



Final Report

Evaluation of DF2000™ Dry Cleaning Solvent in an Acute Fish Toxicity Test

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Research Services Team

This report was prepared by the Local Hazardous Waste Management Program in King County (LHWMP), Washington, a coalition of local governments. Our customers are residents, businesses and institutions with small quantities of hazardous wastes. LHWMP's mission is: to protect and enhance public health and environmental quality in King County by reducing the threat posed by the **production, use, storage and disposal** of hazardous materials.

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ACRONYMS AND ABBREVIATIONS

CAS	Chemical Abstract Service
DSL	Domestic Substances List
EPA	United States Environmental Protection Agency
Ecology	Washington State Department of Ecology
°F	Degrees Fahrenheit
GC-FID	Gas Chromatography with Flame Ionization Detector
GCMS	Gas Chromatography-Mass Spectrometry
KCEL	King County Environmental Laboratory
LC ₅₀	The median lethal test concentration that kills 50 percent of test organisms
LHWMP	Local Hazardous Waste Management Program in King County
mg/L	Milligrams per liter
µg/mL	Micrograms per milliliter
mL	Milliliter
MSDS	Material Safety Data Sheet
NFPA	National Fire Protection Association
NWTPH	Northwest Total Petroleum Hydrocarbon
PBiT	Persistent, Bioaccumulative and inherently Toxic
PERC	Perchloroethylene
ppm	Parts per million
RDL	Reporting Detection Limit
VOC	Volatile Organic Compound

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EXECUTIVE SUMMARY

The Local Hazardous Waste Management Program in King County, Washington (LHWMP) works with local dry cleaners to help them appropriately store, use, and dispose of their process chemicals and waste streams.

Several new solvents are appearing in the King County dry cleaning market as businesses transition from using perchloroethylene (PERC). The most frequently used alternative to PERC in King County is a high flash point hydrocarbon called DF2000TM.

The chemical composition and toxicological properties of substances assigned the same Chemical Abstract Service (CAS) number as DF2000TM vary considerably, depending on the raw material and production process. Consequently, a sample of DF2000TM solvent was analyzed for the presence of relatively toxic, light aromatic hydrocarbons.

Relatively little aquatic toxicology information is available for DF2000TM. Consequently, LHWMP staff collaborated with the King County Environmental Laboratory (KCEL) to derive an LC₅₀ for this solvent using an acute fish toxicity test. In this case, the LC₅₀ is defined as the median lethal concentration of solvent that kills 50 percent of the test fish within 96 hours.

DF2000TM was confirmed to be a complex mixture of aliphatic hydrocarbons with carbon chain lengths predominantly between C-10 and C-12. Although substituted alkanes of less than C-10 were present at low concentrations, neither benzene nor toluene were detected.

It was not possible to define an LC₅₀ because DF2000TM failed to kill fish at the highest tested concentration (5000 mg/L). Consequently, this solvent is less toxic to fish than PERC (LC₅₀ = 5.0 mg/L) and Solvon K4TM, which is an acetal product also used in dry cleaning operations (LC₅₀ = 45.7 mg/L). This lack of toxicity likely reflects DF2000TM's low water solubility. Based on this low toxicity, unused or off-specification DF-2000TM that requires disposal would not designate as Dangerous Waste in Washington state.

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INTRODUCTION

Dry cleaning solvents

In 2010, the Local Hazardous Waste Management Program in King County (LHWMP) conducted a survey of the dry cleaning industry, which revealed that PERC is the most commonly-used solvent in King County.^(1,2) However, 21 percent of shops were using a relatively high flashpoint “hydrocarbon” solvent.^(1,2)

Modern petroleum-based hydrocarbon dry cleaning solvents include Shell Hydroclene™, ExxonMobil DF2000™, and Chevron-Phillips EcoSolv™.⁽³⁾ The most frequently used hydrocarbon solvent in King County is DF2000™, which is a hydrotreated aliphatic hydrocarbon. The Chemical Abstract Service (CAS) number for DF2000™ is 64742-48-9 and the flash point is 147 °F.^(3,4) Although the flash points of these modern hydrocarbons are relatively high, they are more flammable than PERC and are generally classified by the National Fire Protection Association (NFPA) as Class IIIA solvents (i.e., flash points at or above 140 °F and below 200 °F).

The CAS number assigned to DF2000™ is typically used to describe “Naphtha, Hydrotreated Heavy (Heavy Aromatic Distillates)” or “Naphtha (petroleum), hydrotreated heavy; low boiling point hydrogen-treated naphtha”. According to the United States Environmental Protection Agency (EPA), the composition and physical properties of substances with the CAS number assigned to DF2000™ can vary considerably, depending on the raw material and the production processes.⁽⁵⁾ Consequently, it can prove challenging to identify toxicological data that describes the properties of a specific hydrocarbon product. For example, a search of The Pharos Project database⁽⁶⁾ using CAS number 64742-48-9 revealed that this substance is regarded as a mutagen and a carcinogen in a European Union classification system. However, the supporting documentation⁽⁷⁾ states “*The classification as a carcinogen need not apply if it can be shown that the substance contains less than 0.1 % w/w benzene*”. Therefore, it is important to determine whether DF2000™ and similar dry cleaning solvents contain hazardous aromatic hydrocarbons, like benzene.

According to the Material Safety Data Sheets (MSDSs) for these hydrocarbon dry cleaning solvents, acute exposure can precipitate skin and eye irritation as well as central nervous system effects, such as drowsiness and dizziness, and even death.^(4,8) As volatile organic compounds (VOCs), these solvents may contribute to atmospheric ozone formation.⁽⁹⁾

The MSDS for DF2000™ states that “no specific ecological data are available for this product”.⁽⁴⁾ A search of The Pharos Project database⁽⁶⁾ revealed that substances with CAS number 64742-48-9 appear on Environment Canada’s Domestic Substances List (DSL) as being “Persistent, Bioaccumulative and inherently Toxic (PBiT) to aquatic organisms”. However, it was not possible to identify the specific studies used to inform this PBiT designation. No aquatic toxicity data were available for this CAS number in either the EPA’s ECOTOX database⁽¹⁰⁾ or the German GESTIS Substance database.⁽¹¹⁾

Previous DF2000™ fish bioassay

In August 2012, LHWMP and the King County Environmental Laboratory (KCEL) conducted a fish toxicity test on a sample of unused DF2000™ solvent according to the Washington State Department of Ecology's (Ecology's) *Biological Testing Methods for the Designation of Dangerous Waste*.⁽¹²⁾ This test involved exposing juvenile rainbow trout to DF2000™ for 96 hours at two concentrations (100 mg/L and 10 mg/L) in a “non-renewal” static acute fish toxicity bioassay (i.e., Part A: Method 80-12). DF2000™ failed to kill fish at the highest test concentration of 100 mg/L.

Current study

The goal of this study was to evaluate: 1) the hydrocarbon composition of DF2000™ solvent and 2) the toxicity of DF2000™ at higher test concentrations than were used previously, based on measured concentrations in the test vessels.

METHODS

Sample collection and storage

LHWMP staff collected a sample of unused DF2000™ product from a previously unopened five-gallon container that was purchased from a local supplier. The lot number was 051021 and the LHWMP-assigned sample number was SW121213-P01.

DF2000™ was decanted from the product container into three pre-cleaned 500 milliliter amber glass bottles via a glass filter funnel. The filled containers were delivered to KCEL at room temperature. A copy of the chain-of-custody form is included in Appendix A. Sample containers were then refrigerated in the dark at $4 \pm 2.0^\circ\text{C}$ until test initiation.

Analysis of DF2000™ solvent

All organic analyses were conducted by KCEL staff.

A sample of unused DF2000™ solvent was diluted in methylene chloride to a concentration of 200 µg/mL (ppm). This sample was analyzed via Gas Chromatography with Flame Ionization Detector (GC-FID) according to the Northwest Total Petroleum Hydrocarbon-Dx (NWTPH-Dx) method.⁽¹³⁾

Because the FID is a non-specific detector, DF-2000™ was also analyzed the sample via Purge and Trap Gas Chromatography-Mass Spectrometry (GCMS) using EPA Method EPA SW846 8260C (volatile analysis). DF2000™ solvent was diluted in methanol to yield a concentration of 10.74 mg/L. One mL of this solution was then diluted with water to yield an on-column concentration of 214.8 µg/mL. Five mL of this solution was drawn into the Purge and Trap system and analyzed.

Fish bioassay

KCEL staff conducted the fish bioassay using juvenile rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static renewal acute toxicity test between December 14th and December 17th, 2013. The experimental protocol (KCEL Standard Operating Procedure 406v2) was derived from EPA's *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*.⁽¹⁴⁾ This protocol differed from the standard Ecology waste characterization method in that the concentrations of DF2000™ in the test vessels were measured analytically and the test solutions were renewed after 48 hours.

Complete methodological details are provided in KCEL's *Report on LC50 Toxicity Testing Conducted on DF2000 Dry Cleaning Solvent*, which is provided in Appendix A.

Briefly, the test was conducted using a serial dilution of DF2000™ with nominal concentrations of 0 (control), 312.5, 625, 1250, 2500, and 5000 mg/L. Ten rainbow trout were placed randomly into each test vessel; duplicates were prepared at each test

concentration. After 48 hours, 80 percent of the test solution from each vessel was renewed with fresh DF2000TM solution at the appropriate concentration.

Samples of test solution (at nominal concentrations of 0, 1250, and 5000 mg/L) were collected for chemical analysis for DF2000TM at 0, 48 (before renewal), 48 (after renewal) and 96 hours. Extraction and analysis were performed according to the NWTPH-Dx method.⁽¹³⁾ Samples were extracted with methylene chloride for approximately 18 hours using EPA Method SW846 3520C (Continuous Liquid-Liquid Extraction) and then dried with sodium sulfate and concentrated. Analysis was performed via GC-FID. DF2000TM was quantified using two different methods, to account for differences in volatility between the individual peaks (with potential loss of the more volatile, earlier eluting peaks). The first method involved generating a calibration curve for the entire range of peaks within the chromatographic envelope. The second method involved generating individual calibration curves for eight of the more predominant peaks within the envelope of peaks.

