

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)

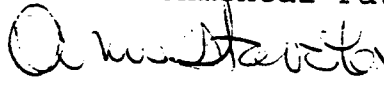
- 1. CHEMICAL: Pirate® AC 303,630 Shaughnessey #: 129093
- 2. TEST MATERIAL: CL 303,630 (AC 303,630) Purity: 94.5%
- 3. CITATION:

Authors: Ward, G. Scott, McElwee, C., Lintott, D., Wisk, Joseph D.
Title: Early Life-Stage Toxicity of AC 303,630 in Rainbow Trout (*Oncorhynchus mykiss*)
Study Completion Date: November 19, 1993
Laboratory: Toxikon Environmental Sciences, 106 Coastal Way, Jupiter, Florida 33477
Laboratory Report ID: J9201015
Sponsor: American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ 08543-0400
MRID No.: 434928-19
DP Barcode: D210808

- 4. REVIEWED BY: William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:  Date: 12/16/96

- 5. APPROVED BY: Ann Stavola, Section Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:  Date: 12/22/96

6. CONCLUSIONS: For the first 77 days of the exposure phase of the study, dissolved oxygen (DO) was maintained above 75% saturation (with one exception). However, after day 77, DO levels dropped, but stayed above 54% saturation. Further, true toxicological effects based on mortality first appeared at the 7.64 µg/L level on day 38 of the study. This study has therefore been classified as Core with a calculated MATC of 5.3 µg/L based on the survival of juvenile rainbow trout. The NOEC was 3.68 µg/L.

7. ADEQUACY OF THE STUDY:

- A. Classification: Core
- B. Rationale: See 6. above.
- C. Reparability: N/A

8. MAJOR GUIDELINE DEVIATIONS:

1. Water temperature should not deviate by more than 2°C from appropriate temperature (12°C for rainbow trout). Test temperature ranged from 9.1 - 16°C. throughout test.
2. The dissolved oxygen (DO) level was maintained above 54% saturation. Guideline criteria require that DO concentration must be above 75% saturation. ASTM guidelines recommend maintaining above 60% saturation.
3. Replicate data was not included for hatching time or time to swim-up.

9. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
<p>Species: Any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See SEP for complete listing.</p>	<p>Test species was Rainbow trout (<i>Oncorhynchus mykiss</i>)</p>
<p>Source</p>	<p>Mt Lassen Trout Farm, Red Bluff, CA</p>
<p>Age at beginning of test: Embryos 2 to 24 hours old.</p>	<p>Eggs distributed to to test chambers ~4 hr. after fertilization.</p>
<p>Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.</p>	<p>80 embryos (40 per embryo chamber) were distributed to each replicate, 2 replicates per concentration. (2 additional chambers with 40-embryos were added to each replicate control.) 30 alevins & fish per treatment were released.</p>

Guideline Criteria	Reported Information
Post Hatch: % 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.	Hatching success was > 50% in each control and the greatest % hatch was 1.54 times that of another cup.
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 fed 2-3 times a day up to 1 day before test termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Survival was monitored daily until test termination at 61 days
Controls: Avg. survival at end of test must be \geq 80%. Survival in any control chamber must not be < 70%.	Control survival ranged from 97 to 100% in control and solvent controls.
Controls: Negative control and carrier control (when applicable) are required.	Solvent control of 2.3 to 2.9 μ l Dimethylformamide (DMF)/L was used.

Comments:**B. Physical System:**

Guideline Criteria	Reported Information
<p>Test Water:</p> <ol style="list-style-type: none"> 1) May be natural or reconstituted; 2) Natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. 3) Hardness of 40 to 48 mg/L as CaCO₃ and pH of 7.2 to 7.6 is recommended. 	Dilution water was reconstituted treated water. Hardness was measured at 44 to 80 mg/L as CaCO ₃ . The pH at the beginning of the test was 7.1 - 7.2.

