



Final Report

Evaluation of Solvon K4™ 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, Washington, a coalition of local governments. Our customers are residents, businesses and institutions with small quantities of hazardous wastes. The Program'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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CONTENTS

Executive Summary	1
Introduction.....	3
Dry cleaning solvents.....	3
Solvon K4 TM toxicity testing	3
Methods.....	5
Sample collection and storage	5
Test procedures	5
Results	7
Conclusions.....	9
Acknowledgments	11
References	13
Appendix A: Report on LC50 Toxicity Testing Conducted on Solvon K4 Dry Cleaning Solvent	15

Tables

Table 1. Fish toxicity testing results for Solvon K4TM product	7
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ACRONYMS AND ABBREVIATIONS

°C	Degrees Centigrade
CAS	Chemical Abstract Service
CETIS	Complex Effluents Toxicity Information System
DW	Dangerous Waste
EHW	Extremely Hazardous Waste
EPA	United States Environmental Protection Agency
Ecology	Washington State Department of Ecology
°F	Degrees Fahrenheit
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
NFPA	National Fire Protection Association
PERC	Perchloroethylene

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EXECUTIVE SUMMARY

Dry cleaners are transitioning from using perchloroethylene (PERC) as their cleaning solvent to several non-chlorinated alternatives. A new dry cleaning solvent, called Solvon K4TM, is gaining market share in King County and elsewhere in the United States. This solvent, which is also known as butylal, is part of a dry cleaning system developed in Europe, known as System K4TM.

Relatively little toxicological information is available for Solvon K4TM. Consequently, the objective of this study was 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.

This study identified the first LC₅₀ for Solvon K4TM product in rainbow trout: 45.7 mg/L. Consequently, this solvent is less toxic to fish than PERC (LC₅₀ = 5.0 mg/L), but more toxic than DF-2000TM, which is a high flash point hydrocarbon product also used in dry cleaning operations (LC₅₀ >100 mg/L).

Based on this LC₅₀, unused or off-specification Solvon K4TM product that requires disposal would designate as Dangerous Waste (DW) with the waste code WT02 in Washington state. This LC₅₀ may also be used to designate waste streams originating from System K4TM dry cleaning and other processes that generate butylal-containing wastes.

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INTRODUCTION

Dry cleaning solvents

Kerosene and other solvents have been used to dry clean fabrics since the mid-19th century. Stoddard solvent was introduced in 1925, but this petroleum distillate's flammability (flash point: 104 °F) prompted the industry to switch to perchloroethylene (PERC) in the 1960s. PERC (also known as tetrachloroethylene or "PCE") is essentially non-flammable and this chlorinated hydrocarbon is still regarded as one of the most efficient dry cleaning solvents.⁽¹⁾

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 still the most commonly-used solvent in King County.⁽²⁾ However, subsequent to the survey, a new dry cleaning technology appeared in King County, called "System K4TM". As of October 2013, five System K4TM dry cleaners are operating in King County.

System K4TM was developed in Germany by Kreussler GmbH. The dry cleaning solvent, Solvon K4TM, is composed primarily of butylal, which is a diether acetal. Synonyms for butylal include dibutoxymethane, 1-(butoxymethoxy)butane, and formaldehyde dibutyl acetal. The Chemical Abstract Service (CAS) number is 2568-90-3.⁽³⁾

According to Kreussler GmbH, n-butyl alcohol (1-butanol) and formaldehyde are present in Solvon K4TM at <0.5% and <0.05%, respectively.⁽³⁾ With a flash point of 143.6 °F,⁽³⁾ Solvon K4TM is regarded as a National Fire Protection Association (NFPA) Class IIIA solvent.

The extent of worker exposure to butylal and the long-term health effects associated with using this solvent have not been well-characterized. While the solvent is reportedly slightly biodegradable, there is little published information concerning its aquatic toxicity.⁽³⁾⁽⁴⁾

Solvon K4TM toxicity testing

In September 2011, LHWMP conducted a fish toxicity test on a sample of unused Solvon K4TM product according to the Washington State Department of Ecology's (Ecology's) *Biological Testing Methods for the Designation of Dangerous Waste*.⁽⁵⁾ This test involved exposing juvenile rainbow trout to Solvon K4TM 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). Solvon K4TM killed fish at 100 mg/L, but not at 10 mg/L (unpublished data). Consequently, unused or off-specification Solvon K4TM product that requires disposal would designate as a Dangerous Waste (DW) with the waste code WT02 in Washington state.

Although this previous study was adequate for waste characterization, it was not of sufficient quality to describe this solvent's aquatic toxicity. The principal shortcomings of this previous study are: 1) only two concentrations of Solvon K4TM were tested and 2)

the reported test concentrations reflected the nominal solvent concentrations (i.e., it was assumed that 100 percent of the Solvon K4TM added to the test system went into solution and that none was lost during the test).

Consequently, LHWMP collaborated with the King County Environmental Laboratory (KCEL, Seattle, WA) to derive an LC₅₀ from multiple test concentrations, based on measured concentrations of Solvon K4TM in the test vessels.

METHODS

Sample collection and storage

A sample of unused Solvon K4TM product was collected from a previously unopened 30-pound product container on July 20th, 2012. This solvent had been delivered to a local dry cleaner just prior to installation of a new System K4TM machine. The lot number on the product container was 6F20023001 and the LHWMP-assigned sample number was SW072012-P01.

Solvon K4TM 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.

Test procedures

Testing was conducted using juvenile rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static renewal acute toxicity test between December 13th and December 17th, 2012. The experimental protocol (KCEL Standard Operating Procedure 406v2) was derived from the United States Environmental Protection Agency's (EPA's) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*.⁽⁶⁾ This protocol differed from the standard Ecology waste characterization method in that six concentrations of Solvon K4TM product (five test solutions and one control) 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 Solvon K4 Dry Cleaning Solvent*, which is provided in Appendix A.

Briefly, the test was conducted using a serial dilution of Solvon K4TM with nominal concentrations of 0 (control), 12.5, 25, 50, 100, and 200 mg/L. Ten rainbow trout were placed randomly into each test jar; duplicates were prepared at each test concentration. After 48 hours, 80 percent of the test solution from each jar was renewed with fresh Solvon K4TM solution at the appropriate concentration.

Samples of test solution were collected for chemical analysis for Solvon K4TM at 0, 48 (before renewal), 48 (after renewal) and 96 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.

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RESULTS

As shown in Table 1, 100 percent of fish survived for 96 hours at nominal Solvon K4TM test concentrations between 0 (control) and 50 mg/L. However, all fish exposed to nominal concentrations of 100 and 200 mg/L were killed within 2 hours of test initiation.

