10	7.54	24.0		5.80		0.015
0.13	7.43	24.2		5.80		0.015
0.16	7.57	24.3		5.90		0.015
0.20	7.58	24.2		5.95		0.015
0.23	7.59	24.4		6.00		0.020
0.26	7.85	24.0	1.0	6.00	2.0	0.020

[a] mg/L dissolved uranium (nominal concentrations).

Table 8. Basic water chemistry for a 48 h Ceriodaphnia dubia static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings on renewal sample. Test organisms were not fed during the test. Work was performed for Westinghouse Savannah River Company, Aiken, SC. Upper Three Runs Creek water served as the control and diluent. 13 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)
Control	7.55	24.6	6.00	0.015
0.10	7.46	24.4	6.15	0.015
0.13	7.43	24.6	6.20	0.015
0.16	7.41	24.5	6.30	0.015
0.20	7.10	24.6	6.35	0.015
0.23	7.30	24.5	6.40	0.015
0.26	7.53	24.1	6.40	0.015

[a]mg/L dissolved uranium (nominal concentrations).

APPENDIX 3

Water Chemistry Data for Chronic Toxicity Test

Table 1. Summary of initial water chemistry for a seven-day Ceriodaphnia dubia static renewal chronic toxicity test conducted on hydrogen uranyl phosphate. Work was performed for Westinghouse Savannah River Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 8 - 15 July 1989.

Concentration	Dissolved Oxygen (mg/L)[a]	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.62 ± 0.26 (7.23-7.90) n = 7	24.4 ± 0.6 (24.0-25.7) n = 7	6.07 ± 0.14 (5.90-6.25) n = 7	0.015 ± 0.000 --- n = 7	1.3 ± 0.8 (0.0-2.0) n = 7	3.6 ± 0.5 (3.0-4.0) n = 7
0.0002	7.53 ± 0.35 (6.81-7.84) n = 7	24.5 ± 0.6 (24.0-25.5) n = 7	5.95 ± 0.13 (5.70-6.10) n = 7	0.015 ± 0.000 --- n = 7	---	---
0.0006	7.47 ± 0.38 (6.71-7.89) n = 7	24.6 ± 0.5 (24.0-25.1) n = 7	5.92 ± 0.10 (5.80-6.10) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.002	7.47 ± 0.36 (6.72-7.78) n = 7	24.5 ± 0.7 (24.0-25.1) n = 7	5.91 ± 0.12 (5.70-6.10) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.006	7.49 ± 0.31 (6.87-7.77) n = 7	24.4 ± 0.5 (24.0-25.2) n = 7	5.91 ± 0.12 (6.70-6.10) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---

[a] mg/L as dissolved uranium (nominal concentrations).

[b] mg/L as $CaCO_3$.

Table 1 (cont.). Summary of initial water chemistry for a seven-day Ceriodaphnia dubia static renewal chronic toxicity test conducted on hydrogen uranyl phosphate.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
0.02	7.49 ± 0.31 (6.87-7.77) n = 7	24.5 ± 0.5 (24.0-25.2) n = 7	5.92 ± 0.11 (5.70-6.05) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.06	7.51 ± 0.28 (6.98-7.78) n = 7	24.5 ± 0.5 (24.0-25.4) n = 7	5.94 ± 0.09 (5.75-6.05) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.12	7.59 ± 0.33 (7.02-8.05) n = 7	24.4 ± 0.5 (24.0-25.2) n = 7	5.99 ± 0.10 (5.80-6.10) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.20	7.67 ± 0.37 (6.95-8.19) n = 7	24.3 ± 0.4 (24.0-25.0) n = 7	6.06 ± 0.10 (5.90-6.20) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	1.7 ± 1.8 (0.0-4.0) n = 7	2.9 ± 1.0 (2.0-4.0) n = 7

[a] mg/L as dissolved uranium (nominal concentrations).

[b] mg/L as $CaCO_3$.

Table 2. Summary of basic water chemistry for seven-day Ceriodaphnia dubia chronic static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings. Work was performed for Westinghouse Savannah River Company, Savannah River Laboratory, Aiken, SC. Values are the mean, standard deviation, range and number of observations. Upper Three Runs Creek water served as the control and diluent. 8 - 15 July 1989.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
Control	7.54 ± 0.18 (7.27-7.76) n = 7	24.2 ± 0.4 (24.0-24.9) n = 7	6.21 ± 0.19 (6.00-6.50) n = 7	0.015 ± 0.000 --- n = 7	1.7 ± 1.1 (0.0-3.0) n = 7	3.3 ± 0.9 (2.0-5.0) n = 7
0.0002	7.45 ± 0.36 (6.91-7.81) n = 7	24.2 ± 0.2 (24.0-24.5) n = 7	6.09 ± 0.12 (5.95-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.0006	7.45 ± 0.36 (6.95-7.89) n = 7	24.3 ± 0.3 (24.0-24.8) n = 7	6.11 ± 0.15 5.90-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.002	7.41 ± 0.33 (6.90-7.71) n = 7	24.3 ± 0.3 (24.0-24.7) n = 7	6.15 ± 0.11 (6.05-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.006	7.36 ± 0.40 (6.73-7.73) n = 7	24.2 ± 0.2 (24.0-24.5) n = 7	6.17 ± 0.10 (6.05-6.30) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---

[a] mg/L as dissolved uranium (nominal concentrations).

