

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

1. **CHEMICAL:** Azoxystrobin **PC Code No.:** 128810

2. **TEST MATERIAL:** ICIA5504 **Purity:** 96.2%

3. **CITATION:**

Authors: Jon E. Rhodes, Yuan Yang and David Abram

Title: Early Life Stage Toxicity of ICIA5504 to
the Fathead Minnow (*Pimephales promelas*)
Under Flow Through Conditions

Study Completion Date: August 22, 1994

Laboratory: ABC Laboratories, Inc., Columbia, Missouri

Laboratory Report ID: 41594

Sponsor: Zeneca Limited, Wilmington, DE

MRID No.: 436781-20

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: 4/03/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *H. T. Craven*

Date: 6/21/96

6. **CONCLUSIONS:** This study is scientifically sound and
fulfills the guideline requirements for a fish early life-
stage toxicity test.

Results synopsis:

Most sensitive endpoint: length

NOEC: 147 ppb

LOEC: 193 ppb

MATC: 168 ppb

7. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

(1)

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

1. **CHEMICAL:** ^{Azoxystrobin} Sulfentrazone

128810
PC Code No.: 129081-

2. **TEST MATERIAL:** ICIA5504

Purity: 96.2%

3. **CITATION:**

Authors: Jon E. Rhodes, Yuan Yang and David Abram
Title: Early Life Stage Toxicity of ICIA5504 to the Fathead Minnow (*Pimephales promelas*) Under Flow Through Conditions

Study Completion Date: August 22, 1994

Laboratory: ABC Laboratories, Inc., Columbia, Missouri.

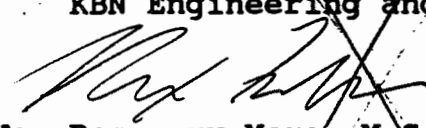
Laboratory Report ID: 41594

Sponsor: Zeneca Limited, Wilmington, DE

MRID No.: 436781-20

DP Barcode: D217072, D217076

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,
KBN Engineering and Applied Sciences, Inc.

Signature: 

Date: 1/19/96

APPROVED BY: Rosemary Mora, M.S., Toxicologist,
KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalawat for RM

Date: 1/22/96

5. **APPROVED BY:**

Signature:

Date:

6. **CONCLUSIONS:** This study is scientifically sound, fulfills the guideline requirements for a fish early life-stage toxicity test, and can be classified as Core. Based on mean measured concentrations, the NOEC and LOEC for fathead minnows exposed to ICIA5504 were 147 and 193 ppb, respectively. The MATC was 168 ppb.

7. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

8. MAJOR GUIDELINE DEVIATIONS:

1. The hardness of the dilution water (140-154 mg/L as CaCO_3) was higher than recommended (40-48 mg/L as CaCO_3).
2. The pH of the dilution water (8.1-8.3) was higher than recommended (7.2-7.6).

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

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	<i>Pimephales promelas</i>
Source: Commercial fishery, wild, or brood stock.	In-house brood stock
Age at beginning of test: Embryos 2 to 24 hours old.	≤24 hours old at initiation
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	30 eggs/cup, 4 cups per treatment or control 20 fish/replicate, 4 replicates per treatment or control
Posthatch: % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	86.1% egg hatch in dilution water and 87.6% in solvent control. 1.2 times
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 at least twice a day. Food was withheld 24 hours before study termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Positive counts of surviving fry were made at least once every two days.

Guideline Criteria	Reported Information
Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $< 70\%$.	Average of 100% and 98.8% survival in the dilution water and solvent control groups, respectively. Survival in each control group replicate was $\geq 95\%$.
Controls: Negative control and carrier control (when applicable) are required.	Dilution water and solvent control (< 0.10 mL/L).

Comments: None.

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) Natural well water. 2) Water was filtered, partially sterilized with UV radiation and screened for contaminants. 3) Hardness of 140-154 mg/L as CaCO_3 , pH of 8.1 to 8.3.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For fathead minnow, 25°C is recommended.	Range from 25.0 to 25.8°C .
Photoperiod: Recommend 16L/8D.	16 h light, 8 h 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. Treatment concentrations of 45, 90, 140, 180, 360, and 720 $\mu\text{g/L}$.