Fish survival was monitored during the test and recorded at 0, 24, 48, 72, and 96 hours. Dissolved oxygen, temperature and pH were recorded for the samples and controls at 0, 24, 48, 72 and 96 hours.

RESULTS

Analysis of DF2000TM solvent

A chromatogram from the GC-FID analysis of the DF2000TM solvent is presented in Figure 1. This solvent displayed chromatographically as a mound that contained multiple peaks. Therefore, DF2000TM was confirmed to be a multicomponent hydrocarbon, with carbon chain lengths predominantly between C-10 and C-12.

Analysis via GCMS confirmed the GC-FID analysis (see Figure 2). Although several peaks representing substituted alkanes were identified below C-10 in the GCMS analysis, neither toluene nor benzene was detected in the solvent sample.

Fish bioassay

As shown in Table 1, 100 percent of fish survived for 96 hours at all test concentrations. The DF2000TM formed an immiscible layer at the surface of the test vessel.

Nominal Concentration (mg/L)	Measured Concentration (mg/L) ^a	Percent Fish Survival				Percent Fish Survival at Test End
		0 h	24 h	48 h	96 h	
0	<RDL ^b	100	100	100	100	100
312.5	--	100	100	100	100	100
625	--	100	100	100	100	100
1250	<RDL ^b	100	100	100	100	100
2500	--	100	100	100	100	100
5000	<RDL ^b	100	100	100	100	100

^aSample collected at 0 h (i.e., test initiation)
^bReporting Detection Limit

When samples were collected at test initiation (i.e., 0 h), none of the DF2000TM concentrations exceeded the Reporting Detection Limit (RDL) of 236 µg/L. A more detailed description of the analytical results is provided in Appendix A and chromatograms for samples collected from the test vessels are presented in Appendix B.

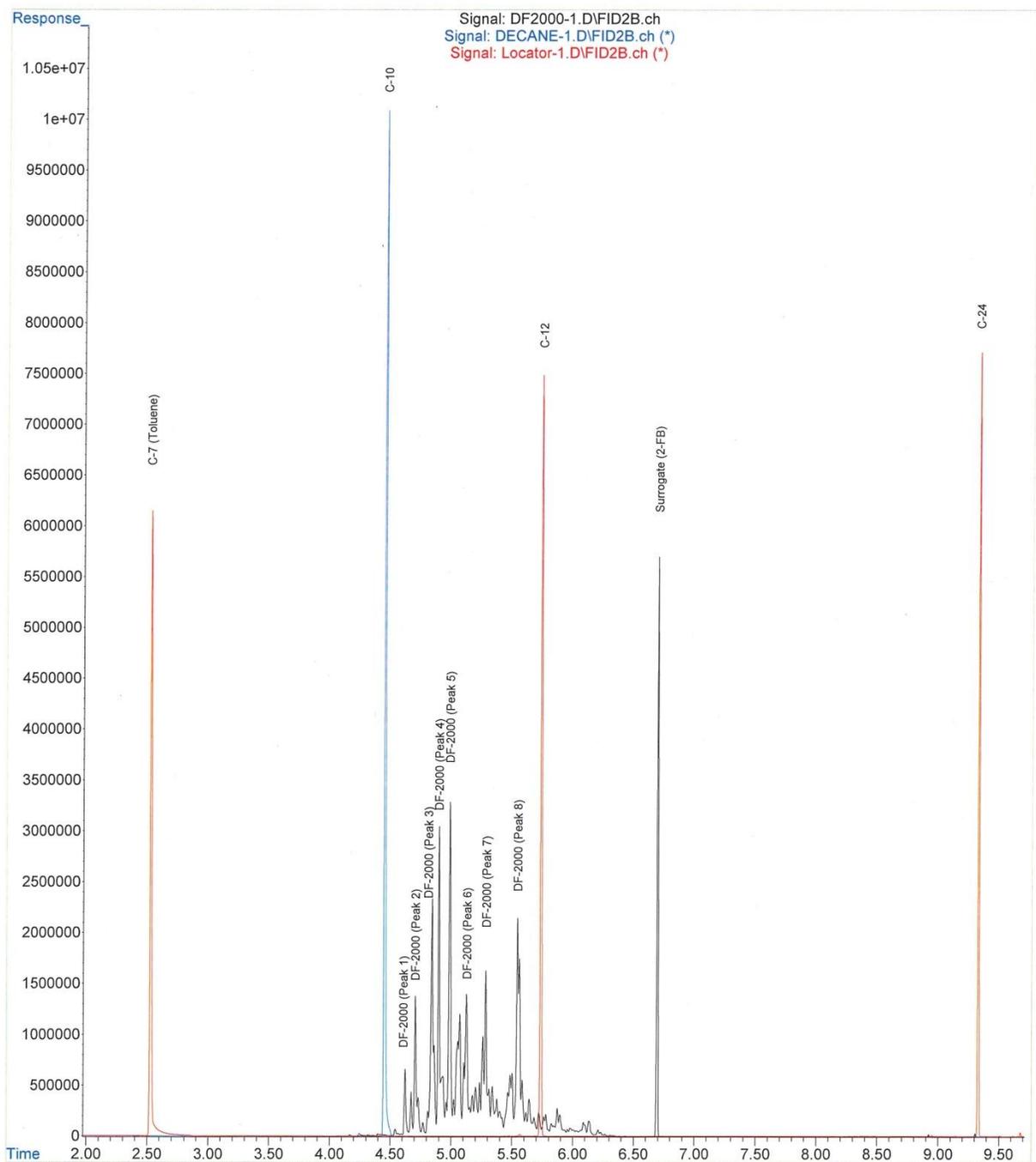


Figure 1. Chromatogram from GC-FID analysis of DF2000™ solvent

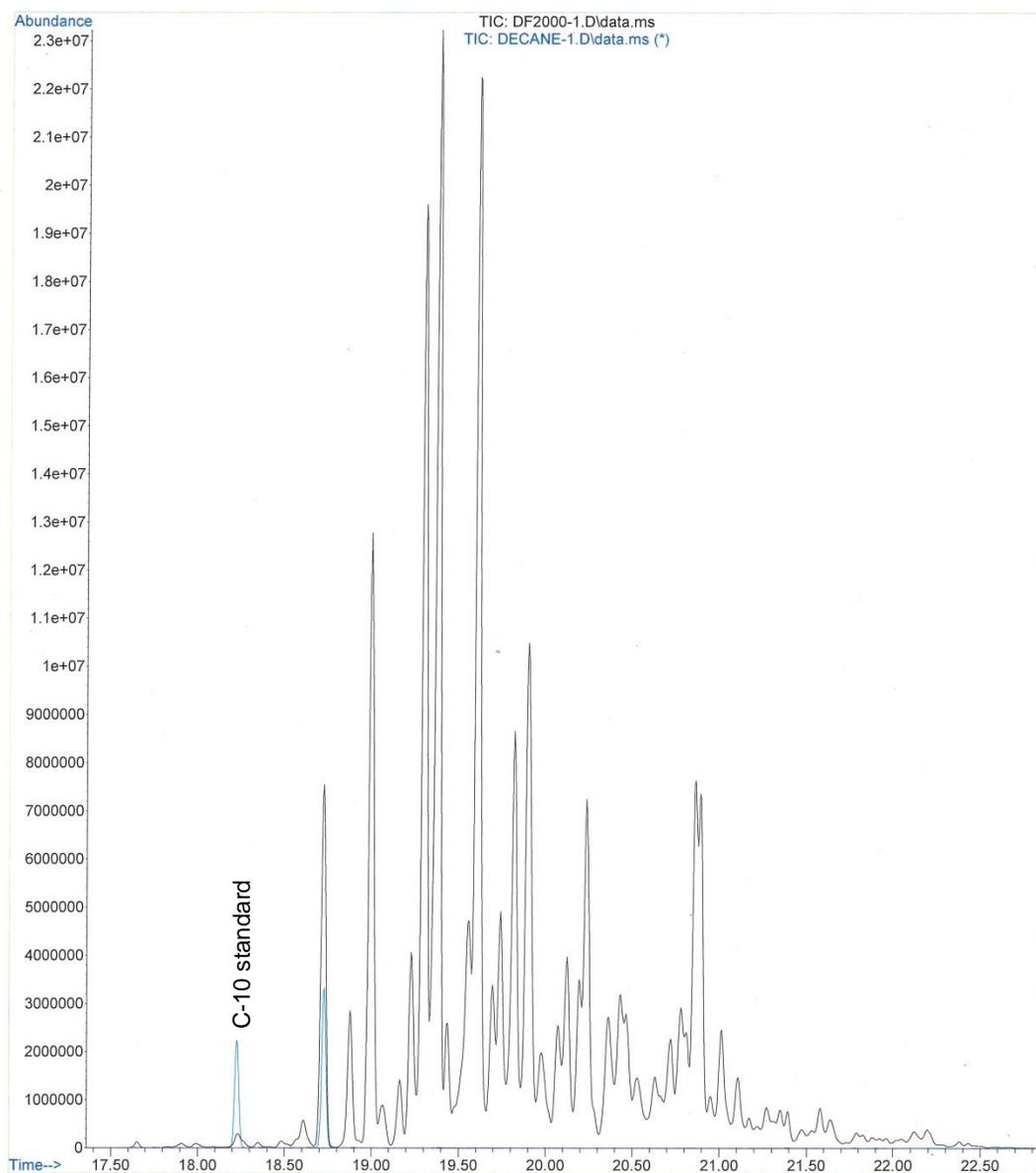


Figure 2. Chromatogram from GCMS analysis of DF2000™ solvent

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CONCLUSIONS

DF2000TM solvent was confirmed to be a multicomponent petroleum hydrocarbon that does not contain relatively toxic aromatic hydrocarbons, like toluene and benzene.