[b] mg/L as $CaCO_3$.

Table 2 (cont.). Summary of basic water chemistry for seven-day Ceriodaphnia dubia chronic static renewal toxicity test conducted on hydrogen uranyl phosphate; 24 h readings.

Concentration (mg/L)[a]	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Conductivity (mS/cm)	Alkalinity (mg/L)[b]	Hardness (mg/L)[b]
0.02	7.29 ± 0.39 (6.80-7.75) n = 7	24.3 ± 0.2 (24.0-24.6) n = 7	6.21 ± 0.12 (6.05-6.35) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.06	7.16 ± 0.63 (6.04-7.69) n = 7	24.3 ± 0.5 (24.0-24.8) n = 7	6.26 ± 0.11 (6.10-6.40) n = 7	0.016 ± 0.002 (0.015-0.020)	---	---
0.12	7.23 ± 0.52 (6.32-7.79) n = 7	24.2 ± 0.3 (24.0-24.7) n = 7	6.31 ± 0.12 (6.15-6.50) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	---	---
0.20	7.63 ± 0.38 (6.90-8.04) n = 7	24.2 ± 0.2 (24.0-24.5) n = 7	6.31 ± 0.12 (6.20-6.55) n = 7	0.016 ± 0.002 (0.015-0.020) n = 7	3.6 ± 3.5 (0.0-11.0) n = 7	2.6 ± 0.9 (2.0-4.0) n = 7

[a] mg/L as dissolved uranium (nominal concentrations).

[b] mg/L as $CaCO_3$.

ATTACHMENT XIII

Test Report No. 80084 Final, Acute and Chronic Toxicity of Hydrogen Uranyl Phosphate to Ceriodaphnia dubia. AnalytiKEM Inc., Rock Hill, SC 29730 (January 5, 1990).

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AnalytiKEM An American NuKEM Company

AnalytiKEM Inc.
454 S. Anderson Road, BTC 532
Rock Hill, SC 29730
803/329-9690

TEST REPORT NO. 80084 FINAL

JANUARY 5, 1990

PREPARED FOR:

WESTINGHOUSE SAVANNAH RIVER COMPANY
ENVIRONMENTAL PROTECTION DIVISION
SAVANNAH RIVER SITE
AIKEN, SC 29808-0001

ATTENTION: JOHN L. KEYES

NJ CERTIFICATION NO. NJ 04012

NY CERTIFICATION NO. NY 10815

SC CERTIFICATION NO. SC 94004

NC CERTIFICATION NO. NC 258

REVIEWED & APPROVED BY: Patricia de Andino

NAME: PATRICIA de ANDINO

TITLE: QUALITY ASSURANCE MANAGER

ACUTE AND CHRONIC TOXICITY OF HYDROGEN URANYL PHOSPHATE TO CERIODAPHNIA DUBIA

Report To:

WESTINGHOUSE SAVANNAH RIVER COMPANY

Savannah River Site

Aiken, South Carolina

December 1989

Submitted By:

SHEALY ENVIRONMENTAL SERVICES, INC.
Columbia, SC
(803) 254-9915

SCDHEC Laboratory Certification No. 26103

NCDEM Laboratory Certification No. 301

Richard L. Shealy, President

TABLE OF CONTENTS

Supplement: "Test Report No A 90084 Supplemental", By Analytikem, octoba 30, 1989.

LIST OF TABLES

I. INTRODUCTION

Acute and chronic toxicity tests were conducted August 17 - 19, 1989 and September 2 - 9, 1989, respectively, for the Savannah River Site to assess the acute and chronic toxicity of the hydrogen uranyl phosphate to Ceriodaphnia dubia.

II. METHODS

Dilution water for the toxicity tests was collected August 9, 1989 (Lab I.D. 89-2249) from Upper Three Runs Creek at the northside of a bridge on Road 2-1 on the Savannah River Site by SHEALY ENVIRONMENTAL SERVICES, INC.'s personnel and returned iced to the laboratory the same day. The water was filtered with a plankton net (37 um mesh). Only one batch of water was used for the acute and chronic testing because of the appearance of sporatic toxicity in the Upper Three Runs Creek water collected at that location. Ceriodaphnia for the acute and chronic tests had been cultured in water from Upper Three Runs Creek since October 25, 1988.