Guideline Criteria	Reported Information
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature.	Test temperature ranged from 9.1 - 16°C. throughout test. Appropriate temperature is 12°C. for rainbow trout.
Photoperiod: Recommend 16L/8D.	8 hours light for 1st wk. followed by 16 hours light for second week and beyond.
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.	A modified proportional vacuum-siphon diluter volumetrically calibrated to provide a 50% dilution. 5 toxicant concentrations with dilution factor of 0.5 was used.
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) Mixing chamber was used. 2) Aeration was not used for mixing. 3) An average high nominal concentration of 8.29 µg ai/L was provided to each chamber. 4) Flow splitting accuracy was within 5% of desired volumes.
Test Vessels: All glass or glass with stainless steel frame.	Test vessels were glass petri dishes.
Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	80 mm diameter glass petri dishes encircled by 35-µm Nitex mesh with a height of 18 cm. Calculated volume of dish = 904 ml ((4cm ²)π x 18cm).
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.	5.8 volume additions were provided every 24 hrs. DO was maintained above 54% saturation.

Guideline Criteria	Reported Information
<p>Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	<p>Dilution water was aerated.</p>

Comments: No comments.

C. Chemical System:

Guideline Criteria	Reported Information
<p>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.</p>	<p>5 replicated concentrations plus solvent control were used. - Toxicant conc. was measured in each replicate once each week as ¹⁴C - AC 303,630 equivalents/L. - One concentration adversely affected a life stage and one concentration did not.</p>
<p>Other Variables: 1) DO must be measured at each conc. at least once a week; 2) Freshwater parameters in a control and one concentration must be analyzed once a week.</p>	<p>1) DO was measured at initiation and once a week thereafter. 2) Freshwater parameters measured once a week.</p>
<p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>Dimethylformamide was used. Concentration was maintained at 0.0023 to 0.0029 ml/L.</p>

Comments:

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
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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 Endpoints included: - Number embryos hatched - Mortality of embryos, larvae, and juveniles - Measurement of growth - Incidence of pathological or histological effects - Observations of other effects. Data Endpoints <u>not</u> included: - Time to hatch - Time to swim-up
Raw data included? (Y/N)	No. Replicate data was not included for hatching time or time to swim-up.

Effects Data:

Toxicant Conc. ($\mu\text{g/L}$)		Percent Hatch		Time to Hatch ^a		Survival #'s (37 days)		Total Length (mm)		Wet weight (gm)	
Nom.	Meas.	A	B	A	B	A	B	A	B	A	B
Ctrl	-	90	88			30	30	40.4	39.3	1.0	0.98
Solv	-	74	84			30	29	38.5	39.2	0.9 9	1.0
0.624	0.459	75	90			30	30	39.5	40.5	1.0 4	1.19
1.25	0.907	86	86			30	30	44.2	39.0	1.4 8	1.12
2.5	1.78	70	90			30	30	40.2	40.8	1.0 5	1.07
4.99	3.68	80	85			30	30	38.5	37.2	0.8 1	1.12
9.98	7.64	79	80			30	30	35	-	0.5 7	-

a Replicate data on time to hatch was not submitted. Study claimed that hatching begun on day 24 and was $\geq 95\%$ completed by day 33.

Toxicity Observations:

Hatching success throughout the test ranged from 79% at at the 7.64 $\mu\text{g/L}$ level to 86% at the 0.907 $\mu\text{g/L}$ level. Survival of juvenile fish ranged from 2% at 7.64 $\mu\text{g/L}$ to 100% at the 1.78

µg/L level. No abnormalities in behavior or physical appearance were noted in the report.

Statistical Results:

Statistical Method: Analysis of variance (ANOVA) - Dunnett's Test

NOEL: 3.68 µg/L LEL: 7.64 µg/L

MATC: 5.30 µg/L

Most sensitive endpoint: Reduction in survival

Comments: None

11. Reviewer's Statistical Results: The reviewer verified the results of the statistical analysis.

Statistical Method: Analysis of variance (ANOVA) - Dunnett's test.

NOEL: 3.68 µg/L LEL: 7.64 µg/L MATC: 5.30 µg/L

Most sensitive endpoint: Survival of juvenile rainbow trout

Comments: DO level was maintained above 54% saturation. Guideline criteria require that DO concentration must be above 75% saturation. ASTM guidelines recommend maintaining above 60% saturation. For the first 77 days of the exposure phase of the study, dissolved oxygen (DO) was maintained above 75% saturation (with one exception). However, after day 77, DO levels dropped, but stayed above 54% saturation. Further, true toxicological effects based on mortality first appeared at the 7.64 µg.L level on day 38 of the study. The study is therefore classified as Core.

