DRAFT

Detailed Summary of Research Design for the Whole Effluent Toxicity Method Blank Study

I. INTRODUCTION

The objective of the WET MDL study was to perform an inter laboratory study that measured the chronic toxicity to Ceriodaphnia of a sample that was non toxic. The non toxic sample was treated as if it were an effluent and the objective of the testing was to determine the chronic toxicity of the sample, which is the objective of NPDES permit-related toxicity testing. Regulatory Management Inc. was hired to supervise the preparation of the non toxic sample that was used for the study.

Regulatory Management Inc. selected the standard, moderately hard, synthetic dilution water, prepared from deionized water and reagent grade chemicals, as the formulation for the non toxic sample. The procedure for preparing the standard synthetic dilution water is given in *Short Term Methods for Estimating the Toxicity of Effluents and Receiving Waters to Fresh Water Organisms, (Third Edition)*, EPA/600/4-91/002, July 1994, Section 7.2.3. This formulation is specified as the preferred dilution water for NPDES permit-related toxicity testing and is also used for culturing *Ceriodaphnia dubia.*

Since the testing and culture of these organisms depends on a dilution water that is non toxic, the standard synthetic freshwater formulation was considered an appropriate non toxic sample. The standard synthetic freshwater that was prepared for this study did not include selenium. For culturing and testing of *Ceriodaphnia dubia*, sodium selenate is added to give a concentration of two micrograms per liter of selenium. Since the purpose of the non toxic sample was not to culture or provide dilution water for testing, selenium was not added. Since the standard NPDES testing does not rely on the presence of selenium in the tested sample, the decision was made to leave selenium out of the non toxic sample formulation and to rely on the addition of selenium from the dilution water supplied by the WET testing laboratories to provide the necessary concentrations of the micro nutrient, selenium.

The study required over 120 gallons (450 liters) of test solution. To provide a single, identical source of dilution water, five 110 liter covered Nalgene tanks were connected to a single outlet. Five separate 110 liter batches of standard synthetic freshwater were prepared and dispensed through a single outlet.

The samples were prepared by the laboratory staff at Chadwick Ecological. The laboratory staff routinely performs NPDES testing for *Ceriodaphnia dubia*. The samples were prepared in the same laboratory facility, using the same source of deionized water that Chadwick Ecological uses to prepare their dilution and culture water.

Samples were dispensed into new one gallon cubitainers. The cubitainers were from a single lot that had been tested for chemical contamination through a travel blank procedure. The cubitainers were rinsed with the standard synthetic dilution water dispensed from the five tanks, the rinsate was discarded, and the cubitainers were filled completely. No headspace was left. The cubitainers are commonly used to transport WET samples for NPDES testing. The standard procedure for NPDES sampling usually requires that the sample containers be rinsed with sample, filled with sample, and shipped to the WET testing laboratory. The dispensing procedure for the non-toxic sample imitated this process.

The samples were packed, three separate one gallon cubitainers in each box and the boxes were shipped by second day UPS to the study participants. The samples were not iced. Since the standard synthetic freshwater does not contain nutrients, has a holding time of fourteen days, and does not require refrigeration, no problems with sample integrity were expected. When the non-toxic samples were shipped to the WET testing laboratories from the clients, the samples were iced and shipped overnight. The samples were iced to simulate normal sampling handling practices. However, no sample deterioration over time was expected.

Samples were shipped a contract laboratory for chemical analysis on the day the samples were prepared, and on the first and last days that samples were shipped to the WET testing laboratory. These samples documented that the sample was properly prepared, met the specifications for mod hard dilution water, had no toxic contaminants, and had experienced no sample deterioration over the shipping and handling period.

The entire process of selecting, preparing, dispensing, and shipping the non toxic samples was carefully monitored and documented. The objective was to prepare a standard non toxic solution and to protect and document the integrity of the sample through the beginning of the WET testing procedure. Every step in the process was supervised and potential error sources were identified and acceptability verified. Care was taken to deliver identical non toxic samples to all participating clients. The study design focused on eliminating any toxicity or variability due to the sample, so that the inter laboratory variability would be a true measure of the variability in the WET testing procedure.