Table 1. Fish toxicity testing results for Solvon K4TM product						
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	--	100	100	100	100	100
12.5	7.11	100	100	100	100	100
25	15.2	100	100	100	100	100
50	30.9	100	100	100	100	100
100	67.5	0 ^b	--	--	--	0
200	135.0	0 ^c	--	--	--	0
^a Sample collected at 0 h (i.e., test initiation) ^b All fish killed within 2 hours of test initiation ^c All fish killed within 50 minutes of test initiation						

Chemical analysis of the test solutions at test initiation (i.e., 0 h) revealed that the measured concentrations were 57 to 68 percent of the nominal concentrations, which may reflect the performance limit of the analytical method for this chemical. (Spike blank recoveries of 62% and 73% were noted when duplicate blanks of laboratory water were spiked with Solvon K4TM). As described in Appendix A, the measured Solvon K4TM concentrations gradually declined over the first 48 hours, likely due to volatilization and/or formation of an immiscible solvent layer at the surface. However, after the solutions were renewed at 48 hours, the measured concentrations were restored to those measured at test initiation. By 96 hours the measured concentrations were again in the range found at 48 hours, before the test solutions were renewed.

An LC₅₀ was calculated using Complex Effluents Toxicity Information System (CETISTM) software, version 1.8.6.6 (Endpoint: 96 h survival rate; Analysis: binomial method). Based on the measured concentrations at test initiation, the LC₅₀ for Solvon K4TM was 45.7 mg/L. The lower- and upper- 95 percent confidence limits on the LC₅₀ were 38.4 and 54.4 mg/L, respectively.

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CONCLUSIONS

The LC₅₀ derived for Solvon K4TM product in this study (45.7 mg/L) is consistent with LHWMP's previous evaluation, in which this solvent killed 100 percent of fish at a nominal concentration of 100 mg/L, but not at 10 mg/L. This latest finding confirmed that unused or off-specification Solvon K4TM product that requires disposal would designate as Dangerous Waste (DW) with the waste code WT02 in Washington state.

LHWMP has also preliminarily evaluated another solvent alternative to PERC, called DF-2000TM. This product is manufactured by ExxonMobil, and is a non-chlorinated hydrotreated aliphatic hydrocarbon with carbon numbers predominantly in the range of C₆-C₁₃. The CAS number for DF-2000TM is 64742-48-9 and the flash point is 147 °F.⁽⁷⁾⁽⁸⁾ This class of dry cleaning solvents has gained considerable market share over the last decade. As of 2010, 21 percent of shops in King County were using a high flashpoint aliphatic hydrocarbon solvent.⁽²⁾ When DF-2000TM product was tested according to Ecology's *Biological Testing Methods for the Designation of Dangerous Waste*,⁽⁵⁾ all fish exposed to 100 mg/L survived (i.e., the LC₅₀ is >100 mg/L, based on the nominal concentration). Consequently, unused or off-specification DF-2000TM product that requires disposal would not designate as Dangerous Waste in Washington state (unpublished data).

The LC₅₀ value for PERC in fish presented in Ecology's *Washington Dangerous Waste Designation Tool*,⁽⁹⁾ 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.⁽¹⁰⁾

Consequently, the potency of unused dry cleaning solvent products towards rainbow trout is: PERC > Solvon K4TM > DF-2000TM. It is noteworthy that LC₅₀ values for Solvon K4TM and DF-2000TM are not currently available in Ecology's *Washington Dangerous Waste Designation Tool*.⁽⁹⁾

In conclusion, the LC₅₀ value derived for Solvon K4TM demonstrates that this solvent is intermediate in toxicity between PERC and the aliphatic hydrocarbon, DF-2000TM. Using Washington state's *Dangerous Waste Regulations*,⁽¹¹⁾ this LC₅₀ may also be used to designate waste streams derived from System K4TM dry cleaning and other processes that generate butylal-containing wastes.

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APPENDIX A:

**REPORT ON LC50 TOXICITY TESTING
CONDUCTED ON SOLVON K4 DRY CLEANING
SOLVENT**

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**REPORT ON LC50 TOXICITY TESTING
CONDUCTED ON SOLVON K4
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 13, 2012
KCEL Test Numbers: #6535 (*Oncorhynchus mykiss*: 96-Hour Acute Toxicity Test)
Report Date: October 7, 2013

INTRODUCTION

SolvonK4 is a recently developed organic solvent used in dry cleaning machines. SolvonK4 was developed as an alternative to the wide spread use of perchloroethylene, which has been subjected to increased regulatory scrutiny. At present there appears to be little information on the toxicity of SolvonK4, hence, this test was conducted to estimate the LC50 of the solvent.

Sample

A sample of SolvonK4 Dry Cleaning Solvent, SW072012-19-P01 collected on 7/20/12 was received by the King County Environmental Laboratory (KCEL); Aquatic Toxicology Section on 7/20/12. The sample was delivered in 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 12-12) and organics are measured annually (last analyzed on 2-12). Hardness, alkalinity, conductivity and pH are measured monthly.

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

Parameter	Value	Units
Temperature	13.3	$^{\circ}\text{C}$, adjusted as necessary
Conductivity	252	$\mu\text{mhos/cm}$
pH	7.99	
Total Hardness (calc.)	95	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	

To conduct the test the well water was diluted 10% with Milli-Q deionized water to keep the hardness between 80 – 100 mg/L as CaCO_3 .

METHODS

The acute toxicity test #6535 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), 12.5, 25, 50, 100, and 200 mg/L SolvonK4.

Test Organisms

Swim-up (swim-up on 11-21-12) rainbow trout (*Oncorhynchus mykiss*) were purchased from Trout Lodge located in Sumner, Washington on 11-29-12. The trout were acclimated for a period of 14 days in well water with a mean temperature of 13.3 °C, a minimum of 13.3 °C and a maximum of 13.3 °C in a flow-through system at KCEL. During acclimation the fish were fed Ziegler's 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)
6535	22	2.8	0.28	0.47

As indicated in the table the mean weight of the trout used in the test was 0.28 g with a mean standard length of 2.8 cm. The loading in each jar was 0.47 g/L.

Rainbow Trout – 96-Hour Static Renewal Acute Toxicity Test

For test #6535, 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 dilution water was well water diluted 10% with Milli-Q deionized water to keep the hardness between 80 – 100 mg/L as CaCO₃. The test solutions were maintained at 12 ± 1.0°C for 96-hours in an environmental chamber (Hotpack Model 08082, s/n 79719).

Ten rainbow trout were placed randomly into each test jar. Assignment of fish to the test jars was random, as was placement of the test jars 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:8 h D. The test was initiated at 1030 h on 12-13-12 and ended at 1000 h on 12-17-12.

