

US EPA ARCHIVE DOCUMENT

MRID No. 437880-01

**DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4**

1. **CHEMICAL:** Dichlorvos (DDVP) **PC Code No.:** 084001

2. **TEST MATERIAL:** DDVP technical **Purity:** 98%

3. **CITATION:**

Author: Jay W. Davis
Title: DDVP Technical Grade: Toxicity to Embryos and Larvae of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions

Study Completion Date: August 10, 1995

Laboratory: Toxikon Environmental Sciences, Jupiter, FL

Laboratory Report ID: J9403007m

Sponsor: AMVAC Chemical Corporation, Los Angeles, CA

MRID No.: 437880-01

DP Barcode: D220208

4. **REVIEWED BY:** Rosemary Mora, M.S., Associate Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalawat for RGM Date: 2/27/96 3/12/96

APPROVED BY: Mark Mossler, M.S., Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature: M. Mossler Date: 2/27/96

5. **APPROVED BY:**

Signature: L. T. C. Date: 3/25/96

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater fish early life-stage test. Based on the most sensitive parameter (survival), the NOEC and LOEC for rainbow trout exposed to DDVP technical are 5.2 and 10.1 µg ai/L, respectively. The MATC is 7.2 µg ai/L.

7. **ADEQUACY OF THE STUDY**

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.



2017794

8. MAJOR GUIDELINE DEVIATIONS:

1. Measured concentrations were somewhat variable throughout the test period. The ratios of the highest to the lowest measured concentration within the same treatment were 1.9, 2.0, 1.9, 1.7, and 1.6 for the nominal concentrations of 6.3, 12.5, 25, 50, and 100 µg ai/L, respectively.
2. Levels of test material near the limit of detection were reported for some of the control solutions on 3 out of 17 sampling occasions. These results were attributed to interference in detection.
3. Test temperature ranged from 8.9 to 14.4 °C. Test temperature should not deviate 2°C from the target temperature.
4. Only 2 true replicates per group were used during the study; 4 replicates are recommended.
5. Dilution water was chlorinated municipal freshwater which was filtered and aerated prior to use; the use of dechlorinated water is discouraged.
6. The dissolved oxygen concentrations during the test were ≥68% saturation. The test system should maintain D.O. concentrations above 75% of saturation.

9. MATERIALS AND METHODS:**A. Biological System**

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	<i>Oncorhynchus mykiss</i>
Source: Commercial fishery, wild, or brood stock.	Eggs and milt were obtained from Mount Lassen Trout Farm, Red Bluff, CA.
Age at beginning of test: Embryos 2 to 24 hours old.	19 hours post-fertilization at test initiation

Guideline Criteria	Reported Information
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	Embryo exposure: 40 eggs/incubation chamber, 2 chambers per replicate, 2 replicates per treatment Larval exposure: 30 fish per replicate, 2 replicates per treatment
Posthatch: % of embryos that produce live fry must be \geq 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	Percentage hatch was 94% in dilution water control group, 95% in solvent control group, and evenly distributed between replicates.
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish were fed three to four times daily. Feeding was terminated the day prior to test termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Larval survival was recorded daily.
Controls: Avg. survival at end of test must be \geq 80%. Survival in any control chamber must not be <70%.	Average survival in both controls was 90%. Survival in each control or solvent control replicate was \geq 87%.
Controls: Negative control and carrier control (when applicable) are required.	A dilution water control and a solvent control (1.5-1.6 μ L TEG/L) were used.

Comments: Only two true replicates for each treatment and control group were used for the embryo exposure and larval growth periods.

B. Physical System

Guideline Criteria	Reported Information
Test Water: 1) May be natural (well or spring) or reconstituted water. 2) Water should be sterilized with UV radiation and screened for contaminants. 3) Hardness of 40-48 mg/L as CaCO ₃ , pH of 7.2-7.6	1) Town of Jupiter water which was aerated and filtered (carbon and 5- μm) to remove chlorine. Water was aerated, filtered, and screened for contaminants, but not UV-sterilized prior to use. 3) Mean hardness of 60-76 mg/L as CaCO ₃ , pH range of 6.9-7.3.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For rainbow trout, 10°C is recommended.	Range of 8.9-14.4°C Mean of 10.3 ±0.4°C
Photoperiod: Recommend 16L/8D.	Total darkness until swim-up at which time a 4-day transition period to full light was initiated. After this transition the photoperiod was 16 hours of light and 8 hours of dark.
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	Intermittent-flow proportional diluter. Nominal concentrations of 6.3, 12.5, 25, 50, and 100 µg ai/L.

Guideline Criteria	Reported Information
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) A mixing chamber was used. 2) Exposure solutions were not aerated. 3) Appropriate mixing was not confirmed by analysis. 4) Not reported.
Test Vessels: All glass or glass with stainless steel frame.	Glass tanks (40 x 29.5 x 20 cm).
Embryo Cups: 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	13 x 142 mm glass petri dishes encircled by 355- μm Nitex mesh for a resulting height of 17 cm.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain D.O. at above 75% of saturation and maintain the toxicant level.	7.5 volume replacements/24 h. D.O. and chemical concentrations were monitored.
Aeration: Dilution water should be aerated to insure D.O. concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	D.O. $\geq 68\%$ (7.5 mg/L) of saturation at all times.