The study was not designed to measure the true variability that is present in the measurement system that is used by dischargers for routine NPDES testing. Error sources that are routinely accepted without verification in routine NPDES testing were eliminated. Some of the factors that are expected to cause variability in routine NPDES testing that were eliminated include: **Sample handling.** The non toxic solution is not unusually sensitive to normal handling or allowable holding times. Effluent samples contain nutrients and are biologically active and sample integrity is sensitive to holding times and temperatures. **Hardness and pH**. All of the dilutions had identical pH, hardness, and ionic strength. Effluent samples can have a three to ten fold difference in hardness from control to 100 percent sample. **Ammonia impacts**. The non toxic dilution water did not contain ammonia. Effluent samples that contain ammonia will exhibit greater toxicity during the test than in environmental situations when the test temperature is greater than the stream temperature.

The second objective for the careful supervision and documentation of the non toxic sample preparation process was to make it possible for other researchers to duplicate the experiment and validate the results. The standard solutions described by EPA are a good basis for other WET method evaluations. This study combines a structured approach to preparing and documenting the delivery of a solution of known toxicity to the laboratory with EPA formulations that have wide technical acceptance. The following sections describe, in detail, the procedures used for preparing, dispensing, and shipping the non toxic samples for this study.

II. SAMPLE PREPARATION

A. TANKS

The solution was prepared and dispensed in five 30 gallon tanks. The tanks were new Nalgene Model 54102-0030 cylindrical flat bottomed HDPE tanks, with covers and dispensing spigots and valves. The tanks were 46 centimeters in diameter and 76 centimeters tall. The tanks were graduated in ten liter increments from ten to one hundred and ten liters. The covers were slightly larger than the tank and had a 30 mm flange that extended over the outside of the tank. The dispensing spigot and valve were standard Nalgene 6422-0010 low density polypropylene needle valve spigots with Teflon O-rings with adapters for 5/16 plastic tubing.

1. CLEANING

The new Nalgene tanks and covers were cleaned using the procedure given at *Short Term Methods for Estimating the Toxicity of Effluents and Receiving Waters to Fresh Water Organisms, (Third Edition)*, EPA/600/4-91/002, July 1994, Section 5.3. The tanks were rinsed three times with tap water and left full of tap water for a minimum of 48 hours. The tanks were then scrubbed with Alconox powdered detergent , followed by a double rinse with tap water to remove soap residue. The tanks were then rinsed with a freshly prepared ten percent (volume:volume) hydrochloric acid solution. The tanks were then triple rinsed with deionized water. The tanks were stored with covers in place. The tanks were double rinsed with deionized water and the rinsate discarded before the tanks were filled with deionized water.

2. CONNECTING TANKS

Each tank had a spigot and needle valve. A short piece of 5/16 " ID tygon tubing was slipped over the spigot and secured with a hose clamp. A polyethylene adapter was slipped inside the tygon tubing to reduce the tubing size to ¼ " ID. The adapters were connected to ¼ " tygon tubing and a series of four polyethylene "y" fittings that brought the five tanks down into a single outlet point. The single outlet tubing was controlled by an external pinch clamp. All of the tubing and fittings were double rinsed with tap water, soaked at least twelve hours in 10 percent hydrochloric acid and triple rinsed with deionized water.

In operation, the flow was controlled by the external pinch clamp. The flow rate from each tank was controlled with the needle valve on the dispensing spigot to try to keep water levels in the five tanks at approximately the same level during the dispensing process.

B. SOLUTION PREPARATION

1. D.I. WATER QUALITY

The deionized water was supplied by CORE laboratories, in Aurora, Colorado. All of the deionized water used by Chadwick Ecological for cultures and for dilution water is routinely hauled from CORE in five gallon carboys. The water was hauled from CORE on September 17th and 18th . The tanks were filled to the 100 liter line from the five gallon carboys. The conductivity of each tank was measured. The water in the tanks had a resistance of between 2.45 and 4.73 meg ohms. The water in each tank was measured to determine the quality of the deionized water and to document that the residue on the tanks from the cleaning process, or the transport and fill operation was minimal. A resistance of greater than 2 megohms indicates that essentially all ions were absent. The tanks were left full of water and covered until September 22.

There is additional data on the quality of the deionized water. CORE laboratories runs a method blank in each analytical run for every parameter analyzed n their laboratory. The method blanks are run on deionized water. We have compiled a complete listing of the parameters that had method blanks run on September 17, and September 18, 1997. This list of parameters verifies that all of the parameters on the list were below detection.