12. COMPLETION OF ONE-LINER FOR STUDY: YES

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
1	control	a	36	0.90	1.55630	-0.04576
2	control	a	35	0.88	1.54407	-0.05552
3	control	a	39	1.12	1.59106	0.04922
4	control	a	39	0.76	1.59106	-0.11919
5	control	a	35	1.15	1.54407	0.06070
6	control	a	41	1.24	1.61278	0.09342
7	control	a	37	1.70	1.56820	0.23045
8	control	a	40	1.06	1.60206	0.02531
9	control	a	40	0.98	1.60206	-0.00877
10	control	a	42	0.71	1.62325	-0.14874
11	control	a	43	1.13	1.63347	0.05308
12	control	a	49	1.03	1.69020	0.01284
13	control	a	40	0.64	1.60206	-0.19382
14	control	a	38	0.88	1.57978	-0.05552
15	control	a	39	0.88	1.59106	-0.05552
16	control	a	40	0.84	1.60206	-0.07572
17	control	a	43	1.10	1.63347	0.04139
18	control	a	41	0.96	1.61278	-0.01773
19	control	a	42	0.91	1.62325	-0.04096
20	control	a	41	1.02	1.61278	0.00860
21	control	a	39	0.91	1.59106	-0.04096
22	control	a	43	1.16	1.63347	0.06446
23	control	a	40	0.94	1.60206	-0.02687
24	control	a	41	1.23	1.61278	0.08991
25	control	a	42	0.97	1.62325	-0.01323
26	control	a	43	1.31	1.63347	0.11727
27	control	a	41	0.85	1.61278	-0.07058
28	control	a	40	0.81	1.60206	-0.09151
29	control	a	42	1.01	1.62325	0.00432
30	control	b	37	0.87	1.56820	-0.06048
31	control	b	44	0.97	1.64345	-0.01323
32	control	b	36	0.93	1.55630	-0.03152
33	control	b	40	0.90	1.60206	-0.04576
34	control	b	39	0.88	1.59106	-0.05552
35	control	b	40	0.92	1.60206	-0.03621
36	control	b	40	1.01	1.60206	0.00432
37	control	b	39	1.16	1.59106	0.06446
38	control	b	42	0.90	1.62325	-0.04576
39	control	b	41	0.91	1.61278	-0.04096
40	control	b	42	0.70	1.62325	-0.15490
41	control	b	40	0.82	1.60206	-0.08619
42	control	b	40	1.31	1.60206	0.11727
43	control	b	37	1.03	1.56820	0.01284
44	control	b	41	1.33	1.61278	0.12385
45	control	b	40	0.98	1.60206	-0.00877
46	control	b	35	1.23	1.54407	0.08991
47	control	b	40	0.93	1.60206	-0.03152
48	control	b	39	0.97	1.59106	-0.01323
49	control	b	39	0.86	1.59106	-0.06550
50	control	b	40	1.31	1.60206	0.11727
51	control	b	37	1.07	1.56820	0.02938
52	control	b	39	0.72	1.59106	-0.14267
53	control	b	35	1.14	1.54407	0.05690
54	control	b	41	1.08	1.61278	0.03342
55	control	b	38	1.11	1.57978	0.04532

