

# **Joint Exhibit 51**

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

**Data Requirement:** EPA DP Barcode 420905  
EPA MRID 49307520  
EPA Guideline Non-guideline (OECD 215)

**Test material:** Chlorthal-dimethyl **Purity:** 98.3%  
**Common name:** DCPA  
**Chemical name:** IUPAC: 2,3,5,6-tetrachloroterephthalate-1,4-dicarboxylate  
CAS name: 1,4-dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate  
CAS No.: 1861-32-1  
Synonyms: chlorthal


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Environmental Scientist, CDM Smith, Inc.

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**Date:** 07/31/16

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Senior Science Advisor, EPA/OPP/EFED/ERB 4

**Signature:**   
**Date:** 02/17/2019

**EPA PC Code** 078701

**Date Evaluation Completed:** 02/17/2019

**CITATION:** Manson, P.S. 2004. Chlorthal-dimethyl (DCPA): Prolonged toxicity test to juveniles *Oncorhynchus mykiss* under semi-static conditions. Unpublished study performed by Covance Laboratories Ltd., Harrogate, North Yorkshire, England. Laboratory Study No. 1708/034-D2149. Study sponsored by AMVAC Chemical UK Ltd., Guildford, Surrey, England. Study initiated September 19, 2003 and submitted January 29, 2014.

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## EXECUTIVE SUMMARY:

The 28-day toxicity of technical grade chlorthal-dimethyl (DCPA; 98.3% active ingredient; a.i.) to juvenile rainbow trout (*Oncorhynchus mykiss*) was studied under static-renewal conditions. Fingerling rainbow trout (ca. 5 cm) were exposed to nominal concentrations of 0.031, 0.098, 0.313, 1.0 and 3.2 mg ai/L, plus a negative and solvent (dimethylformamide; DMF; 0.1 mL/L) control. There was a single aquarium containing 10 fish in the control and DCPA treatments. Concentrations decreased between renewal periods (3-day renewal period). Reviewer-calculated mean-measured concentrations were 0.0126, 0.0128, 0.0341, 0.138, and 0.373 mg ai/L based on filtered samples, representing 41, 13, 11, 14% and 12% of nominal, respectively. In unfiltered water samples, mean-measured concentrations were 0.0251, 0.0655, 0.205, 0.546, and 1.39 mg ai/L, representing 81, 67, 65, 55 and 43% of nominal.

A single fish from the mean-measured 0.138 mg ai/L treatment level was euthanized *in extremis* on Day 20. No other mortality occurred during the study. The 28-day LC<sub>50</sub> was >0.373 mg ai/L. From Days 4 through 28, the study authors noted mild to moderate clinical signs of toxicity (*i.e.*, increased cough frequency, swimming abnormally and/or lying on the bottom of the tank) in a maximum of 30, 100 and 100% of fish from the 0.0341, 0.138, and 0.373 mg ai/L treatment levels, respectively.

For the negative control, solvent control, 0.0126, 0.0128, 0.0341, 0.138, and 0.373 mg ai/L treatment levels, tank-average specific growth rates ( $r_2$ ) for length were 1.49, 1.47, 1.49, 1.43, 0.81, 0.39 and 0.40, respectively, and  $r_2$  values for wet weight were 3.80, 3.78, 3.85, 3.41, 1.53, -0.95 and -0.36, respectively. Differences in growth rate were statistically-significant ( $p < 0.05$ ) compared to the control for both fork length and wet weight at the  $\geq 0.0341$  mg ai/L treatment levels. Therefore, following a 28-day exposure of fingerling rainbow trout to technical grade DCPA under static-renewal conditions, the NOAEC and LOAEC for growth (19.5% reduction in Day 28 fork length and 50.8% reduction in 0 – 28 day fork length change; 49.3% reduction in Day 28 wet weight and 70.2% reduction in 0 – 28 day wet weight change), based on individual fish, were 0.0128 and 0.0341 mg ai/L, respectively, using arithmetic mean-measured filtered concentrations. The 28-day EC<sub>50</sub> value for fork length was 0.0617 mg ai/L while the 28-day LC<sub>50</sub> exceeded the highest treatment concentration, *i.e.*, LC<sub>50</sub> >0.373 mg ai/L.

This study is scientifically sound and is classified as supplemental due to the poor recovery (*i.e.*, 11 – 41% of nominal) of DCPA in filtered water samples from each of the DCPA treatments and the lack of replication.

## Results Synopsis

Test Organism Size/Age (mean Weight or Length): fingerling (group means of 4.94 to 5.13 cm and 1.75 to 2.00 g)

Test Type: static-renewal

### IN TERMS OF ARITHMETIC MEAN-MEASURED FILTERED CONCENTRATIONS

LC <sub>50</sub> : >0.373 mg ai/L	95% C.I.: N/A
Slope: N/A	95% C.I.: N/A
EC <sub>50</sub> (wet weight $r_2$ ): Not calculable	95% C.I.: N/A
EC <sub>50</sub> (fork length $r_2$ ): 0.0617 mg ai/L	95% C.I.: 0.0285-0.134 mg ai/L
NOAEC (wet weight $r_3$ ): 0.0128 mg ai/L	
LOAEC (wet weight $r_3$ ): 0.0341 mg ai/L	
NOAEC (fork length $r_3$ ): 0.0128 mg ai/L	
LOAEC (fork length $r_3$ ): 0.0341 mg ai/L	

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## I. MATERIALS AND METHODS

**GUIDELINE(S) FOLLOWED:** The study protocol followed OECD Guideline for Testing of Chemicals, No. 215, "Fish, Juvenile Growth Test" (2000).

This study does not follow any current U.S. EPA OCSP guideline. Notable deviation(s) from OECD Guideline No. 215 included:

- The test design was insufficient to maintain consistent concentrations over each of the renewal periods; recoveries of chlorthal-dimethyl declined to 11 – 41% of nominal in filtered samples) in test media (all levels) between renewal periods.

This deviation affects the classification of this study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the United Kingdom Statutory Instrument No. 3106 (1999), The Good Laboratory Practice Regulations (1999) and the OECD Principles on GLP ENV/MC/CHEM (98) 17 (1998).

### **A. MATERIALS:**

**1. Test Material** Chlorthal-dimethyl (DCPA)

**Description:** Grey powder

**Lot No./Batch No. :** 010616-2

**Purity:** 98.3%

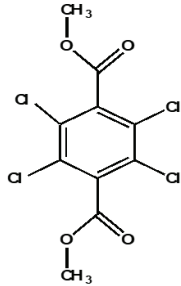
**Stability of compound under test conditions:** Concentrations of chlorthal-dimethyl were determined in freshly-prepared test media on Days 0, 7, 14 and 21 and in aged media on Days 3, 10, 17 and 24. Results demonstrated that concentrations of the test material declined significantly (*ca.* 43 to 81% of nominal in unfiltered water; 11 – 40% of nominal in filtered water) between renewal periods.

**Storage conditions of Test chemicals:** Not reported

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**Physicochemical properties of DCPA.**

Parameter	Values	Comments
Water solubility at 25°C	0.2 to 0.5 mg/L	USEPA 2011 <sup>1</sup>
Vapor pressure	2.5 x 10 <sup>-6</sup> torr	
Structure		
pK <sub>a</sub>	No dissociation constant at pH 2 - 12	
Log K <sub>ow</sub>	4.28 – 4.40	
K <sub>oc</sub>	1,863-3,503 L/kg	

<sup>1</sup>USEPA 2011. Registration Review – Preliminary Problem Formulation for the Ecological Risk Assess of Dimethyl2,3,5,6-Tetrachloroterephthalate (DCPA). DP Barcode D388337.

**2. Test organism:**

<b>Species:</b>	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
<b>Age at test initiation:</b>	Juvenile
<b>Size:</b>	Fingerling; group means (t <sub>0</sub> ) – 4.94 to 5.13 cm and 1.75 to 2.00 g
<b>Source:</b>	Brow Well Fisheries Limited Skipton, North Yorkshire, UK

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: Preliminary solubility assessments using acetone, dimethyl formamide (DMF) and Tween 80 determined that the test material was most soluble in DMF. Following the selection of DMF as the most appropriate carrying agent for preparation of the test media, a further stability trial was conducted to establish the stability of chlorthal-dimethyl in the test media and stock solutions over an extended period. Solvent stocks and test media were prepared at 10 mg/mL and 1.0 mg/mL, respectively, and maintained under test conditions for 72 hours (the longest expected exposure period during the test). Samples collected at 0, 24, 48 and 72 hours demonstrated that the nominal concentrations of chlorthal-dimethyl in DMF solvent were achieved and maintained (range of 8.56 to 9.66 mg/mL), but that in the test medium, some variation was seen in the recoveries reflecting the non-homogenous distribution of particulate (range of 0.611 to 0.926 mg/L). It was reported by the study authors that given the theoretical aqueous solubility of the test material (0.2 to 0.5 mg/L at 25°C), this variation was acceptable.

c. Definitive study:

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**Table 1: Experimental Parameters**

Parameter	Details	Remarks
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	≥14 days Fish were held in flow-through tank under testing conditions The fish were fed a proprietary food at a rate of 1 to 4% of their mean wet weight per day Healthy; mortality <5% over a 7-day period preceding the test	Fish originated from one holding tank (batch number P03/5). Water quality during holding: pH 7.0 to 7.3; hardness 74 to 87 mg/L as CaCO <sub>3</sub> , dissolved oxygen 86 to 94% saturation; temperature 13.2 to 13.6°C; residual chlorine 0.03 to 0.04 mg/L.
Number of organisms in each treatment at test initiation	10 fish per level	
Biomass loading rate	≤1.67 g fish/L (measured on Day 19)	According to OECD guidelines, the loading rate should not typically exceed 1.0 g/fish/L. It was reported that since DO levels were maintained above 60% saturation for the duration of the test, the impact of the high loading rate is considered not to have affected the integrity of the test or the conclusions drawn from the test.
<u>Concentration of test material</u> Nominal: geometric mean-measured (unfiltered): geometric mean-measured (filtered): mean-measured, unfiltered; reviewer-calculated: mean-measured, filtered; reviewer-calculated:	0.031, 0.098, 0.313, 1.0 and 3.2 mg ai/L 0.025, 0.056, 0.184, 0.475 and 1.00 mg ai/L 0.019, 0.018, 0.031, 0.079 and 0.318 mg ai/L 0.0251, 0.0655, 0.205, 0.546 and 1.39 mg ai/L 0.0126, 0.0128, 0.0341, 0.138, and 0.373 mg ai/L	The concentrations of chlorthal-dimethyl were determined in media from each level on Days 0, 7, 14 and 21 (freshly prepared) and on Days 3, 10, 17 and 24 (old test media). Samples from filtered and unfiltered media were analyzed and results were reported in terms of nominal, geometric mean-measured, and arithmetic mean-measured unfiltered and filtered concentrations. Concentrations were unstable between the sampled renewal periods. However, since samples were not collected before and after each renewal period, the reviewer was unable to calculate time-weighted average concentrations.

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Parameter	Details	Remarks
Solvent (type, percentage, if used)	Dimethylformamide (DMF), 0.1 mL/L	2500 µL DMF per 25 L of test media
<u>Number of replicates</u> control: solvent control: treated ones:	1 1 1 per level	
<u>Test condition</u> static renewal/flow-through:  type of dilution system for flow through method:  flow rate:  renewal rate for static renewal:	Static renewal  N/A   Three times per week (every Monday, Wednesday and Friday)	
Aeration, if any	None reported	
Duration of the test	28 days	
<u>Test vessel</u> type/material: (glass/stainless steel)  size:  fill volume:	Glass  30 L  25 L	
Source of dilution water	Mains dechlorinated water	Water quality of the laboratory mains supply (April 2003): alkalinity 33.4 mg/L HCO <sub>3</sub> ; hardness 27 mg/L CaCO <sub>3</sub> ; pH 7.5. Analysis of select metals and pesticides were also provided.