2. CHEMICALS

The chemicals used to prepare the synthetic freshwater were purchased specifically for this study. The chemicals were from Sigma Chemical. All results for this study are on a single lot number of each chemical. The chemicals were used to make one trial batch of mod hard dilution water and the solutions for the study. The following chemicals were used:

Magnesium Sulfate, anhydrous
Sigma M-7506
Lot #106H1028

Potassium Chloride
Sigma P-3911
Lot #27H0545
ACS Reagent Grade

Sodium Bicarbonate
Sigma S-6014
Lot #17H0063
ACS Reagent Grade

Calcium Sulfate Dihydrate
Sigma C-7411
Lot # 116H1002
ACS Reagent Grade

3. WEIGHING

The chemicals were weighed on an AND ER-182A electronic four place balance to an accuracy of 10 micrograms. The balance was calibrated with a 0.5 gram and a 5.0 gram reference weight (Rice Lake Weighing Systems, Class S, Serial #H640) before the chemicals were weighed. The balance was accurate to +/- .0005 grams. The 0.5000 gram weight was measured at 0.5001 grams and the 5.0000 gram weight was measured at 5.0003 grams. The balance was within the accuracy specifications of the check weights, the balance was linear in the 0.5 to 5.0 gram range, and weights were within the range of demonstrated linearity. The balance is under a maintenance contract and had been serviced within one year. The samples were weighed on aluminum weighing pans to an accuracy of two places (10 micrograms)

Approximately five liters of deionized water were removed from each of the Nalgene tanks. Approximately 1800 ml of deionized water was placed in a two liter beaker. 0.18 grams of Potassium Chloride, 2.88 grams of Magnesium Sulfate and 4.66 grams of Sodium Bicarbonate were weighed out in aluminum weighing pans. This makes a double batch of solution. The weighing pans were emptied into the two liter beaker and rinsed with deionized water to obtain a quantitative transfer. The volume in the beaker was brought back to approximately two liters, based on the graduations on the beaker.

The two liter beaker was stirred with a magnetic stirrer until all of the materials were dissolved. The two liter beaker was taken back to the Nalgene tank. The contents of the beaker was added to the Nalgene tank and the beaker was double rinsed into the tank This procedure was repeated. Each 100 liter tank requires two double batches and a single batch. The procedure was repeated for each of the five Nalgene tanks. All of these chemicals were in the Nalgene tanks and aeration begun at 0945 on September 22, 1997.

4. AERATION

The procedure calls for aeration for twenty four hours after the first three chemicals are added. Each tank was aerated and mixed by the aeration process. Each tank had a new Size 4 medium bubble Whisper air stone placed at the 25 liter line. The airstones were connected to stainless steel gang valves for air control by ¼ inch silicone air tubing from a single roll (#12610). The gang valves were connected to a diaphragm type air pump. (One tank had an individual pump, a Second Nature Challenger I air pump and the other four tanks were aerated by a single Perfecto 600 air pump) The gang valves were adjusted to get a moderate air flow and good mixing without much surface agitation. The covers were place back on the Nalgene tanks, over the air lines. The covers fit over the tanks and fit loosely enough to keep from restricting the air lines.

On September 23, approximately five liters of deionized water were removed from each of the Nalgene tanks. Approximately 1800 ml of deionized water was placed in a two liter beaker. 2.64 grams of Calcium Sulfate dihydrate were weighed out in an aluminum weighing pan. This makes a double batch of solution. The weighing pan was emptied into the two liter beaker and rinsed with deionized water to obtain a quantitative transfer. The volume in the beaker was brought back to approximately two liters, based on the graduations on the beaker. The two liter beaker was stirred with a magnetic stirrer until all of the materials were dissolved. The two liter beaker was taken back to the Nalgene tank. The contents of the beaker was added to the Nalgene tank and the beaker was double rinsed into the tank This procedure was repeated. Each 100 liter tank requires two double batches and a single batch. The procedure was repeated for each of the five Nalgene tanks. The calcium sulfate was added by 1100 on September 23, 1997. Deionized water was added to the 110 liter graduation mark on each of the Nalgene tanks and the aeration restarted.

D. DISPENSING

The samples were dispensed into new one gallon polyethylene cubitainers, with 38 mm screw cap opening. All of the cubitainers were from a single lot. Containers from this lot had been subjected to a trip blank procedure to verify that the lot of containers was suitable. The five Nalgene tanks were connected together and terminated in a single ¼ inch ID tygon tube. The flow was controlled by an external plastic pinch clamp. Each of the Nalgene tanks had a needle valve spigot, which was adjusted to keep tanks levels approximately even during the dispensing operation. The caps were removed and each cubitainer was rinsed with approximately one liter of the dispensed solution. The rinsate was discarded and the cubitainer filled completely (no headspace). The caps were rinsed in the dispensed stream of sample water and were placed on the cubitainers. Dispensing started after 10:30 am on September 24, 19997 and was completed at 16:30 the same afternoon.