Comments: Test water was not sterilized. The test system did not maintain the recommended D.O. level on a few occasions.

C. Chemical System

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	<ul style="list-style-type: none"> - Yes; control, solvent control and five concentrations. - Yes; chemical analysis of samples collected at 17 intervals, generally within one week of the next. - Yes, based on mean measured concentrations reported.
Other Variables: D.O. must be measured at each conc. at least once a week.	Yes; D.O. and pH were measured at test initiation, weekly thereafter, and at test termination.
Solvents: Should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Triethylene glycol (1.5-1.6 μ L/L).

Comments: None.

10. REPORTED RESULTS

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Data include: - Number of eggs hatched; - 61-day post-hatch survival; - 61-day post-hatch length; - 61-day post-hatch wet weight.

Guideline Criteria	Reported Information
Raw data included? (Y/N)	Yes; except daily embryo survival data and time to hatch data.

Effects Data

Toxicant Concentration ($\mu\text{g ai/L}$)		Mean Percent Hatch	Percent Post-Hatch Survival (61 days)	Total Length (mm)	Wet Weight (g)
Nominal	Measured				
Control	≤ 0.35	94	90	36.6	0.63
Solvent	≤ 0.35	95	90	37.1	0.63
6.3	5.2	96	87	36.3	0.66
12.5	10.1	95	72	35.4	0.60
25	23.3	98	75	36.9	0.69
50	55.2	96	75	36.6	0.68
100	103	92	73	36.0	0.66

Toxicity Observations: None reported.Statistical Results:

Statistical Method: Williams' test

NOEC: 5.2 $\mu\text{g ai/L}$ LOEC: 10.1 $\mu\text{g ai/L}$ MATC: 7.2 $\mu\text{g ai/L}$

Most sensitive endpoint: Larval survival.

Comments: Hatch, survival, and growth data for the exposure groups were compared to data for the pooled control.**11. REVIEWER'S STATISTICAL RESULTS:**

Statistical Methods: Dunnett's and Williams' tests

NOEC: 5.2 $\mu\text{g ai/L}$ LOEC: 10.1 $\mu\text{g ai/L}$ MATC: 7.2 $\mu\text{g ai/L}$

Most sensitive endpoint: Larval survival.

Comments: Hatch, survival, and length data from the exposure groups were compared to the data for the pooled control.

12. **REVIEWER'S COMMENTS:** Although the ratios of the highest to the lowest measured concentration within the same treatment were between 1.6 and 2.0, the overall exposure concentrations were near nominal ranges with fairly low standard errors of the mean (between 12 and 15%). Therefore, this study is scientifically sound and fulfills the guideline requirements for a freshwater fish early life-stage toxicity test. Based on mean measured concentrations, the MATC for rainbow trout exposed to DDVP technical was between 5.2 and 10.1 $\mu\text{g ai/L}$. The geometric mean MATC was 7.2 $\mu\text{g ai/L}$.

Hatch

DDVP: Survival of Exposed Rainbow Trout

File: 43788001.hat Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

$H_0: \text{GRP1 MEAN} = \text{GRP2 MEAN}$

GRP1 (SOLVENT CRTL) MEAN =	0.9500	CALCULATED t VALUE =	-0.4649
GRP2 (BLANK CRTL) MEAN =	0.9625	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0125		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Hatch

DDVP: Survival of Exposed Rainbow Trout
File: 43788001.hat Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	1	7	9	11	0

Calculated Chi-Square goodness of fit test statistic = 5.1945
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Hatch

DDVP: Survival of Exposed Rainbow Trout
File: 43788001.hat Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.034

W = 0.958

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: DDVP: *Hatch* Survival of Exposed Rainbow Trout
 FILE: 43788001.hat
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	0.9500	0.9500
1	GRPS 1&2 POOLED	2	0.9000	0.9000
1	GRPS 1&2 POOLED	3	0.9500	0.9500
1	GRPS 1&2 POOLED	4	1.0000	1.0000
1	GRPS 1&2 POOLED	5	0.9800	0.9800
1	GRPS 1&2 POOLED	6	0.9800	0.9800
1	GRPS 1&2 POOLED	7	0.9800	0.9800
1	GRPS 1&2 POOLED	8	0.9100	0.9100
2	5.15 ppb	1	0.9300	0.9300
2	5.15 ppb	2	0.9800	0.9800
2	5.15 ppb	3	0.9500	0.9500
2	5.15 ppb	4	1.0000	1.0000
3	10.1 ppb	1	0.9500	0.9500
3	10.1 ppb	2	1.0000	1.0000
3	10.1 ppb	3	0.9300	0.9300
3	10.1 ppb	4	0.9300	0.9300
4	23.3 ppb	1	1.0000	1.0000
4	23.3 ppb	2	0.9300	0.9300
4	23.3 ppb	3	0.9800	0.9800
4	23.3 ppb	4	1.0000	1.0000
5	55.2 ppb	1	1.0000	1.0000
5	55.2 ppb	2	0.9000	0.9000
5	55.2 ppb	3	0.9500	0.9500
5	55.2 ppb	4	1.0000	1.0000
6	103 ppb	1	0.9300	0.9300
6	103 ppb	2	0.9300	0.9300
6	103 ppb	3	0.9800	0.9800
6	103 ppb	4	0.8500	0.8500