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
56	control	b	38	0.69	1.57978	-0.16115
57	control	b	39	1.04	1.59106	0.01703
58	control	b	43	0.74	1.63347	-0.13077
59	solvent	c	35	1.27	1.54407	0.10380
60	solvent	c	36	1.35	1.55630	0.13033
61	solvent	c	40	1.25	1.60206	0.09691
62	solvent	c	39	1.00	1.59106	0.00000
63	solvent	c	43	0.74	1.63347	-0.13077
64	solvent	c	37	0.79	1.56820	-0.10237
65	solvent	c	41	0.90	1.61278	-0.04576
66	solvent	c	42	1.18	1.62325	0.07188
67	solvent	c	41	0.75	1.61278	-0.12494
68	solvent	c	37	0.69	1.56820	-0.16115
69	solvent	c	34	0.99	1.53148	-0.00436
70	solvent	c	35	1.15	1.54407	0.06070
71	solvent	c	42	0.88	1.62325	-0.05552
72	solvent	c	37	1.21	1.56820	0.08279
73	solvent	c	43	0.72	1.63347	-0.14267
74	solvent	c	43	1.16	1.63347	0.06446
75	solvent	c	35	0.86	1.54407	-0.06550
76	solvent	c	40	1.13	1.60206	0.05308
77	solvent	c	39	0.89	1.59106	-0.05061
78	solvent	c	39	1.18	1.59106	0.07188
79	solvent	c	37	0.83	1.56820	-0.08092
80	solvent	c	35	1.19	1.54407	0.07555
81	solvent	c	37	0.74	1.56820	-0.13077
82	solvent	c	37	0.89	1.56820	-0.05061
83	solvent	c	42	1.00	1.62325	0.00000
84	solvent	c	37	1.03	1.56820	0.01284
85	solvent	c	35	1.09	1.54407	0.03743
86	solvent	c	38	1.13	1.57978	0.05308
87	solvent	c	39	0.74	1.59106	-0.13077
88	solvent	c	40	1.10	1.60206	0.04139
89	solvent	d	42	0.89	1.62325	-0.05061
90	solvent	d	45	1.19	1.65321	0.07555
91	solvent	d	41	0.95	1.61278	-0.02228
92	solvent	d	45	1.01	1.65321	0.00432
93	solvent	d	40	0.68	1.60206	-0.16749
94	solvent	d	42	0.71	1.62325	-0.14874
95	solvent	d	38	1.09	1.57978	0.03743
96	solvent	d	45	1.11	1.65321	0.04532
97	solvent	d	36	1.30	1.55630	0.11394
98	solvent	d	31	0.85	1.49136	-0.07058
99	solvent	d	39	1.01	1.59106	0.00432
100	solvent	d	38	1.31	1.57978	0.11727
101	solvent	d	39	1.12	1.59106	0.04922
102	solvent	d	42	1.42	1.62325	0.15229
103	solvent	d	40	0.94	1.60206	-0.02687
104	solvent	d	35	0.90	1.54407	-0.04576
105	solvent	d	39	0.92	1.59106	-0.03621
106	solvent	d	39	0.42	1.59106	-0.37675
107	solvent	d	40	1.11	1.60206	0.04532
108	solvent	d	32	1.47	1.50515	0.16732
109	solvent	d	37	0.98	1.56820	-0.00877
110	solvent	d	43	1.24	1.63347	0.09342

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
111	solvent	d	43	1.00	1.63347	0.00000
112	solvent	d	39	1.09	1.59106	0.03743
113	solvent	d	35	1.30	1.54407	0.11394
114	solvent	d	35	0.54	1.54407	-0.26761
115	solvent	d	39	1.31	1.59106	0.11727
116	solvent	d	38	1.11	1.57978	0.04532
117	solvent	d	41	1.05	1.61278	0.02119
118	0.459	e	41	0.98	1.61278	-0.00877
119	0.459	e	38	1.48	1.57978	0.17026
120	0.459	e	45	0.97	1.65321	-0.01323
121	0.459	e	40	1.22	1.60206	0.08636
122	0.459	e	41	1.40	1.61278	0.14613
123	0.459	e	35	1.30	1.54407	0.11394
124	0.459	e	36	0.65	1.55630	-0.18709
125	0.459	e	40	1.55	1.60206	0.19033
126	0.459	e	42	1.03	1.62325	0.01284
127	0.459	e	41	0.86	1.61278	-0.06550
128	0.459	e	47	1.19	1.67210	0.07555
129	0.459	e	35	1.18	1.54407	0.07188
130	0.459	e	36	1.02	1.55630	0.00860
131	0.459	e	40	1.28	1.60206	0.10721
132	0.459	e	42	0.75	1.62325	-0.12494
133	0.459	e	37	0.78	1.56820	-0.10791
134	0.459	e	35	0.86	1.54407	-0.06550
135	0.459	e	40	1.35	1.60206	0.13033
136	0.459	e	41	0.65	1.61278	-0.18709
137	0.459	e	45	1.15	1.65321	0.06070
138	0.459	e	40	0.97	1.60206	-0.01323
139	0.459	e	38	0.74	1.57978	-0.13077
140	0.459	e	40	0.90	1.60206	-0.04576
141	0.459	e	35	0.86	1.54407	-0.06550
142	0.459	e	38	1.07	1.57978	0.02938
143	0.459	e	44	1.14	1.64345	0.05690
144	0.459	e	40	0.70	1.60206	-0.15490
145	0.459	e	36	1.17	1.55630	0.06819
146	0.459	e	38	0.87	1.57978	-0.06048
147	0.459	f	38	1.26	1.57978	0.10037
148	0.459	f	45	1.12	1.65321	0.04922
149	0.459	f	40	1.59	1.60206	0.20140
150	0.459	f	44	1.62	1.64345	0.20952
151	0.459	f	42	1.42	1.62325	0.15229
152	0.459	f	41	0.92	1.61278	-0.03621
153	0.459	f	42	1.33	1.62325	0.12385
154	0.459	f	40	0.83	1.60206	-0.08092
155	0.459	f	35	1.07	1.54407	0.02938
156	0.459	f	44	1.24	1.64345	0.09342
157	0.459	f	43	0.94	1.63347	-0.02687
158	0.459	f	39	1.29	1.59106	0.11059
159	0.459	f	40	1.20	1.60206	0.07918
160	0.459	f	40	1.27	1.60206	0.10380
161	0.459	f	40	1.56	1.60206	0.19312
162	0.459	f	44	1.28	1.64345	0.10721
163	0.459	f	43	1.16	1.63347	0.06446
164	0.459	f	40	1.29	1.60206	0.11059
165	0.459	f	40	0.96	1.60206	-0.01773