It was not possible to derive an LC₅₀ for DF2000TM because no fish mortality was observed at the highest test concentration of 5000 mg/L. ExxonMobil states that the solubility of DF2000TM in water is less than 0.01% at 77 °F.⁽⁴⁾ Consequently, this limited water solubility is likely responsible for the lack of fish mortality.

In a previous study, LHWMP and KCEL derived an LC₅₀ of 45.7 mg/L in rainbow trout for another solvent alternative to PERC, called Solvon K4TM.⁽¹⁶⁾ This product is part of a relatively new dry cleaning process called System K4TM. Solvon K4TM is composed primarily of butylal, which is a diether acetal.⁽¹⁵⁾

The LC₅₀ value for PERC in fish presented in Ecology's *Washington Dangerous Waste Designation Tool*,⁽¹⁷⁾ is 5.0 mg/L. This value is consistent with data presented in an EPA document, which states that the 96-hour LC₅₀ values for rainbow trout are 5.0-5.8 mg/L.⁽¹⁸⁾

Consequently, the potency of unused dry cleaning solvents towards rainbow trout is: PERC > Solvon K4TM > DF2000TM. Unlike the other two solvents, unused or off-specification DF2000TM product that requires disposal would not designate as Dangerous Waste in Washington state.

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APPENDIX A:

**REPORT ON LC50 TOXICITY TESTING CONDUCTED ON
DF2000 DRY CLEANING SOLVENT**

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**REPORT ON LC50 TOXICITY TESTING
CONDUCTED ON DF2000
DRY CLEANING SOLVENT**

**KING COUNTY DEPARTMENT OF NATURAL RESOURCES AND PARKS
WATER AND LAND RESOURCES DIVISION
ENVIRONMENTAL LABORATORY SECTION
322 WEST EWING STREET
SEATTLE, WASHINGTON 98119**

Test Date: December 14, 2013
KCEL Test Numbers: #6973 (*Oncorhynchus mykiss*: 96-Hour Acute Toxicity Test)

Report Date: March 19, 2014

INTRODUCTION

DF2000 is a dry cleaning fluid manufactured by the Exxon-Mobil Chemical Company. Material Safety Data Sheets state the solubility of DF2000 in water as being negligible. An attempt was made to estimate the LC50 for this dry cleaning fluid.

Sample

A sample of DF2000 Dry Cleaning Solvent, SW121213-P01 collected on 12/12/13 was received by the King County Environmental Laboratory (KCEL), Aquatic Toxicology Section on 12/12/13. The sample was delivered in 2-500 mL amber glass bottles and was refrigerated in the dark at $4 \pm 2.0^\circ\text{C}$ until test initiation. A copy of the chain-of custody is included as an Appendix to this report.

CONTROL WATER

The control water for the test with rainbow trout is freshwater obtained from a 95 ft. deep well located at the KCEL. Stock cultures of rainbow trout are held and acclimated in a flow-through system of well water (WW) for at least 7 days prior to use in tests.

The WW is analyzed for metals monthly (last analyzed 12-13) and organics are measured annually (last analyzed on 2-13). Hardness, alkalinity, conductivity and pH are measured monthly.

Physical-chemical characteristics of the WW are listed in the following table:

Parameter	Value	Units
Conductivity	265	$\mu\text{mhos/cm}$
pH	7.78	
Total Hardness (calc.)	100	mg/L as CaCO_3
Total Alkalinity	80	mg/L as CaCO_3
Total Cd	< 2	$\mu\text{g/L}$
Total Cr	< 3	$\mu\text{g/L}$
Total Cu	< 4	$\mu\text{g/L}$
Total Ni	< 5	$\mu\text{g/L}$
Total Pb	< 20	$\mu\text{g/L}$
Total Zn	< 5	$\mu\text{g/L}$
Total Mercury	< 0.05	$\mu\text{g/L}$ (measured 2-10)
Volatile Organics	45 cmpds not detectable	
Organic Analysis (BNA'S):	68 cmpds not detectable	
Bis(2-Ethylhexyl)Phthalate	15.7	$\mu\text{g/L}$
Pesticides & PCB's:	28 cmpds not detected	

METHODS

The acute toxicity test #6973 was conducted using the general guidelines in US EPA -821-02-012 (October 2002, 5th edition) "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to

Freshwater and Marine Organisms". The test was conducted using a serial dilution with nominal concentrations of: 0 (well water control), 0.312, 0.625, 1.25, 2.5, and 5 g/L DF2000.

Test Organisms

Swim-up (swim-up on 11-18-13) rainbow trout (*Oncorhynchus mykiss*) were purchased from Trout Lodge located in Sumner, Washington on 12-4-13. The trout were acclimated for a period of 10 days in well water with a mean temperature of 13.5°C, a minimum of 13.3°C and a maximum of 13.7°C in a flow-through system at KCEL. During acclimation the fish were fed Zieglers Salmon Starter twice daily. Feed was withheld 48 hours prior to the start of the test.

Physical data (based on a randomly chosen control jar at the end of the test) on trout used in the tests is shown in the table below.

Test #	Age (days-post swim-up at start of test)	Mean Standard Length (cm)	Mean Weight (grams)	Loading Wt./Vol. (g/L)
6973	26	3.2	0.35	0.58

As indicated in the table the mean weight of the trout used in the test was 0.35 g with a mean standard length of 3.2 cm. The loading in each jar was 0.58 g/L.

Rainbow Trout – 96-Hour Static Renewal Acute Toxicity Test

For test #6973, test chambers were 2-gallon glass wide-mouth jars (Anchor Hocking-Heritage Hill) with inside measurements of 25 cm (height) and 23.8 cm (dia.). The liquid level at a volume of 6 L was 15 cm. The test solutions were maintained at $12 \pm 1.0^\circ\text{C}$ for 96-hours in an environmental chamber (Hotpack Model 08082, s/n 79719).

Ten rainbow trout were placed into the test chamber. Assignment of fish to the test chamber was random, as was placement of the test chambers in the environmental chamber. Test solutions were renewed (80% renewal) at 48-hours.

Survival was monitored during the test and recorded at 0, 24, 48, 72, and 96 hours. Dissolved oxygen, temperature and pH were recorded for the samples and controls at 0, 24, 48, 72 and 96 hours. These values can be found on the attached photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. Temperature of the environmental chamber was monitored at 15-minute intervals using an Onset Tidbit data logger. The photoperiod was 16h L:8h D. The test was initiated at 0835 h on 12-14-13 and ended at 0800 h on 12-18-13.

Test Solution Prep

Test solution preparation followed the general guidelines of US EPA 712-C-96-118 (April 1996). DF2000 has very low solubility in water and forms an immiscible layer on the surface of water. In order to obtain large volumes of test solution sufficient for accommodating fish loading rates and maximum saturation a stirred stock solution approach was employed. Vigorous mixing overnight on a magnetic mixer did not seem to be effective presumably because of the volatile nature of DF2000. It was decided to hand mix the DF2000 into the test chambers. Test solutions were prepared at 0 and 48 hours (renewal) of the 96 hour static-renewal exposure period. The volume of DF2000 to add for each test concentration was based on its density of 769 kg/m^3 (0.769 g/ml) @ 15°C which was obtained from Material Safety Data Sheets.

On Day 0 the appropriate amount of DF2000 was added to the glass test chamber (2 gal. Anchor Hocking-Heritage Hill) containing 6 L of well water as indicated below (2 reps per test concentration). The solution was hand mixed for 1 minute and left undisturbed for 1 minute followed by the addition of 10 rainbow trout.

Nominal Sample Conc (g/L)	WW (L/ test chamber)	mls DF2000/ test chamber)*	Number of Reps
0	6	0	2
0.3125	↓	2.44	2
0.625	↓	4.88	2
1.25	↓	9.75	2
2.5	↓	19.5	2
5	↓	39	2

*based on the density of DF2000 of 769 kg/m³ (0.769 g/ml)

48 Hour Renewal

Test solutions were renewed (80% renewal) at 48-hours. Solutions were renewed by siphoning 4.8 L from the test chamber and replacing with newly prepped solution. Renewal solution was prepared by 1 minute of hand mixing of the appropriate amount of DF2000 into well water followed by 1 minute of settling then siphoning into the test chamber. The siphon discharged at the bottom of the test chamber to minimize agitation.

Sampling of Test Solutions for DF2000

Samples for organic analysis were taken at 0, 48 (before renewal), 48 (renewal solution) and 96 hours. Only the 0, 1.25 and 5 g/L concentrations were sampled for organic analysis to limit the number of samples to be analyzed. Samples for 48-hours (before renewal) and 96-hours were taken directly from the test chamber (mid depth) by siphoning into a 1 liter glass amber bottle (discharge at the bottom of the bottle) and filled with no headspace.

Samples for 0 and 48-hour (renewal solution) were sampled after 1 minute of hand mixing the appropriate amount of the DF2000 into a surrogate test chamber then left undisturbed for 1 minute to allow the solution to cease spinning. Samples were taken at mid depth in the test chamber by siphon as previously stated. The samples were stored in the dark at 4 ± 2.0°C and turned in for analysis at the conclusion of the 96-hour test.

Organic Analysis

DF2000 is a multicomponent hydrocarbon solution. The chemical makeup lent itself to NWTPH-Dx (Washington State's Total Petroleum Hydrocarbon) protocols for extraction and analysis.

Samples were extracted by EPA 3520C (Continuous Liquid-Liquid Extraction) and analyzed by GC-FID (Gas Chromatograph with a Flame Ionization Detector). The DF2000 chromatographed as a multiple peaked mound spanning parts of both the gasoline and diesel ranges.

Because it is likely that there are significant differences in the volatility between the individual peaks (and therefore potential bias for the loss of the earlier eluting peaks), DF2000 was analyzed by two different quantitation methods.