Hatch
 DDVP: Survival of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.007	0.001	0.891
Within (Error)	22	0.034	0.002	
Total	27	0.041		

Critical F value = 2.66 (0.05, 5, 22)
 Since F < Critical F FAIL TO REJECT Ho: All equal

Hatch

DDVP: Survival of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

DUNNETT'S TEST

***** WARNING *****

This data set has unequal replicates. The Bonferroni t-test
 should be used instead of the Dunnett's test.

Hatch

DDVP: Survival of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.956	0.956		
2	5.15 ppb	0.965	0.965	-0.364	
3	10.1 ppb	0.953	0.953	0.156	
4	23.3 ppb	0.977	0.977	-0.883	
5	55.2 ppb	0.962	0.962	-0.260	
6	103 ppb	0.923	0.923	1.402	

Dunnett table value = 2.39 (1 Tailed Value, P=0.05, df=20,5)

Hatch

DDVP: Survival of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	5.15 ppb	4	0.058	6.0	-0.009
3	10.1 ppb	4	0.058	6.0	0.004
4	23.3 ppb	4	0.058	6.0	-0.021
5	55.2 ppb	4	0.058	6.0	-0.006
6	103 ppb	4	0.058	6.0	0.034

Hatch

DDVP: Survival of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	0.956	0.956	0.962
2	5.15 ppb	4	0.965	0.965	0.962
3	10.1 ppb	4	0.953	0.953	0.962
4	23.3 ppb	4	0.977	0.977	0.962
5	55.2 ppb	4	0.962	0.962	0.962
6	103 ppb	4	0.923	0.923	0.923

Hatch

DDVP: ~~Survival~~ of Exposed Rainbow Trout
 File: 43788001.hat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.962				
5.15 ppb	0.962	0.225		1.72	k= 1, v=22
10.1 ppb	0.962	0.225		1.80	k= 2, v=22
23.3 ppb	0.962	0.225		1.83	k= 3, v=22
55.2 ppb	0.962	0.225		1.84	k= 4, v=22
103 ppb	0.923	1.404		1.85	k= 5, v=22

s = 0.039

Note: df used for table values are approximate when v > 20.

DDVP: Survival of Exposed Rainbow Trout Larvae
File: 43788001.sur Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

H_0 : GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.9000	CALCULATED t VALUE =	-0.0000
GRP2 (BLANK CRTL) MEAN =	0.9000	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-0.0000		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

DDVP: Survival of Exposed Rainbow Trout Larvae
File: 43788001.sur Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

DDVP: Survival of Exposed Rainbow Trout Larvae
File: 43788001.sur Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.013

W = 0.937

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: DDVP: Survival of Exposed Rainbow Trout Larvae
 FILE: 43788001.sur
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	0.9000	0.9000
1	GRPS 1&2 POOLED	2	0.9000	0.9000
1	GRPS 1&2 POOLED	3	0.9300	0.9300
1	GRPS 1&2 POOLED	4	0.8700	0.8700
2	5.15 ppb	1	0.8300	0.8300
2	5.15 ppb	2	0.9000	0.9000
3	10.1 ppb	1	0.6700	0.6700
3	10.1 ppb	2	0.7700	0.7700
4	23.3 ppb	1	0.7700	0.7700
4	23.3 ppb	2	0.7300	0.7300
5	55.2 ppb	1	0.7700	0.7700
5	55.2 ppb	2	0.7300	0.7300
6	103 ppb	1	0.7000	0.7000
6	103 ppb	2	0.7700	0.7700

DDVP: Survival of Exposed Rainbow Trout Larvae
 File: 43788001.sur Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.080	0.016	9.574
Within (Error)	8	0.013	0.002	
Total	13	0.093		

Critical F value = 3.69 (0.05, 5, 8)
 Since F > Critical F REJECT Ho: All equal

DDVP: Survival of Exposed Rainbow Trout Larvae
 File: 43788001.sur Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED	MEAN CALCULATED IN		T STAT	SIG
		MEAN	ORIGINAL UNITS			
1	GRPS 1&2 POOLED	0.900	0.900			
2	5.15 ppb	0.865	0.865		0.991	
3	10.1 ppb	0.720	0.720		5.098	*
4	23.3 ppb	0.750	0.750		4.248	*
5	55.2 ppb	0.750	0.750		4.248	*

6

103 ppb

0.735

0.735

4.673 *

Bonferroni t table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

DDVP: Survival of Exposed Rainbow Trout Larvae

File: 43788001.sur Transform: NO TRANSFORMATION

BONFERRONI t-TEST

TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	5.15 ppb	2	0.102	11.4	0.035
3	10.1 ppb	2	0.102	11.4	0.180
4	23.3 ppb	2	0.102	11.4	0.150
5	55.2 ppb	2	0.102	11.4	0.150
6	103 ppb	2	0.102	11.4	0.165