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) Mixing chamber used. 2) No aeration of exposure solutions. 3) Mixing confirmed by analysis. 4) Not reported.
Test Vessels: All glass or glass with stainless steel frame.	15.5 x 31 x 25 cm glass aquaria.
Embryo Cups: 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	9-cm diameter glass jars with 40-mesh Nitex-screen bottoms.
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.	7.8 volume replacements/24 h. DO and chemical concentrations confirmed by analysis.
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	DO $\geq$ 80% (6.3 mg/L) of saturation at all times.

Comments: Hardness and pH of the dilution water were higher than recommended.

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.	- Yes; control, solvent control and mean measured conc. of 51.1, 96.3, 147, 193, 374, and 750 µg/L. - Yes. - Yes.
Other Variables: DO must be measured at each conc. at least once a week.	Yes, DO $\geq$ 80% at all times.
Solvents: Should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Acetone (<0.1 mL/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 hatch; - Time to hatch (days 3-5); - 28-day posthatch survival; - 28-day posthatch length; - 28-day posthatch wet weight; - Morphological and behavioral observations.
Raw data included? (Y/N)	Yes.

Effects Data:

Toxicant Concentration (ppb)		Mean Percent Hatch	Percent Post-Hatch Survival (28 days)	Standard Length (mm)	Blotted Wet weight (g)
Nominal	Measured				
Control	<17	86.1	100	22.8	0.190
Solvent	<17	87.6	98.8	23.0	0.203
45	51.1	89.4	98.8	22.8	0.194
90	96.3	81.9	98.8	22.6	0.190
140	147	82.6	98.8	22.6	0.192
180	193	86.0	98.8	22.2	0.190
360	374	88.1	97.5	20.9	0.161
720	750	33.6	0	--	--

Toxicity Observations: No physical or behavioral abnormalities were observed during the study.

Statistical Results:

Statistical Method: Contingency table methods were used to analyze egg hatchability and survival data. ANOVA was used for continuous scale measurements (length and wet weight) with Dunnett's test for control comparisons.

NOEC: 147 µg/L LOEC: 193 µg/L MATC: 168 µg/L

Most sensitive endpoint: 28-day standard length

Comments: Hatch, survival, and growth data from the treatment groups were compared to data from the pooled control.

11. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: ANOVA (nested) and Dunnett's test for pooled mean comparisons.

NOEC: 147 ppb LOEC: 193 ppb MATC: 168 ppb
Most sensitive endpoint: 28-day standard length

Comments: Hatch, survival, and growth data from the treatment groups were compared to the data from the pooled control. An ANOVA was performed using a nested design with vessels nested within aquaria and aquaria nested within treatments.

12. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for a fish early life-stage toxicity test using the fathead minnow. Based on mean measured concentrations, the NOEC and LOEC for fathead minnows exposed to ICIA5504 were 147 and 193 ppb, respectively. The MATC was 168 ppb. This study is classified as Core.