The first quantitation method (reported as DF2000) is calculated using the whole range of peaks starting from the beginning of the mound to the end for calibration. Additionally, eight of these peaks are used for the second quantitation method (reported as DF2000_peak#). The peaks are in order of most volatile

(peak 1) to the least volatile (peak 8) and each peak had a separate calibration curve based upon its response at different concentrations. Each sample was quantitated using both methods. Values for both quantitation methods can be found in the data files at the end of the report.

Using the NWTPH-DX protocol for extractions, the quality control consisted of a method blank (MB) and spiked blank (SB) for every twenty samples; and a laboratory duplicate (LD) for every ten samples. Each sample and QC sample were spiked with a known amount of surrogate (2-Fluorobiphenyl) and the spiked blank sample was also spiked with a known amount of DF2000.

The method blank was free of any interference and the spiked blank and all surrogates recoveries were within control limits. Neither of the two sample/LD (lab duplicate) pairs calculated an RPD (relative percent difference) value because both the samples and their lab duplicates were <RDL (reporting detection limit) in both cases. The RDL is considered the PQL (practical quantitation limit) for this analysis.

The QC shows that the NWTPH-Dx method is an appropriate analysis for DF2000. However the sample analysis shows that between DF2000's low solubility in water and high volatility, the compound was subject to a high degree of loss during the Aquatox experiments.

Quality Assurance

The reference toxicant testing for the lot of fish used in this test was conducted on 12-14-13 (Test #6972). Cadmium nitrate was used as a reference toxicant for rainbow trout. The precision table located at the end of this report is maintained to monitor the sensitivity of these organisms to the reference toxicant and thereby provide an indication of their overall sensitivity to other compounds. The LC50 for the reference toxicant test (#6972) was 1.52 µg Cd/L. The LC50 was within the control limits of 0.98 to 3.06 µg Cd/L).

Temperature, pH and dissolved oxygen measurements remained within acceptable limits (USEPA, 2002) throughout the reference toxicant test for rainbow trout (#6972) and sample test (#6973). Some of the hardness values were in the range of 101 to 104 mg/L as CaCO₃, slightly above the recommended maximum of 100 mg/L. The test met acceptability criteria regarding control mortality.

Physical-chemical methods are outlined in the table below:

Parameter	Method
Water Quality Tests	APHA (1992); US EPA (1991).
Temperature	Standard Mercury Thermometer (calibrated with a certified thermometer traceable to NBS records) and Onset, Tidbit (v2) UTBI-001 Temperature Logger (KCEL #436v1).
Dissolved Oxygen	YSI membrane electrode method (Method #4500-0 G; KCEL #434).
pH	Beckman 690 meter with automatic temperature compensation and Ross combination electrode (Method #4500-H; APHA 1992; KCEL #433).
Total Alkalinity	Potentiometric Method (Method #2320 B; KCEL #319v4).
Total Hardness	By calculation (Method #2340 B; KCEL #612v4).
Conductivity	Orion Model #122 Meter with 012210 conductivity cell (Method 2510B; KCEL #435).
Total Ammonia	Phenate Method (Standard Methods SM 4500 - NH ₃ -G; KCEL #330v4).
Unionized Ammonia	Calculated from total ammonia, pH and ionization constants (APHA Method #417 G).
Pesticides and PCB's	Continuous liquid extraction method (EPA Method #608; KCEL #733).
Organic Analysis	Continuous liquid extraction method for BNA's (EPA Method #625; KCEL #731).
Volatile Organics	Purge and trap method (EPA Method #624; KCEL #732).
Total Metals	ICP for Cd, Cr, Cu, Ni, Pb and Zn (EPA Method #200.7; KCEL #612v4); for Hg analysis (KCEL #604v5, 601v4, 605v0).

RESULTS

Organic Analysis

Results of the organic analysis of test solutions for DF2000 is shown in the table below. Based on the first quantitation method, DF2000 is calculated using the whole range of peaks starting from the beginning of the mound to the end for calibration. Peaks 1 through 8 are used for the second quantitation method. The peaks are in order of most volatile (peak 1) to the least volatile (peak 8)

Organic analysis of test solutions (nominal concentrations 0, 1.25, and 5 g/L) showing results of both quantitation methods.

Nominal Concentration 0 g/L	0 h L59393-1 measured µg/L	48 h Before Renewal L59393-2 measured µg/L	48 h Renewal Sol'n L59393-3 measured µg/L	96 h L59393-4 measured µg/L
DF2000*	< RDL	< RDL	< RDL	< RDL
Peak 1	< RDL	< RDL	< RDL	< RDL
Peak 2	< RDL	< RDL	< RDL	< RDL
Peak 3	< RDL	< RDL	< RDL	< RDL
Peak 4	< RDL	< RDL	< RDL	< RDL
Peak 5	< RDL	< RDL	< RDL	< RDL
Peak 6	< RDL	< RDL	< RDL	< RDL
Peak 7	< RDL	< RDL	< RDL	< RDL
Peak 8	< RDL	< RDL	< RDL	< RDL

Nominal Concentration 1.25 g/L	0 h L59393-5 measured µg/L	48 h Before Renewal L59393-6 measured µg/L	48 h Renewal Sol'n L59393-7 measured µg/L	96 h L59393-8 measured µg/L
DF2000*	< RDL	888	261	< RDL
Peak 1	< RDL	< RDL	< RDL	< RDL
Peak 2	< RDL	< RDL	238	< RDL
Peak 3	< RDL	< RDL	243	< RDL
Peak 4	< RDL	< RDL	240	< RDL
Peak 5	< RDL	< RDL	241	< RDL
Peak 6	< RDL	300	249	< RDL
Peak 7	< RDL	786	249	< RDL
Peak 8	< RDL	1730	259	< RDL

Nominal Concentration 5 g/L	0 h L59393-9 measured µg/L	48 h Before Renewal L59393-10 measured µg/L	48 h Renewal Sol'n L59393-11 measured µg/L	96 h L59393-12 measured µg/L
DF2000*	< RDL	803	1530	< RDL
Peak 1	< RDL	631	1040	< RDL
Peak 2	< RDL	620	992	< RDL
Peak 3	< RDL	684	1130	< RDL
Peak 4	< RDL	704	1270	< RDL
Peak 5	< RDL	721	1270	< RDL
Peak 6	< RDL	778	1390	< RDL
Peak 7	< RDL	816	1670	< RDL
Peak 8	< RDL	903	1920	< RDL

RDL < 236 µg/L

*DF2000 based on whole range of peaks

Only the 0, 1.25 and 5 g/L concentrations were sampled for organic analysis to limit the number of samples to be analyzed.

Based on the whole range of peaks the 0-hour measured concentration of DF2000 (< RDL) for the 5 g/L and 1.25 µg/L concentrations were not close to the expected nominal concentrations. Possibly a reflection of the very low solubility and volatile nature of DF2000 in water. It was difficult to ensure that a maximum amount of DF2000 was going into solution.

The 0 h and 48 h renewal solutions were prepped in the same manner, yet the amount measured in solution for the 48 h solution (1.25 and 5 g/L nominal concentrations) were greater than that measured at 0 h even though the amounts were very small. Another inconsistency was more DF2000 was measured at 48 h before renewal than measured at 0 h.

Clearly the amounts of DF2000 measured in solution were orders of magnitude lower than the expected nominal concentrations.

The testing of DF2000 proved to be problematic for reasons such as:

Volatile nature of DF2000

Negligible solubility of DF2000 in water

Inconsistencies of hand mixing of DF2000 into solution

DF2000 sampling methodology at the various time intervals

These are a few of the factors that may be responsible for the low measured concentrations of DF2000.

Rainbow Trout Survival

The following table contains 24-hour survival percentages for rainbow trout exposed to various concentrations of DF2000 during the 96-hour test.

Nominal Concentration (g/L)	Measured Concentration (µg/L)	% Survival (2 reps/conc, 10 fish/rep)				% Survival at Test End
		0 h	24 h	48 h	96 h	
0	<RDL	100	100	100	100	100
0.3125	--	100	100	100	100	100
0.625	--	100	100	100	100	100
1.25	<RDL	100	100	100	100	100
2.5		100	100	100	100	100
5	<RDL	100	100	100	100	100

As the table above shows for the DF2000 sample SW121213-P01 there was 100% survival at all concentrations tested at the end of the 96 hour test. The LC50 was indeterminate.

Water Quality

The following table contains measurements of Temperature, pH and Dissolved Oxygen taken throughout the 96 h test. Measurement of Total Hardness, Total Alkalinity and Conductivity are taken from samples collected at the beginning (0-h) and end (96-h) of the test.

Parameter		0 g/L	0.3125 g/L	0.625 g/L	1.25 g/L	2.5 g/L	5 g/L
Temperature (°C)	Mean	11.9	11.9	11.9	12.0	12.1	12.0
	Min.	11.6	11.4	11.7	11.7	11.9	11.7
	Max.	12.0	12.2	12.0	12.3	12.2	12.1
pH	Mean	7.76	7.84	7.87	7.88	7.83	7.79
	Min.	7.56	7.68	7.62	7.62	7.60	7.60
	Max.	8.04	8.06	8.15	8.10	8.11	8.15
D.O. (mg/L)	Mean	8.7	9.0	9.0	9.2	9.1	9.1
	Min.	8.0	8.0	8.4	8.5	8.2	8.2
	Max.	10.2	10.1	10.0	10.0	10.1	10.0
Tot. Hard (mg/L as CaCO ₃)	0h	102	102	102	100	101	101
	96h	104	101	101	101	101	100
Tot. Alk (mg/L as CaCO ₃)	0h	81	80	80	80	80	80
	96h	81	81	81	80	81	80
Cond (µmhos/cm)	0h	252	252	253	254	253	254
	96h	259	259	259	257	259	256

Additional water quality and QC data are listed on the attached photocopied pages from the laboratory notebook.

TESTED BY:

King County Environmental Laboratory
322 West Ewing Street
Seattle WA 98119

REFERENCES

APHA. 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition. American Public Health Association, American Waterworks Association, Water Pollution Control Association. Washington D.C.