DDVP: Survival of Exposed Rainbow Trout Larvae

File: 43788001.sur Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.900	0.900	0.900
2	5.15 ppb	2	0.865	0.865	0.865
3	10.1 ppb	2	0.720	0.720	0.740
4	23.3 ppb	2	0.750	0.750	0.740
5	55.2 ppb	2	0.750	0.750	0.740
6	103 ppb	2	0.735	0.735	0.735

DDVP: Survival of Exposed Rainbow Trout Larvae

File: 43788001.sur Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.900				
5.15 ppb	0.865	0.992		1.86	k= 1, v= 8
10.1 ppb	0.740	4.535	*	1.96	k= 2, v= 8
23.3 ppb	0.740	4.535	*	2.00	k= 3, v= 8
55.2 ppb	0.740	4.535	*	2.01	k= 4, v= 8
103 ppb	0.735	4.676	*	2.02	k= 5, v= 8

s = 0.041

Note: df used for table values are approximate when v > 20.

File:c:\epa\aquatic\43788001.out Page 1
DDVP Technical Grade: Effects to the ELS of Rainbow Trout

OBS	LEVEL	REP	LEN	WT
1	CONTROL	1	38.2	0.75
2	CONTROL	1	37.5	0.64
3	CONTROL	1	38.1	0.73
4	CONTROL	1	33.0	0.47
5	CONTROL	1	38.9	0.74
6	CONTROL	1	37.7	0.66
7	CONTROL	1	35.1	0.62
8	CONTROL	1	36.8	0.61
9	CONTROL	1	37.0	0.62
10	CONTROL	1	36.2	0.62
11	CONTROL	1	39.4	0.71
12	CONTROL	1	36.6	0.59
13	CONTROL	1	36.8	0.63
14	CONTROL	1	37.0	0.64
15	CONTROL	1	35.9	0.59
16	CONTROL	1	35.4	0.54
17	CONTROL	1	38.0	0.70
18	CONTROL	1	38.2	0.71
19	CONTROL	1	34.7	0.61
20	CONTROL	1	34.0	0.53
21	CONTROL	1	38.1	0.66
22	CONTROL	1	39.0	0.75
23	CONTROL	1	34.3	0.55
24	CONTROL	1	35.5	0.64
25	CONTROL	1	35.0	0.51
26	CONTROL	1	35.2	0.59
27	CONTROL	1	37.1	0.63
28	CONTROL	1	35.1	0.57
29	CONTROL	2	38.1	0.71
30	CONTROL	2	37.0	0.63
31	CONTROL	2	35.9	0.57
32	CONTROL	2	38.2	0.76
33	CONTROL	2	35.6	0.55
34	CONTROL	2	38.0	0.75
35	CONTROL	2	36.1	0.63
36	CONTROL	2	37.1	0.70
37	CONTROL	2	36.4	0.65
38	CONTROL	2	37.6	0.66
39	CONTROL	2	37.0	0.63
40	CONTROL	2	39.1	0.78
41	CONTROL	2	35.3	0.54
42	CONTROL	2	37.7	0.67
43	CONTROL	2	35.5	0.53
44	CONTROL	2	37.8	0.70
45	CONTROL	2	35.2	0.55
46	CONTROL	2	35.4	0.55
47	CONTROL	2	37.6	0.69
48	CONTROL	2	38.0	0.66
49	CONTROL	2	36.0	0.63
50	CONTROL	2	34.9	0.53
51	CONTROL	2	34.8	0.52
52	CONTROL	2	39.1	0.72
53	CONTROL	2	33.9	0.46
54	CONTROL	2	35.4	0.50
55	SOL_CONT	1	40.7	0.87
56	SOL_CONT	1	36.3	0.62
57	SOL_CONT	1	44.2	0.76
58	SOL_CONT	1	35.0	0.52
59	SOL_CONT	1	35.3	0.54
60	SOL_CONT	1	37.2	0.64
61	SOL_CONT	1	47.1	0.85
62	SOL_CONT	1	36.4	0.56
63	SOL_CONT	1	35.0	0.52
64	SOL_CONT	1	37.2	0.66

File:c:\epa\aquatic\43788001.out Page 2
DDVP Technical Grade: Effects to the ELS of Rainbow Trout