A. Preparation of Test Solutions

Uranium concentrations were prepared by ANALYTIKEM, INC. on July 31, 1989 (Lab I.D. No. 89-2178), and sent to SHEALY ENVIRONMENTAL SERVICES, INC. for use in the toxicity tests. These hydrogen uranyl phosphate solutions were only used for the initial range-finding tests August 3 - 5, 1989 and were found to be too low. Subsequent uranium solutions were prepared from a stock uranium solution of 1.05 ppm urannium (from 2000 ppm hydrogen uranyl phosphate filtered through

0.45 um filter) prepared by ANALYTIKEM, INC. August 4, 1989 (Lab I.D. No. 89-2201).

B. Range-Finding Tests

Range-finding tests were conducted August 3 - 5 and August 15 - 22, 1989, with concentrations ranging from 0.012 to 0.3 ppm uranium (Table 1). These tests were used to determine test concentrations for the definitive acute and chronic toxicity tests.

C. Acute Toxicity Test

Test methods conformed to those described in USEPA (1985a; see Table 2). The 48-hour acute toxicity test was conducted August 17 - 19, 1989, with the following uranium concentrations: 0.10, 0.15, 0.20, 0.25, and 0.30 ppm uranium. For the control, 100% dilution water was used.

All organisms used in the toxicity tests were from SHEALY ENVIRONMENTAL SERVICES, INC.'s in-house cultures with the original stock culture obtained from the USEPA Newton Laboratory April 20, 1987, Lab I.D. No. 87-271. Ceriodaphnia from in-house cultures are identified and preserved monthly. Standard toxicant tests with the EPA reference toxicant cadmium chloride and laboratory reagent grade cadmium chloride are performed twice monthly on Ceriodaphnia cultured in water from Upper Three Runs Creek and in conjunction with the chronic toxicity tests. The results of these tests demonstrated that the condition of the cultures were within the acceptable range (Central Tendency = 0.138 ppm, Upper Limit = 0.25, Lower Limit = 0.03

Table 1: Summary of results of range-finding tests with hydrogen uranyl phosphate conducted August 3 - 5 and August 15 -22, 1989.

Test Date/Test Type	Test Concentrations	Results
August 3 - 5, 1989 Acute	0.02, 0.04, 0.06, 0.08, and 0.10 ppm uranium. (All concentrations prepared by ANALYTIKEM, INC.)	No mortality. Need higher concentrations.
August 3 - 5, 1989 Chronic	0.012, 0.018, 0.024, 0.036 and 0.048 ppm uranium. (All solutions prepared by ANALYTIKEM, INC.)	Test terminated - higher concentrations needed based on results for Acute Range-Find Test.
August 15 - 22, 1989 Chronic 7-day	0.05, 0.10, 0.15, 0.20, and 0.30 ppm uranium.	Need lower concentrations. Chronic toxicity at lowest concentration of 0.05 ppm uranium.

Table 2: Summary of test conditions for the acute toxicity bioassay with Ceriodaphnia dubia

1.	Temperature:	25 ± 1°C
2.	Light Intensity:	Ambient laboratory levels
3.	Photoperiod:	16 h light/8 h dark
4.	Size of test vessel:	100 ml beakers
5.	Volume of test solutions:	50 ml
6.	Age of test organisms:	2-24 hour neonates
7.	No. animals per test vessel:	1
8.	No. replicate test vessels per concentration:	2
9.	Total no. organisms per concentration:	20
10.	Feeding regime:	No feeding required.
11.	Aeration:	None, unless D.O. falls below 40% saturation, at which time gentle single-bubble aeration started.
12	Dilution water:	Upper Three Runs Creek Water at the Savannah River Site Road 2-1.
13.	Test duration:	48 hours (Static, nonrenewal)
14.	Effect measured:	Mortality - no movement of appendages on gentle prodding.

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5 of 5

ppm). Test solutions and the controls were prepared in 50 ml quantities in all-glass test chambers. All concentrations and the controls were tested in duplicate with ten Ceriodaphnia dubia neonates (less than 24 hours old) each. The hydrogen uranyl phosphate compound was prepared by SHEALY ENVIRONMENTAL SERVICES, INC.'s personnel using a procedure provided by Dr. John Pickett by mixing uranyl nitrate and phosphoric acid on a 1 mole U to 1 mole PO_4 ratio and neutralized to a pH of 6 - 7 standard units with sodium hydroxide. The compound was stirred for 15 minutes and the precipitate filtered through a #40 Whatman filter paper. The compound was then rinsed three times with deionized water and dried overnight at 105°C. This compound was sent to ANALYTIKEM, INC., for preparation of a uranium stock solution for testing. All of the definitive acute and chronic toxicity tests were conducted using this stock solution of 1.05 ppm uranium which was obtained by filtering 2000 ppm hydrogen uranyl phosphate through a 0.45 um filter. The test concentrations were prepared by dosing the dilution water with the appropriate aliquot of the uranium stock solution using Class A volumetric pipets. After all testing was completed, the uranium stock solution was preserved with 10% metals grade nitric acid and returned to ANALYTIKEM, INC. for verification that the uranium stock had not dissipated. The uranium content was verified on September 29, 1989 to be 1.0 ppm uranium.