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OBS	REP	AQUA	LENGTH	WEIGHT
1	A	A	25.2293	0.186
2	A	A	20.9299	0.243
3	A	A	25.2345	0.198
4	A	A	22.4554	0.222
5	A	A	26.6627	0.221
6	A	A	22.0560	0.235
7	A	A	24.0864	0.327
8	A	A	23.4791	0.173
9	A	A	21.8816	0.203
10	A	A	23.8615	0.225
11	A	A	22.3407	0.189
12	A	A	22.9652	0.158
13	A	A	22.3712	0.187
14	A	A	24.0787	0.178
15	A	A	24.1097	0.219
16	A	A	21.2817	0.199
17	A	A	19.9646	0.249
18	A	A	24.9022	0.165
19	A	A	23.5524	0.127
20	A	A	22.5508	0.222
21	A	A	21.9989	0.182
22	A	A	23.0075	0.183
23	A	A	24.0989	0.125
24	A	A	20.1572	0.230
25	A	A	23.4847	0.187
26	A	A	21.9039	0.142
27	A	A	22.4768	0.153
28	A	A	22.5797	0.209
29	A	A	25.8398	0.259
30	A	A	22.9692	0.194
31	A	A	19.9756	0.188
32	A	A	22.2147	0.211
33	A	A	24.6306	0.270
34	A	A	22.0818	0.188
35	A	A	26.6074	0.223
36	A	A	22.3188	0.175
37	A	A	26.4421	0.126
38	A	A	21.8623	0.207
39	A	A	20.0783	0.179
40	A	A	22.9441	0.196
41	A	A	23.2707	0.129
42	A	A	22.5075	0.234
43	A	A	24.1451	0.273
44	A	A	24.8856	0.261
45	A	A	21.1683	0.202
46	A	A	25.0169	0.198
47	A	A	23.8496	0.147
48	A	A	24.4989	0.148
49	A	A	21.0152	0.202
50	A	A	23.9201	0.137
51	A	A	22.3192	0.155
52	A	A	19.3893	0.233
53	A	A	20.7982	0.110
54	A	A	25.1269	0.117
55	A	A	20.9408	0.159
56	A	A	23.2732	0.159

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TRI=C
(continued)C-Control
V-Vehicle Control

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OBS	REP	AQUA	LENGTH	WEIGHT
57	C	B	21.4116	0.208
58	C	B	19.7697	0.169
59	C	B	21.3046	0.138
60	C	B	21.5177	0.187
61	C	B	21.7545	0.131
62	C	B	25.3912	0.262
63	C	B	20.6534	0.134
64	C	B	22.0696	0.115
65	C	B	20.5812	0.230
66	C	B	21.6079	0.279
67	C	B	22.2849	0.171
68	C	B	22.7104	0.087
69	C	B	20.8177	0.135
70	C	B	23.7987	0.285
71	C	B	23.2637	0.168
72	C	B	18.6360	0.251
73	C	B	22.8293	0.212
74	C	B	23.8585	0.222
75	C	B	20.0000	0.161
76	C	B	25.5458	0.114
77	C	B	21.8389	0.133
78	C	B	24.1151	0.274
79	C	B	26.1744	0.169
80	C	B	20.7379	0.163

TRI=V

OBS	REP	AQUA	LENGTH	WEIGHT
81	E	C	24.6412	0.219
82	E	C	27.0560	0.179
83	E	C	22.9636	0.221
84	E	C	22.6717	0.312
85	E	C	25.2727	0.255
86	E	C	23.8506	0.198
87	E	C	23.2903	0.188
88	E	C	23.0618	0.201
89	E	C	24.1542	0.171
90	E	C	23.7231	0.223
91	E	C	22.5768	0.250
92	E	C	23.4396	0.184
93	E	C	24.5271	0.243
94	E	C	24.6936	0.223
95	E	C	22.2250	0.201
96	E	C	22.3434	0.254
97	E	C	22.7018	0.185
98	E	C	21.9455	0.201
99	E	C	22.6099	0.246
100	E	C	24.3677	0.185
101	E	C	22.5634	0.188
102	E	C	19.5334	0.211
103	E	C	22.4789	0.214
104	E	C	24.6330	0.244
105	E	C	22.3552	0.186

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TRI=V
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
106	F	C	22.1168	0.171
107	F	C	23.9768	0.102
108	F	C	20.9256	0.178
109	F	C	23.2119	0.168