OBS	LEVEL	REP	LEN	WT
65	SOL_CONT	1	35.7	0.60
66	SOL_CONT	1	38.4	0.70
67	SOL_CONT	1	38.6	0.69
68	SOL_CONT	1	35.7	0.56
69	SOL_CONT	1	37.8	0.63
70	SOL_CONT	1	37.3	0.63
71	SOL_CONT	1	39.1	0.76
72	SOL_CONT	1	38.5	0.48
73	SOL_CONT	1	37.6	0.68
74	SOL_CONT	1	37.0	0.62
75	SOL_CONT	1	34.8	0.58
76	SOL_CONT	1	38.1	0.68
77	SOL_CONT	1	38.0	0.63
78	SOL_CONT	1	36.6	0.62
79	SOL_CONT	1	36.6	0.65
80	SOL_CONT	1	34.7	0.47
81	SOL_CONT	1	37.1	0.61
82	SOL_CONT	2	40.0	0.84
83	SOL_CONT	2	35.8	0.62
84	SOL_CONT	2	39.3	0.69
85	SOL_CONT	2	37.7	0.66
86	SOL_CONT	2	38.3	0.75
87	SOL_CONT	2	35.2	0.58
88	SOL_CONT	2	35.2	0.59
89	SOL_CONT	2	35.4	0.67
90	SOL_CONT	2	35.0	0.57
91	SOL_CONT	2	36.1	0.65
92	SOL_CONT	2	36.2	0.61
93	SOL_CONT	2	37.1	0.70
94	SOL_CONT	2	37.0	0.69
95	SOL_CONT	2	37.4	0.66
96	SOL_CONT	2	38.1	0.64
97	SOL_CONT	2	34.6	0.53
98	SOL_CONT	2	36.2	0.60
99	SOL_CONT	2	38.5	0.70
100	SOL_CONT	2	33.9	0.46
101	SOL_CONT	2	36.0	0.58
102	SOL_CONT	2	34.0	0.51
103	SOL_CONT	2	36.3	0.66
104	SOL_CONT	2	35.0	0.54
105	SOL_CONT	2	36.5	0.65
106	SOL_CONT	2	36.8	0.62
107	SOL_CONT	2	39.0	0.69
108	SOL_CONT	2	33.8	0.45
109	TRT1	1	37.0	0.70
110	TRT1	1	38.2	0.71
111	TRT1	1	37.9	0.76
112	TRT1	1	37.8	0.79
113	TRT1	1	38.1	0.74
114	TRT1	1	37.9	0.87
115	TRT1	1	36.7	0.76
116	TRT1	1	36.0	0.61
117	TRT1	1	34.5	0.57
118	TRT1	1	33.5	0.42
119	TRT1	1	37.3	0.71
120	TRT1	1	33.0	0.45
121	TRT1	1	36.7	0.68
122	TRT1	1	35.8	0.64
123	TRT1	1	35.2	0.71
124	TRT1	1	34.9	0.58
125	TRT1	1	34.0	0.59
126	TRT1	1	38.1	0.73
127	TRT1	1	36.8	0.67
128	TRT1	1	36.4	0.65

OBS	LEVEL	REP	LEN	WT
129	TRT1	1	34.5	0.60
130	TRT1	1	34.8	0.60
131	TRT1	1	37.2	0.70
132	TRT1	1	35.5	0.59
133	TRT1	1	38.4	0.79
134	TRT1	2	36.4	367.00
135	TRT1	2	37.7	0.74
136	TRT1	2	40.2	0.83
137	TRT1	2	36.2	0.74
138	TRT1	2	36.7	0.62
139	TRT1	2	37.1	0.63
140	TRT1	2	35.6	0.67
141	TRT1	2	39.7	0.82
142	TRT1	2	36.1	0.63
143	TRT1	2	35.0	0.57
144	TRT1	2	39.7	0.82
145	TRT1	2	35.7	0.60
146	TRT1	2	31.1	0.38
147	TRT1	2	37.1	0.67
148	TRT1	2	37.8	0.75
149	TRT1	2	35.2	0.61
150	TRT1	2	38.7	0.78
151	TRT1	2	34.2	0.47
152	TRT1	2	36.0	0.68
153	TRT1	2	33.8	0.55
154	TRT1	2	36.5	0.63
155	TRT1	2	38.2	0.68
156	TRT1	2	35.4	0.61
157	TRT1	2	35.0	0.57
158	TRT1	2	35.7	0.65
159	TRT1	2	36.8	0.64
160	TRT1	2	33.8	0.50
161	TRT2	1	35.0	0.52
162	TRT2	1	35.8	0.57
163	TRT2	1	32.5	0.48
164	TRT2	1	35.0	0.54
165	TRT2	1	32.8	0.42
166	TRT2	1	37.8	0.67
167	TRT2	1	34.7	0.62
168	TRT2	1	35.6	0.61
169	TRT2	1	31.6	0.39
170	TRT2	1	32.0	0.38
171	TRT2	1	36.2	0.67
172	TRT2	1	35.5	0.59
173	TRT2	1	38.9	0.76
174	TRT2	1	34.2	0.46
175	TRT2	1	34.0	0.48
176	TRT2	1	32.5	0.46
177	TRT2	1	31.0	0.32
178	TRT2	1	37.5	0.54
179	TRT2	1	36.0	0.61
180	TRT2	1	36.3	0.71
181	TRT2	2	36.8	0.68
182	TRT2	2	36.8	0.71
183	TRT2	2	35.5	0.60
184	TRT2	2	34.1	0.53
185	TRT2	2	37.4	0.72
186	TRT2	2	36.1	0.59
187	TRT2	2	37.0	0.82
188	TRT2	2	38.2	0.77
189	TRT2	2	35.8	0.68
190	TRT2	2	37.0	0.67
191	TRT2	2	36.1	0.64
192	TRT2	2	31.8	0.48