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Parameter	Details	Remarks
<p><u>Water parameters</u>  hardness:  pH:  dissolved oxygen:  temperature:  photoperiod:  other measurements:  interval of water quality measurements:</p>	<p>63 to 87 mg/L as CaCO<sub>3</sub>  7.1 to 7.9  62 to 103% of saturation  12.6 to 15.0°C  16 hour light:8 hour dark  Residual chlorine – ≤0.05 mg/L  The pH, hardness, residual chlorine and dissolved oxygen concentrations of the test media were determined at study initiation and three times per week thereafter – the DO and pH were determined in all media and the total hardness and residual chlorine concentrations were determined in the control medium. The ambient, min and max temperatures of the water were recorded daily in the control and 3.2-mg/L media.</p>	<p>An out-of-range temperature recording of 17.2°C was taken on Day 14. Ambient temperature range recorded using a mercury thermometer at the Day-14 observation time was 14.6°C (old media) and 13.6°C (new media).</p>



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Parameter	Details	Remarks
<u>Feeding</u> type/source of feed: amount given: frequency of feeding:	Fish were fed a proprietary food at a rate between 3 and 4% of the initial mean wet weight per day. The amount of feed was adjusted to take into account fish mortality as it occurred. Uneaten food and debris were cleaned, by siphoning, at least one hour after each feed.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	Mean range of 89.7 to 94.2% of nominal Days 0, 7, 14, 21 and 28 Not specified 0.005 mg ai/L	Based upon recoveries of chlorthal-dimethyl from new and old solvent stock solutions [Days 0, 7, 14 and 21 (freshly prepared) and Days 7, 14, 21 and 28 (old stock solutions)]. The stock solutions were renewed weekly during the test.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

**2. Observations:**

**Table 2: Observations**

Parameters	Details	Remarks
		<i>Criteria</i>
Parameters measured including the sublethal effects/toxicity symptoms	- Mortality and clinical signs of toxicity - Wet weight - Fork length	
Observation intervals:	Fish were observed daily for toxic symptoms. Growth parameters were measured at the beginning and end of the exposure period to assess growth rates.	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

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## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL EFFECTS

A single fish from the nominal 1.0 mg ai/L treatment level was euthanized *in extremis* on Day 20. No other mortality occurred during the study. The 28-day LC<sub>50</sub> was >3.2 mg/L, the highest nominal exposure concentration tested (equivalent to >1.00 and >0.318 mg ai/L for unfiltered and filtered geometric mean-measured media concentrations, respectively).

Beginning on Day 4 and continuing through Day 28, the study author reported “mild to moderate” clinical signs of toxicity in a maximum of 30, 100 and 100% of fish from the 0.313, 1.0 and 3.2 mg ai/L treatment levels, respectively. Effects included increased cough frequency, swimming abnormally and/or lying on the bottom of the tank.

Tank-average specific growth rates ( $r_2$ ) and ‘pseudo’-specific growth rates ( $r_3$ ) for length and wet weight were calculated (see Reported Statistics). For the negative control, solvent (DMF) control, 0.031, 0.098, 0.313, 1.0 and 3.2 mg ai/L treatment levels,  $r_2$  values for wet weight were 3.8045, 3.7763, 3.8490, 3.4084, 1.5337, -0.9480 and -0.3644, respectively (**Table 3**);  $r_2$  values for fork length were 1.4926, 1.4657, 1.4886, 1.4306, 0.8093, 0.3899 and 0.3974, respectively (**Table 4**). Differences in  $r_3$  values were statistically-significant for both weight wet and fork length at the  $\geq 0.313$  mg/L treatment levels. The NOAEC and LOAEC for both length and wet weight growth rates were 0.098 and 0.313 mg ai/L, respectively, using nominal concentrations. In terms of unfiltered geometric mean-measured concentrations, the NOAEC and LOAEC were 0.056 and 0.184 mg ai/L, respectively. In terms of filtered geometric mean-measured concentrations, the NOAEC and LOAEC were 0.018 and 0.031 mg ai/L, respectively.

Using nominal concentrations and calculated  $r_2$  values, the study author calculated 28-day EC<sub>50</sub> (with 95% C.I.) were 0.25 (0.22 to 0.28) mg ai/L for wet weight and 0.66 (0.1 to 12.9) mg ai/L for fork length. In terms of unfiltered mean-measured media concentrations, the 28-day EC<sub>50</sub> (with 95% C.I.) were 0.14 (0.10 to 0.19) mg ai/L for wet weight and 0.31 (0.11 to 0.99) mg ai/L for fork length. In terms of filtered geometric mean-measured media concentrations, the 28-day EC<sub>50</sub> (with 95% C.I.) were 0.03 (0.02 to 0.05) mg ai/L for wet weight and 0.08 (not calculable) mg ai/L for fork length.

**Table 3: Effect of Technical Grade Chlorthal-dimethyl (DCPA) on Wet Weight of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) over 28-Day Exposure Period.**

Treatment Geometric Mean-measured (and Nominal) conc. (mg ai/L)	Mean wet weight at start of exposure (g) $W_1$	Mean wet weight at end of exposure (g) $W_2$	Change in wet weight		Tank-Average Specific Growth Rate ( $r_2$ )
			g	%	
Negative control	1.9232 ± 0.3105	5.6334 ± 1.499	3.7103	192.9	3.8045
Solvent control	1.8170 ± 0.3736	5.3556 ± 1.366	3.5386	194.7	3.7763
0.025 (0.031)	1.8245 ± 0.2910	5.4112 ± 1.091	3.5866	196.6	3.8490
0.056 (0.098)	1.9936 ± 0.459	5.3253 ± 1.799	3.3316	167.1	3.4084

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0.184 (0.313)	1.7483 ± 0.331	2.8545 ± 1.157	1.1062	63.3	1.5337
0.475 (1.0)	1.9799 ± 0.927	1.4526 ± 0.307	-0.5273	-26.6	-0.9480
1.00 (3.2)	1.8632 ± 0.442 (1.8722 ± 0.446)*	1.6794 ± 0.368	-0.1838	-9.9	-0.3644

<sup>(a)</sup> Mean-measured concentrations reported reflect unfiltered sample results.

\* Reviewer-calculated value

**Table 4: Effect of Technical Grade Chlorthal-dimethyl (DCPA) on Fork Length of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) of 28-Day Exposure Period.**

Treatment Geometric Mean-measured (and Nominal) conc. (mg ai/L) <sup>(a)</sup>	Mean length at start of exposure (cm)	Mean length at end of exposure (cm)	Change in mean fork length		Tank-Average Specific Growth Rate (r <sub>2</sub> )
			cm	%	
Negative control	5.13 ± 0.16	7.75 ± 0.60	2.62	51.1	1.4926
Solvent control	5.04 ± 0.28	7.67 ± 0.66	2.63	52.2	1.4657
0.025 (0.031)	4.98 ± 0.21	7.56 ± 0.42	2.58	51.8	1.4886
0.056 (0.098)	5.06 ± 0.35	7.58 ± 0.85	2.52	49.8	1.4306
0.184 (0.313)	4.95 ± 0.19	6.24 ± 0.70	1.29	26.1	0.8093
0.475 (1.0)	4.94 ± 0.19	5.51 ± 0.25	0.57	11.6	0.3899
1.00 (3.2)	5.09 ± 0.34	5.69 ± 0.39	0.60	11.8	0.3974

<sup>(a)</sup> Mean-measured concentrations reported reflect unfiltered sample results.

**B. REPORTED STATISTICS:**

Tank-average specific growth rates (r<sub>2</sub>) and 'pseudo'-specific growth rates (r<sub>3</sub>) for fork length and wet weight were calculated using the following formulae:

$$r_2 = 100 * [\text{mean}(\log_e W_2) - \text{mean}(\log_e W_1)] / (t_2 - t_1)$$

$$r_3 = 100 * [\log_e W_2 - \text{mean}(\log_e W_1)] / (t_2 - t_1)$$

where:

r<sub>2</sub> = tank-average specific growth rate

r<sub>3</sub> = 'pseudo' specific growth rate

W<sub>1</sub>, W<sub>2</sub> = weight/length of a particular fish at times t<sub>1</sub> and t<sub>2</sub> respectively

log<sub>e</sub>W<sub>1</sub> = log of the weight/length of an individual fish at the start of the study period

log<sub>e</sub>W<sub>2</sub> = log of the weight/length of an individual fish at the end of the study period

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$t_1, t_2$  = time (days) at start and end of study period

The tank-average specific growth rates ( $r_2$ ) for weight and length were used to estimate the  $EC_{50}$ . The control was taken as the baseline and a value for each concentration was calculated as a percentage of the control value.

The 'pseudo' specific growth rates ( $r_3$ ) for weight and length were analyzed to estimate the NOAEC using one-way analysis of variance (ANOVA). Levene's test for equality of variances was performed ( $\alpha = 0.01$ ), no evidence of heterogeneity was indicated, and pairwise comparisons of the test concentrations with control were made using Dunnett's test (one-sided, decreasing;  $\alpha = 0.05$ ).

A two-sided two-sample t-test was also performed to compare control with solvent control; no significant differences were indicated (p values = 0.842 and 0.951 for length and weight, respectively). The 28-day  $LC_{50}$  was not calculated due to lack of significant concentration related mortality during the growth test.

## IN TERMS OF NOMINAL CONCENTRATIONS

$LC_{50}$ : >3.2 mg/L	95% C.I.: N/A
$EC_{50}$ (wet weight $r_2$ ): 0.25 mg/L	95% C.I.: 0.22 to 0.28 mg/L
$EC_{50}$ (fork length $r_2$ ): 0.66 mg/L	95% C.I.: 0.1 to 12.9 mg/L
NOAEC (wet weight $r_3$ ): 0.098 mg/L	
LOAEC (wet weight $r_3$ ): 0.313 mg/L	
NOAEC (fork length $r_3$ ): 0.098 mg/L	
LOAEC (fork length $r_3$ ): 0.313 mg/L	

## IN TERMS OF UNFILTERED MEAN-MEASURED CONCENTRATIONS

$LC_{50}$ : >1.00 mg ai/L	95% C.I.: N/A
$EC_{50}$ (wet weight $r_2$ ): 0.14 mg ai/L	95% C.I.: 0.10 to 0.19 mg ai/L
$EC_{50}$ (fork length $r_2$ ): 0.31 mg ai/L	95% C.I.: 0.11 to 0.99 mg ai/L
NOAEC (wet weight $r_3$ ): 0.056 mg ai/L	
LOAEC (wet weight $r_3$ ): 0.184 mg ai/L	
NOAEC (fork length $r_3$ ): 0.056 mg ai/L	
LOAEC (fork length $r_3$ ): 0.184 mg ai/L	

## IN TERMS OF FILTERED MEAN-MEASURED CONCENTRATIONS

$LC_{50}$ : >0.3180 mg ai/L	95% C.I.: N/A
$EC_{50}$ (wet weight $r_2$ ): 0.03 mg ai/L	95% C.I.: 0.02 to 0.05 mg ai/L
$EC_{50}$ (fork length $r_2$ ): 0.08 mg ai/L	95% C.I.: not calculable
NOAEC (wet weight $r_3$ ): 0.018 mg ai/L	
LOAEC (wet weight $r_3$ ): 0.031 mg ai/L	
NOAEC (fork length $r_3$ ): 0.018 mg ai/L	
LOAEC (fork length $r_3$ ): 0.031 mg ai/L	

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## C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer analyzed R<sub>2</sub> and R<sub>3</sub> growth rates based on length and wet weight using CETISTM statistical software (version 1.8.7.12) with database backend settings implemented by EFED on 10/20/15. The R<sub>2</sub> data were used to estimate tank-level growth rates based on total length only using non-linear regression. Due to negative tank-level growth rates (R<sub>2</sub>) based on wet weight, IC<sub>x</sub> values could not be estimated. The pseudo-specific growth rates (R<sub>3</sub>) in the negative and solvent (DMF) controls were compared using a two-sample t-test assuming equal variance. No differences were detected and all subsequent analyses were conducted by comparing treatment data to the negative control only. Data were then tested for normality using the Shapiro-Wilk's test ( $\alpha = 0.01$ ) and for homogeneity of variance using Bartlett's test ( $\alpha = 0.01$ ). Both endpoints met these assumptions and the NOAEC and LOAEC were therefore estimated using analysis of variance (ANOVA) followed by Dunnett's multiple means comparison test and Williams test. All toxicity values were based on the arithmetic mean-measured concentrations for filtered samples.