Dissolved oxygen, water temperature, pH, conductivity, total alkalinity and hardness measurements were made in conjunction with the test. Temperature was maintained at 25°C ± 1°C in all test chambers.

The test organisms were placed singly in the test vessels each containing 50 ml of solution. Transfer of the neonates was accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia. Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding.

D. Chronic Toxicity Bioassay

Test methods conformed to those described in USEPA (1989; see Table 3). The 7-day chronic toxicity bioassay was performed September 2 - 9, 1989, as six treatments exposing 10 test organisms each. The first treatment was the control (100% filtered Upper Three Runs Creek Water). The uranium solutions were 0.020, 0.035, 0.050, 0.065, and 0.080 ppm uranium. test solutions were prepared from the stock uranium daily by dos the dilution water with the appropriate aliquot using Class A volumetric pipets.

Dissolved oxygen, water temperature, pH, and conductivity measurements were made daily in conjunction with the test. Temperature was maintained at 25oC ± 1oC in all test chambers during the test.

The test organisms were exposed to each treatment in individual test chambers. The test organi s were placed singly in the test vessels each containing 15 ml of solution. Transfer of the neonates was

Table 3: Summary of test conditions for the chronic toxicity bioassays with Ceriodaphnia dubia conducted September 2 - 9, 1989.

1.	Temperature:	25°C ± 1°C
2.	Light intensity:	Ambient laboratory levels
3.	Photoperiod:	16 h light/8 h dark
4.	Size of test vessel:	1 ounce SOLO plastic disposable cups
5.	Volume of test solution:	15 ml
6.	Age of test organisms:	2-24 hour neonates and all released within the same eight hour period
7.	No. animals per test vessel:	1
8.	No. replicate test vessels per concentration:	10
9.	Total no. organisms per concentration:	10
10.	Feeding regime:	Selenastrum capricornutum at the rate of 500,000 cells per ml test solution per day
11.	Aeration:	None
12.	Dilution water:	Upper Three Runs Creek Water collected at the Savannah River Site Road 2-1; Filtered through plankton net.
13.	Test duration:	7 days
14.	Effect measured:	Mortality - no movement of appendages on gentle prodding and number of offspring produced.
15.	Test acceptability:	80% or greater control survival and an average of 15 or more young/surviving female

accomplished using an eye dropper where the organism was never removed from solution.

Test chambers were examined every 24 hours for immobile Ceriodaphnia and number of offspring produced. Immobile animals were examined with a stereoscope (60X) and were considered dead if no appendage activity could be observed after gentle prodding. Each day after reproduction counts had been recorded each female was transferred to a new cup with fresh solution. The organisms were between 2 and 8 hours old at the start of the test. All Ceriodaphnia were fed the green alga Selenastrum capricornutum at a rate of approximately 500,000 cells per ml. per day in each solution. Selenastrum cultures were obtained from Carolina Biological Supply Company and cultured in natural spring water and Alga-Gro media in 1-liter cotton-plugged Erlenmeyer flasks and maintained under bright fluorescent lighting for 4 - 5 days. Test chambers were incubated for temperature control with photoperiod held at 16 hours of light and 8 hours of darkness. Randomization of test animals in the incubator and on the test trays was established based on random number tables.

III. RESULTS

A. Acute Toxicity Bioassay

The results of the 48-hour acute toxicity bioassay are given in Table 4. Mortality occurred in the 0.15 (30% mortality), 0.20 (55% mortality), 0.25 (70% mortality), and 0.30 (100% mortality) ppm uranium solutions.

Table 4: Number and percentage of Ceriodaphnia showing effect (death) during the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia. Concentrations prepared from 1.05 ppm uranium stock (2000 ppm hydrogen uranyl phosphate). Ten test organisms per replicate.

Test Concentration	Replicate	Number Dead After 24 Hours	48 Hours	% Mortality
Control	A	0	0	0%
	B	0	0	
Uranium (ppm)				
0.10	A	0	0	0%
	B	0	0	
0.15	A	0	2	30%
	B	0	4	
0.20	A	0	4	55%
	B	0	7	
0.25	A	2	8	70%
	B	4	6	
0.30	A	3	10	100%
	B	5	10	

No mortality occurred in the 0.10 ppm uranium or the control. These data were used to determine a 48-hour LC50 (median lethal concentration) value with the Probit Method (EPA, 1989). This calculation resulted in a 48-hour LC50 of 0.19 ppm uranium with 95% confidence limits of 0.17 and 0.21 ppm uranium.

Water chemistry data taken in conjunction with the acute bioassay are given in Table 5. All parameters monitored were within acceptable limits for bioassay purposes.