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
166	0.459	f	40	1.23	1.60206	0.08991
167	0.459	f	36	1.34	1.55630	0.12710
168	0.459	f	40	0.78	1.60206	-0.10791
169	0.459	f	45	1.13	1.65321	0.05308
170	0.459	f	40	1.15	1.60206	0.06070
171	0.459	f	41	1.24	1.61278	0.09342
172	0.459	f	40	0.86	1.60206	-0.06550
173	0.459	f	40	1.16	1.60206	0.06446
174	0.459	f	39	1.24	1.59106	0.09342
175	0.459	f	35	1.11	1.54407	0.04532
176	0.459	f	40	1.13	1.60206	0.05308
177	0.907	g	41	1.07	1.61278	0.02938
178	0.907	g	47	1.48	1.67210	0.17026
179	0.907	g	44	1.01	1.64345	0.00432
180	0.907	g	43	1.38	1.63347	0.13988
181	0.907	g	45	1.41	1.65321	0.14922
182	0.907	g	46	1.63	1.66276	0.21219
183	0.907	g	44	1.25	1.64345	0.09691
184	0.907	g	45	1.65	1.65321	0.21748
185	0.907	g	45	1.58	1.65321	0.19866
186	0.907	g	43	1.67	1.63347	0.22272
187	0.907	g	44	1.68	1.64345	0.22531
188	0.907	g	43	1.64	1.63347	0.21484
189	0.907	g	47	1.72	1.67210	0.23553
190	0.907	g	42	1.80	1.62325	0.25527
191	0.907	g	44	1.18	1.64345	0.07188
192	0.907	h	44	0.52	1.64345	-0.28400
193	0.907	h	40	1.10	1.60206	0.04139
194	0.907	h	38	1.24	1.57978	0.09342
195	0.907	h	30	0.64	1.47712	-0.19382
196	0.907	h	40	0.91	1.60206	-0.04096
197	0.907	h	45	1.32	1.65321	0.12057
198	0.907	h	43	1.56	1.63347	0.19312
199	0.907	h	42	1.26	1.62325	0.10037
200	0.907	h	35	0.87	1.54407	-0.06048
201	0.907	h	41	1.44	1.61278	0.15836
202	0.907	h	43	1.24	1.63347	0.09342
203	0.907	h	42	1.57	1.62325	0.19590
204	0.907	h	35	0.64	1.54407	-0.19382
205	0.907	h	38	1.17	1.57978	0.06819
206	0.907	h	38	1.42	1.57978	0.15229
207	0.907	h	37	1.47	1.56820	0.16732
208	0.907	h	35	1.48	1.54407	0.17026
209	0.907	h	37	0.82	1.56820	-0.08619
210	0.907	h	39	0.83	1.59106	-0.08092
211	0.907	h	33	1.23	1.51851	0.08991
212	0.907	h	39	1.09	1.59106	0.03743
213	0.907	h	40	1.06	1.60206	0.02531
214	0.907	h	40	1.70	1.60206	0.23045
215	0.907	h	40	0.97	1.60206	-0.01323
216	0.907	h	44	1.18	1.64345	0.07188
217	0.907	h	34	0.91	1.53148	-0.04096
218	0.907	h	42	0.76	1.62325	-0.11919
219	0.907	h	35	0.86	1.54407	-0.06550
220	0.907	h	37	1.01	1.56820	0.00432