The filled cubitainers were randomly placed in specially designed boxes for shipping. Each box contained three one gallon cubitainers. The dispensed solution was at room temperature. There was no attempt to cool the samples or to keep the samples cool during shipment. Since the samples contained no nutrients and were not biologically active, there was no reason to believe that sample handling and holding times were important to maintaining sample integrity. The boxes containing the three cubitainers were shipped by second day UPS to the clients.

The clients were instructed to refrigerate the samples on arrival. The clients were instructed to ship the first sample to the WET testing laboratory via next day delivery service in the original cubitainer. The non toxic samples were placed in a cooler, iced down like regular samples, and shipped to the WET testing laboratories. A second cubitainer was shipped on Tuesday for the second renewal and the third cubitainer was shipped on Friday for the third renewal. There were four sets of samples where Chadwick acted as the client. Those samples were refrigerated from the time they were dispensed until they were mailed to the participating WET labs.

III. ANALYSIS

A. DEIONIZED WATER

The deionized water was supplied by Core laboratories Inc., Aurora, Colorado. The Core Laboratories deionized water system produces water quality equivalent to ASTM type II. There are four exchange tanks, arranged in a specific order per manufacturers recommendation. The order, starting from the water main, is as follows:

1) Carbon bed for dechlorination, organic removal

2) Cation bed - weak base deionizer

3) Anion bed - weak base deionizer

4) Mixed bed - combination anion/cation weak base deionizer

5) 5 micron particulate filter

The water was hauled in five gallon carboys from Core laboratories to Chadwick Ecological in Littleton, Colorado. The 110 liter tanks were filled on September 17, 18, and 19th. The deionized water sat in the tanks over the weekend. The preparation of the mod hard dilution water began on Monday, September 22. The conductivity of the deionized water is measured daily and recorded in the Reagent Water Monitoring Logbook. The measured conductivities for September 17th to September 19th were 0.77, 0.76, and 0.58 µmhos/cm. Conductivity was measured at 25° C. the cell constant was 0.0609. The conductivity meter was calibrated daily.

Core Laboratories is a full service laboratory. The deionized water is indirectly tested as part of the laboratory quality assurance system as laboratory blanks and method blanks for a each parameter tested. The quality assurance program would stop all chemical analysis if detectable concentrations of analyte were found in any of the laboratory blank or method blank samples. These quality assurance measurements provide additional documentation of the quality of the deionized water used for the WET MDL study. An evaluation of quality assurance records for September 17th to September 19th indicates that the deionized water was analyzed for the following parameters:

Parameter	**MDL**
Aluminum	.05 µg/L
Antimony	.002
Arsenic	.005
Boron	.01
Barium	.001
Beryllium	.001
Calcium	.10
Cadmium	.0005
Cobalt	.005
Chromium	.005
Copper	.005
Iron	.03
Lead	.002
Magnesium	.10
Manganese	.001
Molybdenum	.005
Mercury	.0002
Sodium	1.0
Nickel	.005
Selenium	.01
Silica	.05
Silver	.005
Strontium	.001
Tin	.01
Tellurium	.01
Vanadium	.005
Zinc	.005

Parameter		Detection Limit
EPA 524 organics	59 cpds - ND	0.5 - 1.0 µg/L
EPA 608 organics	18 cpds - ND	.003 - .083 µg/L
EPA 8260 organics	56 cpds -ND	5 - 10 µg/L
EPA 8270 organics	144 cpds - ND	10 - 50 µg/L

There were five organic compounds detected in the deionized water during this three day period. 2 Hexone, methylene chloride, acetone, vinyl acetate, and dibromofloromethane. Only methylene chloride at 7 µg/L and dibromofloromethane at 10 µg/L were detected in more than one sample. These compounds were detected infrequently and at low levels in multiple analyses and different EPA methods. The methlyene chloride and acetone are common air contaminants in the laboratory and were probably not present in the deionized water.

The laboratory quality assurance records provide documented quality data for over 125 metals and organic compounds. This data supplements the general laboratory data, conductivity, that is usually used to determine the suitability of the deionized water. The general laboratory data and the quality assurance data indicate that the deionized water was suitable for the preparation of the mod hard dilution water.