### IN TERMS OF ARITHMETIC MEAN-MEASURED FILTERED CONCENTRATIONS

LC <sub>50</sub> : >0.373 mg ai/L	95% C.I.: N/A
Slope: N/A	95% C.I.: N/A
EC <sub>50</sub> (wet weight r <sub>2</sub> ): Not calculable	95% C.I.: N/A
EC <sub>50</sub> (fork length r <sub>2</sub> ): 0.0617 mg ai/L	95% C.I.: 0.0285-0.134 mg ai/L
NOAEC (wet weight r <sub>3</sub> ): 0.0128 mg ai/L	
LOAEC (wet weight r <sub>3</sub> ): 0.0341 mg ai/L	
NOAEC (fork length r <sub>3</sub> ): 0.0128 mg ai/L	
LOAEC (fork length r <sub>3</sub> ): 0.0341 mg ai/L	

## D. STUDY DEFICIENCIES:

According to the study report, new test media appeared to be "homogeneous dispersions: while 3-day old test media (*i.e.*, just prior to renewal) appeared to be "non-homogeneous dispersions" with fine particulate materials on the vase of the aquaria. In situations where test material has limited solubility and there is evidence that the compound is not in complete solution, water samples used for analytical verification of exposure should be centrifuged and/or filtered. Based on measured concentrations from filtered samples, exposure concentrations were 0.0126, 0.0128, 0.0341, 0.138, and 0.373mg ai/L representing 41, 13, 11, 14, and 12% of nominal, respectively, indicating that the functional solubility limit of DCPA under the conditions tested was lower than the reported 0.2 to 0.5 mg ai/L values at 25°C even with the DMF (0.1 mL/L) co-solvent. While the study report indicates that measured concentrations of DCPA in freshly prepared were 0.035, 0.094, 0.276, 0.800, and 2.30 mg ai/L representing 113, 96, 88, 80 and 72% of nominal, respectively, measured concentrations and the presence of suspended particulates and a precipitate in the exposure tanks by after three days of static exposure, indicates that the test design was insufficient to maintain stable concentrations; arithmetic mean-measured concentrations of chlorthal-dimethyl declined (recoveries ranging from 11 to 41% on nominal) in filtered test media (all levels) between renewal periods. It is uncertain whether exposure levels in the two lowest treatment levels could be differentiated statistically based on the mean-measured concentrations.

Although the study is consistent with OECD 215, there is no replication in any of the treatments or the controls.

## E. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions were based on the arithmetic mean-measured filtered concentrations and are reported in the Executive Summary and Conclusions sections of this DER.

# Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

EPA MRID Number 49307520

Aqueous media samples were analyzed for chlorthal-dimethyl concentrations using high performance liquid chromatography with UV Detection (HPLC-UV) at 230 nm. The limit of determination was 0.005 mg ai/L.

In both unfiltered and filtered samples of test media, small amounts of chlorthal-dimethyl were detected above the limit of determination (0.005 mg/L) in the control and solvent control samples on Day 21 (new media) and Day 24 (old media). As no adverse effects were observed in the control fish, these two detections were thought to have no effect on the outcome of the study. These values were not included in the statistical analysis.

The first preliminary stability trial was started on October 3, 2003 and the definitive test was completed on November 14, 2003. Chemical analysis was completed on the December 4, 2003.

The OECD Test Guideline 215 is intended to provide information on the effects of the test substance on growth rates and to develop a regression-based model with which to estimate the concentration that would cause an X% variation in in growth rate (*i.e.*, EC<sub>x</sub>). According to the study guideline, data generated from the study can be compared with control values in order to determine the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). However, while there are 10 fish per control and treatment, there is no replication in either the controls or the different treatments. The test guideline indicates the for estimating a NOEC and LOEC using ANOVA, there should “preferably be replicate tanks at each concentration, and statistical analysis should be at the tank level. Without replicate tanks, no allowance can be made for variability between tanks beyond that due to individual fish.” The guideline goes on to say though that “experience has shown that between-tank variability was very small compared with within-tank (*i.e.*, between fish) variability in the case examined. Therefore a relatively acceptable alternative is to perform statistical analysis at the level of individual fish.” The reference (Pack 1991) used to support this understanding though appears to be meeting notes from an OECD meeting of experts and does not appear to be published in a peer-reviewed journal.

Reviewer analysis is consistent with study author’s summary statistics (*i.e.*, mean ± std dev), reported in **Tables 3 and 4** of this DER; however, the mean and std dev reported for fish wet weight in the 1.00 mg ai/L treatment (1.8632 ± 0.4416) could not be reproduced. Based on the data in the study report, the reviewer-calculated mean ± std dev is 1.8722 ± 0.44597 representing a coefficient of variation of 23.82%.

Mean changes in fork length and wet from Day 0 to Day 28 by treatment are summarized in **Table 5**.

**Table 5: Effect of Technical Grade Chlorthal-dimethyl (DCPA) on Mean Change Fork Length and Wet Weight of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) and Tank Average Specific Growth Rate (r<sub>2</sub>) based on Wet Weight and Fork Length over 28-Day Exposure Period.**

Nominal (Filtered Mean Measured mg ai/L)	Change in Fork Length from Day 0 to Day 28 (mean ± std dev.)	Change in Wet Weight from Day 0 to Day 28 (mean ± std dev.)	Tank Average Specific Growth Rate r <sub>2</sub> Weight	Tank Average Specific Growth Rate r <sub>2</sub> Length
Negative Control	2.62 ± 0.545	3.710 ± 1.391	3.8383	1.4735
Solvent Control	2.63 ± 0.842	3.538 ± 1.649	3.8606	1.4997
0.031 (0.0126)	2.58 ± 0.437	3.587 ± 1.103	3.8827	1.4909
0.098 (0.0128)	2.52 ± 0.549	3.332 ± 1.459	3.509	1.4434
0.313 (0.0341)	1.29 ± 0.801*	1.106 ± 1.300*	1.7509	0.8271
1.0 (0.138)	0.589 ± 0.341*	-0.550 ± 1.040*	-1.106	0.390
3.2 (0.373)	0.600 ± 0.350*	-0.193 ± 0.379*	-0.388	0.398

\*statistically significant (p<0.05) different from negative control.

# Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Based on the study author's equation for tank average specific growth rate ( $r_2$ ), for on wet weight, the values are 3.8383, 3.8606, 3.8827, 3.509, 1,7509, -1.106, and -0.388 for the negative, solvent controls and 0.031, 0.098, 0.313, 1.0, and 3.2 mg ai/L treatments, respectively (**Table 5**). The  $r_2$  values for fork length are 1.4735, 1.4997, 1.4909, 1.4434, 0.8271, 0.390, and 0.398 for the negative, solvent controls and 0.031, 0.098, 0.313, 1.0, and 3.2 mg ai/L treatments, respectively (**Table 5**).

Although each of the treatments contained 10 fish, there is a single treatment unit per treatment; therefore, none of the controls nor DCPA-treatments are replicated. If individual fish are treated as pseudo replicates, statistical analysis using SAS® (SAS Institute, Cary, NC; version 9.4; **Appendix A**) PROC ANOVA indicated statistically significant ( $p < 0.05$ ) differences from the negative control for fork length, wet weight, 0-28 day change in fork length and 0 – 28 day change in wet weight of fish treated with DCPA at 0.313, 1,0 and 3.2 mg ai/L. Based on such an analysis, the NOAEC and LOAEC would be nominal exposure values of 0.098 and 0.313 mg ai/L, respectively, representing mean-measured concentrations of 0.0128 and 0.0341 mg ai/L, respectively, for both Day 28 fork length, Day 28 wet weight, and 0 – 28 day changes in fork length and wet weight measurement endpoints. For Day 28 fork length, the LOAEC is based on a difference of 19.5%; the change in length from Day 0 to Day 28 represents a 50.8% difference (reduction) relative to the negative control. For Day 28 wet weight, the LOAEC is based on a 49.3% difference from the negative control while the weight change from Day 0 to Day 28 represents a difference of 70.2% (reduction) relative to the negative control.

The EPA reviewer did not verify the study authors analysis of 'pseudo' specific growth rate ( $r_3$ ) since its relevancy as a measurement endpoint is uncertain.

## F. CONCLUSIONS:

This study is scientifically sound but is classified as supplemental due to the low recoveries (11 – 41% of nominal) of DCPA across treatments and the lack of suitable replication. Following a 28-day exposure to fingerling rainbow trout, the NOAEC and LOAEC for growth (19.5% reduction in Day 28 fork length and 50.8% reduction in 0 – 28 day fork length change; 49.3% reduction in Day 28 wet weight and 70.2% reduction in 0 – 28 day wet weight change), based on individual fish, were 0.0128 and 0.0341 mg ai/L, respectively, using arithmetic mean-measured filtered concentrations. The 28-day  $EC_{50}$  value for fork length was 0.0617 mg ai/L while the 28-day  $LC_{50}$  exceeded the highest treatment concentration, *i.e.*,  $LC_{50} > 0.373$  mg ai/L.

## IN TERMS OF ARITHMETIC MEAN-MEASURED FILTERED CONCENTRATIONS

$LC_{50}$ : $>0.373$ mg ai/L	95% C.I.: N/A
Slope: N/A	95% C.I.: N/A
$EC_{50}$ (wet weight $r_2$ ): Not calculable	95% C.I.: N/A
$EC_{50}$ (fork length $r_2$ ): 0.0617 mg ai/L	95% C.I.: 0.0285-0.134 mg ai/L
NOAEC (wet weight $r_3$ ): 0.0128 mg ai/L	
LOAEC (wet weight $r_3$ ): 0.0341 mg ai/L	
NOAEC (fork length $r_3$ ): 0.0128 mg ai/L	
LOAEC (fork length $r_3$ ): 0.0341 mg ai/L	

## III. REFERENCES:

- Pack S. (1991). Statistical issues concerning the design of tests for determining the effects of chemicals on the growth rate of fish. Room Document 4, OECD *Ad Hoc* Meeting of Experts on Aquatic Toxicology, WRC Medmenham, UK, 10-12 December 1991.

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

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**Appendix A (SAS® Output)**

MEAN LENGTH AT DAY 0 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	5.13	0.16364
2	0.031	0	10	4.98	0.20976
3	0.098	0	10	5.06	0.35024
4	0.313	0	10	4.95	0.19003
5	1	0	10	4.94	0.18974
6	3.2	0	10	5.09	0.34140
7	solvent	0	10	5.04	0.27968

MEAN LENGTH AT DAY 28 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	7.75000	0.60415
2	0.031	0	10	7.56000	0.42216
3	0.098	0	10	7.58000	0.85479
4	0.313	0	10	6.24000	0.70269
5	1	0	10	5.51111	0.24721
6	3.2	0	10	5.69000	0.38715
7	solvent	0	10	7.67000	0.65498



**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

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MEAN WEIGHT AT DAY 0 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	1.92316	0.31052
2	0.031	0	10	1.82453	0.29101
3	0.098	0	10	1.99364	0.45936
4	0.313	0	10	1.74828	0.33145
5	1	0	10	1.97986	0.92709
6	3.2	0	10	1.87219	0.44597
7	solvent	0	10	1.81703	0.37359

MEAN WEIGHT AT DAY 28 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	5.63344	1.49903
2	0.031	0	10	5.41117	1.09126
3	0.098	0	10	5.32516	1.79858
4	0.313	0	10	2.85451	1.15656
5	1	0	10	1.45258	0.30739
6	3.2	0	10	1.67939	0.36796
7	solvent	0	10	5.35558	1.36623

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

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MEAN CHANGE IN LENGTH BY DAY 28 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	2.62000	0.54528
2	0.031	0	10	2.58000	0.43665
3	0.098	0	10	2.52000	0.54934
4	0.313	0	10	1.29000	0.80062
5	1	0	10	0.58889	0.34075
6	3.2	0	10	0.60000	0.34960
7	solvent	0	10	2.63000	0.84202

MEAN CHANGE IN WEIGHT BY DAY 28 BY TREATMENT

Obs	TREAT	_TYPE_	_FREQ_	MEAN	STD
1	0	0	10	3.71028	1.39109
2	0.031	0	10	3.58664	1.10336
3	0.098	0	10	3.33152	1.45895
4	0.313	0	10	1.10623	1.30014
5	1	0	10	-0.54978	1.04002
6	3.2	0	10	-0.19280	0.37854
7	solvent	0	10	3.53855	1.64906

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR DAY 28 FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