Test Solution Prep

Test solution preparation followed the general guidelines of US EPA 712-C-96-118 (April 1996). SolvonK4 has very low solubility in water and forms an immiscible layer on the surface of water. In order to obtain large volumes, sufficient for fish loading rates with maximum saturation a stirred stock solution approach was employed in sealed test vessels. Test solutions were prepared at 0 and 48 hours of the 96 hour static-renewal exposure period.

One day prior to test initiation (12/12/12), 6-7 L of well water (temp approx. 13.3°C) diluted 10% with Milli-Q deionized water was measured into a 2 gal Anchor Hocking-Heritage Hill glass jar containing a 3 x 0.5 in. teflon coated stir bar. The appropriate amount of SolvonK4 was added to each jar as indicated below (2 reps per test concentration). Seven liters of test solution was prepared for 0 and 200 mg/L test concentrations to allow for alkalinity and hardness samples at 0 hours (1 liter was removed for these samples). A rubber gasket was placed around the lip of the jar, the jar was covered with its lid and a lead weight was placed on top of the lid. To further enhance the seal, plastic packaging wrap was stretched around the lip of the jar. The jar was placed on a magnetic mixer in a 12°C environmental chamber for approximately 22 hours. The speed of the magnetic mixer was increased until a funnel-shaped vortex was seen in the water column (too high of a rate would cause the stir bar to spin out).

Nominal Sample Conc (mg/L)	WW (L/ jar) on Day 0-1	grams SolvonK4/ jar)	Number of Reps
0	7 L**	0 *	2
12.5	6 L	0.075	2
25	↓	1.5	2
50	↓	0.3	2
100	↓	0.6	2
200	7 L	1.4*	2

*Day 0-1: Add 7 L WW for control. For 200 mg/L add 1.4 g SolvonK4/7 L WW.

Extra 1 liter needed for WQ samples.

**6 L for 48-hour renewal

48 Hour Renewal

Test solutions were renewed (80% renewal) at 48-hours. One day prior to solution renewal (12/14/12), test renewal solutions were prepared similarly to Day 0-1. Only the 0, 12.5, 25 and 50 mg/L nominal sample concentrations were prepared since there were no survivors in the 100 and 200 mg/L concentrations. The solutions were mixed overnight for approximately 20.5-hours.

Solutions were renewed by siphoning 4.8 L from the test jar and replacing with newly prepped solution. As the renewal solution was needed the mag mixer was stopped and after 1 minute new solution was replaced in the test jar. A beaker was used to transfer the renewal solution at the surface of the solution in the test jar with minimal agitation.

Sampling of Test Solutions for SolvonK4

Samples for organic analysis were taken at 0, 48 (before renewal), 48 (after renewal) and 96 hours. Samples for 0-hour were sampled after 1 minute from stopping the mag mix to allow the solution to cease spinning. The 48-hour (after renewal) sample was sampled immediately following solution replacement. Samples were taken with a pipette at mid depth in the test jar and transferred to a 40 mL glass amber VOA vial with septa cap and no headspace. Samples were taken from both A and B replicates, but only the A reps were turned in for analysis to limit the amount of samples for analysis. The samples were stored in the dark at $4 \pm 2.0^{\circ}\text{C}$ until analyzed.

Organic Analysis

The extraction batch (WG122953) consisted of a method blank (MB), spike blank (SB), spike blank duplicate (SBD), matrix spike (MS), and six samples. All samples were spiked with surrogates and the SB, SBD and MS were spiked with dibutoxymethane to determine precision and accuracy of the extraction and method. The one liter samples were prepped by continuous liquid-liquid extraction using methylene chloride (EPA METHOD 3520C). The samples were concentrated to a 1 ml final volume for analyses.

The extracts were analyzed using gas chromatography mass spectroscopy electron impact (GC/MS/EI). The instrument was calibrated using dibutoxymethane standards with concentrations from 100 ppm to 1 ppm. All samples were analyzed for dibutoxymethane (SW846-8720D). Positive results had the correct retention time and spectra as compared to the standards. Results where dibutoxymethane exceeded the calibration range, the extracts diluted and re-analyzed.

Quality Assurance

The reference toxicant testing for the lot of fish used in this test was conducted on 12-17-12 (Test #6539). 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 (#6539) was 1.91 µg Cd/L. The LC50 was within the control limits of 0.99 to 2.87 µg Cd/L.

Temperature, pH and dissolved oxygen measurements remained within acceptable limits (USEPA, 2002) throughout the reference toxicant test for rainbow trout (#6539) and sample test (#6535). 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 SolvonK4 is shown in the table below.

Organic analysis of test solutions (SolvonK4)

Nominal Concentration (mg/L)	0 h		48 h Before Renewal		48 h After Renewal		96 h	
	Sample #	Measured (mg/L)	Sample #	Measured (mg/L)	Sample #	Measured (mg/L)	Sample #	Measured (mg/L)
0	L57134-1	--	L57134-9	--	L57134-13	0.320	L57134-17	--
12.5	-2	7.110	-10	1.750	-14	6.830	-18	1.470
25	-3	15.200	-11	4.360	-15	13.300	-19	3.820
50	-4	30.900	-12	7.270	-16	23.500	-20	7.050
100	-5	67.500	-7*	38.200				
200	-6	135.000	-8*	41.700				

*Taken at 24 h (mortalities were removed on Day 0 after death)

The 0-hour measured concentration of SolvonK4 was in the range of 57 – 68% of the nominal concentration. Possibly a reflection of the low solubility and volatile nature of SolvonK4 in water. Through the first 48 hours the gradual decline over time of the measured concentration is apparent.

Solution renewal at 48 hours restored measured concentrations in the test jars to nearly the levels found at 0 hours. By 96 hours the measured concentrations in the test jars were again in the range found at 48-hours before test solution renewal.

It appears that a slight contamination of SolvonK4 occurred in the control (0.320 mg/L) at the 48-hour renewal. This did not affect the outcome of the test as there was 100% survival in the control at the end of the test.

Rainbow Trout Survival

The following table contains 24-hour survival percentages for rainbow trout exposed to various concentrations of SolvonK4 during the 96-hour test.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)*	% Survival (2 reps/conc, 10 fish/rep)				% Survival at Test End
		0 h	24 h	48 h	96 h	
0	--	100	100	100	100	100
12.5	7.11	100	100	100	100	100
25	15.2	100	100	100	100	100
50	30.9	100	100	100	100	100
100	67.5	0 ^a	--	--	--	0
200	135.0	0 ^b	--	--	--	0

*Taken at 0 hours

^a all dead by 1230 h on 12/13/12

^b all dead by 1120 h on 12/13/12

As the table above shows for the SolvonK4 sample SW072012-19-P01 there was 100% survival in the control, 12.5, 25, and 50 mg/L nominal test concentrations at the end of the 96 hour test. At a nominal concentration of 100 mg/L all fish were dead within 2 hours of the start of the test. At a nominal concentration of 200 mg/L all fish were dead within 50 minutes of test initiation.