B. PILOT STUDY

A pilot study was conducted to verify that the source chemicals, the deionized water, and the cubitainers would not introduce toxicity. The objective of the study was to prepare the mod hard dilution water according to the procedures in the EPA manual, dispense the samples into the cubitainers, and analyze the samples for the chemicals added and for metals, nutrients, and semi volatile organic chemicals that should have been absent. The dispensed samples in the cubitainers were kept at room temperature throughout the study to demonstrate that chemicals were not leached out of the cubitainers and sample integrity was maintained. The objective of the study was to document through extensive chemical analysis that the chemicals, materials, and study design were appropriate and that a consistent non toxic sample could be prepared and delivered for analysis.

The chemical analysis performed during the pilot study far exceeded the analysis required by the EPA manual for the preparation of the mod hard dilution water. The preparation of a non toxic mod hard dilution water is considered to be a relatively simple task and the only chemical analysis required by the EPA manual is pH, alkalinity, and hardness. Our study indicated that most laboratories should be able to prepare non toxic mod hard dilution water with a normal degree of care. Since this study involved dispensing mod hard water into cubitainers and holding the samples at room temperature for at least two days, the pilot study evaluated the suitability of the cubitainers and the impact of prolonged room temperature storage (up to 14 days) on a large suite of chemical parameters. Our conclusion is that new cubitainers are an acceptable container for shipping samples and that sample container interactions with mod hard dilution water are minimal.

The preliminary studies indicated that the chemicals, dispensing containers and apparatus, and sample containers were appropriate for the study and would not be expected to add toxicity to the mod hard dilution water. The study showed that the cubitainers were acceptable sample containers and that even with prolonged room temperature storage, sample container interactions with the sample were minimal. The pilot study also confirmed that standard industry practices were appropriate. The cornerstone of the EPA test procedures are that mod hard dilution water is non toxic and that samples can be stored temporarily and shipped to WET testing laboratories without impacting sample integrity. This study spent more than $5000 to document the absence of chemicals that would cause toxicity, to demonstrate that there was no reason to suspect chemical contamination by the sample containers, and that there were no sample container interactions or sample deterioration of mod hard dilution water over time.

A ten liter batch of mod hard dilution water was prepared. The initial chemicals were added on August 4, 1997 and the sample was dispensed into three randomly selected cubitainers. (The cubitainers were selected from a single lot of cubitainers that had been reserved for this study.) One container was taken immediately to CORE Laboratories for analysis. The other two cubitainers were stored at room temperature for analysis on August 11th and August 14th. (The study protocol called for shipping unrefrigerated samples on Wednesday for Friday delivery to clients. The sample instructions called for sample refrigeration until Monday, when samples would be placed on ice in a cooler and shipped to the WET laboratories.) In the worst case, our samples should have been subjected to an unrefrigerated condition for a maximum of 3 days. The pilot study samples were unrefrigerated for six and nine days respectively. Table 1 summarizes the results of the chemical analysis.

Table 1

PARAMETER	8/6/97	8/11/97	8/14/97
Magnesium	12.2 mg/L	14.8 mg/L	15.4 mg/L
Sodium	31.9	26.3	27.9
Potassium	2.7	2.3	2.3
Calcium	17.2	19	19.8
Sulfate	96	99	97
Chloride	1.1	2.1	1.9
Hardness	93	108	113
Cadmium	1.6 µg/L	1.0 µg/L	0.6 µg/L
Chromium	6	<5	<5
Lead	4	<3	<3
Zinc	<5	9	22
Bis(2ethylhexyl-phthalate)	70	40	30
Nitrite	0.01 mg/L	<0.01 mg/L	<0.01
Phosphorous	0.02	<0.01	<.01
Ammonia	<0.01 mg/L	<0.01 mg/L	<.01 mg/L
Nitrate	<0.10	<0.10	<0.10
Copper	<05 µg/L	<5 µg/L	<5 µg/L
Iron	<30	<30	<30
Manganese	<10	<10	<10
Mercury	<0.2	<0.2	<0.2
Nickel	<5	<5	<5
Selenium	<5	<5	<5
Silver	<5	<5	<5

Table 1 is divided into three general categories. There are the chemicals that were added and expected to be present, chemicals that were detected, but not expected to be present, and chemicals that were not detected and not expected to be present.

The samples were delivered to the laboratory at room temperature with no preservation, with full chain of custody documentation. The samples were divided into aliquots and preserved by the laboratory in accordance with standard laboratory practice for each analyte or group of analytes. The metals samples were digested prior to analysis. All analyses were performed using EPA standard methods, using the most sensitive methods generally available.