U.S. E.P.A. 1991. Code of Federal Regulations, 40CFR, Appendix A, July 1991 U.S. Environmental Protection Agency, Office of Federal Registry, Washington, D.C.

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US EPA. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th edition. EPA-821-02-012, October 2002. US Environmental Protection Agency, Office of Water (4303T), Washington, DC.

US EPA. 1996 (April). Ecological Effects Test Guidelines: OPPTS 850.1000 Special Considerations for Conducting Aquatic Laboratory Studies.

Bench Sheets

Chain-of-Custody

Supporting Chemistry

**DF2000 96-Hour Acute Static Renewal Test
Rainbow Trout**

Test #: 6973
Test Date: 12-14-13

ORGANISMS

500 fish received from Treat Lodge Lot # (Swim-up date): 11-18-13 Shipped via Pick up Arrived at KCEL at 1415 h on 12-4-13 in 1 box double Plastic Bag.
0 dead removed. At Arrival: pH —, D.O. >20 mg/L, Temp 8.0 °C. Into Tank # 1 Hold in tank with new well water and aeration for 10 days. Feed 2X/day with Ziegler's Salmon starter. Refer to culture log for feeding & holding information.

DILUTION WATER/TOXICANT

1. New Well Water (NWW) 12-12-13, filtered through nylon netting. Hardness should be between 80-100 mg/L. At start TH ≈ 98 mg/L. Dilute — w/ MilliQ DI.
2. DF2000: Sample # SW 12123-701 Collected on 12-12-13 By SW
Rec'd by KCEL 12-12-13 Stored in the dark at 4 ± 2°C

SOLUTIONS

Code	Sample Conc (g/L)	NWW (L/ jar)	ml DF2000/ jar
Blue	0	6 L (NWW only)	0 (NWW only)
Green	0.3125	↓	2.44
Yellow	0.625	↓	4.88
Orange	1.25	↓	9.75
Red	2.5	↓	19.5
White	5	↓	39

Can see DF2000 on surface layer of water after mixing + settling

PROCEDURE

1. Add 6 L NWW to each of 2 jars/trtmt; place in 12°C EC # 8556, East & west shelf. Bring to 12°C.
2. Dispense DF2000 into test chamber and mix by hand stirring. Let settle 1 min.
3. Add 10 fish to test chamber. Start count verified by cy & —.
4. Take 0 h organics sample (on 0, 1.25 and 5 g/L). Take 0h sample for pH, DO, Temp, Tot. Alk, Tot. Hard, Cond.
5. **Start** test at 0835 h on 12-14-13. Place Tidbit temp recorder (SN 9716077, East shelf; SN 9716078, west shelf) in beaker w/WW into EC. into EC 0940
6. Remove dead fish daily; record #/ weight/ length/ time dead. Record survival daily. Measure Temp, pH & DO daily in all treatments.
7. Take 48 h organics sample before and after renewal on 0, 1.25 and 5 g/L test chambers.
8. Renew solutions (≈ 80%) at 48h:
 - a) Siphon 4.8 L from each jar.
 - b) Replace 6 L with renewal solution by siphoning from container prepped with new solution (prepped at renewal).
9. **End** test at 0800 h on 12-18-13. Measure Temp, pH and DO in all trtmts. Sample for Organics, Tot. Alk, Tot. Hard and Cond.

**DF2000-Hour Acute Static Renewal Test
Rainbow Trout**

Test #: 6973
Test Date: 12-14-13

Load Rate = [(Wt)(# Fish)] / Vol = (0.350 g)(10) / 6 L = 0.58 g/L

Where: Wt = Mean Wt in g; Vol = Total Test Vol in L; # Fish = #Fish/Rep

Chemistry

Code	Rep	Temp (°C) SN: 3A2302					pH					D.O. (mg/L)				
		0h	24h	48h	72h	96h	0h	24h	48h	72h	96h	0h	24h	48h	72h	96h
Blue	A	11.9	11.9	11.9	11.9	12.0	7.982	7.580	7.732	7.768	7.562	10.0	8.2	9.0	8.0	8.0
	B	11.6	11.9	11.8	11.8	11.8	8.039	7.677	7.815	7.853	7.618	10.2	8.6	9.0	8.2	8.1
Grn	A	12.2	11.5	12.1	11.4	11.7	8.055	7.707	7.761	7.904	7.681	10.1	8.9	9.0	8.6	8.0
	B	12.2	12.2	12.0	12.0	12.0	8.064	7.747	7.791	7.947	7.744	10.1	8.9	9.2	8.6	8.4
Yell	A	11.9	11.9	11.7	11.9	11.8	8.043	7.661	7.836	7.965	7.820	10.0	9.1	9.0	8.6	8.5
	B	11.8	12.0	12.0	11.9	11.7	8.145	7.616	7.910	7.952	7.801	9.9	9.0	9.0	8.6	8.4
Orng	A	12.0	12.3	12.3	12.1	11.9	8.083	7.667	7.821	7.973	7.891	10.0	9.1	9.1	9.2	8.6
	B	11.9	12.0	11.9	11.9	11.7	8.100	7.616	7.804	7.980	7.859	9.9	9.1	9.3	9.3	8.5
Red	A	11.9	12.1	12.0	12.2	12.1	8.105	7.694	7.812	7.911	7.766	10.1	8.9	9.1	9.3	8.2
	B	11.9	12.2	12.2	12.1	11.9	8.088	7.603	7.751	7.883	7.672	10.1	9.0	9.3	9.2	8.2
Wht	A	11.9	12.0	11.9	12.1	11.9	8.096	7.596	7.670	7.822	7.705	10.0	9.1	9.2	9.0	8.2
	B	11.7	12.1	12.0	12.1	12.0	8.149	7.640	7.710	7.827	7.652	10.0	9.1	9.2	8.9	8.5
Analyst:		GY	GY	GY	GY	GY	GY	GY	JA	GY	GY	GY	GY	JA	GY	GY

Code	Sample Conc (g/L)	Sample #		T. Alkalinity (mg/L as CaCO ₃)		T. Hardness (mg/L as CaCO ₃)		Conductivity (µmhos/cm)	
		0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
Blue	0	4.59347-1	-7	80.5	80.8	102	104	252	259
Green	0.3125	-2	-9	80.2	80.7	102	101	252	259
Yellow	0.625	-3	-7	80.1	80.5	102	101	253	259
Orange	1.25	-4	-10	80.2	80.2	100	101	254	257
Red	2.5	-5	-11	80.0	80.8	101	101	253	259
White	5.0	-6	-12	80.2	80.0	101	100	254	256
Analyst:		GY	GY						

DF2000 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6973
Test Date: 12-14-13

Random # Beaker Position					
Code	Rep	Random Jar #	Code	Rep	Random Jar #
Blue	A	5	Orange	A	6
	B	2		B	11
Green	A	1	Red	A	8
	B	4		B	3
Yellow	A	10	White	A	9
	B	12		B	7

NOTES

West well	East well
Blue A, B	Yellow A, B
Green A, B	Orange B
Orange A	Red A
Red B	White A, B

Control A End of Test wts

fish #	length cm	weight (g)
1	3.3	0.370
2	3.3	0.356
3	3.1	0.297
4	3.2	0.387
5	3.3	0.422
6	3.2	0.339
7	3.5	0.439
8	3.0	0.270
9	3.0	0.263
10	3.2	0.348
	\bar{x} 3.2	0.350

King County Environmental Lab Analytical Report

Project: 421193
 Locator: LAB
 Descrpt: LAB LOCATOR
 Sample: L59393-1
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrpt: LAB LOCATOR
 Sample: L59393-2
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrpt: LAB LOCATOR
 Sample: L59393-3
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Parameters	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units
OR WDOE NWTPH-DX															
DF2000		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_1		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_2		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_3		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_4		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_5		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_6		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_7		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L
DF2000_Peak_8		<MDL	24	236	ug/L		<MDL	24	236	ug/L		<MDL	24	236	ug/L

King County Environmental Lab Analytical Report

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-4
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-5
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-6
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Parameters	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units
OR WDOE NWTPH-DX															
DF2000 Peak_1	24	<MDL	24	236	ug/L	71	<RDL	24	236	ug/L	888	<RDL	24	236	ug/L
DF2000 Peak_2		<MDL	24	236	ug/L	58	<RDL	24	236	ug/L	45	<RDL	24	236	ug/L
DF2000 Peak_3		<MDL	24	236	ug/L	61	<RDL	24	236	ug/L	42	<RDL	24	236	ug/L
DF2000 Peak_4		<MDL	24	236	ug/L	62	<RDL	24	236	ug/L	65	<RDL	24	236	ug/L
DF2000 Peak_5		<MDL	24	236	ug/L	59	<RDL	24	236	ug/L	120	<RDL	24	236	ug/L
DF2000 Peak_6		<MDL	24	236	ug/L	58	<RDL	24	236	ug/L	150	<RDL	24	236	ug/L
DF2000 Peak_7		<MDL	24	236	ug/L	63	<RDL	24	236	ug/L	300	<RDL	24	236	ug/L
DF2000 Peak_8		<MDL	24	236	ug/L	66	<RDL	24	236	ug/L	786	<RDL	24	236	ug/L
			24	236	ug/L			24	236	ug/L	1730		24	236	ug/L

King County Environmental Lab Analytical Report

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-7
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-8
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-9
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Parameters	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units
OR WDOE NWTPH-DX															
DF2000 Peak 1	261	<RDL	24	236	ug/L	43	<MDL	24	236	ug/L	71	<RDL	24	236	ug/L
DF2000 Peak 2	230	<RDL	24	236	ug/L			24	236	ug/L	58	<RDL	24	236	ug/L
DF2000 Peak 3	238		24	236	ug/L			24	236	ug/L	62	<RDL	24	236	ug/L
DF2000 Peak 4	243		24	236	ug/L			24	236	ug/L	61	<RDL	24	236	ug/L
DF2000 Peak 5	240		24	236	ug/L			24	236	ug/L	60	<RDL	24	236	ug/L
DF2000 Peak 6	241		24	236	ug/L			24	236	ug/L	60	<RDL	24	236	ug/L
DF2000 Peak 7	249		24	236	ug/L	25	<RDL	24	236	ug/L	61	<RDL	24	236	ug/L
DF2000 Peak 8	249		24	236	ug/L	36	<RDL	24	236	ug/L	65	<RDL	24	236	ug/L
DF2000 Peak 8	259		24	236	ug/L	52	<RDL	24	236	ug/L	67	<RDL	24	236	ug/L