**Class Level Information**

Class	Levels	Values
TREAT	7	0 0.031 0.098 0.313 1 3.2 solvent

**Number of Observations Read** 70

**Number of Observations Used** 69

ANOVA FOR DAY 28 FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

Dependent Variable: L28

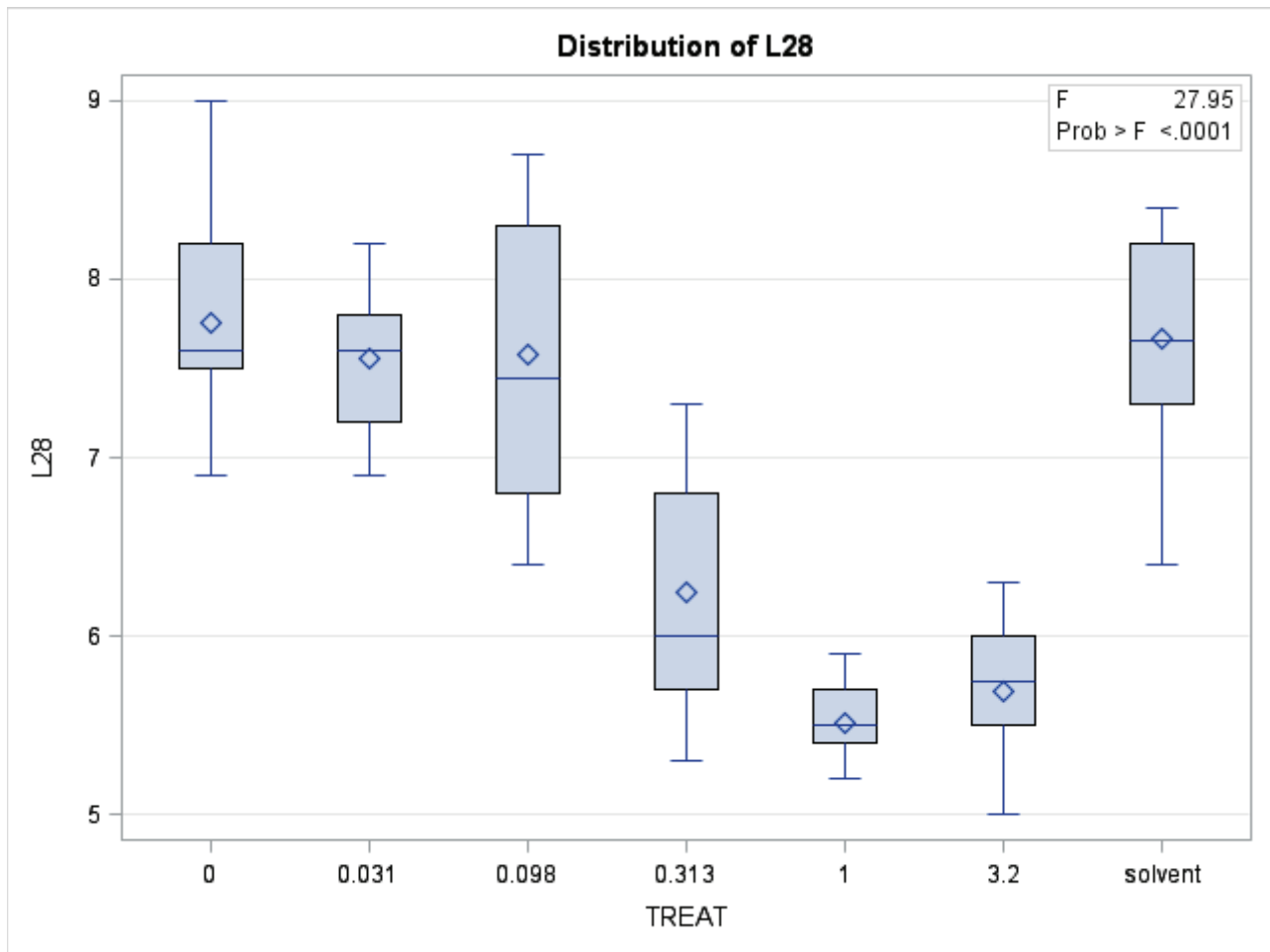
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	58.45500966	9.74250161	27.95	<.0001
Error	62	21.60788889	0.34851434		
Corrected Total	68	80.06289855			

R-Square	Coeff Var	Root MSE	L28 Mean
0.730114	8.584662	0.590351	6.876812

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	6	58.45500966	9.74250161	27.95	<.0001

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

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**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR DAY 28 FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

Bonferroni (Dunn) t Tests for L28

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

<b>Alpha</b>	0.05
<b>Error Degrees of Freedom</b>	62
<b>Error Mean Square</b>	0.348514
<b>Critical Value of t</b>	3.16828

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
0 - solvent	0.0800	-0.7565	0.9165
0 - 0.098	0.1700	-0.6665	1.0065
0 - 0.031	0.1900	-0.6465	1.0265
0 - 0.313	1.5100	0.6735	2.3465 ***
0 - 3.2	2.0600	1.2235	2.8965 ***
0 - 1	2.2389	1.3795	3.0983 ***
solvent - 0	-0.0800	-0.9165	0.7565
solvent - 0.098	0.0900	-0.7465	0.9265
solvent - 0.031	0.1100	-0.7265	0.9465
solvent - 0.313	1.4300	0.5935	2.2665 ***
solvent - 3.2	1.9800	1.1435	2.8165 ***
solvent - 1	2.1589	1.2995	3.0183 ***
0.098 - 0	-0.1700	-1.0065	0.6665
0.098 - solvent	-0.0900	-0.9265	0.7465
0.098 - 0.031	0.0200	-0.8165	0.8565
0.098 - 0.313	1.3400	0.5035	2.1765 ***
0.098 - 3.2	1.8900	1.0535	2.7265 ***
0.098 - 1	2.0689	1.2095	2.9283 ***

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
0.031 - 0	-0.1900	-1.0265	0.6465	
0.031 - solvent	-0.1100	-0.9465	0.7265	
0.031 - 0.098	-0.0200	-0.8565	0.8165	
0.031 - 0.313	1.3200	0.4835	2.1565	***
0.031 - 3.2	1.8700	1.0335	2.7065	***
0.031 - 1	2.0489	1.1895	2.9083	***
0.313 - 0	-1.5100	-2.3465	-0.6735	***
0.313 - solvent	-1.4300	-2.2665	-0.5935	***
0.313 - 0.098	-1.3400	-2.1765	-0.5035	***
0.313 - 0.031	-1.3200	-2.1565	-0.4835	***
0.313 - 3.2	0.5500	-0.2865	1.3865	
0.313 - 1	0.7289	-0.1305	1.5883	
3.2 - 0	-2.0600	-2.8965	-1.2235	***
3.2 - solvent	-1.9800	-2.8165	-1.1435	***
3.2 - 0.098	-1.8900	-2.7265	-1.0535	***
3.2 - 0.031	-1.8700	-2.7065	-1.0335	***
3.2 - 0.313	-0.5500	-1.3865	0.2865	
3.2 - 1	0.1789	-0.6805	1.0383	
1 - 0	-2.2389	-3.0983	-1.3795	***
1 - solvent	-2.1589	-3.0183	-1.2995	***
1 - 0.098	-2.0689	-2.9283	-1.2095	***
1 - 0.031	-2.0489	-2.9083	-1.1895	***
1 - 0.313	-0.7289	-1.5883	0.1305	
1 - 3.2	-0.1789	-1.0383	0.6805	

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR DAY 28 WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

**Class Level Information**

Class	Levels	Values
TREAT	7	0 0.031 0.098 0.313 1 3.2 solvent

**Number of Observations Read** 70

**Number of Observations Used** 69

ANOVA FOR DAY 28 WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

Dependent Variable: W28

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	207.9081329	34.6513555	23.64	<.0001
Error	62	90.8680930	1.4656144		
Corrected Total	68	298.7762259			

R-Square	Coeff Var	Root MSE	W28 Mean
0.695866	30.30234	1.210626	3.995155

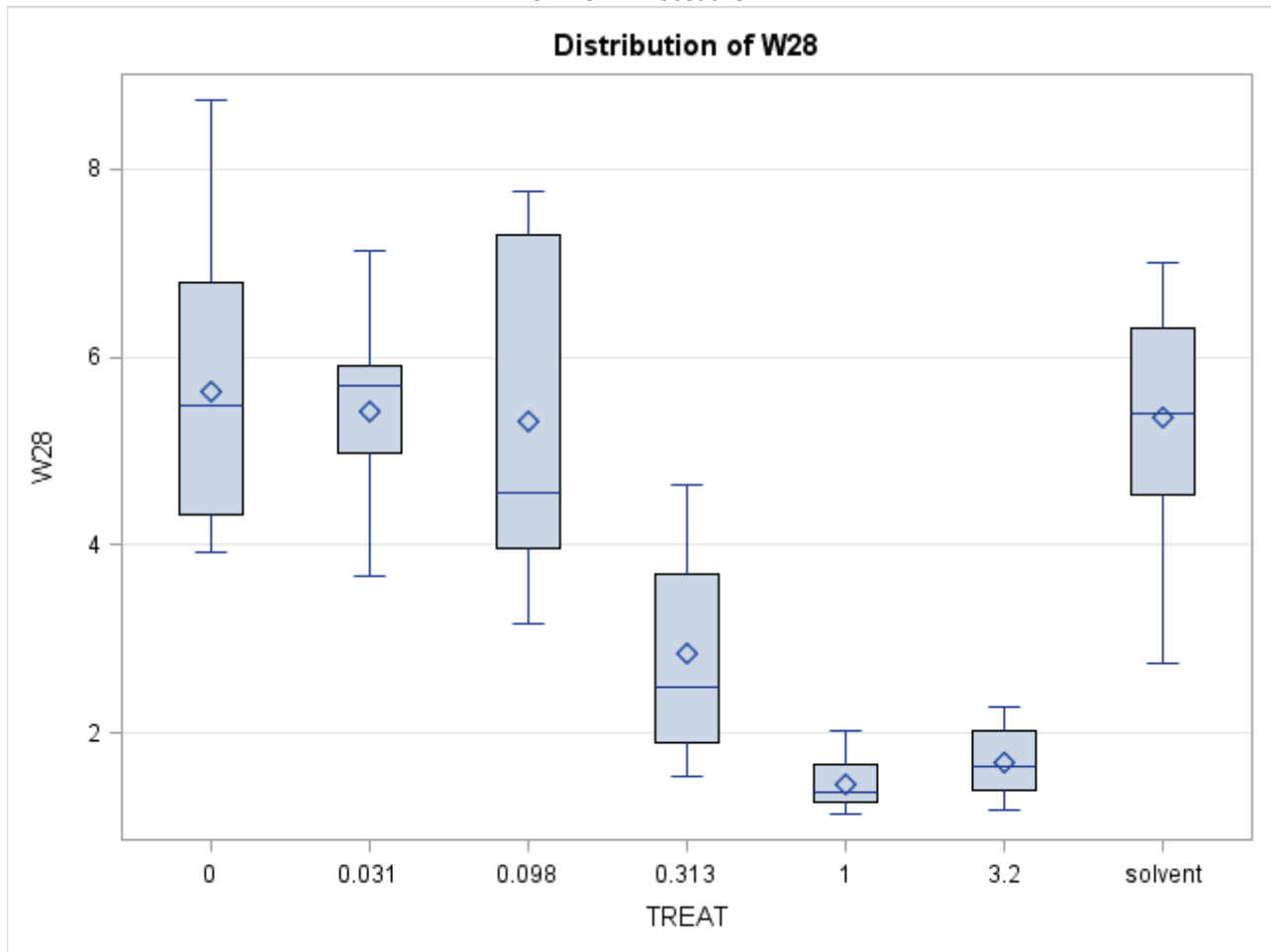
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	6	207.9081329	34.6513555	23.64	<.0001

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

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ANOVA FOR DAY 28 WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure





**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR DAY 28 WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

Bonferroni (Dunn) t Tests for W28

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

<b>Alpha</b>	0.05
<b>Error Degrees of Freedom</b>	62
<b>Error Mean Square</b>	1.465614
<b>Critical Value of t</b>	3.16828

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
0 - 0.031	0.2223	-1.4931	1.9376
0 - solvent	0.2779	-1.4375	1.9932
0 - 0.098	0.3083	-1.4071	2.0236
0 - 0.313	2.7789	1.0636	4.4943 ***
0 - 3.2	3.9540	2.2387	5.6694 ***
0 - 1	4.1809	2.4185	5.9432 ***
0.031 - 0	-0.2223	-1.9376	1.4931
0.031 - solvent	0.0556	-1.6597	1.7709
0.031 - 0.098	0.0860	-1.6293	1.8013
0.031 - 0.313	2.5567	0.8413	4.2720 ***
0.031 - 3.2	3.7318	2.0164	5.4471 ***
0.031 - 1	3.9586	2.1963	5.7209 ***
solvent - 0	-0.2779	-1.9932	1.4375
solvent - 0.031	-0.0556	-1.7709	1.6597
solvent - 0.098	0.0304	-1.6849	1.7458
solvent - 0.313	2.5011	0.7857	4.2164 ***
solvent - 3.2	3.6762	1.9609	5.3915 ***
solvent - 1	3.9030	2.1407	5.6653 ***