OBS	LEVEL	REP	LEN	WT
193	TRT2	2	35.6	0.68
194	TRT2	2	34.8	0.64
195	TRT2	2	35.4	0.59
196	TRT2	2	35.8	0.60
197	TRT2	2	36.8	0.69
198	TRT2	2	38.0	0.70
199	TRT2	2	32.8	0.48
200	TRT2	2	36.6	0.58
201	TRT2	2	36.1	0.66
202	TRT2	2	38.1	0.71
203	TRT2	2	36.7	0.61
204	TRT3	1	35.5	0.79
205	TRT3	1	35.3	0.62
206	TRT3	1	37.0	0.71
207	TRT3	1	36.4	0.73
208	TRT3	1	36.0	0.67
209	TRT3	1	40.8	0.90
210	TRT3	1	39.4	0.82
211	TRT3	1	37.4	0.75
212	TRT3	1	37.0	0.72
213	TRT3	1	36.1	0.74
214	TRT3	1	39.1	0.82
215	TRT3	1	32.7	0.44
216	TRT3	1	34.1	0.52
217	TRT3	1	37.5	0.64
218	TRT3	1	37.0	0.75
219	TRT3	1	36.2	0.76
220	TRT3	1	37.5	0.74
221	TRT3	1	37.6	0.70
222	TRT3	1	35.4	0.57
223	TRT3	1	36.1	0.65
224	TRT3	1	37.0	0.70
225	TRT3	1	36.3	0.62
226	TRT3	1	35.4	0.54
227	TRT3	2	36.2	0.67
228	TRT3	2	37.4	0.72
229	TRT3	2	38.4	0.74
230	TRT3	2	35.7	0.63
231	TRT3	2	38.5	0.78
232	TRT3	2	36.0	0.69
233	TRT3	2	37.2	0.73
234	TRT3	2	37.4	0.68
235	TRT3	2	34.8	0.56
236	TRT3	2	37.1	0.60
237	TRT3	2	35.1	0.63
238	TRT3	2	36.1	0.61
239	TRT3	2	38.0	0.76
240	TRT3	2	39.0	0.74
241	TRT3	2	37.8	0.80
242	TRT3	2	37.0	0.67
243	TRT3	2	36.8	0.69
244	TRT3	2	36.2	0.67
245	TRT3	2	41.3	0.96
246	TRT3	2	37.8	0.72
247	TRT3	2	36.5	0.64
248	TRT3	2	36.8	0.67
249	TRT4	1	37.5	0.74
250	TRT4	1	36.8	0.69
251	TRT4	1	35.8	0.70
252	TRT4	1	37.7	0.73
253	TRT4	1	39.0	0.79
254	TRT4	1	37.1	0.72
255	TRT4	1	37.1	72.00
256	TRT4	1	35.6	0.61

OBS	LEVEL	REP	LEN	WT
257	TRT4	1	36.4	0.71
258	TRT4	1	37.5	0.64
259	TRT4	1	33.8	0.56
260	TRT4	1	34.6	0.57
261	TRT4	1	36.9	0.64
262	TRT4	1	38.0	0.76
263	TRT4	1	35.1	0.64
264	TRT4	1	39.4	0.86
265	TRT4	1	38.2	0.73
266	TRT4	1	35.5	0.65
267	TRT4	1	38.5	0.69
268	TRT4	1	39.0	0.85
269	TRT4	1	35.0	0.53
270	TRT4	1	35.8	0.65
271	TRT4	1	32.4	0.42
272	TRT4	2	36.5	0.65
273	TRT4	2	35.5	0.77
274	TRT4	2	34.8	0.55
275	TRT4	2	34.5	0.55
276	TRT4	2	36.2	0.60
277	TRT4	2	35.4	0.59
278	TRT4	2	33.9	0.58
279	TRT4	2	33.7	0.54
280	TRT4	2	36.2	0.70
281	TRT4	2	37.0	0.63
282	TRT4	2	40.1	0.84
283	TRT4	2	38.9	0.86
284	TRT4	2	37.8	0.69
285	TRT4	2	39.9	0.80
286	TRT4	2	35.8	0.67
287	TRT4	2	39.9	0.79
288	TRT4	2	37.3	0.75
289	TRT4	2	38.0	0.82
290	TRT4	2	37.8	77.00
291	TRT4	2	36.9	0.70
292	TRT4	2	34.0	0.55
293	TRT4	2	34.3	0.53
294	TRT5	1	35.4	0.64
295	TRT5	1	37.2	0.76
296	TRT5	1	37.8	0.72
297	TRT5	1	36.2	0.58
298	TRT5	1	34.1	0.64
299	TRT5	1	32.4	0.55
300	TRT5	1	36.1	0.67
301	TRT5	1	36.2	0.67
302	TRT5	1	36.0	0.58
303	TRT5	1	36.8	0.75
304	TRT5	1	34.7	0.56
305	TRT5	1	33.5	0.50
306	TRT5	1	40.0	0.90
307	TRT5	1	35.4	0.64
308	TRT5	1	36.1	0.66
309	TRT5	1	36.8	0.83
310	TRT5	1	36.5	0.71
311	TRT5	1	36.8	0.66
312	TRT5	1	32.3	0.46
313	TRT5	1	37.9	0.77
314	TRT5	1	38.8	0.82
315	TRT5	2	38.0	0.81
316	TRT5	2	35.5	0.63
317	TRT5	2	37.8	0.77
318	TRT5	2	36.7	0.71
319	TRT5	2	36.3	0.68
320	TRT5	2	35.8	0.71