The LC50 (binomial-graphical method) for SolvonK4 based on the measured concentrations taken at 0 hours at each test concentration was 45.7 mg/L. The measured concentration of SolvonK4 at each test concentration was used to calculate the LC50 because of the difference in comparison to the nominal concentration.

Water Quality

The following table contains measurements of Temperature, pH and Dissolved Oxygen taken throughout the 96 h test (or up to the time of 100% mortality). 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 (unless otherwise noted).

Parameter		0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L ^a	200 mg/L ^a
Temperature (°C)	Mean	11.9	11.8	11.8	12.0	11.8	11.7
	Min.	11.3	11.3	11.1	11.7	11.6	11.1
	Max.	12.1	12.2	12.1	12.3	12.1	12.0
pH	Mean	7.80	7.94	7.98	8.02	8.17	8.21
	Min.	7.72	7.85	7.89	7.93	8.14	8.19
	Max.	8.06	8.15	8.14	8.18	8.19	8.22
D.O. (mg/L)	Mean	9.3	9.6	9.9	9.9	10.0	9.9
	Min.	8.6	9.3	9.6	9.5	9.6	9.5
	Max.	10.1	9.9	10.1	10.3	10.4	10.4
Tot. Hard (mg/L as CaCO ₃)	0h	91				--	87
	96h	92				93	--
Tot. Alk (mg/L as CaCO ₃)	0h	72				--	72
	96h	74				74	--
Cond (µmhos/cm)	0h	231				230	228
	96h	234				230 ^a	232 ^b

^a Taken at 0 and 24 hours

^b Taken on Day 0, 1733 h

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.

Washington State Department of Ecology. Biological Testing Methods for the designation of Dangerous Waste. DOE 80-12, revised June 2009.

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

3/2/20

Phone # 206.268.8499 Fax #

206.268.8491

REMARKS

Fish Biology

PO#

Rush charges authorized by

☐ Will call with instructions

SIGNATURE	PRINT NAME	COMPANY	DATE	TIME
Relinquished by: <i>S.G. Whittaker</i>	<i>Steve Whittaker</i>	<i>RH-SKC</i>	<i>7/20/12</i>	<i>2:25 PM</i>
Received by: <i>[Signature]</i>	<i>Lynne Cox</i>	<i>KCEL</i>	<i>7/20/12</i>	<i>1425</i>
Relinquished by:				
Received by:				

Fax (206) 283-5044

SolvonK4 96-Hour Acute Static Renewal Test Proj 421193
Rainbow Trout

Test #: 6535
 Test Date: 12-13-12

ORGANISMS

650 fish received from Trout Lodge Lot # (Swim-up date): 11-21-12 Shipped via Pick up Arrived at KCEL at 1340 h on 11-29-12 in 1 Box double plastic Bag.
0 dead removed. At Arrival: pH —, D.O. 7.20 mg/L, Temp 11.0 °C. Into Tank # 1 Hold in tank with new well water and aeration for — days. Feed 2X/day with —.
Ziegler's #1. Refer to culture log for feeding & holding information.

DILUTION WATER/TOXICANT

1. New Well Water (NWW) 12-12-12, filtered through nylon netting. Hardness should be between 80-100 mg/L. At start TH ≈ 100 mg/L. Dilute 10% w/ MilliQ DI.
2. Solvon K4: Sample # SW072012-19-PT Collected on 7-20-12 By Steve Whittaker
 Rec'd by KCEL 7-20-12 Stored in the dark at $4 \pm 2^\circ\text{C}$

SOLUTIONS					
Code	Sample Conc (mg/L)	NWW (L/jar)	g SolvonK4/jar		
Blue	0	6 L (NWW only)	0 *		
Green	12.5	↓	0.075		
Yellow	25	↓	0.15		
Orange	50	↓	0.3		
Red	100	↓	0.6		
White	200	↓	1.2*		

Day 0-1: Add 7 L NWW for control. White add 1.4 g SolvonK4/7 L NWW. Extra needed for WQ. On Day 0 siphon test chamber down to 6 L.

PROCEDURE

1. On day 0-1: Add 6 L NWW (dilute with DW if necessary) to each of 2 jars/trtmt. Add Solvon K4 as indicated.
2. Place jars randomly on mag mix in 12°C EC # 8555, East & West shelf. Seal jars with lid and gasket. * Let mix overnight. Start mixing at 1015 h on 12-12-12. EC set pt 11.4°C. mag mix heats up soln.
3. On Day 0: Remove lid and gasket. Stop mixer and let stand 1 minute. Take 0 h organics sample. Take 0h sample for pH, DO, Temp, Tot. Alk, Tot. Hard, Cond. End mixing 0845 - 0915 on 12-13-12
4. Add 10 fish/jar, one at a time to randomize, using dip net. Start count verified by ky & —.
5. Start test at 1030 h on 12-13-12. Place Tidbit temp recorder (SN 9716077, East shelf; SN 9716078, West shelf) in beaker w/WW into EC. 8556
6. Remove dead fish daily; record #/ weight/ length/ time dead. Record survival daily. Measure Temp, pH & DO daily in all trtmts.
7. At 24 hours prep renewal solutions as in step 1 above. Place jars on mag mix in 12°C EC 8555. Seal jars with lid and gasket. Let mix overnight. Setup at — h on —. seal lip with plastic
8. Renew solutions ($\approx 80\%$) at 48h: start mixing 1015/12/14/12 Packaging wrap
 a) Siphon 4.8 L from each jar.
 b) Replace 6 L with renewal solution by pouring using 1L bucket through funnel and tubing into jar. ky
9. End test at 1000 h on 12-17-12. Measure Temp, pH and DO in all trtmts. Sample for Tot. Alk, Tot. Hard, Cond.

* sealed lip with plastic packing wrap.

SolvonK4 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6535
 Test Date: 12-13-12

SolvonK4 Sampling

1. Sample Solvon K4 solution from each test jar at 0, 24, 48, 72 and 96 h. Place sample in 40 mL glass vial with septa cap.