110	F	C	21.4264	0.174
111	F	C	25.0731	0.177
112	F	C	19.1818	0.245
113	F	C	26.2058	0.211
114	F	C	24.2372	0.280
115	F	C	21.9732	0.152
116	F	C	25.2293	0.212
117	F	C	24.7420	0.173
118	F	C	23.0361	0.110
119	F	C	22.8958	0.136
120	G	D	20.5612	0.280
121	G	D	24.3864	0.279
122	G	D	24.5412	0.176
123	G	D	22.7046	0.209
124	G	D	22.1927	0.181
125	G	D	23.4666	0.173
126	G	D	21.4719	0.157
127	G	D	21.4825	0.174
128	G	D	22.0397	0.202
129	G	D	24.0107	0.190
130	G	D	22.0161	0.107
131	G	D	24.1165	0.246
132	G	D	21.5721	0.190
133	G	D	21.1898	0.179
134	G	D	25.5072	0.191
135	G	D	22.4789	0.261
136	G	D	24.1404	0.221
137	G	D	25.5123	0.262
138	G	D	18.5209	0.244
139	G	D	21.5551	0.142
140	H	D	23.6066	0.201
141	H	D	25.1637	0.238
142	H	D	19.8731	0.173
143	H	D	24.7420	0.244
144	H	D	19.1410	0.264
145	H	D	23.7221	0.196
146	H	D	22.5753	0.121
147	H	D	21.5551	0.126
148	H	D	24.9166	0.225
149	H	D	22.4192	0.218
150	H	D	24.5718	0.094
151	H	D	23.8615	0.166
152	H	D	24.6952	0.131
153	H	D	22.8517	0.252
154	H	D	17.4724	0.283
155	H	D	25.4747	0.194
156	H	D	21.8474	0.270
157	H	D	22.3090	0.227
158	H	D	21.6503	0.240
159	H	D	20.0297	0.204

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TRT=C

TRT=V

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	80	22.773013	1.8466718	18.6360000	26.6627000
WEIGHT	80	0.1904125	0.0476729	0.0870000	0.3270000

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	2	C V
REP	8	A B C D E F G H
AQUA	4	A B C D

Number of observations in data set = 159

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General Linear Models Procedure

Dependent Variable: LENGTH	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model		7	23.00469091	3.28638442	0.98	0.4482
Error		151	506.51704250	3.35441750		
Corrected Total		158	529.52173341			

R-Square	C.V.	Root MSE	LENGTH Mean
0.043444	8.004399	1.8315069	22.881255

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	1.84185256	1.84185256	0.55	0.4598
AQUA(TRT)	2	14.09709980	7.04854990	2.10	0.1289
REP(TRT*AQUA)	4	7.06573855	1.76643464	0.53	0.7163
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	1.83008972	1.83008972	0.55	0.4613
AQUA(TRT)	2	13.83344936	6.91672468	2.06	0.1308
REP(TRT*AQUA)	4	7.06573855	1.76643464	0.53	0.7163

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General Linear Models Procedure

Dependent Variable: WEIGHT	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model		7	0.02185756	0.00312251	1.44	0.1947
Error		151	0.32827574	0.00217401		
Corrected Total		158	0.35013330			

R-Square	C.V.	Root MSE	WEIGHT Mean
0.062426	23.73485	0.0466263	0.1964465

No significant ($P < 0.05$) between control + vehicle control.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	1	0.00586241	0.00586241	2.70	0.1026
AQUA(TRT)	2	0.00508209	0.00254104	1.17	0.3135
REP(TRT*AQUA)	4	0.01091305	0.00272826	1.25	0.2903
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	0.00566165	0.00566165	2.60	0.1087
AQUA(TRT)	2	0.00510807	0.00255403	1.17	0.3117
REP(TRT*AQUA)	4	0.01091305	0.00272826	1.25	0.2903

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TTEST PROCEDURE

Variable: LENGTH

TRT	N	Mean	Std Dev	Std Error	Minimum	Maximum
C	80	22.77430125	1.84667180	0.20646418	18.63600000	26.66270000
V	79	22.98956329	1.81967290	0.20472920	17.47240000	27.05600000

For H0: Variances are equal, F' = 1.03 DF = (79,78) Prob>F' = 0.8969

Variable: WEIGHT

TRT	N	Mean	Std Dev	Std Error	Minimum	Maximum
C	80	0.19041250	0.04767286	0.00532999	0.08700000	0.32700000
V	79	0.20255696	0.04595531	0.00517038	0.09400000	0.31200000