B. Chronic Toxicity Bioassay

The results of the 7-day chronic toxicity test are given in Table 6. Mortality occurred in the control (10% mortality), 0.020 ppm (20% mortality), 0.035 ppm (10% mortality), 0.050 ppm (30% mortality), 0.065 ppm (40% mortality) and 0.080 ppm (70% mortality) uranium concentrations. Reproduction in the control averaged 17.4 offspring per female.

Table 5: Water quality data recorded in conjunction with the 48-hour static renewal bioassay to determine the acute toxicity of hydrogen uranyl phosphate to Ceriodaphnia dubia, August 17 - 19, 1989.

Exposure Period	Parameter	Test Concentrations (Nominal Uranium Concentration) Control	0.10 ppm	0.15 ppm	0.20 ppm	0.25 ppm	0.30 ppm
0 Hours	D.O. (mg/l)	8.00	8.00	8.05	8.10	8.10	8.20
	Temp. (^{o}C)	24.5	24.5	24.5	24.5	24.5	24.5
	pH (SU)	6.89	6.80	6.81	6.72	6.69	6.71
	Cond. (umhos/cm)	22.0					21.0
	Tot. Hard. (mg/l)	5.1					3.9
	Tot. Alk. (mg/l)	3.0					3.0
24 Hours	D.O. (mg/l)	7.90	8.00	8.00	8.05	8.00	7.95
	Temp. (^{o}C)	25.2	25.2	25.2	25.2	25.2	25.2
48 Hours	D.O. (mg/l)	8.00	7.90	8.00	8.00	8.00	8.00
	Temp. (^{o}C)	25.5	25.5	25.5	25.5	25.5	25.5
	pH (SU)	7.11	7.00	6.99	7.04	7.09	6.89

TABLE 6: REPRODUCTION/MORTALITY DATA FOR THE CHRONIC TOXICITY TEST FOR HYDROGEN URANYL PHOSPHATE (Tested 09/02 - 09/09/89)
(CONCENTRATIONS IN TERMS OF PPM OF URANIUM)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
Control	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	4	0	0	0	0	3	0	0	0	0
	4	5	2	0	4	0	0	0	0	2	2
	5	0	5	2	5	5	D/0	7	2	3	5
	6	2	10	5	6	4	-	2	3	8	10
	7	10	4	16	6	7	-	10	9	6	0
	TOTAL	21	21	23	21	16	3	19	14	19	17
	ADULT	L	L	L	L	L	D	L	L	L	L

$\bar{X}$= 17.4 S.D.= 5.7 CV= 33.0%

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
0.020 ppm Uranium	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0		0	0	0	0
	3	0	0	D/0	0	0	0	0	0	0	0
	4	7	D/4	-	3	6	0	3	0	0	10
	5	0	-	-	9	0	3	0	10	6	2
	6	8	-	-	0	8	4	4	0	0	4
	7	11	-	-	10	3	10	9	4	6	0
	TOTAL	26	4	0	22	17	17	16	14	12	16
	ADULT	L	D	D	L	L	L	L	L	L	L

$\bar{X}$= 14.4 S.D.= 7.7 CV= 53.4%

TABLE 6: REPRODUCTION/MORTALITY DATA FOR THE CHRONIC TOXICITY TEST FOR HYDROGEN URANYL PHOSPHATE (Tested 09/02 - 09/09/89) CONTINUED
(CONCENTRATIONS IN TERMS OF PPM OF URANIUM)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
0.035 ppm Uranium	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	5	3	0	0	0	0	0	D/3	7	6
	5	0	4	2	5	4	2	0	-	3	0
	6	10	4	4	0	4	3	0	-	0	0
	7	3	3	10	4	10	10	5	-	8	11
	TOTAL	18	14	16	9	18	15	5	3	18	17
	ADULT	L	L	L	L	L	L	L	D	L	L

$\bar{X}$= 13.3 S.D.= 5.6 CV= 42.2%

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
0.050 ppm Uranium	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	4	0	3	2	0	0	3	0	0
	4	2	3	0	3	3	D/6	0	0	5	1
	5	2	0	4	3	4	-	3	3	0	D/5
	6	6	4	3	4	4	-	3	5	2	-
	7	10	D/7	4	7	6	-	6	10	9	-
	TOTAL	20	18	11	20	19	6	12	21	16	6
	ADULT	L	D	L	L	L	D	L	L	L	D

$\bar{X}$= 14.9 S.D.= 5.8 CV= 38.7%

TABLE 6: REPRODUCTION/MORTALITY DATA FOR THE CHRONIC TOXICITY TEST FOR HYDROGEN URANYL PHOSPHATE (Tested 09/02 - 09/09/89) CONTINUED

(CONCENTRATIONS IN TERMS OF PPM OF URANIUM)

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
0.065 ppm Uranium	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	4	0	0	0	0	0	0	0
	4	3	D/0	0	5	4	0	4	0	0	0
	5	4	-	D/4	0	4	2	0	5	4	6
	6	3	-	-	2	D/3	5	2	6	0	3
	7	5	-	-	2	-	7	6	D/10	5	0
	TOTAL	15	0	8	9	11	14	12	21	9	9
	ADULT	L	D	D	L	D	L	L	D	L	L