0 hour sampled at 0845
 24 hour sampled at 1045
 48 hour sampled at 0600 (Before Renewal) 0650-0635 (after Renewal)
 72 hour sampled at 0820
 96 hour sampled at 0945

MEASUREMENTS

Code	Cumulative Survival (#Alive/Rep)						Tot # Alive
	Sample Conc (mg/L)	Rep	24 h	48 h	72 h	96 h	
Blue	0	A	10	10	10	10	
		B	10	10	10	10	
Green	12.5	A	10	10	10	10	
		B	10	10	10	10	
Yellow	25	A	10	10	10	10	
		B	10	10	10	10	
Orange	50	A	10	10	10	10	
		B	10	10	10	10	
Red	100	A	0	0	0	0	0
		B	0	0	0	0	0
White	200	A	0	0	0	0	0
		B	0	0	0	0	0
		Analyst:	Gy	Gy	Gy	Gy	

s = stressed

Code	Rep		Daily #Dead/Rep										Mean
			1	2	3	4	5	6	7	8	9	10	
white	A	Date	12-13										
		Time	1120										
		cm	3.1	3.2	3.0	2.9	3.0	3.3	2.9	2.9	3.0	3.0	
		g	0.341	0.387	0.279	0.234	0.277	0.398	0.223	0.207	0.241	0.258	
white	B	Date	12-13										
		Time	1120										
Red	A	Date	12-13										
		Time	1733h										
Red	B	Date	12-13										
		Time	1733h										
		Date											
		Time											
		Date											
		Time											

Day 0 12-13-12 1035 Nearly all Dead white A/B

SolvonK4 96-Hour Acute Static Renewal Test Rainbow Trout

Test #: 6535
Test Date: 12-13-12

[illegible]

$$\text{Load Rate} = [(\text{Wt})(\# \text{ Fish})] / \text{Vol} = (0.284 \text{ g})(10) / 6 \text{ L} = 0.47 \text{ g/L}$$

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

Chemistry

[illegible]

SolvonK4 96-Hour Acute Static Renewal Test
Rainbow Trout

Test #: 6535
 Test Date: 12-13-12

Code	Sample Conc (mg/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	57125-1	-3	71.5	74.4	91	92.1	231	234
Green	12.5							-	-
Yellow	25							-	-
Orange	50							-	-
Red	100							230	*230
White	200	-2	-4	71.9	73.8	86.8	93.3	228	*232
-4 taken at 24h on Red								Analyst:	JA JA

*Day 0, 1733h
 *24h

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

NOTES

withhold feed 48h prior to test start

West

Green A, B
 Blue B
 Red B
 Yellow A
 Orange A

East

Blue A
 Yellow B
 Orange B
 Red A
 White A/B

Day 0 @ 1733h, "orange" trtmt fish at surface, darker color.
 "Red" trtmt fish all dead Dead at 1230

Solvon K4 sampling

product organic	Sample #	0h	48h Before Renew	48h After Renew	96h
Blue	57134-1	-9	-13	-17	
Green	-2	-10	-14	-18	
Yellow	-3	-11	-15	-19	
Orange	-4	-12	-16	-20	
Red	-5	-7	-	-	
White	-6	-8	-	-	

sampled Rep A
 Turned into organics

-19, -20 Sampled for organics Dup, however, taken at 1120h about 1.5h after 1st sample taken.
 -7, -8 taken at 24h

CETIS Analytical Report

 Report Date: 09 Jan-13 14:39 (p 1 of 2)
 Test Code: 6535RTASK4 | 18-1716-6639

Fish 96-h Acute Survival Test				King County Metro Services, WQ Lab	
Analysis ID:	15-3613-2587	Endpoint:	96h Survival Rate	CETIS Version:	CETISv1.8.6
Analyzed:	09 Jan-13 14:35	Analysis:	Binomial Method	Official Results:	Yes
Batch ID:	03-0533-3864	Test Type:	Survival (96h)	Analyst:	GY
Start Date:	13 Dec-12 10:30	Protocol:	EPA/821/R-02-012 (2002)	Diluent:	Well Water
Ending Date:	17 Dec-12 10:00	Species:	Oncorhynchus mykiss	Brine:	Not Applicable
Duration:	95h	Source:	Trout Lodge Fish Farm	Age:	22
Sample ID:	04-8442-1450	Code:	1CDFAF4A	Client:	Internal Lab
Sample Date:	20 Jul-12	Material:	Other sample type	Project:	Special Studies
Receive Date:	20 Jul-12 14:25	Source:			
Sample Age:	146d 10h	Station:			
Batch Note:	NWW diluted 10% with Milli-Q DI				

Binomial/Graphical Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.66	0	45.67	38.35	54.39

Test Acceptability Criteria

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

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/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
7.11		2	1	1	1	0	0	0.0%	0.0%	20	20
15.2		2	1	1	1	0	0	0.0%	0.0%	20	20
30.9		2	1	1	1	0	0	0.0%	0.0%	20	20
67.5		2	0	0	0	0	0		100.0%	0	20
135		2	0	0	0	0	0		100.0%	0	20

96h Survival Rate Detail

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	1	1
7.11		1	1
15.2		1	1
30.9		1	1
67.5		0	0
135		0	0

96h Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2
0	Dilution Water	10/10	10/10
7.11		10/10	10/10
15.2		10/10	10/10
30.9		10/10	10/10
67.5		0/10	0/10
135		0/10	0/10

CETIS Analytical Report

Report Date: 09 Jan-13 14:39 (p 2 of 2)
Test Code: 6535RTASK4 | 18-1716-6639

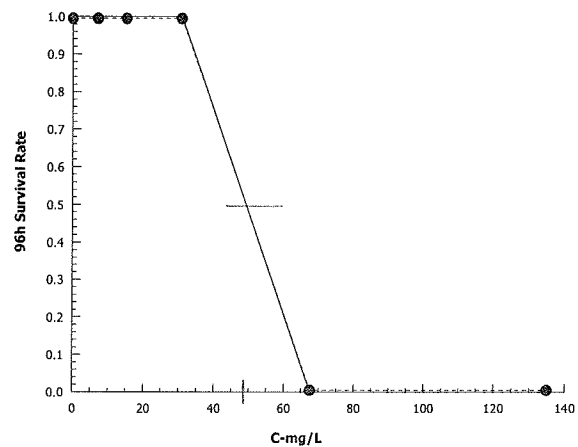
Fish 96-h Acute Survival Test

King County Metro Services, WQ Lab

Analysis ID: 15-3613-2587 Endpoint: 96h Survival Rate
Analyzed: 09 Jan-13 14:35 Analysis: Binomial Method