OBS	LEVEL	REP	LEN	WT
321	TRT5	2	36.2	0.61
322	TRT5	2	34.7	0.62
323	TRT5	2	37.7	0.85
324	TRT5	2	33.2	0.55
325	TRT5	2	37.7	0.77
326	TRT5	2	36.4	0.63
327	TRT5	2	38.5	0.79
328	TRT5	2	32.5	0.51
329	TRT5	2	35.4	0.69
330	TRT5	2	35.7	0.67
331	TRT5	2	36.3	0.64
332	TRT5	2	37.1	0.65
333	TRT5	2	34.8	0.56
334	TRT5	2	33.8	0.60
335	TRT5	2	35.0	0.52
336	TRT5	2	32.2	0.46
337	TRT5	2	37.5	0.66

DDVP Technical Grade: Effects to the ELS of Rainbow Trout

	LEVEL			
	CONTROL	SOL_CONT	TRT1	TRT2
LEN	MEAN	MEAN	MEAN	MEAN
WT	0.63	0.63	7.70	0.60

(CONTINUED)
 DDVP Technical Grade: Effects to the ELS of Rainbow Trout

	LEVEL		
	TRT3	TRT4	TRT5
LEN	MEAN	MEAN	MEAN
WT	36.89	36.60	35.95

DDVP Technical Grade: Effects to the ELS of Rainbow Trout

LEVEL=CONTROL

Variable	N	Mean	Std Dev	CV
REP	54	1.481	0.504	34.044
LEN	54	36.602	1.529	4.176
WT	54	0.627	0.080	12.740

LEVEL=SOL_CONT

Variable	N	Mean	Std Dev	CV

Variable	N	Mean	Std Dev	CV
REP	54	1.500	0.505	33.646
LEN	54	37.04	2.334	6.300
WT	54	0.630	0.091	14.434

LEVEL=TRT1

Variable	N	Mean	Std Dev	CV
REP	52	1.519	0.505	33.208
LEN	52	36.500	1.804	4.970
WT	52	7.701	50.803	659.679

LEVEL=TRT2

Variable	N	Mean	Std Dev	CV
REP	43	1.535	0.505	32.881
LEN	43	35.447	1.959	5.526
WT	43	0.596	0.113	18.893

LEVEL=TRT3

Variable	N	Mean	Std Dev	CV
REP	45	1.489	0.506	33.953
LEN	45	36.387	1.578	4.271
WT	45	0.695	0.095	13.707

LEVEL=TRT4

Variable	N	Mean	Std Dev	CV
REP	45	1.489	0.506	33.953
LEN	45	36.602	1.863	5.089
WT	45	3.956	15.395	389.119

LEVEL=TRT5

Variable	N	Mean	Std Dev	CV
REP	44	1.523	0.505	33.181
LEN	44	35.950	1.811	5.038
WT	44	0.663	0.105	15.780

LEVEL=TRT6

Variable	N	Mean	Std Dev	CV
REP	44	1.523	0.505	33.181
LEN	44	35.950	1.811	5.038
WT	44	0.663	0.105	15.780

LEVEL=TRT7

Variable	N	Mean	Std Dev	CV
REP	44	1.523	0.505	33.181
LEN	44	35.950	1.811	5.038
WT	44	0.663	0.105	15.780

Class	Levels	Values
LEVEL	7	CONTROL SOL_CONT TRT1 TRT2 TRT3 TRT4 TRT5

Number of observations in data set = 337

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure
Type I Estimable Functions for: LEVEL

Coefficients

0

LEVEL

CONTROL	L2
SOL_CONT	L3
TRT1	L4
TRT2	L5
TRT3	L6
TRT4	L7
TRT5	-L2-L3-L4-L5-L6-L7

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure

Dependent Variable: LEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	85.308364	14.218061	4.11	0.0005
Error	330	1142.561903	3.462309		

Corrected Total

R-Square	C.V.	Root MSE	LEN Mean
0.069477	5.107435	1.8607	36.432

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure
Least Squares Means

LEVEL	LEN	LSMEAN	Number
CONTROL	36.6018519	1	
SOL_CONT	37.0444444	2	
TRT1	36.3000000	3	
TRT2	35.4465116	4	
TRT3	36.8866667	5	
TRT4	36.6022222	6	

File:c:\epa\aquatic\43788001.out Page 9
 TRT5 35.9500000 7
 Pr > |T| HO: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6	7
1	0.2174	0.4044	0.0026	0.4488	0.9992	0.0855	
2	0.2174	0.0403	0.0001	0.6747	0.2399	0.0040	
3	0.4044	0.0403	-0.0267	0.1224	0.4256	0.3591	
4	0.0026	0.0001	0.0267	0.0003	0.0038	0.2079	
5	0.4488	0.6747	0.1224	0.0003	0.4689	0.0182	
6	0.9992	0.2399	0.4256	0.0038	0.4689	0.0992	
7	0.0855	0.0040	0.3591	0.2079	0.0182	0.0992	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: LEN

NOTE: This test controls the type I experimentwise error rate.