$\bar{X}$= 10.8 S.D.= 5.5 CV= 50.5%

L=Live
D=Dead

Conc.	Day	A	B	C	D	E	F	G	H	I	J
0.080 ppm Uranium	1	0	0	0	0	0	D/0	0	0	0	0
	2	0	0	0	0	0	-	0	0	0	0
	3	0	0	2	0	2	-	0	0	0	0
	4	4	5	4	0	0	-	7	0	0	8
	5	0	D/0	5	D/0	7	-	3	0	D/0	0
	6	D/3	-	4	-	2	-	4	0	-	0
	7	-	-	0	-	0	-	4	D/0	-	D/5
	TOTAL	7	5	15	0	11	0	18	0	0	13
	ADULT	D	D	L	D	L	D	L	D	D	D

$\bar{X}$= 6.9 S.D.= 7.0 CV= 101%

Average reproduction in the uranium solutions was as follows:

		Offspring Per Female	% Mortality
Control	=	17.4	10
0.020 ppm U	=	14.4	20
0.035 ppm U	=	13.3	10
0.050 ppm U	=	14.9	30
0.065 ppm U	=	10.8	40
0.080 ppm U	=	6.9	70

U = uranium

The reproduction data were tested for normality using the Chi-Square Goodness of Fit Test and for homogeneous variances using Bartlett's test. Log transformed data were found to be normally distributed (Chi-Square = 8.14, critical value = 55.76) and with homogeneous variances (Bartlett's Test p value = 0.822, p = 0.01). Statistical analyses of the results using Dunnett's Multiple Comparison Procedure indicated chronic toxicity at the 0.065 uranium concentration. The no observed effect concentration (NOEC) was 0.050 uranium while the lowest observed effect concentration (LOEC) was 0.065 uranium. The chronic value (ChV), taken as the geometric mean of the NOEC and LOEC, was 0.057 uranium. Acute toxicity was observed at the 0.080 ppm uranium concentration.

Water chemistry data taken in conjunction with the chronic toxicity test are given in Table 7. All parameters monitored were within acceptable limits for bioassay purposes.

IV. REFERENCES

Peltier, W.H., & C.I. Weber, United States Environmental Protection Agency 1985a. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA 600/4-85/013. 216 pp.

Weber, C.I., W.H. Peltier, et. al. United States Environmental Protection Agency 1989. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Second Edition 600/4-89/001. 249 pp.

TABLE 7: WATER CHEMISTRY DATA FOR THE CHRONIC TOXICITY TEST FOR HYDROGEN URANYL PHOSPHATE (TESTED 09/02 - 09/09/89) CONTINUED

Conc.	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
0.050 ppm Uranium	D.O. (ppm)	7.55	7.80	7.90	7.70	7.70	7.70	7.80	7.40	8.10	7.80	8.00	8.00	8.15	7.80	
	pH (SU)	6.80		6.73		6.55		6.36		6.20		6.11		6.03		
0.065 ppm Uranium	D.O. (ppm)	7.60	7.90	8.10	7.60	7.90	7.70	7.95	7.60	8.20	7.70	8.00	8.05	8.10	7.90	
	pH (SU)	6.76		6.70		6.57		6.38		6.18		6.14		6.00		
0.080 ppm Uranium	D.O. (ppm)	7.70	7.80	8.20	7.70	7.90	7.70	8.00	7.50	8.30	7.80	8.10	8.00	8.20	7.90	
	pH (SU)	6.57		6.61		6.54		6.40		6.17		6.19		6.00		
	Alk.(ppm CaCO3)	3.0														
	Hard.(ppm CaCO3)	5.0														
	Cond.(umhos/cm)	22.0														

TABLE 7: WATER CHEMISTRY DATA FOR THE CHRONIC TOXICITY TEST FOR HYDROGEN URANYL PHOSPHATE
(TESTED 09/02 - 09/09/89)

Conc.	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	D.O. (ppm)	7.50	7.90	8.00	7.70	7.60	7.60	7.70	7.70	8.00	7.60	8.10	7.90	8.20	7.90	
Control	pH (SU)	6.61		6.83		6.80		6.80		6.25		6.39		6.21		
(0%)	Alk.(ppm CaCO3)	3.0														
	Hard.(ppm CaCO3)	5.1														
	Cond.(umhos/cm)	22.0														

Temperature was maintained at 25 C ± 1 C during test.