Variances
Unequal -1.6355 156.9 0.1040
Equal -1.6351 157.0 0.1040

For H0: Variances are equal, F' = 1.08 DF = (79,78) Prob>F' = 0.7463

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TRT=C

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	159	22.8812553	1.8306839	17.4724000	27.0560000
WEIGHT	159	0.1964465	0.0470748	0.0870000	0.3270000

TRT=I

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	78	22.8411577	1.6970182	18.4346000	26.4049000
WEIGHT	78	0.1942308	0.0440601	0.1050000	0.2950000

T-Test: Control & Vehicle control not significantly different. Pool controls

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	79	22.5598468	2.6831935	11.9047000	28.0153000
WEIGHT	79	0.1896709	0.0598009	0.0190000	0.3220000

TRT=III

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	78	22.5600000	2.0427299	13.4881000	25.5458000
WEIGHT	78	0.1916410	0.0489442	0.0470000	0.3000000

TRT=IV

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	79	22.2032911	1.9285446	16.6194000	27.1963000
WEIGHT	79	0.1897468	0.0473418	0.0700000	0.3310000

TRT=V

Variable	N	Mean	Std Dev	Minimum	Maximum
LENGTH	78	20.9133449	1.9673128	15.3664000	24.6197000
WEIGHT	78	0.1606026	0.0466240	0.0600000	0.2910000

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General Linear Models Procedure

Class Level Information

Class Levels Values
TRT 6 C I II III IV V
REP 8 A B C D E F G H
AQUA 4 A B C D

Number of observations in data set = 551

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General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	313.47936170	11.61034673	2.84	0.0001
Error	523	2140.21088653	4.09218143		
Corrected Total	550	2453.69024823			

TREATMENTS
Control
I - 51.1 ppb
II - 96.3
III - 147
IV - 193
V - 374

	R-Square	C.V.	Root MSE	LENGTH Mean
	0.127758	9.027539	2.0229141	22.408257

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	231.43513151	46.28702630	11.31	0.0001
AQUA(TRT)	8	43.11264083	5.38908010	1.32	0.2323
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	231.22299951	46.24459990	11.30	0.0001
AQUA(TRT)	8	42.16878186	5.34609773	1.31	0.2336
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953

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General Linear Models Procedure

Dependent Variable: WEIGHT	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model		27	0.11263924	0.00417182	1.72	0.0140
Error		523	1.26687493	0.00242232		
Corrected Total		550	1.37951417			

	R-Square	C.V.	Root MSE	WEIGHT Mean
	0.081651	26.11729	0.0492171	0.188465

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.07430602	0.01486120	6.14	0.0001
AQUA(TRT)	8	0.01642720	0.00230340	0.95	0.4739
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.07412713	0.01482543	6.12	0.0001
AQUA(TRT)	8	0.01624119	0.00228015	0.94	0.4818
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761

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OBS	REP	AQUA	LENGTH	WEIGHT
57	C	B	21.4116	0.208
58	C	B	19.7697	0.169
59	C	B	21.3046	0.138
60	C	B	21.5777	0.187
61	D	B	21.7545	0.151
62	D	B	25.3912	0.262
63	D	B	20.6534	0.134
64	D	B	22.0696	0.115
65	D	B	20.5812	0.230
66	D	B	21.6079	0.279
67	D	B	22.2849	0.171
68	D	B	22.7104	0.087
69	D	B	20.8177	0.135
70	D	B	23.7987	0.285
71	D	B	25.2637	0.168
72	D	B	18.6360	0.251

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TRT=C
(continued)