The analysis of the materials added indicates that all of the analytes were present in the expected amounts. The measured concentration differed slightly from the theoretically expected concentration because the test solutions were prepared using the approximate graduations in the plastic ware, rather than using volumetrically measured volumes. Mod hard dilution water is usually prepared in graduated plastic ware and the volumes (110 liters) in the final study were not easily measured with volumetric glassware.

There was no clear cut pattern of increasing or decreasing strength for any of the parameters. The variation between samples appears to be the normal variation in the chemical analysis of these parameters. The samples were within the acceptable ranges for mod hard dilution water.

The analysis detected trace quantities of materials that were not added and were expected to be absent. Cadmium, chromium, and lead were detected at or just above the detection limit. One possible source of these metals was in the preservation or digestion acids and in the digestion procedure. There is good chance that measurements at this level are unreliable or were added in the analysis process and were not present in the sample. The methods used were selected to have the maximum practical sensitivity. The presence of these metals at these concentrations was not expected to impact the study. Nitrite and Total Phosphorous were detected in at least one of the three samples at or near the detection limit. The presence of these compounds at these concentrations was not expected to impact the study.

Zinc was detected in low concentrations, but was substantially higher than the detection limit of 5 µg/L. The zinc measurements were considered reliable and the presence of zinc in the 0 to 20 µg/L range in the samples was verified. Zinc concentrations in this range were not expected to impact the study.

The organic chemicals were measure by EPA Method 624, semi volatile organics by GC/MS. The detection limit was approximately 10 µg/L for the nearly fifty organic compounds that were measured. The semi volatile organics were selected to give a broad spectrum coverage of chemicals associated with plastics, since our solutions were exposed to plastics during the preparation, dispensing, and storage of samples. The only chemical detected was bis-2-ethylhexyl phthalate, a common plastiscizer, at concentrations in the 40 to 70 µg/L range. Bis-2-ethylhexyl phthalate is not toxic and was not expected to impact the study at these concentrations.

The pilot study demonstrated that the deionized water, the chemicals, the sample preparation and dispensing system, and the cubitainers would not introduce toxicity into the mod hard dilution water. The room temperature storage of the samples for an extended period of time had no impact on sample integrity. The pilot study indicated that a non toxic sample could be prepared with the chemicals and system that we intended to use, and that shipment and sample handling would not change the toxicity of the sample.

The pilot study suggested some minor improvements that were incorporated into the final study. The cubitainers were not rinsed with sample in the pilot study. The cubitainers in the final study were rinsed with 500 to 1000 ml of sample, and the rinsate discarded before the cubitainers were filled. The bis-2-ethylhexyl phthalate is characteristic of new plastics, and since the cubitainers could not easily be acid cleaned like the other plastics in the study, the same rinse procedure normally used for effluent samples was used. The metals analysis indicated that the preservation and digestion acids were potential sources for metals. The quality of the acids was specified and the QA/QC procedures were changed to verify that metals in detectable concentrations were absent in the acids. The pilot study did identify a higher than expected variability in the measurement of the general inorganic components that were added. This variability was noted again in the final study and was greater in the WET testing laboratories.

C. CHEMICAL DATA ON FINAL SAMPLE SOLUTIONS

The Study required some minor changes in the sample dispensing system. The single ten liter plastic container was replaced with five 110 liter containers that were connected through a series of tygon tubes to a single dispensing tube. The EPA specified cleaning procedure that was used on the ten liter plastic container was used on the 110 liter containers. The 110 liter containers were covered. The aeration systems were the same. A rinse procedure for the new cubitainers was adopted. Each cubitainer was rinsed with 500 to 1000 ml of the mod hard water dispensed from the connected 110 liter containers and discarded, before the cubitainers were filled.

The chemical analysis for the Study paralleled the analysis that was performed for the pilot study. Samples were analyzed for the specific inorganic compounds that were added, metals, nutrients, and semi volatile organics. Three samples were taken at random from the dispensed samples waiting to be shipped. One sample was taken to CORE laboratories for analysis the following day. The remaining samples were stored at room temperature and were taken to the laboratory for analysis on the next Monday and Friday. Table 2 summarizes the results of the chemical analysis.