King County Environmental Lab Analytical Report

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-10
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-11
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Project: 421193
 Locator: LAB
 Descrip: LAB LOCATOR
 Sample: L59393-12
 Matrix: LK FRESH WTR
 ColDate: 12/18/13 0:00
 TimeSpan:
 TotalSolid:
 ClientLoc:
 SampDepth:
 WET Weight Basis

Parameters	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units	Value	Qual	MDL	RDL	Units
OR WDOE NMTPH-DX															
DF2000	803		24	236	ug/L	1530		24	236	ug/L	130	<RDL	24	236	ug/L
DF2000 Peak 1	631		24	236	ug/L	1040		24	236	ug/L	91	<RDL	24	236	ug/L
DF2000 Peak 2	620		24	236	ug/L	992		24	236	ug/L	95	<RDL	24	236	ug/L
DF2000 Peak 3	684		24	236	ug/L	1130		24	236	ug/L	100	<RDL	24	236	ug/L
DF2000 Peak 4	704		24	236	ug/L	1270		24	236	ug/L	110	<RDL	24	236	ug/L
DF2000 Peak 5	721		24	236	ug/L	1270		24	236	ug/L	110	<RDL	24	236	ug/L
DF2000 Peak 6	778		24	236	ug/L	1390		24	236	ug/L	120	<RDL	24	236	ug/L
DF2000 Peak 7	816		24	236	ug/L	1670		24	236	ug/L	120	<RDL	24	236	ug/L
DF2000 Peak 8	903		24	236	ug/L	1920		24	236	ug/L	130	<RDL	24	236	ug/L

Workgroup: WG130406 (dl#379 DF2000) Run ID: R192860

MB:WG130406-1 Matrix: BLANK WTR Listtype:ORORGMISC Method:WDOE NWTPH-DX Project: Pkey:STD
 (Method Blank)

Parameter	MDL	RDL	Units	MB Value	Qual
DF2000		25	250 ug/L	<MDL	
DF2000_Peak_1		25	250 ug/L	<MDL	
DF2000_Peak_2		25	250 ug/L	<MDL	
DF2000_Peak_3		25	250 ug/L	<MDL	
DF2000_Peak_4		25	250 ug/L	<MDL	
DF2000_Peak_5		25	250 ug/L	<MDL	
DF2000_Peak_6		25	250 ug/L	<MDL	
DF2000_Peak_7		25	250 ug/L	<MDL	
DF2000_Peak_8		25	250 ug/L	<MDL	

SB:WG130406-2 MB:WG130406-1 Matrix: BLANK WTR Listtype:ORORGMISC Method:WDOE NWTPH-DX Project: Pkey:STD
 (Spike Blank, Method Blank)

Parameter	MDL	RDL	Units	MB Value	TrueValue	SB Value	% Rec.	Qual	LabLimit
DF2000		25	250 ug/L	<MDL	2500	2230	89		60--110
DF2000_Peak_1		25	250 ug/L	<MDL	2500	2120	85		60--110
DF2000_Peak_2		25	250 ug/L	<MDL	2500	2120	85		60--110
DF2000_Peak_3		25	250 ug/L	<MDL	2500	2130	85		60--110
DF2000_Peak_4		25	250 ug/L	<MDL	2500	2160	86		60--110
DF2000_Peak_5		25	250 ug/L	<MDL	2500	2180	87		60--110
DF2000_Peak_6		25	250 ug/L	<MDL	2500	2200	88		60--110
DF2000_Peak_7		25	250 ug/L	<MDL	2500	2230	89		60--110
DF2000_Peak_8		25	250 ug/L	<MDL	2500	2340	94		60--110

LD:WG130406-3 L59393-5 Matrix: FRESH WTR Listtype:ORORGMISC Method:WDOE NWTPH-DX Project:421193 Pkey:STD
 (Lab Duplicate)

Parameter	MDL	RDL	Units	SAMP Value	LD Value	RPD	Qual	LabLimit
DF2000		24	236 ug/L	71	71	32		0--40
DF2000_Peak_1		24	236 ug/L	58	58	25		0--40
DF2000_Peak_2		24	236 ug/L	61	61	28		0--40
DF2000_Peak_3		24	236 ug/L	62	62	27		0--40
DF2000_Peak_4		24	236 ug/L	59	59	25		0--40
DF2000_Peak_5		24	236 ug/L	58	58	25		0--40
DF2000_Peak_6		24	236 ug/L	63	63	27		0--40
DF2000_Peak_7		24	236 ug/L	62	62	25		0--40
DF2000_Peak_8		24	236 ug/L	66	66	26		0--40

LD:WG130406-4 L59393-9 Matrix: FRESH WTR Listtype:ORORGMISC Method:WDOE NWTPH-DX Project:421193 Pkey:STD
 (Lab Duplicate)

Parameter	MDL	RDL	Units	SAMP Value	LD Value	RPD	Qual	LabLimit
DF2000		24	236 ug/L	71	71	61		0--40
DF2000_Peak_1		24	236 ug/L	58	58	51		0--40
DF2000_Peak_2		24	236 ug/L	62	62	55		0--40
DF2000_Peak_3		24	236 ug/L	61	61	55		0--40
DF2000_Peak_4		24	236 ug/L	60	60	52		0--40
DF2000_Peak_5		24	236 ug/L	60	60	53		0--40

DF2000_Peak_6	24	236 ug/L	61	53	0--40
DF2000_Peak_7	24	236 ug/L	65	53	0--40
DF2000_Peak_8	24	236 ug/L	67	54	0--40

Surrogate:	2-Fluorobiphenyl
(Lab Limits)	70--130
L59393-1	96
L59393-2	96
L59393-3	96
L59393-4	92
L59393-5	90
L59393-6	85
L59393-7	100
L59393-8	93
L59393-9	99
L59393-10	101
L59393-11	103
L59393-12	104
WG130406-1	92
WG130406-2	102
WG130406-3	95
WG130406-4	98

Reference Toxicant Test:

Bench Sheets

Precision Table

Reference Toxicant, Cd, 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6972
Test Date: 12-14-13

ORGANISMS

500 fish received from Trout Lodge Lot # (Swim-up date): 11-18-13 Shipped via Pick up Arrived at KCEL at 1415 h on 12-4-13 in 1 box double plastic bag.
0 dead removed. At Arrival: pH -, D.O. >20 mg/L, Temp 8.0 °C. Into Tank # 1 Hold in tank with new well water and aeration for 10 days. Feed 2X/day with Ziegler's Salmon starter Refer to culture log for feeding & holding information.

DILUTION WATER/TOXICANT

1. New Well Water (NWW) 12-13-13, filtered through nylon netting.
2. Cd Stock Soln: Nominal 20 mg Cd/L, Measured 20.3 mg/L on 12-20-12 Prep 12-5-12
_____ by add _____ g Cd(NO₃)₂·4H₂O (mfr Baker # 1-1226, rec'd _____, opened _____, lot # 049130) ⊆ 1L DW.
LIMS RTA Sample #: WG130384-1 Wkgp #: WG130384

SOLUTIONS

Cd Trtmt (µg/L)	Code	Cd Stock (mL/ jar)	NWW (L/ jar)	Sample #	Cd (µg/L) (Measured)
0	Blue	0 (NWW only)	12 L (NWW only)		
0.75	Green	0.44	⊆ 12L		
1.5	Yellow	0.88	↓	* 59362-1	1.52
3.0	Orange	1.76	↓	* 67	
6.0	Red	3.52	↓		
12.0	White	7.04	↓		

PROCEDURE

1. Add 12 L NWW to each of 2 jars/trtmt; place in 12°C EC # 8555, East & West shelf. Bring to 12°C. Setup at _____ h.
2. Measure DO; if DO << saturation, aerate until DO ≥ 9 mg/L. Stop aeration.
3. Measure Temp, pH & DO. in all trtmts.
4. Add Cd stock soln to jars: Mix: Sample for Cd: sample at 48h Acidify: Analyst: GY
5. Add 10 fish/jar, one at a time to randomize, using dip net. Start count verified by GY & [initials].
6. Start test at 0730 h on 12-14-13. Place Tidbit temp recorder (SN 1118067, East shelf; SN 1118068, West shelf) in beaker w/WW into EC.
7. Remove dead fish daily; record #/ weight/ length/ time dead. Record survival daily. Measure Temp, pH & DO daily in all trtmts.
8. Renew solns (≈ 80%) at 48h:
 - a) Siphon 9.6 L from each jar.
 - b) Filter NWW into 4L graduated cylinder.
 - c) Add Cd stock soln ⊆ 4L aliquot during filling as below:

Cd (µg/L):	0	0.75	1.5	3	6	12
mL Cd Stock:	0	0.15	0.30	0.59	1.18	2.36

- d) Replace ⊆ 12 L/jar with fresh soln by pouring through funnel and tubing into jar.
9. End test at 0725 h on 12-18-13. Measure Temp, pH and DO in all trtmts.