Obs - Observation
Rep - Replicates
Aqua - Aquarium

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73	D	B	22.8293	0.212
74	D	B	23.8585	0.222
75	D	B	20.0000	0.161
76	D	B	25.5458	0.133
77	D	B	21.8389	0.274
78	D	B	24.1151	0.169
79	D	B	26.1744	0.163
80	D	B	20.7379	0.219
81	D	B	24.6412	0.179
82	D	B	27.0560	0.221
83	D	B	22.6717	0.312
84	D	B	25.2727	0.255
85	D	B	23.8506	0.198
86	D	B	23.2903	0.188
87	D	B	23.0618	0.201
88	D	B	24.1542	0.171
89	D	B	23.7231	0.223
90	D	B	22.5176	0.250
91	D	B	23.4396	0.184
92	D	B	24.5271	0.243
93	D	B	22.6956	0.223
94	D	B	22.2250	0.201
95	D	B	24.3434	0.254
96	D	B	22.7018	0.185
97	D	B	21.9455	0.201
98	D	B	22.6099	0.246
99	D	B	24.3677	0.185
100	D	B	22.5634	0.188
101	D	B	19.5334	0.211
102	D	B	22.4789	0.214
103	D	B	24.6330	0.244
104	D	B	22.3552	0.166
105	D	B	22.1168	0.171
106	D	B	23.9768	0.102
107	D	B	20.9256	0.178
108	D	B	23.2119	0.168
109	D	B	21.4264	0.174
110	D	B	25.0731	0.177
111	D	B		

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134	G	D	25.5072	0.191
135	G	D	22.4789	0.261
136	G	D	22.1404	0.221
137	G	D	25.5123	0.262
138	G	D	18.5209	0.244
139	G	D	21.5551	0.142
140	G	D	23.6066	0.201
141	H	D	23.1637	0.238
142	H	D	19.8731	0.173
143	H	D	24.7420	0.244
144	H	D	19.1410	0.264
145	H	D	23.7221	0.196
146	H	D	22.5753	0.121
147	H	D	21.5551	0.126
148	H	D	24.9166	0.125
149	H	D	22.4192	0.218
150	H	D	24.5718	0.094
151	H	D	23.8615	0.166
152	H	D	24.4952	0.151
153	H	D	22.8517	0.252
154	H	D	17.4724	0.283
155	H	D	25.4747	0.194
156	H	D	21.8474	0.270
157	H	D	22.9090	0.227
158	H	D	21.4503	0.240
159	H	D	20.0297	0.204

TRT=1

OBS	REP	AQUA	LENGTH	WEIGHT
-----	-----	------	--------	--------

160 A A 23.7231 0.2

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TRT=1
(continued)

112	F	C	19.1818	0.245
113	F	C	26.2058	0.211
114	F	C	24.2372	0.280
115	F	C	21.9732	0.152
116	F	C	25.2293	0.212
117	F	C	24.7420	0.173
118	F	C	23.0361	0.110
119	F	C	22.8958	0.136
120	F	C	20.5812	0.280
121	F	C	24.5864	0.279
122	F	C	24.6412	0.176
123	F	C	22.7046	0.209
124	F	C	22.1927	0.181
125	F	C	23.4666	0.173
126	F	C	21.4719	0.157
127	F	C	21.4825	0.174
128	F	C	22.0387	0.202
129	F	C	24.0161	0.190
130	F	C	24.0161	0.246
131	F	C	21.5721	0.190
132	F	C	21.1898	0.179
133	F	C		

TRT=C
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
189	B	A	22.7701	0.228
190	B	A	20.4094	0.239
191	B	A	24.2322	0.129
192	B	A	25.2022	0.196
193	B	A	23.0967	0.167
194	B	A	23.1530	0.202
195	B	A	26.0202	0.164
196	B	A	18.7339	0.116
197	B	A	18.4346	0.219
198	B	A	22.7580	0.269
199	B	A	19.6790	0.204
200	B	A	22.9993	0.227
201	B	A	24.7450	0.237
202	B	A	22.1417	0.200
203	B	A	20.7418	0.257
204	B	A	24.4447	0.179
205	B	A	23.7272	0.243
206	B	A	22.9852	0.143
207	B	A	23.7221	0.200
208	B	A	23.2782	0.232
209	B	A	23.1013	0.160
210	B	A	24.7686	0.220
211	B	A	22.5742	0.254
212	B	A	23.1867	0.139
213	B	A	21.8087	0.202
214	B	A	21.4291	0.147
215	B	A	20.0917	0.216