Alpha= 0.05 Confidence= 0.95 df= 330 MSE= 3.462309
 Critical Value of Studentized Range= 4.195

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

LEVEL Comparison		Simultaneous Lower Confidence Limit	Simultaneous Difference Between Means	Simultaneous Upper Confidence Limit	
SOL_CONT - TRT3		-0.9564	0.1578	1.2720	
SOL_CONT - TRT4		-0.6720	0.4422	1.5564	
SOL_CONT - CONTROL		-0.6197	0.4426	1.5049	
SOL_CONT - TRT1		-0.3281	0.7444	1.8170	
SOL_CONT - TRT5		-0.0266	1.0944	2.2155	
SOL_CONT - TRT2		0.4697	1.5979	2.7262	***
TRT3 - SOL_CONT		-1.2720	-0.1578	0.9564	
TRT3 - TRT4		-0.8793	0.2844	1.4482	
TRT3 - CONTROL		-0.8294	0.2848	1.3990	
TRT3 - TRT1		-0.5372	0.5867	1.7106	
TRT3 - TRT5		-0.2337	0.9367	2.1070	
TRT3 - TRT2		0.2630	1.4402	2.6173	***
TRT4 - SOL_CONT		-1.5564	-0.4422	0.6720	
TRT4 - TRT3		-1.4482	-0.2844	0.8793	
TRT4 - CONTROL		-1.1138	-0.0004	1.1146	
TRT4 - TRT1		-0.8217	0.3022	1.4261	
TRT4 - TRT5		-0.5181	0.6522	1.8226	
TRT4 - TRT2		-0.0215	1.1557	2.3329	
CONTROL - SOL_CONT		-1.5049	-0.4426	0.6197	
CONTROL - TRT3		-1.3990	-0.2848	0.8294	
CONTROL - TRT4		-1.1146	-0.0004	1.1138	
CONTROL - TRT1		-0.7707	0.3019	1.3744	
CONTROL - TRT5		-0.4692	0.6519	1.7729	
CONTROL - TRT2		0.0271	1.1553	2.2836	***
TRT1 - SOL_CONT		-1.8170	-0.7444	0.3281	
TRT1 - TRT3		-1.7106	-0.5867	0.5372	
TRT1 - TRT4		-1.4261	-0.3022	0.8217	

File:c:\epa\aquatic\43788001.out Page 10
 TRT1 - CONTROL -1.3744 -0.3019 0.7707
 TRT1 - TRT5 -0.7807 0.3500 1.4807
 TRT1 - TRT2 -0.2843 0.8535 1.9913

TRT5 - SOL_CONT	-2.2155	-1.0944	0.0266
TRT5 - TRT3	-2.1070	-0.9367	0.2337
TRT5 - TRT4	-1.8226	-0.6522	0.5181
TRT5 - CONTROL	-1.7729	-0.6519	0.4692
TRT5 - TRT1	-1.4807	-0.3500	0.7807
TRT5 - TRT2	-0.6802	0.5035	1.6872
TRT2 - SOL_CONT	-2.7262	-1.5979	-0.4697 ***
TRT2 - TRT3	-2.6173	-1.4402	-0.2630 ***
TRT2 - TRT4	-2.3329	-1.1557	0.0215
TRT2 - CONTROL	-2.2836	-1.1553	-0.0271 ***
TRT2 - TRT1	-1.9913	-0.8535	0.2843

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure

LEVEL Comparison	Simultaneous Lower Confidence Limit	Simultaneous Difference Between Means	Simultaneous Upper Confidence Limit
TRT2 - TRT5	-1.6872	-0.5035	0.6802

DDVP Technical Grade: Effects to the ELS of Rainbow Trout
 1. ANALYSIS OF Length

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: LEN

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 330 MSE= 3.462309
 Critical Value of Dunnett's T= 2.314

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

LEVEL Comparison	Simultaneous Lower Confidence Limit	Simultaneous Difference Between Means	Simultaneous Upper Confidence Limit
SOL_CONT - CONTROL	-0.3861	0.4426	1.2713
TRT3 - CONTROL	-0.5843	0.2848	1.1539
TRT4 - CONTROL	-0.8687	0.0004	0.8695
TRT1 - CONTROL	-1.1384	-0.3019	0.5347
TRT5 - CONTROL	-1.5263	-0.6519	0.2226
TRT2 - CONTROL	-2.0354	-1.1553	-0.2753 ***