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
0.098 - 0	-0.3083	-2.0236	1.4071	
0.098 - 0.031	-0.0860	-1.8013	1.6293	
0.098 - solvent	-0.0304	-1.7458	1.6849	
0.098 - 0.313	2.4707	0.7553	4.1860	***
0.098 - 3.2	3.6458	1.9304	5.3611	***
0.098 - 1	3.8726	2.1102	5.6349	***
0.313 - 0	-2.7789	-4.4943	-1.0636	***
0.313 - 0.031	-2.5567	-4.2720	-0.8413	***
0.313 - solvent	-2.5011	-4.2164	-0.7857	***
0.313 - 0.098	-2.4707	-4.1860	-0.7553	***
0.313 - 3.2	1.1751	-0.5402	2.8905	
0.313 - 1	1.4019	-0.3604	3.1643	
3.2 - 0	-3.9540	-5.6694	-2.2387	***
3.2 - 0.031	-3.7318	-5.4471	-2.0164	***
3.2 - solvent	-3.6762	-5.3915	-1.9609	***
3.2 - 0.098	-3.6458	-5.3611	-1.9304	***
3.2 - 0.313	-1.1751	-2.8905	0.5402	
3.2 - 1	0.2268	-1.5355	1.9891	
1 - 0	-4.1809	-5.9432	-2.4185	***
1 - 0.031	-3.9586	-5.7209	-2.1963	***
1 - solvent	-3.9030	-5.6653	-2.1407	***
1 - 0.098	-3.8726	-5.6349	-2.1102	***
1 - 0.313	-1.4019	-3.1643	0.3604	
1 - 3.2	-0.2268	-1.9891	1.5355	

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

**Class Level Information**

Class	Levels	Values
TREAT	7	0 0.031 0.098 0.313 1 3.2 solvent

**Number of Observations Read** 70

**Number of Observations Used** 69

ANOVA FOR 0 - 28 DAY CHANGE IN FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

Dependent Variable: DELTA\_L

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	54.90557488	9.15092915	26.65	<.0001
Error	62	21.28688889	0.34333692		
Corrected Total	68	76.19246377			

R-Square	Coeff Var	Root MSE	DELTA_L Mean
0.720617	31.66055	0.585950	1.850725

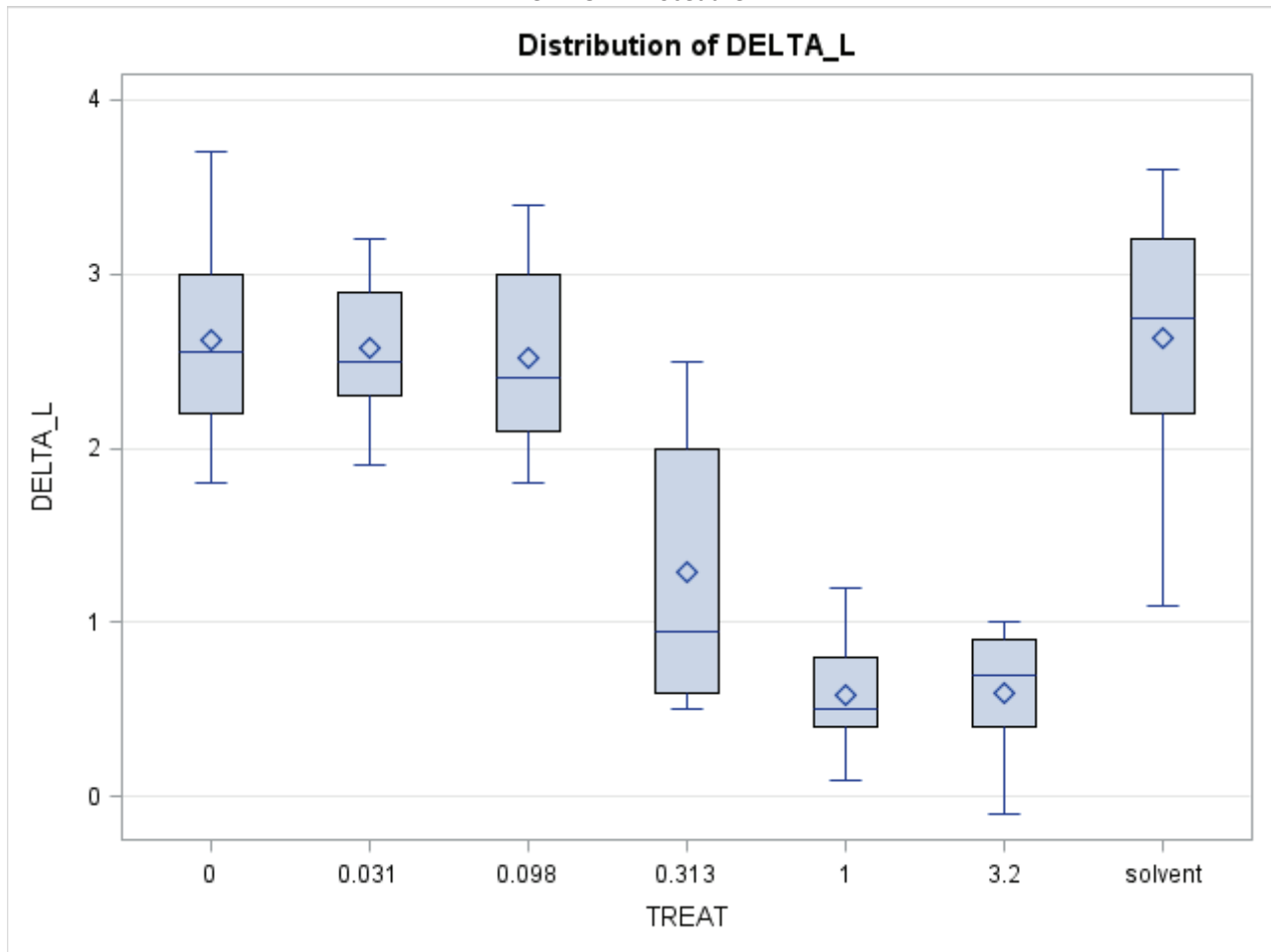
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	6	54.90557488	9.15092915	26.65	<.0001

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure



**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN FORK LENGTH ACROSS TREATMENTS

The ANOVA Procedure

Bonferroni (Dunn) t Tests for DELTA\_L

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

<b>Alpha</b>	0.05
<b>Error Degrees of Freedom</b>	62
<b>Error Mean Square</b>	0.343337
<b>Critical Value of t</b>	3.16828

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
solvent - 0	0.0100	-0.8202	0.8402
solvent - 0.031	0.0500	-0.7802	0.8802
solvent - 0.098	0.1100	-0.7202	0.9402
solvent - 0.313	1.3400	0.5098	2.1702 ***
solvent - 3.2	2.0300	1.1998	2.8602 ***
solvent - 1	2.0411	1.1881	2.8941 ***
0 - solvent	-0.0100	-0.8402	0.8202
0 - 0.031	0.0400	-0.7902	0.8702
0 - 0.098	0.1000	-0.7302	0.9302
0 - 0.313	1.3300	0.4998	2.1602 ***
0 - 3.2	2.0200	1.1898	2.8502 ***
0 - 1	2.0311	1.1781	2.8841 ***
0.031 - solvent	-0.0500	-0.8802	0.7802
0.031 - 0	-0.0400	-0.8702	0.7902
0.031 - 0.098	0.0600	-0.7702	0.8902
0.031 - 0.313	1.2900	0.4598	2.1202 ***
0.031 - 3.2	1.9800	1.1498	2.8102 ***
0.031 - 1	1.9911	1.1381	2.8441 ***

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
0.098 - solvent	-0.1100	-0.9402	0.7202	
0.098 - 0	-0.1000	-0.9302	0.7302	
0.098 - 0.031	-0.0600	-0.8902	0.7702	
0.098 - 0.313	1.2300	0.3998	2.0602	***
0.098 - 3.2	1.9200	1.0898	2.7502	***
0.098 - 1	1.9311	1.0781	2.7841	***
0.313 - solvent	-1.3400	-2.1702	-0.5098	***
0.313 - 0	-1.3300	-2.1602	-0.4998	***
0.313 - 0.031	-1.2900	-2.1202	-0.4598	***
0.313 - 0.098	-1.2300	-2.0602	-0.3998	***
0.313 - 3.2	0.6900	-0.1402	1.5202	
0.313 - 1	0.7011	-0.1519	1.5541	
3.2 - solvent	-2.0300	-2.8602	-1.1998	***
3.2 - 0	-2.0200	-2.8502	-1.1898	***
3.2 - 0.031	-1.9800	-2.8102	-1.1498	***
3.2 - 0.098	-1.9200	-2.7502	-1.0898	***
3.2 - 0.313	-0.6900	-1.5202	0.1402	
3.2 - 1	0.0111	-0.8419	0.8641	
1 - solvent	-2.0411	-2.8941	-1.1881	***
1 - 0	-2.0311	-2.8841	-1.1781	***
1 - 0.031	-1.9911	-2.8441	-1.1381	***
1 - 0.098	-1.9311	-2.7841	-1.0781	***
1 - 0.313	-0.7011	-1.5541	0.1519	
1 - 3.2	-0.0111	-0.8641	0.8419	

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

**Class Level Information**

Class	Levels	Values
TREAT	7	0 0.031 0.098 0.313 1 3.2 solvent

**Number of Observations Read** 70

**Number of Observations Used** 69

ANOVA FOR 0 - 28 DAY CHANGE IN WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

Dependent Variable: DELTA\_W

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	209.5154338	34.9192390	22.28	<.0001
Error	62	97.1604019	1.5671033		
Corrected Total	68	306.6758357			

R-Square	Coeff Var	Root MSE	DELTA_W Mean
0.683182	59.22063	1.251840	2.113858

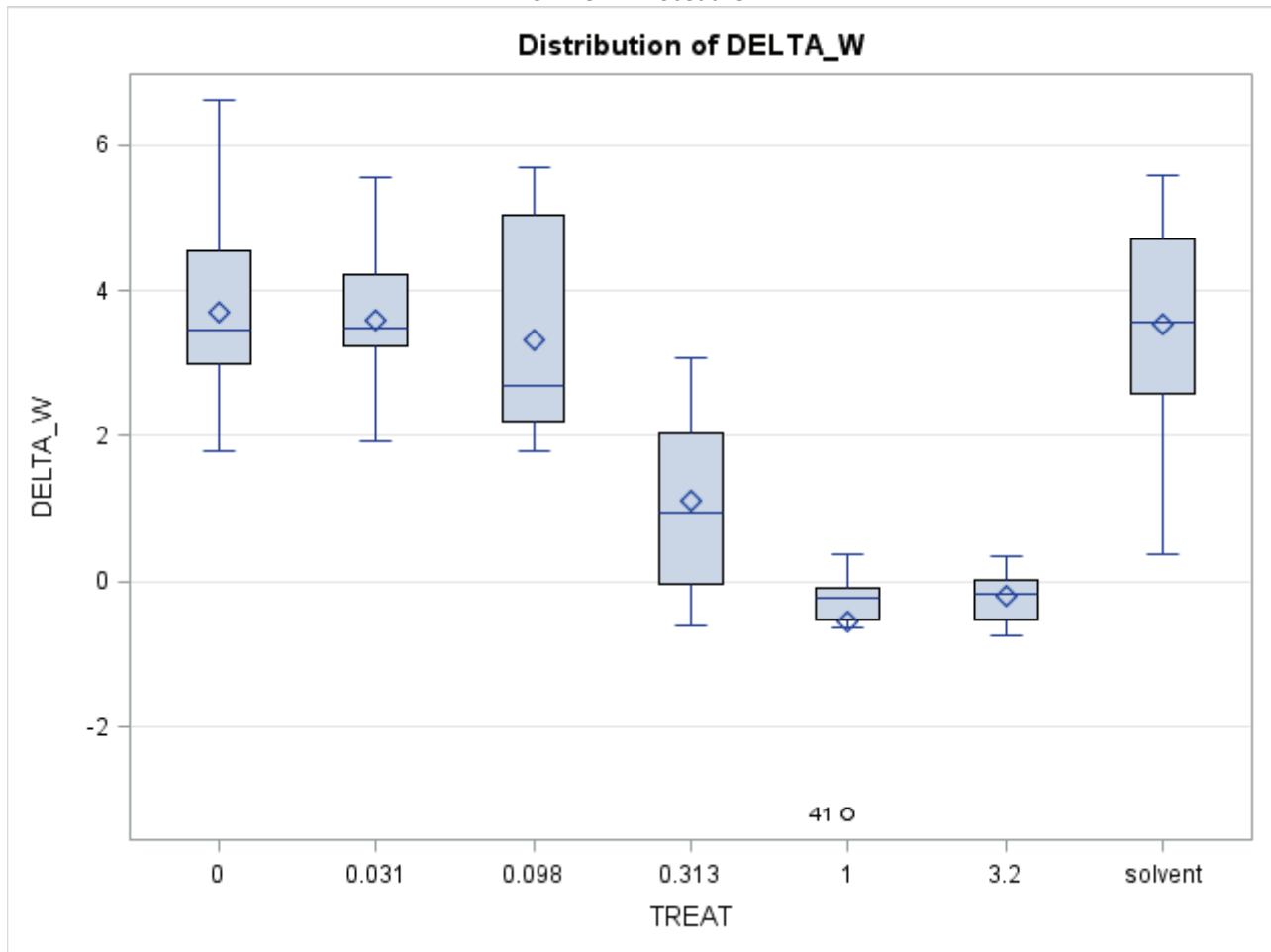
Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	6	209.5154338	34.9192390	22.28	<.0001