CETIS Version: CETISv1.8.6
Official Results: Yes

Graphics



King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C 8270D Dibutoxymethane	Project: 421193 Locator: LAB Descrip: LAB LOCATOR Sample: L57134-1 Matrix: LK FRESH WTR ColDate: 12/14/12 0:00 TimeSpan: TotalSolid: ClientLoc: SampDepth: WET Weight Basis	Project: 421193 Locator: LAB Descrip: LAB LOCATOR Sample: L57134-2 Matrix: LK FRESH WTR ColDate: 12/14/12 0:00 TimeSpan: TotalSolid: ClientLoc: SampDepth: WET Weight Basis	Project: 421193 Locator: LAB Descrip: LAB LOCATOR Sample: L57134-3 Matrix: LK FRESH WTR ColDate: 12/14/12 0:00 TimeSpan: TotalSolid: ClientLoc: SampDepth: WET Weight Basis
	Value	Value	Value
	Qual	Qual	Qual
	MDL	MDL	MDL
	RDL	RDL	RDL
	Units	Units	Units
	<MDL	7110	15200
	240	240	1200
	476	476	2380
	ug/L	ug/L	ug/L

King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C 8270D Dibutoxymethane	Project:	421193	Project:	421193	Project:	421193					
	Locator:	LAB	Locator:	LAB	Locator:	LAB					
	Descrp:	LAB LOCATOR	Descrp:	LAB LOCATOR	Descrp:	LAB LOCATOR					
	Sample:	L57134-4	Sample:	L57134-5	Sample:	L57134-6					
	Matrix:	LK FRESH WTR	Matrix:	LK FRESH WTR	Matrix:	LK FRESH WTR					
	ColDate:	12/14/12 0:00	ColDate:	12/14/12 0:00	ColDate:	12/14/12 0:00					
	TimeSpan:		TimeSpan:		TimeSpan:						
	TotalSolid:		TotalSolid:		TotalSolid:						
	ClientLoc:		ClientLoc:		ClientLoc:						
	SampDepth:		SampDepth:		SampDepth:						
WET Weight Basis			WET Weight Basis			WET Weight Basis					
	Value	Qual	MDL	RDL	Units		Value	Qual	MDL	RDL	Units
	30900		1200	2380	ug/L		67500		2400	4760	ug/L
									</		

King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C-8270D Dibutoxymethane	Project:	421193																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C 8270D Dibutylmethane	Project: 421193															Project: 421193				
	Locator: LAB															Locator: LAB				
	Descrip: LAB LOCATOR															Descrip: LAB LOCATOR				
	Sample: L57134-10															Sample: L57134-12				
	Matrix: LK FRESH WTR															Matrix: LK FRESH WTR				
	ColDate: 12/14/12 0:00															ColDate: 12/14/12 0:00				
	TimeSpan:															TimeSpan:				
	TotalSolid:															TotalSolid:				
	ClientLoc:															ClientLoc:				
	SampDepth:															SampDepth:				
WET Weight Basis															WET Weight Basis					
Value															Value					
Qual															Qual					
MDL															MDL					
RDL															RDL					
Units															Units					
1750															4360					
240															240					
476															476					
ug/L															ug/L					

King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C 8270D Dibutylmethane	Value	Qual	MDL	RDL	Units
	320	<RDL	240	476	ug/L
	Project: 421193				
	Location: LAB				
	Descrip: LAB LOCATOR				
	Sample: L57134-13				
	Matrix: LK FRESH WTR				
	ColDate: 12/14/12 0:00				
	TimeSpan:				
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
RDL					
Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-14					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
RDL					
Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
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Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
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WET Weight Basis					
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Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
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WET Weight Basis					
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Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
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Project: 421193					
Location: LAB					
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Project: 421193					
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Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
RDL					
Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
RDL					
Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					
Sample: L57134-15					
Matrix: LK FRESH WTR					
ColDate: 12/14/12 0:00					
TimeSpan:					
TotalSolid:					
ClientLoc:					
SampDepth:					
WET Weight Basis					
Value					
Qual					
MDL					
RDL					
Units					
Project: 421193					
Location: LAB					
Descrip: LAB LOCATOR					

King County Environmental Lab Analytical Report

Parameters OR EPA 625/SW846 3520C 8270D Dibutoxymethane	Project: 421193				Project: 421193				Project: 421193			
	Locator: LAB				Locator: LAB				Locator: LAB			
	Descrip: LAB LOCATOR				Descrip: LAB LOCATOR				Descrip: LAB LOCATOR			
	Sample: L57134-16				Sample: L57134-17				Sample: L57134-18			
	Matrix: LK FRESH WTR				Matrix: LK FRESH WTR				Matrix: LK FRESH WTR			
	ColDate: 12/14/12 0:00				ColDate: 12/14/12 0:00				ColDate: 12/14/12 0:00			
	TimeSpan:				TimeSpan:				TimeSpan:			
	TotalSolid:				TotalSolid:				TotalSolid:			
	ClientLoc:				ClientLoc:				ClientLoc:			
	SampDepth:				SampDepth:				SampDepth:			
WET Weight Basis				WET Weight Basis				WET Weight Basis				
Value				Value				Value				
23500				<MDL				1470				
MDL				MDL				MDL				
1200				240				240				
RDL				RDL				RDL				
2380				476				476				
Units				ug/L				ug/L				
Qual				Qual				Qual				
<MDL				<MDL				<MDL				
MDL				MDL				MDL				
240				240				240				
RDL				RDL				RDL				
476				476				476				
Units				ug/L				ug/L				

King County Environmental Lab Analytical Report

Project: 421193		Project: 421193	
Locator: LAB		Locator: LAB	
Descrip: LAB LOCATOR		Descrip: LAB LOCATOR	
Sample: L57134-19		Sample: L57134-20	
Matrix: LK FRESH WTR		Matrix: LK FRESH WTR	
ColDate: 12/14/12 0:00		ColDate: 12/14/12 0:00	
TimeSpan:		TimeSpan:	
TotalSolid:		TotalSolid:	
ClientLoc:		ClientLoc:	
SampDepth:		SampDepth:	
WET Weight Basis		WET Weight Basis	
Value		Value	
3820		7050	
Parameters	MDL	RDL	Units
OR EPA 625/SW846 3520C 8270D	240	476	ug/L
Dibutylmethane	240	476	ug/L

King County Environmental Laboratory
LIMSView QC Report - 10/04/13 12:13

Workgroup: WG124688 (BL#85 orgmisc) Run ID: R185616

MB:WG124688-1 Matrix: BLANK WTR Listtype:ORORGMISC Method:EPA 625/SW846 3520C 8270D Project: Pkey:STD
(Method Blank)

Parameter	MDL	RDL	Units	MB Value	Qual
Dibutoxymethane	10	20	ug/L	<MDL	

LD:WG124688-4 L57134-19 Matrix: FRESH WTR Listtype:ORORGMISC Method:EPA 625/SW846 3520C 8270D Project:421193 Pkey:STD
(Lab Duplicate)

Parameter	MDL	RDL	Units	SAMP Value	LD Value	RPD	Qual	LabLimit
Dibutoxymethane	240	476	ug/L	3820	3500	9		0--40