Reference Toxicant, Cd, 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6972
Test Date: 12-14-13

MEASUREMENTS

24

Code	Cumulative Survival (#Alive/Rep)						Tot # Alive
	Cd (µg/L)	Rep	24 h	48 h	72 h	96 h	
Blue	0	A	10	10	10	10	10
	0	B	10	10	10	10	10
Green	0.75	A	10	10	10	10	10
	0.75	B	10	10	10	10	10
Yellow	1.5	A	9	6	4	4	4
	1.5	B	10	7	5	5	5
Orange	3	A	5	1	1	1	1
	3	B	5	0	0	0	0
Red	6	A	3	0	0	0	0
	6	B	3	0	0	0	0
White	12	A	0	0	0	0	0
	12	B	0	0	0	0	0
		Analyst:	GY	JJA	JJA	GY	

s = stressed

Code	Rep	Daily #Dead/Rep										Mean		
		1	2	3	4	5	6	7	8	9	10			
white	A	Date	12-15-13										12-15-30	
		Time	1030										1030	
		cm	3.3	3.3	3.3	3.4	3.1	3.1	3.4	3.5	3.2	3.0	3.3	
		g	0.510	0.469	0.543	0.501	0.380	0.438	0.536	0.574	0.462	0.458	0.487	
white	B	Date	12-15-13										12-15-13	
		Time	1030										1030	
Red	A	Date	12-15-13						12-15-13	12-16-13	12-16-13	12-16-13		
		Time	1030						1030	1000	1000	1000		
Red	B	Date	12-15-13						12-15-13	12-16-13				
		Time	1030						1030	1000				
OR	A	Date	12-15-13					12-15-13	12-16					
		Time	1030					1030	1000					
OR	B	Date	12-15-13					12-15-13	12-16					
		Time	1030					1030	1000					
Yellow	A	Date	12-15-13	12-16	12-16	12-16	12-17	12-17						
		Time	1030	1000	1000	1000	1445	1445						
Yellow	B	Date	12-16	12-16	12-16	12-17	12-17							
		Time	1000	1000	1000	1445	1445							
		Date												
		Time												
		Date												
		Time												

Load Rate = [(Wt)(# Fish)] / Vol = (0.487 g)(10) / 12 L = 0.41 g/L

Where: Wt = Mean Wt in g; Vol = Total Test Vol in L; # Fish = #Fish/Rep

Reference Toxicant, Cd, 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6972
Test Date: 12-14-13

Chemistry

Code	Rep	Temp (°C) SN: 3A2302					pH					D.O. (mg/L)				
		0h	24h	48h	72h	96h	0h	24h	48h	72h	96h	0h	24h	48h	72h	96h
Blue	A	12.4	12.4	12.5	12.5	12.4	8.252	7.677	7.824	7.834	7.731	10.8	8.8	9.0	8.8	8.5
	B	11.9	11.7	12.0	12.1	11.8	8.281	7.820	7.850	7.948	7.734	11.1	9.1	9.0	8.8	8.6
Grn	A	11.7	11.6	11.9	11.9	11.8	8.312	7.800	7.835	8.055	7.717	11.2	9.0	9.1	8.9	8.7
	B	12.3	12.3	12.0	12.6	12.5	8.303	7.810	7.767	8.110	7.627	11.3	9.0	9.0	8.6	8.4
Yell	A	12.0	11.9	12.1	12.2	12.1	8.278	7.838	7.670	8.182	7.808	11.0	8.8	8.9	8.9	9.1
	B	12.1	12.2	11.9	12.3	12.3	8.299	7.888	7.641	8.147	7.799	11.2	9.0	9.1	9.0	9.2
Orng	A	12.0	11.9	12.1	12.2	12.1	8.304	7.770	7.810	8.219	7.939	11.3	8.7	8.9	9.3	9.6
	B	12.4	12.2	12.6	-	-	8.303	7.807	7.721	-	-	11.0	8.7	8.9	-	-
Red	A	12.4	12.3	12.7	-	-	8.328	7.744	7.873	-	-	11.1	8.7	8.9	-	-
	B	12.2	12.1	12.4	-	-	8.286	7.859	7.890	-	-	11.2	8.8	8.8	-	-
Wht	A	12.4	12.4	-	-	-	8.293	7.789	-	-	-	11.4	8.7	-	-	-
	B	11.9	11.8	-	-	-	8.279	7.820	-	-	-	11.4	8.9	-	-	-
Analyst:		GY	GY	JA	GY	GY	GY	GY	JA	JA/GY	JA	GY	GY	JA	JA	JA

west East Well
Blue B
Green A, B
Yellow A
Orange A
Red B
white B

west East Well
Blue A
Yellow B
Orange B
Red A
White A

Random # Beaker Position					
Code	Rep	Random Jar #	Code	Rep	Random Jar #
Blue	A	9	Orange	A	6
	B	3		B	11
Green	A	4	Red	A	12
	B	5		B	1
Yellow	A	2	White	A	10
	B	8		B	7

NOTES

CETIS Analytical Report

Report Date: 18 Dec-13 12:18 (p 1 of 2)
 Test Code: 6972RTAQC | 02-1969-1247

6972
 WG-130384

Fish 96-h Acute Survival Test

King County Metro Services, WQ Lab

Analysis ID: 07-3647-5931	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.6
Analyzed: 18 Dec-13 12:18	Analysis: Linear Regression (MLE)	Official Results: Yes
Batch ID: 11-8576-1280	Test Type: Survival (96h)	Analyst: JA
Start Date: 14 Dec-13 07:30	Protocol: EPA/821/R-02-012 (2002)	Diluent: Well Water
Ending Date: 18 Dec-13 07:25	Species: Oncorhynchus mykiss	Brine: Not Applicable
Duration: 96h	Source: Trout Lodge Fish Farm	Age: 26d ← changed in CETIS 12-20-13 JA
Sample ID: 08-7730-6312	Code: WG130384-1	Client: Internal Lab
Sample Date: 14 Dec-13 07:00	Material: Cadmium nitrate	Project: Reference Toxicant
Receive Date:	Source: Reference Toxicant	
Sample Age: 30m	Station:	

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
66	-18.52	46.05	44.5	0.1828	0.1529	0.9579	3.274	4.757	0.1008	Non-Significant Lack of Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
EC50	1.523	1.275	1.82

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits	Overlap	Decision
Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	5.31E-08	5.15E-05	-0.00010	0.000101	0.00103	0.9992	Non-Significant Parameter
Slope	6.54	1.405	3.787	9.293	4.656	0.0012	Significant Parameter
Intercept	-1.196	0.3478	-1.877	-0.5139	-3.437	0.0074	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	92.82845	92.82845	1	252.5	<0.0001	Significant
Lack of Fit	2.054043	0.684681	3	3.274	0.1008	Non-Significant
Pure Error	1.254652	0.209109	6			
Residual	3.308695	0.367633	9			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	3.309	16.92	0.9508	Non-Significant Heterogeneity
	Likelihood Ratio GOF	3.22	16.92	0.9549	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	65540	4.387	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8526	0.8608	0.0395	Non-normal Distribution
	Anderson-Darling A2 Normality	1.043	2.492	0.0098	Non-normal Distribution

96h Survival Rate Summary

Calculated Variate(A/B)

C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	2	1	1	1	0	0	0.0%	0.0%	20	20
0.75		2	1	1	1	0	0	0.0%	0.0%	20	20
1.5		2	0.45	0.4	0.5	0.05	0.07071	15.71%	55.0%	9	20
3		2	0.05	0	0.1	0.05	0.07071	141.4%	95.0%	1	20
3		2	0	0	0	0	0		100.0%	0	20
12		2	0	0	0	0	0		100.0%	0	20

CETIS Analytical Report

Report Date: 18 Dec-13 12:18 (p 2 of 2)
Test Code: 6972RTAQC | 02-1969-1247

Fish 96-h Acute Survival Test

King County Metro Services, WQ Lab

Analysis ID: 07-3647-5931 Endpoint: 96h Survival Rate
Analyzed: 18 Dec-13 12:18 Analysis: Linear Regression (MLE)

CETIS Version: CETISv1.8.6
Official Results: Yes

96h Survival Rate Detail

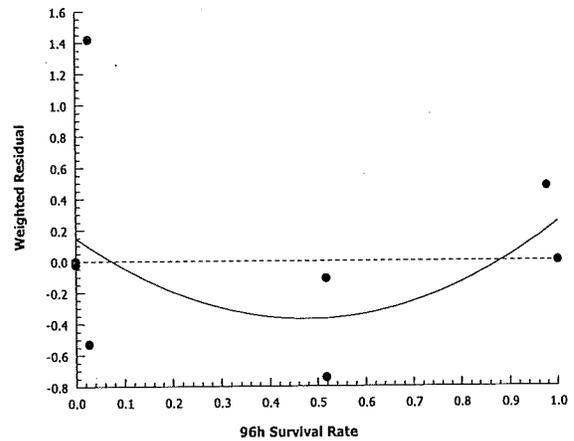
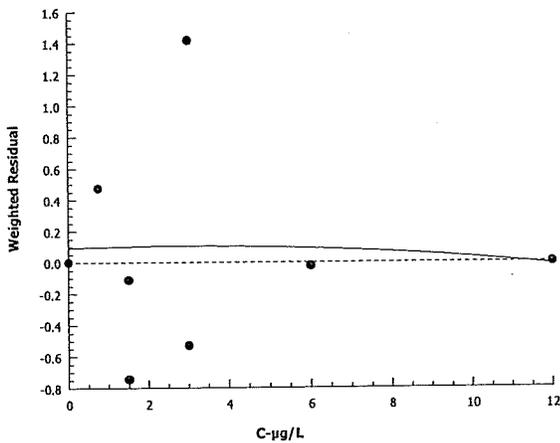
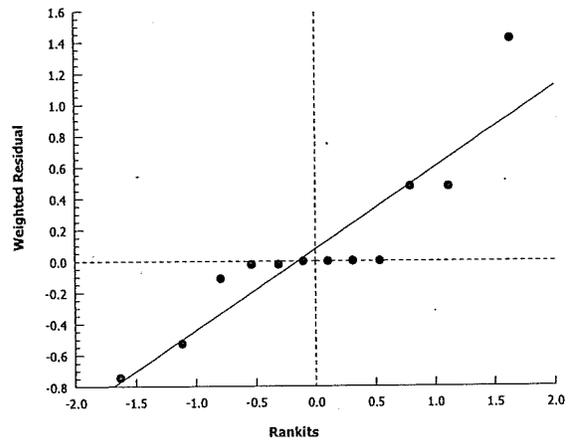
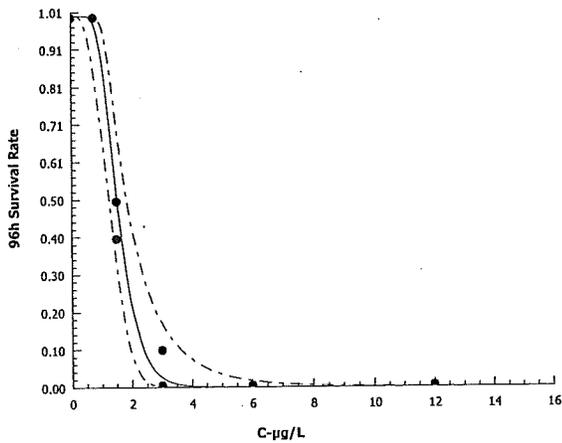
C-µg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
0.75		1	1
1.5		0.4	0.5
3		0.1	0
6		0	0
12		0	0