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure





**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

ANOVA FOR 0 - 28 DAY CHANGE IN WET WEIGHT ACROSS TREATMENTS

The ANOVA Procedure

Bonferroni (Dunn) t Tests for DELTA\_W

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

<b>Alpha</b>	0.05
<b>Error Degrees of Freedom</b>	62
<b>Error Mean Square</b>	1.567103
<b>Critical Value of t</b>	3.16828

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
0 - 0.031	0.1236	-1.6501	1.8974
0 - solvent	0.1717	-1.6020	1.9455
0 - 0.098	0.3788	-1.3950	2.1525
0 - 0.313	2.6041	0.8303	4.3778 ***
0 - 3.2	3.9031	2.1294	5.6768 ***
0 - 1	4.2601	2.4377	6.0824 ***
0.031 - 0	-0.1236	-1.8974	1.6501
0.031 - solvent	0.0481	-1.7256	1.8218
0.031 - 0.098	0.2551	-1.5186	2.0288
0.031 - 0.313	2.4804	0.7067	4.2541 ***
0.031 - 3.2	3.7794	2.0057	5.5532 ***
0.031 - 1	4.1364	2.3141	5.9588 ***
solvent - 0	-0.1717	-1.9455	1.6020
solvent - 0.031	-0.0481	-1.8218	1.7256
solvent - 0.098	0.2070	-1.5667	1.9808
solvent - 0.313	2.4323	0.6586	4.2060 ***
solvent - 3.2	3.7314	1.9576	5.5051 ***
solvent - 1	4.0883	2.2660	5.9107 ***

**Data Evaluation Record on the Toxicity of Chlorthal-dimethyl (DCPA) to Juvenile Rainbow Trout (*Oncorhynchus mykiss*)**

EPA MRID Number 49307520

Comparisons significant at the 0.05 level are indicated by \*\*\*.

TREAT Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
0.098 - 0	-0.3788	-2.1525	1.3950	
0.098 - 0.031	-0.2551	-2.0288	1.5186	
0.098 - solvent	-0.2070	-1.9808	1.5667	
0.098 - 0.313	2.2253	0.4516	3.9990	***
0.098 - 3.2	3.5243	1.7506	5.2980	***
0.098 - 1	3.8813	2.0590	5.7036	***
0.313 - 0	-2.6041	-4.3778	-0.8303	***
0.313 - 0.031	-2.4804	-4.2541	-0.7067	***
0.313 - solvent	-2.4323	-4.2060	-0.6586	***
0.313 - 0.098	-2.2253	-3.9990	-0.4516	***
0.313 - 3.2	1.2990	-0.4747	3.0728	
0.313 - 1	1.6560	-0.1663	3.4783	
3.2 - 0	-3.9031	-5.6768	-2.1294	***
3.2 - 0.031	-3.7794	-5.5532	-2.0057	***
3.2 - solvent	-3.7314	-5.5051	-1.9576	***
3.2 - 0.098	-3.5243	-5.2980	-1.7506	***
3.2 - 0.313	-1.2990	-3.0728	0.4747	
3.2 - 1	0.3570	-1.4654	2.1793	
1 - 0	-4.2601	-6.0824	-2.4377	***
1 - 0.031	-4.1364	-5.9588	-2.3141	***
1 - solvent	-4.0883	-5.9107	-2.2660	***
1 - 0.098	-3.8813	-5.7036	-2.0590	***
1 - 0.313	-1.6560	-3.4783	0.1663	
1 - 3.2	-0.3570	-2.1793	1.4654	

# CETIS Analytical Report

Report Date: 04 Aug-16 06:43 (p 1 of 2)  
 Test Code: 49307520 R2 | 12-7954-3227

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 11-5225-2698	<b>Endpoint:</b> Mean Length	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:26	<b>Analysis:</b> Nonlinear Regression	<b>Official Results:</b> Yes
<b>Batch ID:</b> 12-3135-0895	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b>	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> NA	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

### Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Poisson [W=1/Y]	Off [Y*=Y]

### Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
19	-5.31	28.6	16	0.7840	Yes				Lack of Fit Not Tested

### Point Estimates

Level	mg ai/L	95% LCL	95% UCL
IC5	0.00245	N/A	0.0222
IC10	0.005	N/A	0.0236
IC15	0.00808	N/A	0.0288
IC20	0.0118	0.000405	0.0366
IC25	0.0164	0.00282	0.0464
IC40	0.0376	0.0142	0.0885
IC50	0.0617	0.0285	0.134

### Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	1.62	0.308	1.01	2.22	5.24	0.0135	Significant Parameter
C	1.96	0.692	0.605	3.32	2.83	0.0660	Non-Significant Parameter
D	0.0617	0.0424	-0.0213	0.145	1.46	0.2413	Non-Significant Parameter

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1.250317	1.250317	1	20.1	0.0206	Significant
Residual	0.186176	0.062059	3			

### Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	0.186	7.81	0.9798	Non-Significant Heterogeneity
	Likelihood Ratio GOF	0.188	7.81	0.9795	Non-Significant Heterogeneity
Distribution	Shapiro-Wilk W Normality	0.834	0.513	0.1154	Normal Distribution

### Mean Length Summary

C-mg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	1	1.49	1.49	1.49	0	0	0.0%	0.0%
0.0126		1	1.49	1.49	1.49	0	0	0.0%	0.27%
0.0128		1	1.43	1.43	1.43	0	0	0.0%	4.15%
0.0341		1	0.809	0.809	0.809	0	0	0.0%	45.8%
0.138		1	0.39	0.39	0.39	0	0	0.0%	73.9%
0.373		1	0.397	0.397	0.397	0	0	0.0%	73.4%

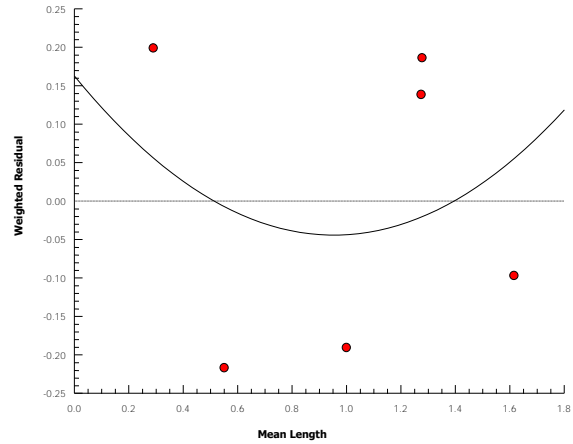
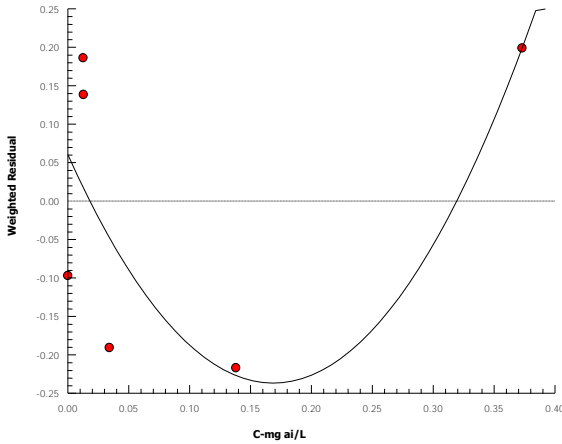
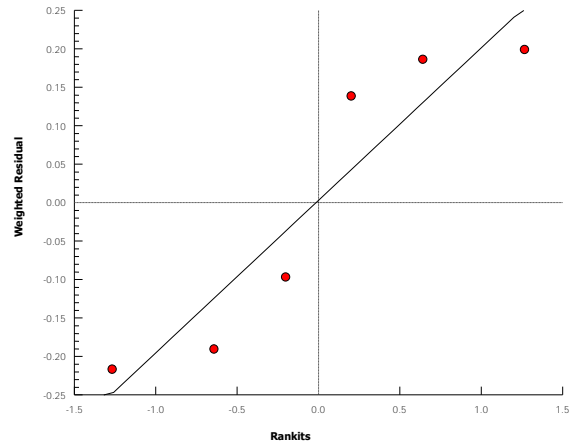
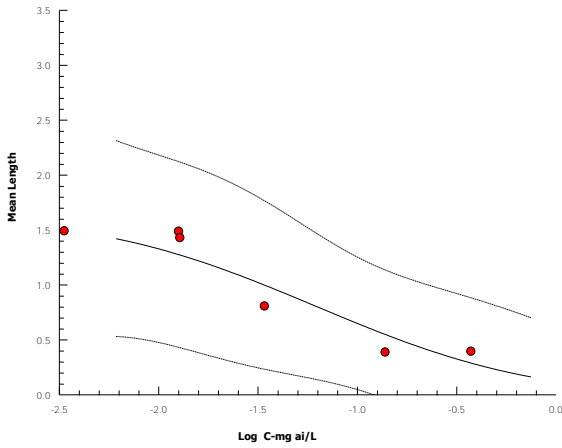
Analysis ID: 11-5225-2698  
Analyzed: 04 Aug-16 6:26

Endpoint: Mean Length  
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Summary Report**

**Report Date:** 04 Aug-16 06:43 (p 1 of 1)  
**Test Code:** 49307520 R2 | 12-7954-3227

**OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)**

**Covance Laboratories Ltd.**

**Batch ID:** 12-3135-0895      **Test Type:** Fish ELS (28-60d) Test      **Analyst:**  
**Start Date:** 03 Oct-03      **Protocol:** Not Applicable      **Diluent:** Dechlorinated & purified tap water  
**Ending Date:**      **Species:** Oncorhynchus mykiss      **Brine:** Not Applicable  
**Duration:** NA      **Source:** Brow Well Fisheries Limited Skipton, UK      **Age:** Juv

**Sample ID:** 14-6668-6306      **Code:** 49307520 R2      **Client:** CDM Smith - J. Marton  
**Sample Date:** 03 Oct-03      **Material:** Chlorthal dimethyl (DCPA)      **Project:** Herbicide  
**Receive Date:**      **Source:** AMVAC Chemical Corporation  
**Sample Age:** NA      **Station:**

**Batch Note:** PC Code 078701, MRID 49307520, OECD 215, mean-measured filtered concentrations, R2 analysis

**Sample Note:** PC Code 078701, MRID 49307520, OECD 215, mean-measured filtered concentrations, R2 analysis

**Point Estimate Summary**

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
11-5225-2698	Mean Length	IC5	0.00245	N/A	0.0222		Nonlinear Regression
		IC10	0.005	N/A	0.0236		
		IC15	0.00808	N/A	0.0288		
		IC20	0.0118	0.000405	0.0366		
		IC25	0.0164	0.00282	0.0464		
		IC40	0.0376	0.0142	0.0885		
		IC50	0.0617	0.0285	0.134		

**Mean Length Summary**

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	1	1.47			1.47	1.47	0	0	0.0%	0.0%
0	Negative Control	1	1.49			1.49	1.49	0	0	0.0%	-1.84%
0.0126		1	1.49			1.49	1.49	0	0	0.0%	-1.56%
0.0128		1	1.43			1.43	1.43	0	0	0.0%	2.39%
0.0341		1	0.809			0.809	0.809	0	0	0.0%	44.8%
0.138		1	0.39			0.39	0.39	0	0	0.0%	73.4%
0.373		1	0.397			0.397	0.397	0	0	0.0%	72.9%

**Mean Length Detail**

C-mg ai/L	Control Type	Rep 1
0	Solvent Blank	1.47
0	Negative Control	1.49
0.0126		1.49
0.0128		1.43
0.0341		0.809
0.138		0.39
0.373		0.397

# CETIS Analytical Report

Report Date: 04 Aug-16 06:55 (p 1 of 6)  
 Test Code: 49307520 R3 | 11-1127-7441

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 14-4747-3150	<b>Endpoint:</b> Mean Length	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C <> T	NA	NA	24.2%	Passes mean length