Conc.	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	D.O. (ppm)	7.60	7.70	8.00	7.60	7.60	7.70	7.70	7.40	8.10	7.70	7.90	7.80	8.20	7.80	
0.020 ppm	pH (SU)	6.47		6.81		6.33		6.32		6.27		6.31		6.19		
Uranium																

Conc.	Parameter	Init.	DAY 1 old	DAY 1 renew	DAY 2 old	DAY 2 renew	DAY 3 old	DAY 3 renew	DAY 4 old	DAY 4 renew	DAY 5 old	DAY 5 renew	DAY 6 old	DAY 6 renew	DAY 7 old	DAY 7 renew
	D.O. (ppm)	7.55	7.80	7.90	7.70	7.70	7.70	7.80	7.40	8.10	7.80	8.00	8.00	8.15	7.80	
0.035 ppm	pH (SU)	6.39		6.52		6.54		6.29		6.25		6.24		6.02		
Uranium																

APPENDIX A

Statistical Analyses of Acute and Chronic Toxicity Tests

H.U.P. September 1989

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	17.4000	5.7388	33.0
2	10	14.4000	7.6913	53.4
3	10	13.3000	5.6184	42.2
4	10	14.9000	5.7629	38.7
5*	10	10.8000	5.4528	50.5

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -6.092432
This difference corresponds to -35.01 percent of control

Between groups sum of squares = 231.320000 with 4 degrees of freedom.

Error mean square = 37.320000 with 45 degrees of freedom.

Bartlett's test p-value for equality of variances = .822

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HUP 8/17 Acute

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.1000	20	0	0.0000	0.0000	0.0175
0.1500	20	6	0.3000	0.3000	0.2188
0.2000	20	11	0.5500	0.5500	0.5673
0.2500	20	14	0.7000	0.7000	0.8167
0.3000	20	20	1.0000	1.0000	0.9335

Chi - Square Heterogeneity = 4.396

Mu = -0.721352
Sigma = 0.132120

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	10.459810	0.910720	(8.674800,	12.244821)
Slope	7.568853	1.279810	(5.060425,	10.077281)