LD:WG124688-5 L57134-20 Matrix: FRESH WTR Listtype:ORORGMISC Method:EPA 625/SW846 3520C 8270D Project:421193 Pkey:STD
(Lab Duplicate)

Parameter	MDL	RDL	Units	SAMP Value	LD Value	RPD	Qual	LabLimit
Dibutoxymethane	240	476	ug/L	7050	6880	3		0--40

Surrogate: (Lab Limits)	2,4,6-Tribromophenol 35--150	2-Fluorobiphenyl 30--120	2-Fluorophenol 27--126	d14-Terphenyl 56--150	d4-2-Chlorophenol 36--117	d5-Nitrobenzene 33--129
L57134-1	106	108	84	103	94	95
L57134-2	108	111	87	109	96	102
L57134-3	104	108	83	103	94	99
L57134-4	105	104	81	100	90	98
L57134-5	111	106	85	104	95	101
L57134-6	110	107	89	112	96	102
L57134-7	112	108	84	107	93	100
L57134-8	110	107	85	106	95	100
L57134-9	105	104	85	104	92	98
L57134-10	106	106	86	107	95	101
L57134-11	108	105	85	105	93	102
L57134-12	112	110	87	110	97	98
L57134-13	118	110	90	115	99	103
L57134-14	107	107	88	112	97	100
L57134-15	111	111	88	115	99	103
L57134-16	110	105	84	107	96	99
L57134-17	107	107	86	108	97	103

L57134-18	117	113	91	116	101	104
L57134-19	115	115	91	111	102	109
L57134-20	118	112	91	109	100	106
WG124688-1	96	105	83	101	91	95
WG124688-4	107	105	86	100	91	98
WG124688-5	111	107	84	99	94	99

Reference Toxicant Test:

Bench Sheets

Precision Table

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

Test #: 6539
Test Date: 12-17-12

ORGANISMS

650 fish received from Treat Lodge Lot # (Swim-up date): 11-21-12 Shipped via Pick up Arrived at KCEL at 1340 h on 11-29-12 in 1 Box double plastic bag.
0 dead removed. At Arrival: pH -, D.O. > 20 mg/L, Temp 11.0 °C. Into Tank # 1 Hold in tank with new well water and aeration for 18 days. Feed 2X/day with Feiglers Salmon starter. Refer to culture log for feeding & holding information. Age at testing: 26d post swim-up

DILUTION WATER/TOXICANT

1. New Well Water (NWW) 12-16-12 (2L milliQ + 18L WW) ← (diluted 10% with milliQ), filtered through nylon netting.
2. Cd Stock Soln: Nominal 20 mg Cd/L, Measured 21 mg/L on 3-13-08 Prep 080228
1 by add - g Cd(NO₃)₂•4H₂O (mfr - # -), rec'd -, opened -, lot # -) ⊆ 1L DW.
LIMS RTA Sample #: WG-124702-1 Wkgrp #: WG-124702

SOLUTIONS

Cd Trtmt (µg/L)	Code	Cd Stock (mL/jar)	NWW (L/jar)	Sample #	Cd (µg/L) (Measured)
0	Blue	0 (NWW only)	6 L (NWW only)		
0.75	Green	0.214	⊆ 6L		
1.5	Yellow	0.428	↓		
3.0	Orange	0.856	↓	* L57271-1	2.96
6.0	Red	1.712	↓		
12.0	White	3.424	↓		

PROCEDURE

- Add 6 L NWW to each of 2 jars/trtmt; place in 12°C EC # 8555, East & West shelf. Bring to 12°C. Setup at - h. set up on 12-16-12
- Measure DO; if DO << saturation, aerate until DO ≥ 9 mg/L. Stop aeration.
- Measure Temp, pH & DO. in all trtmts.
- Add Cd stock soln to jars: ✓ Mix: ✓ Sample for Cd: ✓ Acidify: ✓ Analyst: Gy
- Add 10 fish/jar, one at a time to randomize, using dip net. Start count verified by Gy & -.
- Start test at 1320 h on 12-17-12. Place Tidbit temp recorder (SN 9716078, West shelf; SN 9716077, East shelf) in beaker w/WW into EC.
- Remove dead fish daily; record #/ weight/ length/ time dead. Record survival daily. Measure Temp, pH & DO daily in all trtmts.
- Renew solns (≈ 80%) at 48h: ☒ JA
 - Siphon 4.8 L from each jar.
 - Filter NWW into 4L graduated cylinder.
 - 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	0.143	0.285	0.571	1.141	2.283

d) Replace ⊆ 6 L/jar with fresh soln by pouring through funnel and tubing into jar.

9. End test at 1420 h on 12-17-12. Measure Temp, pH and DO in all trtmts.

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

Test #: 6539
Test Date: 12-17-12

MEASUREMENTS

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	10	10	7	7	7
	1.5	B	10	10	9	9	9
Orange	3	A	9	3	0	0	0
	3	B	10	5	2	1	1
Red	6	A	7	1	0	0	0
	6	B	5	0	0	0	0
White	12	A	8	1	0	0	0
	12	B	6	0	0	0	0
		Analyst:	JA	JA	JA	JA	

s = stressed

Code	Rep		Daily #Dead/Rep										Mean
			1	2	3	4	5	6	7	8	9	10	
White	B	Date	12-18-12	12-18-12	12-18-12	12-18-12	12-19	—	—	—	—	→	
		Time	1045	1045	1045	1045	1030	—	—	—	—	→	
		cm	3.5	3.5 + 3.1 JA	3.0	3.2	2.5	2.7	2.7	3.1	2.8	2.9	2.95
		g	0.546	0.463	0.375	0.426	0.268	0.286	0.280	0.393	0.297	0.332	0.367
White	A	Date	12-18	12-18	12-19	—	—	—	—	—	→	12-20	
		Time	1045	1045	1030	—	—	—	—	—	→	1015	
Red	B	Date	12-18	12-18	12-18	12-18	12-18	12-19	—	—	—	→	
		Time	1045	1045	1045	1045	1045	1030	—	—	—	→	
Red	A	Date	12-18	12-18	12-18	12-19	—	—	—	—	→	12-20	
		Time	1045	1045	1045	1030	—	—	—	—	→	1015	
Orange	A	Date	12-18	12-19	—	—	—	—	→	12-20	12-20	12-20	
		Time	1045	1030	—	—	—	—	→	1015	1015	1015	
Orange	B	Date	12-19	—	—	—	→	12-20	12-20	12-20	10-21		
		Time	1030	—	—	—	→	1015	1015	1015	1420		
Yellow	A	Date	12-20	12-20	12-20								
		Time	1015	1015	1015								
Yellow	B	Date	12-20										
		Time	1015										
		Date											
		Time											
		Date											
		Time											

$$\text{Load Rate} = [(\text{Wt})(\# \text{ Fish})] / \text{Vol} = (0.367 \text{ g})(10) / 6 \text{ L} = 0.61 \text{ g/L}$$