### Equal Variance t Two-Sample Test

Control	vs Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	Solvent Blank	0.736	2.1	0.362	18	0.4710	CDF	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08045461	0.08045461	1	0.542	0.4710	Non-Significant Effect
Error	2.67024	0.1483466	18			
Total	2.750694		19			

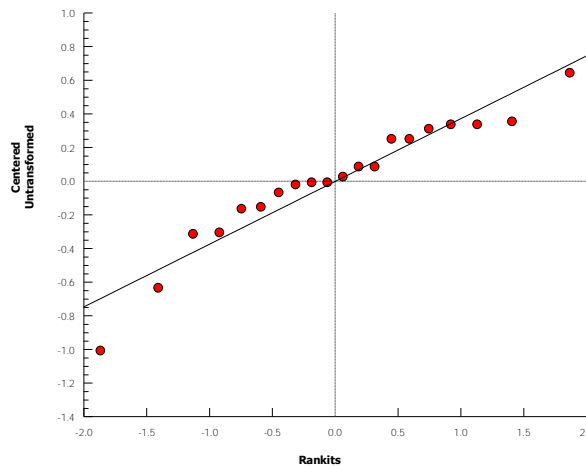
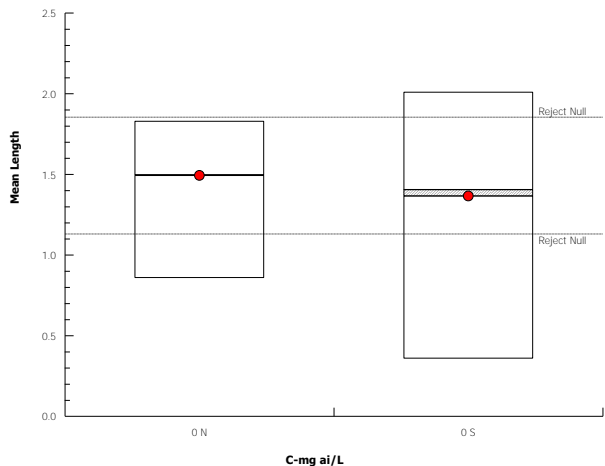
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.01	6.54	0.3125	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.933	0.866	0.1777	Normal Distribution

### Mean Length Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	10	1.37	1.05	1.68	1.41	0.358	2.01	0.141	32.6%	0.0%
0	Negative Control	10	1.49	1.27	1.72	1.5	0.858	1.83	0.0993	21.0%	-9.29%

### Graphics



# CETIS Analytical Report

Report Date: 04 Aug-16 06:55 (p 2 of 6)  
 Test Code: 49307520 R3 | 11-1127-7441

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 09-6778-7018	<b>Endpoint:</b> Mean Length	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	20.9%	0.0128	0.0341	0.02089	

### Dunnett Multiple Comparison Test

Control	vs C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	0.0126	0.0292	2.29	0.312	18	0.8264	CDF	Non-Significant Effect
	0.0128	0.456	2.29	0.312	18	0.6674	CDF	Non-Significant Effect
	0.0341*	5.02	2.29	0.312	18	<0.0001	CDF	Significant Effect
	0.138*	7.89	2.29	0.32	17	<0.0001	CDF	Significant Effect
	0.373*	8.05	2.29	0.312	18	<0.0001	CDF	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	14.00361	2.800723	5	30.3	<0.0001	Significant Effect
Error	4.904995	0.09254708	53			
Total	18.90861		58			

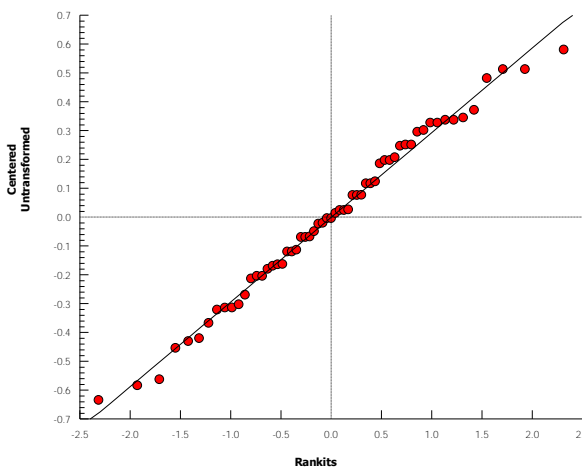
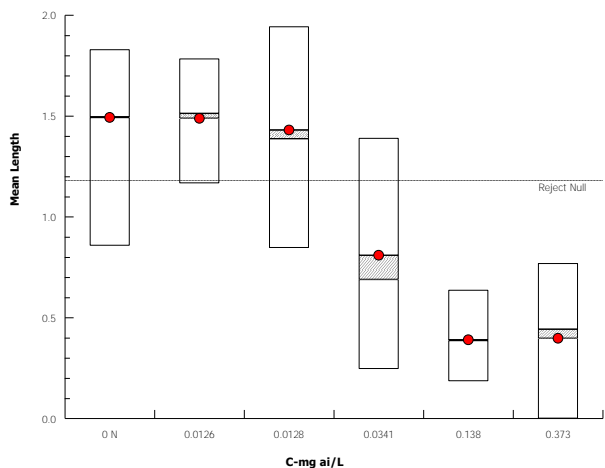
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.5	15.1	0.0615	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.986	0.945	0.7313	Normal Distribution

### Mean Length Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	10	1.49	1.27	1.72	1.5	0.858	1.83	0.0993	21.0%	0.0%
0.0126		10	1.49	1.34	1.63	1.51	1.17	1.78	0.0636	13.5%	0.27%
0.0128		10	1.43	1.14	1.72	1.39	0.847	1.94	0.128	28.2%	4.15%
0.0341		10	0.809	0.523	1.1	0.689	0.246	1.39	0.126	49.4%	45.8%
0.138		9	0.39	0.267	0.513	0.386	0.186	0.637	0.0532	40.9%	73.9%
0.373		10	0.397	0.221	0.574	0.442	-0.0567	0.769	0.078	62.1%	73.4%

### Graphics



OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 06-3930-7453	<b>Endpoint:</b> Mean Length	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	16.3%	0.0128	0.0341	0.02089	

Williams Multiple Comparison Test

Control	vs C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	0.0126	0.0292	1.67	0.228	18	>0.05	CDF	Non-Significant Effect
	0.0128	0.456	1.75	0.238	18	>0.05	CDF	Non-Significant Effect
	0.0341*	5.02	1.77	0.241	18	<0.05	CDF	Significant Effect
	0.138*	7.89	1.78	0.249	17	<0.05	CDF	Significant Effect
	0.373*	8.08	1.79	0.244	18	<0.05	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	14.00361	2.800723	5	30.3	<0.0001	Significant Effect
Error	4.904995	0.09254708	53			
Total	18.90861		58			

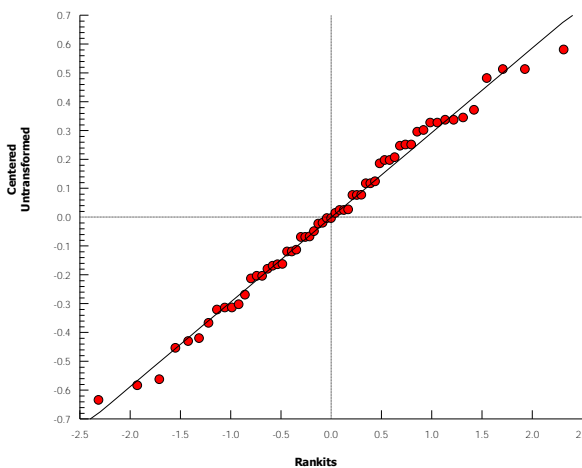
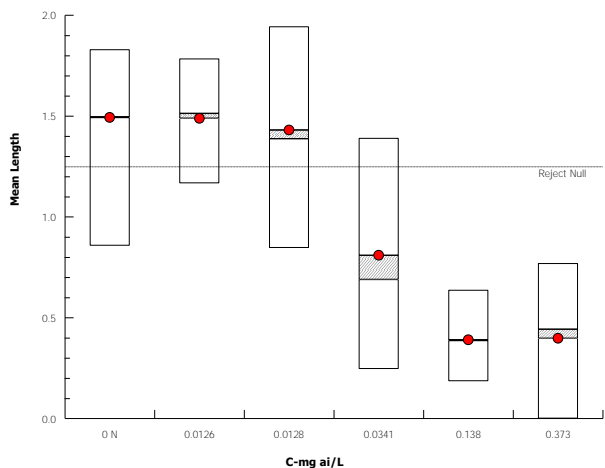
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.5	15.1	0.0615	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.986	0.945	0.7313	Normal Distribution

Mean Length Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	10	1.49	1.27	1.72	1.5	0.858	1.83	0.0993	21.0%	0.0%
0.0126		10	1.49	1.34	1.63	1.51	1.17	1.78	0.0636	13.5%	0.27%
0.0128		10	1.43	1.14	1.72	1.39	0.847	1.94	0.128	28.2%	4.15%
0.0341		10	0.809	0.523	1.1	0.689	0.246	1.39	0.126	49.4%	45.8%
0.138		9	0.39	0.267	0.513	0.386	0.186	0.637	0.0532	40.9%	73.9%
0.373		10	0.397	0.221	0.574	0.442	-0.0567	0.769	0.078	62.1%	73.4%

Graphics





# CETIS Analytical Report

Report Date: 04 Aug-16 06:55 (p 4 of 6)  
 Test Code: 49307520 R3 | 11-1127-7441

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 08-5971-7186	<b>Endpoint:</b> Mean Wet Weight	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C <> T	NA	NA	24.1%	Passes mean wet weight

### Equal Variance t Two-Sample Test

Control	vs Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	Solvent Blank	0.0644	2.1	0.917	18	0.9493	CDF	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003953672	0.003953672	1	0.00415	0.9493	Non-Significant Effect
Error	17.14684	0.9526024	18			
Total	17.1508		19			

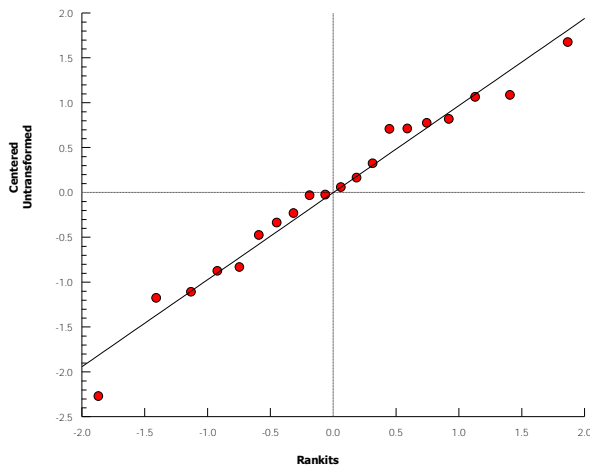
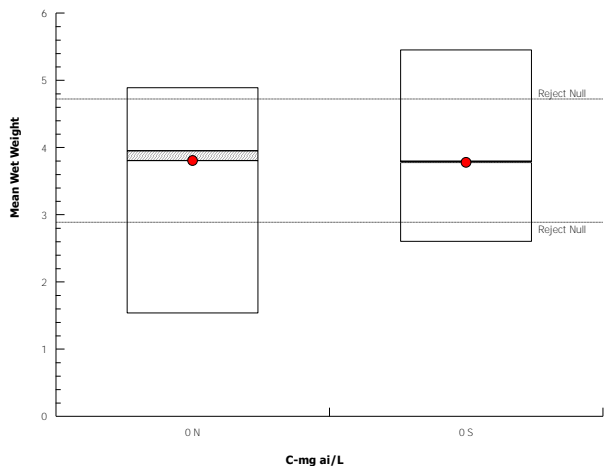
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.28	6.54	0.7201	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.975	0.866	0.8472	Normal Distribution

### Mean Wet Weight Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	10	3.78	3.12	4.43	3.79	2.6	5.45	0.289	24.2%	0.0%
0	Negative Control	10	3.8	3.06	4.54	3.95	1.53	4.89	0.327	27.2%	-0.75%

### Graphics



# CETIS Analytical Report

Report Date: 04 Aug-16 06:55 (p 5 of 6)  
 Test Code: 49307520 R3 | 11-1127-7441

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 17-4027-9539	<b>Endpoint:</b> Mean Wet Weight	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	28.0%	0.0128	0.0341	0.02089	