Theoretical Spontaneous Response Rate = 0.0000

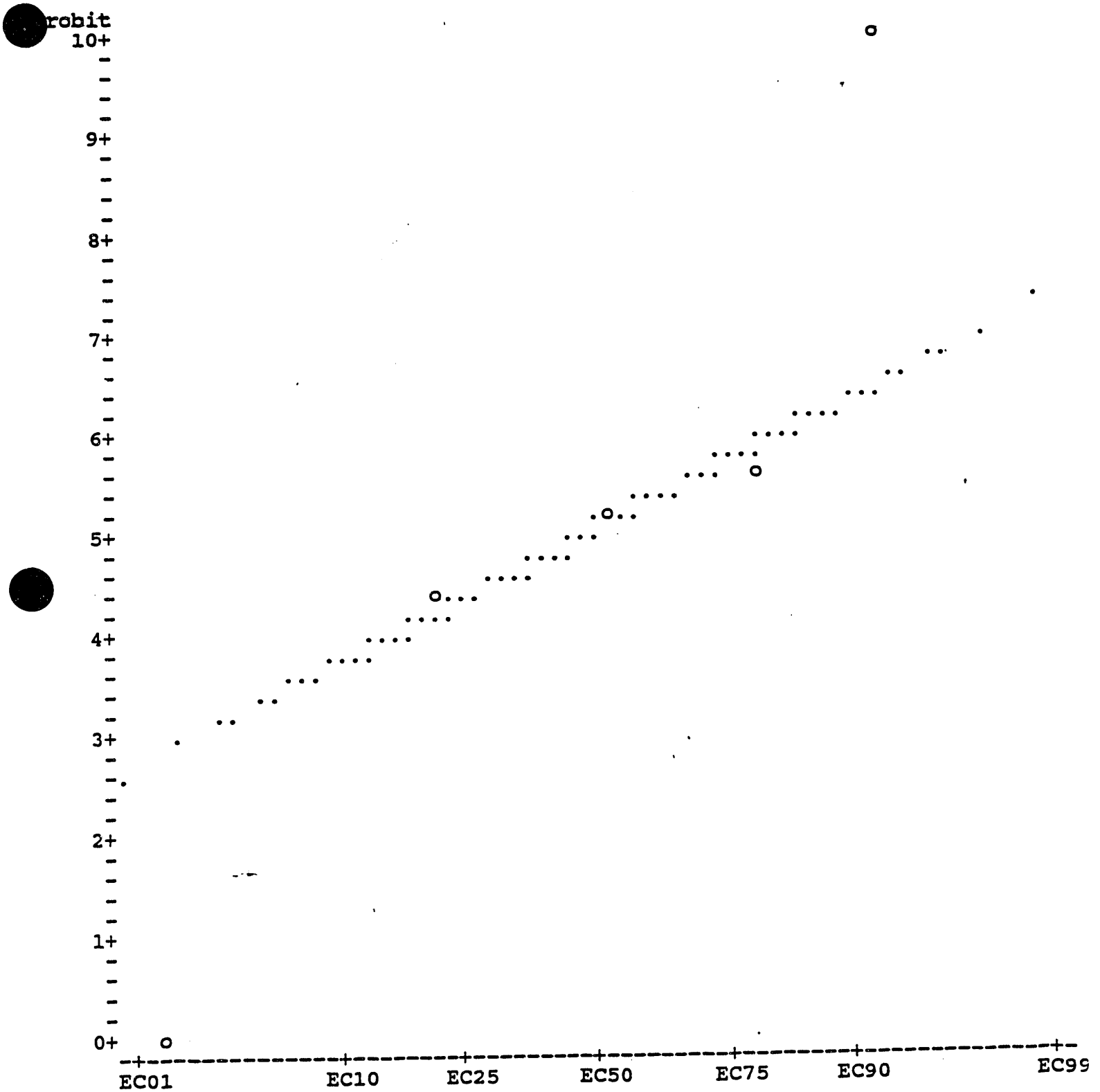
HUP 8/17 Acute

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0936	0.0633	0.1149
EC 5.00	0.1152	0.0858	0.1351
EC10.00	0.1286	0.1007	0.1475
EC15.00	0.1386	0.1120	0.1568
EC50.00	0.1900	0.1706	0.2091
EC85.00	0.2604	0.2336	0.3100
EC90.00	0.2805	0.2488	0.3442
EC95.00	0.3133	0.2723	0.4031
EC99.00	0.3855	0.3207	0.5453

HUP 8/17 Acute

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE



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AnalytiKEM *An American NuKEM Company*

AnalytiKEM Inc.
454 S. Anderson Road, BTC 532
Rock Hill, SC 29730
803/329-9690

TEST REPORT NO. A80084 SUPPLEMENTAL

OCTOBER 30, 1989

PREPARED FOR:

WESTINGHOUSE SAVANNAH RIVER COMPANY
ATOMIC ENERGY DIVISION
SAVANNAH RIVER SITE
AIKEN, SC 29808-0001

ATTENTION: JOHN L. KEYES

NJ CERTIFICATION NO. NJ 04012

NY CERTIFICATION NO. NY 10815

SC CERTIFICATION NO. SC 94004

NC CERTIFICATION NO. NC 258

REVIEWED & APPROVED BY: Patricia deAndino

NAME: PATRICIA deANDINO

TITLE: QUALITY ASSURANCE MANAGER

Test Report No. A80084

METHODOLOGY

Aqueous

Sample Preparation Methods

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW846, Third Edition, USEPA, November 1986.

* Method 3010: Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by Flame Atomic Absorption Spectroscopy or Inductively Coupled Plasma Spectroscopy.

Sample Analysis Methods

Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, USEPA, March 1983.

* Method 200.7: Inductively Coupled Plasma-Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes.

Test Report No. A80084

I. Preparation of Hydrogen Uranyl Phosphate Compound

($HUO_2PO_4.4H_2O$, MW=438 g/Mole)
(Prepared by Shealy Environmental Services, Inc.)

1. Mix uranyl nitrate and phosphoric acid at a 1 mole U to 1 mole PO_4 ratio.
2. Neutralize to pH 6-7 with NaOH.
3. Stir 15 minutes and filter through #40 Whatman Filter Paper.
4. Dry precipitate at 105°C overnight.

II. Preparation of Hydrogen Uranyl Phosphate (HUP) Stock Solution

1. Weigh 1.00 grams HUP and dilute volumetrically to 500ml using laboratory deionized water.
2. Agitate solution for 1 hour to achieve saturation.
3. Filter through 0.45um filter paper.

Test Report No. A80084

Stock Solution Verification

Solution Replicate	Date Prepared	Date Analyzed	Total Uranium (ppm)
1	7/27/89	7/27/89	1.11
2	7/27/89	7/27/89	1.17
3	7/27/89	7/27/89	1.27
4	8/04/89	8/04/89	1.05*
4	8/04/89	9/29/89	1.00*

*This solution used in Bioassy Tests.

AnalytiKEM

Test Report No. A80084

Test Concentration Verification

Serial Dilutions from the HUP Stock Solution were prepared for analysis.

Theoretical Uranium Concentration (ppm)	Observed Uranium Concentration (ppm)	Percent Recovery
0.100	0.102	102
0.080	0.078	98
0.060	0.072	120
0.060	0.050	83
0.048	0.041	85
0.040	0.048	120
0.036	0.032	89
0.024	0.024	100
0.018	0.018	100
0.008	0.009	113

Concentrations below 0.080ppm were concentrated prior to analysis by Inductively Coupled Plasma (ICP).

Test Report No. A80084

III. QUALITY CONTROL DATA

Matrix Spike/Matrix Spike Duplicate Recovery Data

Constituent	Sample Spiked	Concentration of Spike	Recovery Matrix Spike
Uranium	UTR Water	0.12	92
Uranium	UTR Water	0.16	96
Units		(ppm)	(%)

UTR = Water collected from Upper Three Runs Creek. Same water used as dilution water in Bioassay Tests.

DATE
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9/9/93
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