Table 2

PARAMETER	SAMPLE 1	SAMPLE 2	SAMPLE 3
Chloride	1.6 mg/L	1.5 mg/L	1.7 mg/L
TDS	170	190	180
Conductivity	306 µmhos/cm	309 µmhos/cm	309 µmhos/cm
Sulfate	74 mg/L	78 mg/L	71 mg/L
Calcium	14.0	14.5	14.1
Magnesium	13.3	13.8	13.8
Potassium	1.9	2.0	2.1
Sodium	29.3	30.4 mg/L	31.5
Hardness	90 mg/L as CaCO3	93 mg/L as CaCO3	92 mg/L as CaCO3
Alkalinity	66		
Cadmium	0.6 * µg/L	<0. 6 µg/L	<0.6 µg/L
Chromium	<5	<5	<5
Copper	<5	<5	<5
Iron	<30	<30	60
Lead	<3	<3	<3
Manganese	<1	<1	<1
Mercury	<0.2	<0.2	<0.2
Nickel	<5	<5	<5
Selenium	<5	<5	<5
Silver	<0.5	<0.5	<.5
Zinc	<5	<5	<5
Semi Volatiles	ND @ 10 µg/L	Not tested	ND @10 µg/L

D. DISCUSSION OF CHEMICAL DATA

The deionized water was tested alone and as part of the complete system. The deionized water met all the criteria for preparation of the mod hard water. Chemical specific testing documented the absence of metals and organics. The source of the deionized water was a chemical testing laboratory that routinely performs a wide variety of chemical tests and the quality assurance program documents daily that the deionized water is suitable for trace chemical analysis. The quality and documentation of the deionized water exceeds the normal industry standard.

The chemical measurements for all measured parameters in all three samples were within the normal range of variability for chemical measurements. The dispensing system resulted in samples that were for all practical purposes, identical. Since the samples for chemical analysis were taken at random from dispensed samples prior to shipping, it is reasonable to assume that all samples were identical.

The sample measurements for alkalinity and hardness were in the proper range for moderately hard dilution water. The elements and ions that were added were found in the proper concentration ranges. The chemical analysis documented that the sample was properly prepared and met the specification for mod hard dilution water.

The metals analysis detected only one metal, cadmium , at the detection limit of 0.5 µg/L, in one sample out of three. The semi volatile analysis did not detect any organic compounds at the 10 µg/L level. The time series analysis indicated no significant change in any parameter over time.

The chemical analysis on the study samples confirmed the preliminary analysis results. The mod hard water could be prepared and dispensed with little chance for contamination. The integrity of the mod hard water samples was documented for the duration and conditions that the shipped samples were expected to experience. The chemical analysis confirms that the samples were properly prepared, contained no compounds that would indicate that the sample was toxic, and that sample integrity should have been maintained during shipping.

The results of the chemical analysis indicate that all of the samples tested were identical, met the specifications for mod hard dilution water, and contained nothing that would indicate toxicity. The study objective, to prepare and distribute a non toxic sample for chronic Ceriodaphnia testing was accomplished and documented.

The validation that samples were identical, met the specifications for mod hard dilution water, and contained nothing that would indicate toxicity was based on the analysis performed by CORE Laboratories. CORE is an experienced contract laboratory performing chemical analysis, a full level two data package was prepared to support the analysis on these samples, and CORE was audited after the pilot study was completed by RMI. The stability of the samples with respect to the measured parameters was validated by direct analysis during the pilot study and the final study. The stability studies confirmed the EPA guidance that mod hard dilution water can be stored at room temperature and used up to 14 days later with no concern for its suitability as a non toxic dilution and culture water.

Validation by direct chemical analysis under controlled analytical conditions was judged more appropriate than validation by a twenty different WET testing laboratories under a variety of analytical conditions. The chemical analyses performed by the WET laboratories showed a high degree of variability. The variability of the chemical measurements between WET laboratories and between different samples analyzed by the same WET laboratory is cause for concern. The rigorous sample preparation and dispensing procedures, and the triplicate sample validation by CORE labs resulted in identical samples. The analytical results from the WET laboratories were not identical and in many cases were far outside the acceptable range of analytical variability established by CORE laboratories on the preliminary and final study samples.

RMI relied on past experience with laboratories and referrals in the selection of the four laboratories. RMI had direct experience with the CH2M Hill laboratory in Corvallis and SF Analytical in Milwaukee from previous projects. Parametrix in Kirkland, Washington was recommended by a NPDES permit holder in Region X. Northwestern Aquatic Sciences in Newport, Oregon was independently recommended by toxicologists from two different CH2M Hill offices. The laboratories selected by RMI were certified and had a good reputation in the scientific community.