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
221	0.907	h	45	1.23	1.65321	0.08991
222	1.78	i	44	1.30	1.64345	0.11394
223	1.78	i	36	1.26	1.55630	0.10037
224	1.78	i	41	0.99	1.61278	-0.00436
225	1.78	i	40	1.28	1.60206	0.10721
226	1.78	i	41	1.09	1.61278	0.03743
227	1.78	i	37	0.99	1.56820	-0.00436
228	1.78	i	42	1.15	1.62325	0.06070
229	1.78	i	40	0.98	1.60206	-0.00877
230	1.78	i	33	0.88	1.51851	-0.05552
231	1.78	i	42	1.29	1.62325	0.11059
232	1.78	i	36	1.41	1.55630	0.14922
233	1.78	i	35	0.77	1.54407	-0.11351
234	1.78	i	40	0.98	1.60206	-0.00877
235	1.78	i	37	1.15	1.56820	0.06070
236	1.78	i	43	0.91	1.63347	-0.04096
237	1.78	i	42	0.58	1.62325	-0.23657
238	1.78	i	43	1.10	1.63347	0.04139
239	1.78	i	40	0.75	1.60206	-0.12494
240	1.78	i	40	1.41	1.60206	0.14922
241	1.78	i	35	0.77	1.54407	-0.11351
242	1.78	i	43	0.67	1.63347	-0.17393
243	1.78	i	36	0.58	1.55630	-0.23657
244	1.78	i	43	1.30	1.63347	0.11394
245	1.78	i	38	1.16	1.57978	0.06446
246	1.78	i	42	1.22	1.62325	0.08636
247	1.78	i	42	1.24	1.62325	0.09342
248	1.78	i	44	1.37	1.64345	0.13672
249	1.78	i	43	0.76	1.63347	-0.11919
250	1.78	i	44	1.18	1.64345	0.07188
251	1.78	i	44	0.88	1.64345	-0.05552
252	1.78	j	38	1.07	1.57978	0.02938
253	1.78	j	42	0.92	1.62325	-0.03621
254	1.78	j	42	1.12	1.62325	0.04922
255	1.78	j	37	1.03	1.56820	0.01284
256	1.78	j	42	0.86	1.62325	-0.06550
257	1.78	j	41	1.04	1.61278	0.01703
258	1.78	j	43	1.32	1.63347	0.12057
259	1.78	j	41	1.07	1.61278	0.02938
260	1.78	j	38	1.09	1.57978	0.03743
261	1.78	j	41	0.90	1.61278	-0.04576
262	1.78	j	42	1.17	1.62325	0.06819
263	1.78	j	37	0.82	1.56820	-0.08619
264	1.78	j	38	1.22	1.57978	0.08636
265	1.78	j	43	0.99	1.63347	-0.00436
266	1.78	j	39	1.46	1.59106	0.16435
267	1.78	j	41	1.05	1.61278	0.02119
268	1.78	j	40	0.77	1.60206	-0.11351
269	1.78	j	47	1.10	1.67210	0.04139
270	1.78	j	42	1.11	1.62325	0.04532
271	1.78	j	41	0.81	1.61278	-0.09151
272	1.78	j	40	1.40	1.60206	0.14613
273	1.78	j	42	0.84	1.62325	-0.07572
274	1.78	j	38	0.96	1.57978	-0.01773
275	1.78	j	38	1.25	1.57978	0.09691