### Dunnett Multiple Comparison Test

Control	vs C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	0.0126	-0.0957	2.29	1.07	18	0.8619	CDF	Non-Significant Effect
	0.0128	0.852	2.29	1.07	18	0.4865	CDF	Non-Significant Effect
	0.0341*	4.89	2.29	1.07	18	<0.0001	CDF	Significant Effect
	0.138*	9.95	2.29	1.09	17	<0.0001	CDF	Significant Effect
	0.373*	8.97	2.29	1.07	18	<0.0001	CDF	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	222.5754	44.51508	5	41.2	<0.0001	Significant Effect
Error	57.24739	1.080139	53			
Total	279.8228		58			

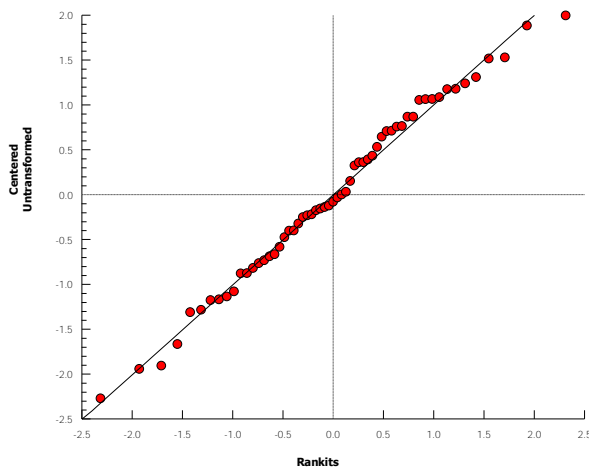
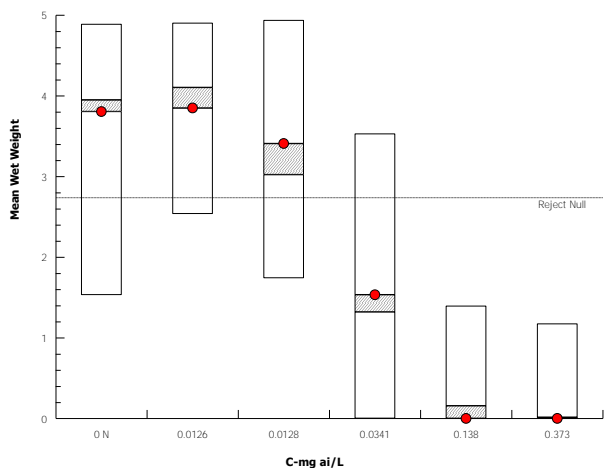
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.95	15.1	0.2243	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.987	0.945	0.7657	Normal Distribution

### Mean Wet Weight Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	10	3.8	3.06	4.54	3.95	1.53	4.89	0.327	27.2%	0.0%
0.0126		10	3.85	3.3	4.4	4.1	2.54	4.9	0.245	20.1%	-1.17%
0.0128		10	3.41	2.55	4.27	3.02	1.74	4.94	0.38	35.3%	10.4%
0.0341		10	1.53	0.484	2.58	1.32	-0.411	3.53	0.464	95.7%	59.7%
0.138		9	-0.948	-1.5	-0.395	-1.1	-1.77	0.291	0.24	-75.9%	125.0%
0.373		10	-0.364	-0.934	0.205	-0.35	-1.53	0.809	0.252	-218.0%	110.0%

### Graphics



# CETIS Analytical Report

Report Date: 04 Aug-16 06:55 (p 6 of 6)  
 Test Code: 49307520 R3 | 11-1127-7441

## OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

<b>Analysis ID:</b> 18-4203-1418	<b>Endpoint:</b> Mean Wet Weight	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 04 Aug-16 6:54	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.9%	0.0128	0.0341	0.02089	

### Williams Multiple Comparison Test

Control	vs C-mg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	0.0126	-0.0957	1.67	0.778	18	>0.05	CDF	Non-Significant Effect
	0.0128	0.852	1.75	0.813	18	>0.05	CDF	Non-Significant Effect
	0.0341*	4.89	1.77	0.825	18	<0.05	CDF	Significant Effect
	0.138*	9.95	1.78	0.85	17	<0.05	CDF	Significant Effect
	0.373*	9.56	1.79	0.833	18	<0.05	CDF	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	222.5754	44.51508	5	41.2	<0.0001	Significant Effect
Error	57.24739	1.080139	53			
Total	279.8228		58			

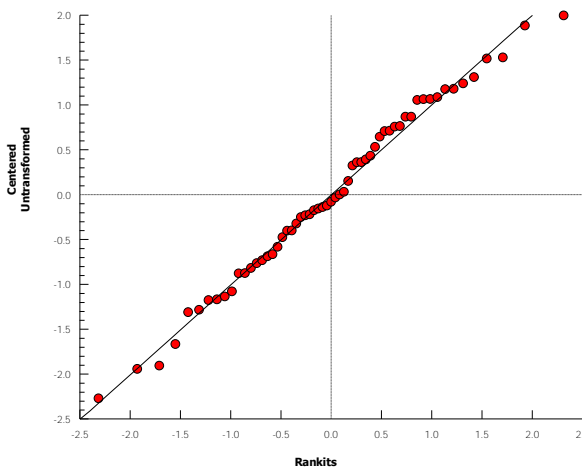
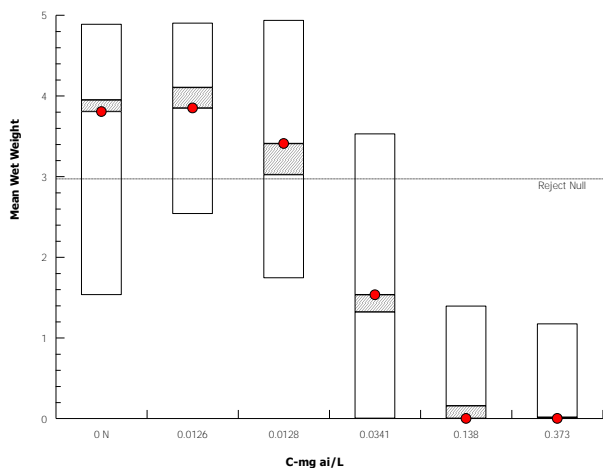
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.95	15.1	0.2243	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.987	0.945	0.7657	Normal Distribution

### Mean Wet Weight Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	10	3.8	3.06	4.54	3.95	1.53	4.89	0.327	27.2%	0.0%
0.0126		10	3.85	3.3	4.4	4.1	2.54	4.9	0.245	20.1%	-1.17%
0.0128		10	3.41	2.55	4.27	3.02	1.74	4.94	0.38	35.3%	10.4%
0.0341		10	1.53	0.484	2.58	1.32	-0.411	3.53	0.464	95.7%	59.7%
0.138		9	-0.948	-1.5	-0.395	-1.1	-1.77	0.291	0.24	-75.9%	125.0%
0.373		10	-0.364	-0.934	0.205	-0.35	-1.53	0.809	0.252	-218.0%	110.0%

### Graphics



**CETIS Summary Report**

**Report Date:** 04 Aug-16 06:55 (p 1 of 2)  
**Test Code:** 49307520 R3 | 11-1127-7441

**OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)**

**Covance Laboratories Ltd.**

<b>Batch ID:</b> 20-9384-6866	<b>Test Type:</b> Fish ELS (28-60d) Test	<b>Analyst:</b>
<b>Start Date:</b> 03 Oct-03	<b>Protocol:</b> Not Applicable	<b>Diluent:</b> Dechlorinated & purified tap water
<b>Ending Date:</b> 04 Aug-16 06:47	<b>Species:</b> Oncorhynchus mykiss	<b>Brine:</b> Not Applicable
<b>Duration:</b> 4689d 7h	<b>Source:</b> Brow Well Fisheries Limited Skipton, UK	<b>Age:</b> Juv

<b>Sample ID:</b> 11-5216-6119	<b>Code:</b> 49307520 R3	<b>Client:</b> CDM Smith - J. Marton
<b>Sample Date:</b> 03 Oct-03	<b>Material:</b> Chlorthal dimethyl (DCPA)	<b>Project:</b> Herbicide
<b>Receive Date:</b> 04 Aug-16 06:47	<b>Source:</b> AMVAC Chemical Corporation	
<b>Sample Age:</b> NA	<b>Station:</b>	

**Batch Note:** PC Code 078701, MRID 49307520, OECD 215, mean-measured concentrations, R3 analysis

**Sample Note:** PC Code 078701, MRID 49307520, OECD 215, mean-measured concentrations, R3 analysis

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-4747-3150	Mean Length	0	>0		24.2%		Equal Variance t Two-Sample Test
09-6778-7018	Mean Length	0.0128	0.0341	0.02089	20.9%		Dunnett Multiple Comparison Test
06-3930-7453	Mean Length	0.0128	0.0341	0.02089	16.3%		Williams Multiple Comparison Test
08-5971-7186	Mean Wet Weight	0	>0		24.1%		Equal Variance t Two-Sample Test
17-4027-9539	Mean Wet Weight	0.0128	0.0341	0.02089	28.0%		Dunnett Multiple Comparison Test
18-4203-1418	Mean Wet Weight	0.0128	0.0341	0.02089	21.9%		Williams Multiple Comparison Test

**Mean Length Summary**

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	10	1.37	1.05	1.68	0.358	2.01	0.141	0.445	32.6%	0.0%
0	Negative Control	10	1.49	1.27	1.72	0.858	1.83	0.0993	0.314	21.0%	-9.29%
0.0126		10	1.49	1.34	1.63	1.17	1.78	0.0636	0.201	13.5%	-9.0%
0.0128		10	1.43	1.14	1.72	0.847	1.94	0.128	0.404	28.2%	-4.75%
0.0341		10	0.809	0.523	1.1	0.246	1.39	0.126	0.4	49.4%	40.7%
0.138		9	0.39	0.267	0.513	0.186	0.637	0.0532	0.16	40.9%	71.4%
0.373		10	0.397	0.221	0.574	-0.0567	0.769	0.078	0.247	62.1%	70.9%

**Mean Wet Weight Summary**

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	10	3.78	3.12	4.43	2.6	5.45	0.289	0.914	24.2%	0.0%
0	Negative Control	10	3.8	3.06	4.54	1.53	4.89	0.327	1.03	27.2%	-0.75%
0.0126		10	3.85	3.3	4.4	2.54	4.9	0.245	0.774	20.1%	-1.92%
0.0128		10	3.41	2.55	4.27	1.74	4.94	0.38	1.2	35.3%	9.74%
0.0341		10	1.53	0.484	2.58	-0.411	3.53	0.464	1.47	95.7%	59.4%
0.138		9	-0.948	-1.5	-0.395	-1.77	0.291	0.24	0.719	-75.9%	125.0%
0.373		10	-0.364	-0.934	0.205	-1.53	0.809	0.252	0.796	-218.0%	110.0%

**CETIS Summary Report**Report Date: 04 Aug-16 06:55 (p 2 of 2)  
Test Code: 49307520 R3 | 11-1127-7441

OPPTS 850.1400 Chronic Fish Early Life Stage (ELS)

Covance Laboratories Ltd.

**Mean Length Detail**

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	1.36	1.36	1.06	1.72	0.358	2.01	1.21	1.45	1.68	1.45
0	Negative Control	1.18	1.33	1.83	1.47	1.42	1.74	1.74	1.83	1.52	0.858
0.0126		1.78	1.22	1.7	1.51	1.51	1.32	1.61	1.61	1.47	1.17
0.0128		1.73	1.06	1.27	1.94	1.51	1.94	1.22	1.78	0.847	1.01
0.0341		0.629	0.689	1.39	1.14	0.246	0.379	0.506	0.689	1.29	1.14
0.138		0.386	0.32	0.186	0.576	0.386	0.637	0.186	0.32	0.514	
0.373		0.595	-0.0567	0.284	0.473	0.595	0.348	0.411	0.473	0.0834	0.769

**Mean Wet Weight Detail**

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Solvent Blank	3.44	3.83	2.6	4.59	2.67	5.45	2.94	3.75	4.55	3.94
0	Negative Control	2.93	3.33	4.87	4.13	3.77	4.51	4.51	4.89	3.57	1.53
0.0126		4.9	2.56	4.49	4.21	4	3.63	4.24	4.21	3.71	2.54
0.0128		4.27	2.53	2.72	4.92	3.33	4.94	2.64	4.72	1.74	2.27
0.0341		0.868	1.28	3.53	2.6	-0.374	-0.411	0.357	1.36	3.42	2.71
0.138		-1.1	-1.68	-1.77	0.291	-1.07	-0.081	-1.35	-1.35	-0.417	
0.373		0.0679	-1.53	-0.688	-0.365	0.398	-0.947	-0.334	0.392	-1.44	0.809