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TRT=1
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
216	C	B	23.3478	0.218
217	C	B	24.2439	0.175
218	C	B	25.1712	0.153
219	C	B	21.9662	0.241
220	D	B	26.0414	0.224
221	D	B	23.3739	0.167
222	D	B	22.8589	0.168
223	D	B	23.1632	0.172
224	D	B	22.3246	0.187
225	D	B	21.3172	0.153
226	D	B	22.9579	0.180
227	D	B	22.2849	0.149
228	D	B	18.8964	0.216
229	D	B	22.5753	0.209
230	D	B	21.7115	0.107
231	D	B	22.3319	0.227
232	D	B	23.1688	0.177
233	D	B	21.1302	0.193
234	D	B	21.9332	0.206
235	D	B	20.0268	0.212
236	D	B	23.9798	0.292
237	D	B	23.8871	0.152

TRT=11

OBS	REP	AQUA	LENGTH	WEIGHT
238	A	A	24.4673	0.227
239	A	A	24.5354	0.247
240	A	A	24.6412	0.160
241	A	A	24.3928	0.273
242	A	A	23.1013	0.169
243	A	A	22.6257	0.117

OBS	REP	AQUA	LENGTH	WEIGHT
244	A	A	23.0872	0.213
245	A	A	21.5291	0.238
246	A	A	19.6194	0.150
247	A	A	22.2092	0.070
248	A	A	24.2349	0.220
249	A	A	25.3072	0.170
250	A	A	25.1249	0.173
251	A	A	24.6620	0.250
252	A	A	23.3478	0.214
253	A	A	23.0163	0.169
254	A	A	21.8090	0.183
255	A	A	21.1744	0.228
256	A	A	21.6500	0.217
257	A	A	15.8884	0.151
258	A	A	25.2509	0.220
259	B	A	19.0751	0.160
260	B	A	26.1461	0.236
261	B	A	21.6114	0.131
262	B	A	24.6174	0.322
263	B	A	24.8708	0.156
264	B	A	20.1995	0.203

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TRT=11
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
265	B	A	22.8987	0.116
266	B	A	21.5913	0.229
267	B	A	23.3377	0.167
268	B	A	22.7447	0.108
269	B	A	23.3422	0.258
270	B	A	22.4757	0.216
271	B	A	22.4467	0.223
272	B	A	21.0574	0.205
273	B	A	19.7743	0.122
274	B	A	20.5206	0.135
275	B	A	21.3113	0.204
276	B	A	23.3405	0.166
277	B	A	19.8014	0.200
278	B	A	19.1410	0.225
279	B	A	25.1246	0.206
280	B	A	23.4680	0.130
281	B	A	26.0202	0.236
282	B	A	23.3405	0.117
283	B	A	23.9781	0.220
284	B	A	23.8769	0.210
285	B	A	20.1701	0.103
286	B	A	21.9377	0.242
287	B	A	18.9853	0.174
288	B	A	21.3367	0.223
289	B	A	23.9496	0.187
290	B	A	22.1872	0.256
291	B	A	20.5432	0.317
292	B	A	26.7853	0.270
293	B	A	22.8742	0.191
294	B	A	25.6604	0.199
295	B	A	24.6065	0.157
296	B	A	23.9853	0.128
297	B	A	20.7002	0.100
298	B	A	24.8813	0.316
299	B	A	24.8695	0.035
300	B	A	28.0153	0.243
301	B	A	21.2205	0.132
302	B	A	22.2048	0.266
303	B	A	24.4899	0.019
304	B	A	19.9858	0.236

305	D	B	19.3960	0.179
306	D	B	19.6906	0.123
307	D	B	11.9047	0.249
308	D	B	25.7737	0.252
309	D	B	24.2975	0.223
310	D	B	25.4616	0.122
311	D	B	23.3673	0.121
312	D	B	25.5187	0.230
313	D	B	23.5956	0.218
314	D	B	20.3183	0.147
315	D	B	21.1191	0.160
316	D	B	14.3204	0.206