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
276	1.78	j	43	1.11	1.63347	0.04532
277	1.78	j	41	0.97	1.61278	-0.01323
278	1.78	j	41	1.30	1.61278	0.11394
279	1.78	j	46	0.74	1.66276	-0.13077
280	1.78	j	42	1.19	1.62325	0.07555
281	1.78	j	39	1.34	1.59106	0.12710
282	3.68	k	42	0.55	1.62325	-0.25964
283	3.68	k	42	1.10	1.62325	0.04139
284	3.68	k	34	0.69	1.53148	-0.16115
285	3.68	k	40	0.94	1.60206	-0.02687
286	3.68	k	41	1.37	1.61278	0.13672
287	3.68	k	38	0.92	1.57978	-0.03621
288	3.68	k	38	0.75	1.57978	-0.12494
289	3.68	k	30	0.81	1.47712	-0.09151
290	3.68	k	40	1.32	1.60206	0.12057
291	3.68	k	36	1.00	1.55630	0.00000
292	3.68	k	34	0.67	1.53148	-0.17393
293	3.68	k	38	0.73	1.57978	-0.13668
294	3.68	k	45	1.01	1.65321	0.00432
295	3.68	k	39	0.95	1.59106	-0.02228
296	3.68	k	34	0.69	1.53148	-0.16115
297	3.68	k	35	0.93	1.54407	-0.03152
298	3.68	k	39	0.60	1.59106	-0.22185
299	3.68	k	38	0.81	1.57978	-0.09151
300	3.68	k	37	0.97	1.56820	-0.01323
301	3.68	k	37	0.54	1.56820	-0.26761
302	3.68	k	30	0.75	1.47712	-0.12494
303	3.68	k	36	0.41	1.55630	-0.38722
304	3.68	k	38	0.80	1.57978	-0.09691
305	3.68	k	38	1.10	1.57978	0.04139
306	3.68	k	34	0.68	1.53148	-0.16749
307	3.68	k	40	0.90	1.60206	-0.04576
308	3.68	k	36	0.63	1.55630	-0.20066
309	3.68	k	43	0.97	1.63347	-0.01323
310	3.68	k	37	0.79	1.56820	-0.10237
311	3.68	k	37	0.84	1.56820	-0.07572
312	3.68	l	40	1.29	1.60206	0.11059
313	3.68	l	43	1.09	1.63347	0.03743
314	3.68	l	43	1.19	1.63347	0.07555
315	3.68	l	43	1.34	1.63347	0.12710
316	3.68	l	40	1.22	1.60206	0.08636
317	3.68	l	44	0.83	1.64345	-0.08092
318	3.68	l	45	1.24	1.65321	0.09342
319	3.68	l	40	1.41	1.60206	0.14922
320	3.68	l	37	1.00	1.56820	0.00000
321	3.68	l	43	0.93	1.63347	-0.03152
322	3.68	l	39	0.98	1.59106	-0.00877
323	3.68	l	42	1.10	1.62325	0.04139
324	3.68	l	43	0.96	1.63347	-0.01773
325	3.68	l	42	1.06	1.62325	0.02531
326	3.68	l	35	1.17	1.54407	0.06819
327	3.68	l	43	1.30	1.63347	0.11394
328	3.68	l	42	1.65	1.62325	0.21748
329	3.68	l	40	1.30	1.60206	0.11394
330	3.68	l	44	1.50	1.64345	0.17609

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OBS	TRTMNT	REP	LENGTH	WEIGHT	LLENGTH	LWEIGHT
331	3.68	1	45	1.02	1.65321	0.00860
332	3.68	1	38	1.22	1.57978	0.08636
333	3.68	1	44	1.18	1.64345	0.07188
334	3.68	1	40	1.44	1.60206	0.15836
335	3.68	1	40	0.67	1.60206	-0.17393
336	3.68	1	42	1.22	1.62325	0.08636
337	3.68	1	40	0.92	1.60206	-0.03621
338	3.68	1	40	1.08	1.60206	0.03342

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PIRATE - Rainbow Trout Early Life Stage Test
 Descriptive statistics for length and weight

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----- TRTMNT=0.459 -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
59	LENGTH		59	40.0338983	8.5850380	2.9300235
	WEIGHT		59	1.1150847	0.0576737	0.2401535
	LLENGTH	log10 length	59	1.6012790	0.0010185	0.0319133
	LWEIGHT	log10 weight	59	0.0368084	0.0096221	0.0980923

N Obs	Variable	Label	Std Error
59	LENGTH		0.3814566
	WEIGHT		0.0312653
	LLENGTH	log10 length	0.0041548
	LWEIGHT	log10 weight	0.0127705

----- TRTMNT=0.907 -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
45	LENGTH		45	40.7555556	16.3252525	4.0404520
	WEIGHT		45	1.2366667	0.1100773	0.3317790
	LLENGTH	log10 length	45	1.6079869	0.0020097	0.0448302
	LWEIGHT	log10 weight	45	0.0748581	0.0166898	0.1291888