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 #: 6539
Test Date: 12-17-12

Chemistry

Code	Rep	Temp (°C) SN: 342302					pH					D.O. (mg/L)				
		0h	24h	48h	72h	96h	0h	24h	48h	72h	96h	0h	24h	48h	72h	96h
Blue	A	11.5	11.5	11.7	11.7	11.7	8.060	7.727	7.799	7.687	7.605	9.7	8.8	8.9	8.5	8.6
	B	12.1	12.0	12.0	12.0	12.0	8.136	7.846	7.907	7.793	7.687	9.9	8.8	9.0	8.6	8.7
Grn	A	12.1	12.1	12.2	12.2	12.1	8.171	7.859	7.911	7.806	7.743	10.0	8.9	9.0	8.7	8.8
	B	11.1	11.7	11.4	11.5	11.6	8.183	7.800	7.927	7.745	7.692	10.0	8.7	9.0	8.5	9.0
Yell	A	11.7	12.0	11.9	11.9	11.9	8.201	7.885	7.994	7.853	7.757	10.1	8.9	9.1	8.9	9.2
	B	11.7	11.8	11.6	11.7	11.7	8.203	7.804	7.923	7.796	7.713	10.0	8.7	9.0	8.6	8.9
Orng	A	11.9	12.1	11.8	11.6	11.7	8.191	7.805	7.915	8.021	—	10.1	8.7	9.0	9.4	—
	B	12.1	11.9	11.7	11.7	11.6	8.187	7.893	7.941	7.997	7.964	10.1	8.9	9.1	9.4	9.8
Red	A	12.1	12.1	11.9	12.0	—	8.200	7.822	7.976	8.114	—	9.9	8.7	9.1	9.6	—
	B	12.2	12.1	12.1	—	—	8.193	7.869	8.085	—	—	9.9	8.8	9.3	—	—
Wht	A	11.6	11.6	11.6	11.4	—	8.193	7.795	8.046	8.127	—	9.9	8.7	9.2	9.7	—
	B	12.0	12.1	12.1	—	—	8.189	7.797	8.075	—	—	9.9	8.7	9.2	—	—
Analyst: Gy		JA	JA	JA	JA	JA	Gy	JA	JA	JA	JA	Gy	JA	JA	JA	JA

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

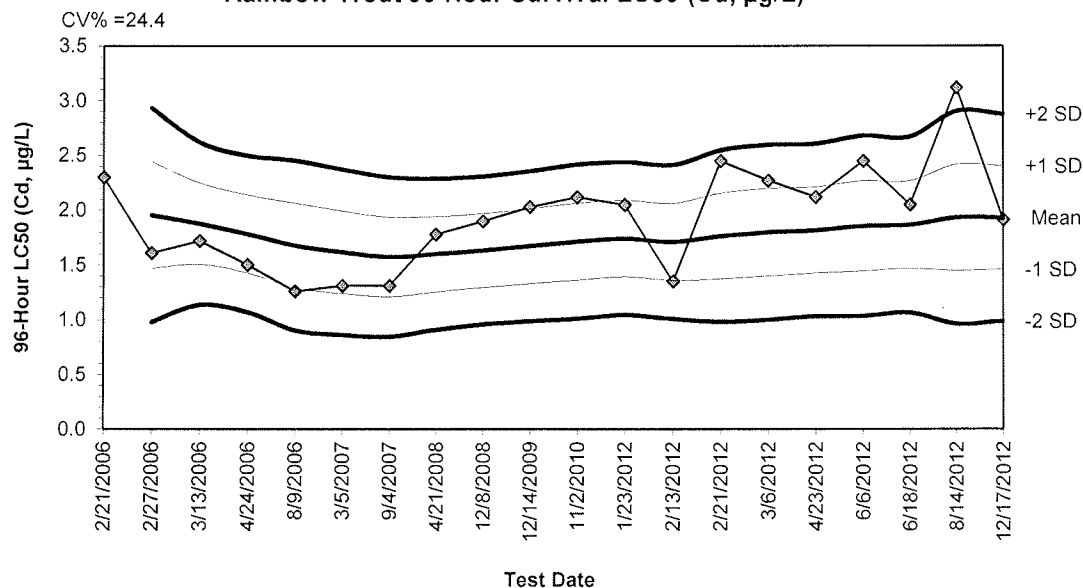
NOTES

This test meets all QC acceptance criteria. Ja 12-27-12

Table 3 of 3

PA = Probit Analysis
MA = Moving Average
(T)SK = (Trimmed) Spearman Karber
GI = Graphical Interpolation
RW = Reconstituted Water
WW = Well Water
* = Value Outside Control Limits

**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
2/21/2006	2.30					
2/27/2006	1.61	1.9550	1.4671	0.9792	2.4429	2.9308
3/13/2006	1.72	1.8767	1.5059	1.1352	2.2474	2.6181
4/24/2006	1.50	1.7825	1.4260	1.0695	2.1390	2.4955
8/9/2006	1.26	1.6780	1.2908	0.9036	2.0652	2.4524
3/5/2007	1.31	1.6167	1.2392	0.8617	1.9942	2.3717
9/4/2007	1.31	1.5729	1.2093	0.8457	1.9364	2.3000
4/21/2008	1.78	1.5988	1.2543	0.9098	1.9432	2.2877
12/8/2008	1.90	1.6322	1.2947	0.9572	1.9697	2.3073
12/14/2009	2.03	1.6720	1.3298	0.9876	2.0142	2.3564
11/2/2010	2.12	1.7127	1.3611	1.0095	2.0643	2.4159
1/23/2012	2.05	1.7408	1.3917	1.0427	2.0899	2.4390
2/13/2012	1.35	1.7108	1.3594	1.0080	2.0621	2.4135
2/21/2012	2.45	1.7636	1.3724	0.9813	2.1547	2.5459
3/6/2012	2.27	1.7973	1.3984	0.9994	2.1963	2.5952
4/23/2012	2.12	1.8175	1.4237	1.0299	2.2113	2.6051
6/6/2012	2.45	1.8547	1.4437	1.0328	2.2657	2.6767
6/18/2012	2.05	1.8656	1.4642	1.0628	2.2669	2.6683
8/14/2012	3.12	1.9316	1.4469	0.9621	2.4163	2.9010
12/17/2012	1.91	1.9305	1.4587	0.9869	2.4023	2.8741