The study objective was to determine the interlaboratory variability is a properly performed test. The study design took special precautions to eliminate or reduce variability, so that the measured variability would be characteristic of test performance. A random selection of certified laboratories would be expected to have a higher degree of variability. While random selection of certified laboratories would have been more representative of the actual conditions that a NPDES permit holder faces with WET testing, the study objective was to eliminate identifiable sources of variability.

The tests were scheduled with the laboratory ten to fourteen days in advance. The laboratories were given written special instructions to eliminate variability due to procedural differences in the testing procedures. The written instructions specifically required:

> The test be performed using EPA most recent guidance (1994 manual.)
>
> Certification that the test procedures used complied with the 1994 manual
>
> The use of the EPA formulation for mod hard dilution water given in Section 7.1.1 as the dilution water and the control water. The diluted mineral water formulation was specifically forbidden.
>
> A parallel reference toxicant test.
>
> Copies of all raw data and bench sheets

The instructions also included specific data reporting procedures. The laboratories were instructed to report the NOEC and LOEC for survival and reproduction, the IC-25 for reproduction, including 95 percent confidence limits around the mean IC-25 estimate, statistically significant difference between any dilution and controls at the $p<.05$ level, and the LC50 and LC1 for survival. The laboratories were asked to express the toxicity results in Toxicity Units (TUc) based on both the IC25 and the NOEC method, since these two statistics are widely used as NPDES permit limits.

IV. SHIPPING AND HANDLING

The study objective was to make this a single blind study. The objective was for samples and tests to be handled in a routine manner in order to assure representative results. Three cubitainers filled with the test solutions were shipped directly to the study participants by second day UPS. The test solutions were shipped on Wednesday, September 24. The samples were picked up at Chadwick Ecological at 5:30 pm. Each package picked up by UPS contained three cubitainers. The packages were not cooled or refrigerated prior to pickup.

The study participants were instructed to refrigerate the cubitainers upon the receipt. The study participants created new chain of custody forms to make it appear that the samples originated at the treatment facility. The study participants were instructed to designate the samples as "reference toxicant samples" or "process control samples" and to make up alphanumeric sample identifiers to identify the sample to the WET laboratories. The study participants scheduled the testing to begin the week of September 29. The study participants labeled the first cubitainer as a sample, placed the sample in a well iced cooler, and shipped the cooler to the WET laboratory using a courier or an overnight delivery service to begin the chronic test on September 30.

The same procedure was used to ship the second cubitainer on Wednesday, October 1, and the third cubitainer on Friday, October 3. The only exceptions to this procedure were the samples that were shipped directly to the WET laboratories. These samples were retained at Chadwick and Associates and were shipped directly to the WET testing labs on the appropriate days.

V. LABORATORY SELECTION AND COORDINATION

The objective of the study was to determine the variability in a properly performed chronic toxicity test on identical, non toxic samples. Laboratory selection is an important factor in studies that evaluate method performance. Experience with previous method development studies indicated that selecting laboratories at random from a universe of certified laboratories often results in some data that is representative of poor laboratory performance not method variability. These data are not representative of the variability that is being measured and usually are removed from the study. Since the number of determinations in this study was less than thirty, the loss of data for poor laboratory performance was considered significant. The laboratory selection process was designed to select certified laboratories that were above average.

The selection process was designed to choose laboratories that had a known history of successful experience with WET testing under the NPDES program. Laboratories with NPDES program experience are usually state certified, are subject to audit, and are aware of the importance of following and documenting compliance with the CFR Part 136 conditions. Laboratories that routinely perform NPDES testing are familiar with the level of care that is necessary when the chronic Ceriodaphnia test is used to certify that effluents are non toxic.

The majority of the laboratories that participated in the study were the regular laboratories that NPDES permit holders use to perform their compliance testing. If there was a bias in the selection of WET testing laboratories, it was towards the selection of laboratories that had lower than average variability in the test, through strict adherence to Part 136 procedural conditions and careful attention to detail organism health and culture. This bias towards lower than average variability was consistent with the study objective of eliminating poor laboratory performance from the measured variability.

There were also four laboratories that were selected by Regulatory Management Inc. Selection of laboratories by the NPDES permit holders that were study participants resulted in a heavy concentration of laboratories in EPA Region XIII and IX. RMI was directed to select four additional laboratories to increase the diversity of laboratory locations. Three laboratories were added from EPA Region X and one was added from EPA Region V.