N Obs	Variable	Label	Std Error
45	LENGTH		0.6023150
	WEIGHT		0.0494587
	LLENGTH	log10 length	0.0066829
	LWEIGHT	log10 weight	0.0192583

----- TRTMNT=1.78 -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
60	LENGTH		60	40.5166667	7.9827684	2.8253793
	WEIGHT		60	1.0570000	0.0478146	0.2186654
	LLENGTH	log10 length	60	1.6065679	0.000954754	0.0308991
	LWEIGHT	log10 weight	60	0.0141365	0.0092210	0.0960260

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PIRATE - Rainbow Trout Early Life Stage Test
 Descriptive statistics for length and weight

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----- TRTMNT=1.78 -----

N Obs	Variable	Label	Std Error
60	LENGTH		0.3647549
	WEIGHT		0.0282296
	LLENGTH	log10 length	0.0039891
	LWEIGHT	log10 weight	0.0123969

----- TRTMNT=3.68 -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
57	LENGTH		57	39.3508772	12.5889724	3.5480942
	WEIGHT		57	0.9917544	0.0718004	0.2679560
	LLENGTH	log10 length	57	1.5931422	0.0016409	0.0405086
	LWEIGHT	log10 weight	57	-0.0203165	0.0155558	0.1247229

N Obs	Variable	Label	Std Error
57	LENGTH		0.4699566
	WEIGHT		0.0354916
	LLENGTH	log10 length	0.0053655
	LWEIGHT	log10 weight	0.0165200

----- TRTMNT=control -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
58	LENGTH		58	39.8620690	6.3666062	2.5232135
	WEIGHT		58	0.9929310	0.0369579	0.1922444
	LLENGTH	log10 length	58	1.5997117	0.000747910	0.0273479
	LWEIGHT	log10 weight	58	-0.0107169	0.0066660	0.0816458

N Obs	Variable	Label	Std Error
58	LENGTH		0.3313142
	WEIGHT		0.0252429
	LLENGTH	log10 length	0.0035910
	LWEIGHT	log10 weight	0.0107206

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PIRATE - Rainbow Trout Early Life Stage Test
 Descriptive statistics for length and weight

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----- TRTMNT=solvent -----

N Obs	Variable	Label	N	Mean	Variance	Std Dev
59	LENGTH		59	38.8644068	10.0502630	3.1702150
	WEIGHT		59	1.0144068	0.0482320	0.2196178
	LLENGTH	log10 length	59	1.5881103	0.0012852	0.0358492
	LWEIGHT	log10 weight	59	-0.0051085	0.0108116	0.1039789

N Obs	Variable	Label	Std Error
59	LENGTH		0.4127268
	WEIGHT		0.0285918
	LLENGTH	log10 length	0.0046672
	LWEIGHT	log10 weight	0.0135369

N P A R I W A Y P R O C E D U R E

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable TRTMNT

TRTMNT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
control	58	8212.0000	9860.0	679.463014	141.586207
solvent	59	9231.0000	10030.0	684.074948	156.457627
0.459	59	11377.5000	10030.0	684.074948	192.838983
0.907	45	10108.5000	7650.0	612.179224	224.633333
1.78	60	10366.5000	10200.0	688.614862	172.775000
3.68	57	8327.5000	9690.0	674.777583	146.096491
7.64	1	7.0000	170.0	97.849140	7.000000

Average Scores were used for Ties

Kruskal-Wallis Test (Chi-Square Approximation)

CHISQ= 29.402 DF= 6 Prob > CHISQ= 0.0001

```

data trout;
options ps=60;
infile 'a:\bill\billrb.dat';
input trtmnt $ rep $ length weight;
run;

data trout; set trout;
llength=log10(length);
lweight=log10(weight);
label llength = 'log10 length';
label lweight = 'log10 weight';
run;

Title 'PIRATE - Rainbow Trout Early Life Stage Test';
proc print data=trout;
quit;

proc sort data=trout;
by trtmnt;
quit;

title2 'Descriptive statistics for length and weight';
proc means n mean var std stderr data=trout;
by trtmnt;
quit;

Title2 'Levenes test to generate residuals for Homogeneity of Variance
testing';
proc glm data=trout;
class rep trtmnt;
model length = trtmnt /ss4;
output out = length p=plength r=rlength;
quit;

data length; set length;

```

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