96h Survival Rate Binomials

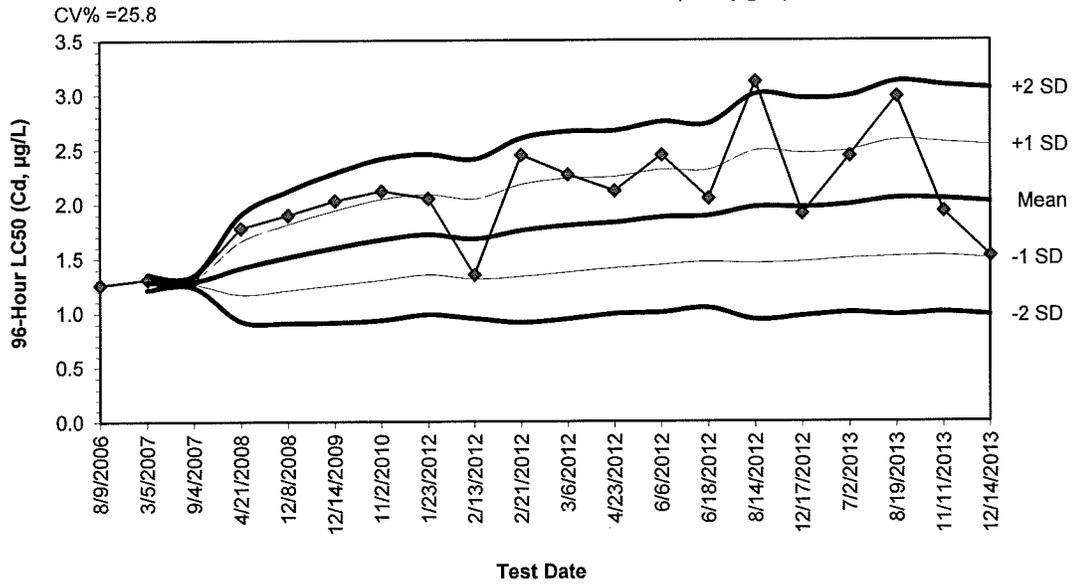
C-µg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	10/10	10/10
0.75		10/10	10/10
1.5		4/10	5/10
3		1/10	0/10
6		0/10	0/10
12		0/10	0/10

Graphics

Log-Normal [NED=A+B*log(X)]



**Control Chart for Acute Reference Toxicant Tests with
Rainbow Trout 96-Hour Survival LC50 (Cd, µg/L)**

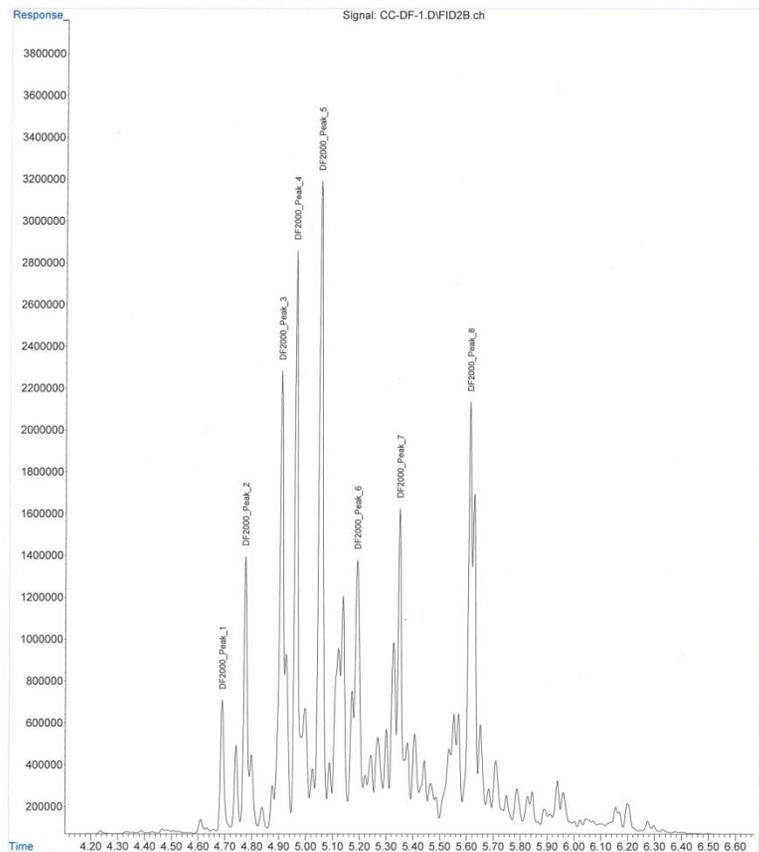


Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
8/9/2006	1.26					
3/5/2007	1.31	1.2850	1.2496	1.2143	1.3204	1.3557
9/4/2007	1.31	1.2933	1.2645	1.2356	1.3222	1.3511
4/21/2008	1.78	1.4150	1.1705	0.9261	1.6595	1.9039
12/8/2008	1.90	1.5120	1.2089	0.9058	1.8151	2.1182
12/14/2009	2.03	1.5983	1.2545	0.9107	1.9422	2.2860
11/2/2010	2.12	1.6729	1.3022	0.9315	2.0435	2.4142
1/23/2012	2.05	1.7200	1.3518	0.9837	2.0882	2.4563
2/13/2012	1.35	1.6789	1.3131	0.9473	2.0447	2.4105
2/21/2012	2.45	1.7560	1.3336	0.9112	2.1784	2.6008
3/6/2012	2.27	1.8027	1.3731	0.9435	2.2324	2.6620
4/23/2012	2.12	1.8292	1.4094	0.9897	2.2489	2.6687
6/6/2012	2.45	1.8769	1.4397	1.0025	2.3141	2.7514
6/18/2012	2.05	1.8893	1.4667	1.0441	2.3119	2.7345
8/14/2012	3.12	1.9713	1.4548	0.9383	2.4879	3.0044
12/17/2012	1.91	1.9675	1.4682	0.9690	2.4668	2.9660
7/2/2013	2.44	1.9953	1.4985	1.0017	2.4921	2.9889
8/19/2013	2.98	2.0500	1.5151	0.9801	2.5849	3.1199
11/11/2013	1.93	2.0437	1.5231	1.0025	2.5643	3.0849
12/14/2013	1.52	2.0175	1.4974	0.9774	2.5376	3.0576

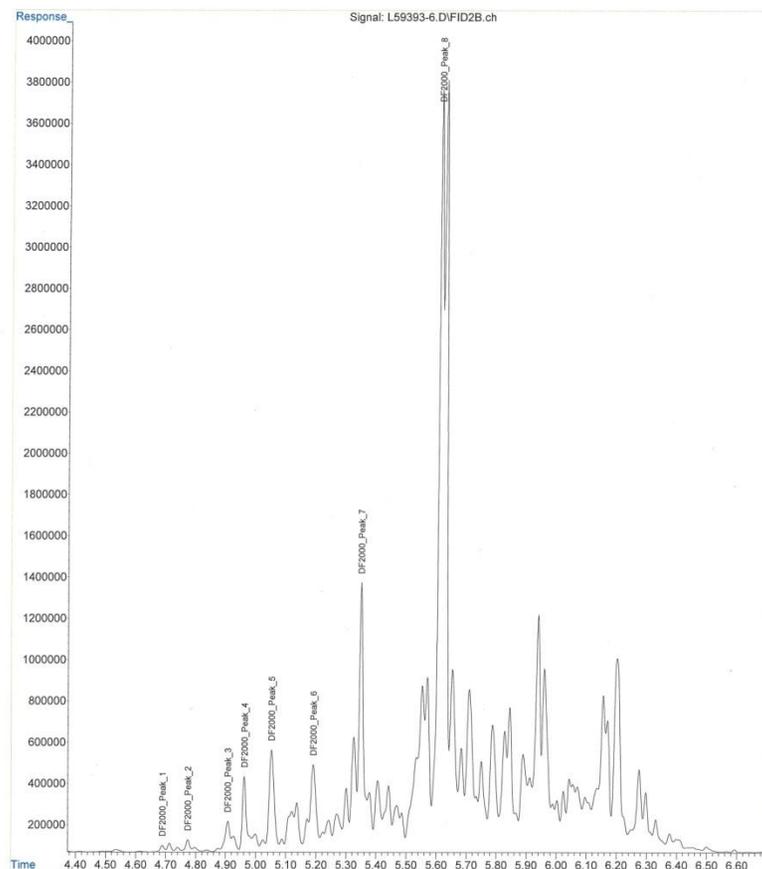
APPENDIX B:

**CHROMATOGRAMS FROM THE ANALYSIS OF DF2000™
SOLVENT VS. FISH BIOASSAY TEST SOLUTION**

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DF2000™ standard at 200 mg/L



Test vessel solution at a nominal concentration of 1250 mg/L, collected after 48 hours. Note that Peaks 1-6 are significantly lower in response compared to Peak 8, indicating loss of more volatile constituents.