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TRT=III

317	A	A	23.6823	0.194
318	A	A	22.6174	0.259
319	A	A	24.3677	0.219
320	A	A	21.8459	0.209
321	A	A	22.7072	0.211
322	A	A	23.1126	0.207
323	A	A	22.7929	0.186
324	A	A	23.8711	0.175
325	A	A	24.0256	0.223
326	A	A	23.8578	0.158
327	A	A	23.0897	0.219
328	A	A	22.6257	0.189
329	A	A	21.8102	0.139
330	A	A	22.4007	0.170
331	A	A	23.4753	0.222
332	A	A	24.5430	0.187
333	A	A	21.8325	0.171
334	A	A	22.0051	0.186
335	A	A	20.5337	0.219
336	A	A	22.9090	0.219
337	A	A	23.4791	0.300
338	A	A	23.5458	0.226
339	A	A	25.2985	0.220
340	A	A	22.7619	0.194
341	A	A	25.3954	0.125
342	A	A	24.1905	0.047
343	A	A	22.4366	0.189
344	A	A	22.8379	0.206
345	A	A	22.7046	0.086
346	A	A	23.8660	0.103
347	A	A	22.5797	0.190
348	A	A	22.5389	0.099
349	A	A	20.5476	0.296
350	A	A	19.3893	0.116
351	A	A	19.9161	0.239
352	A	A	19.8960	0.161
353	A	A	19.1512	0.245
354	A	A	19.0375	0.217
355	A	A	18.3887	0.104
356	A	A	13.4881	0.249
357	A	A	24.6158	0.231
358	A	A	23.3447	0.218
359	A	A	24.4972	0.164
360	A	A	23.3516	0.222
361	A	A	24.3647	0.210
362	A	A	22.1927	0.188
363	A	A	19.9058	0.108
364	A	A	21.9429	0.171
365	A	A	18.9373	0.106
366	A	A	18.7495	

367	C	B	24.3404	0.237
368	C	B	25.3065	0.147
369	C	B	22.0770	0.179
370	C	B	24.0432	0.173
371	C	B	24.3864	0.195
372	C	B	25.3610	0.232

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TRT=III
(continued)

373	C	B	21.9810	0.170
374	C	B	22.4119	0.235
375	C	B	22.7476	0.222
376	D	B	25.3152	0.223
377	D	B	21.9599	0.249
378	D	B	24.1074	0.300
379	D	B	23.1435	0.215
380	D	B	24.6197	0.160
381	D	B	22.9597	0.161
382	D	B	20.7175	0.132
383	D	B	21.5672	0.183
384	D	B	20.5349	0.237
385	D	B	22.4554	0.147
386	D	B	25.4056	0.190
387	D	B	22.7046	0.172
388	D	B	24.5082	0.142
389	D	B	21.5177	0.200
390	D	B	24.3677	0.241
391	D	B	21.0624	0.176
392	D	B	21.8180	0.204
393	D	B	21.1825	0.161
394	D	B	23.5459	0.238

TRT=IV

395	A	A	27.1963	0.244
396	A	A	23.1126	0.176
397	A	A	26.7236	0.125
398	A	A	21.8269	0.313
399	A	A	22.8322	0.109
400	A	A	24.2402	0.172
401	A	A	22.4768	0.192
402	A	A	23.0967	0.351
403	A	A	23.8506	0.204
404	A	A	18.4522	0.193
405	A	A	21.8120	0.256
406	A	A	20.1693	0.204
407	A	A	24.7335	0.235
408	A	A	23.9445	0.178
409	A	A	22.6099	0.181
410	A	A	20.3107	0.095
411	A	A	22.7322	0.218
412	A	A	22.2045	0.175
413	A	A	18.0841	0.203
414	A	A	23.2399	0.200
415	A	A	24.3677	0.196
416	A	A	23.5013	0.239
417	A	A	23.0978	0.237
418	A	A	25.2525	0.206
419	A	A	21.9366	0.199
420	A	A	23.5997	0.198
421	A	A	21.4359	0.221

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TRT=IV
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
422	B	A	20.3806	0.201
423	B	A	21.4355	0.200
424	B	A	21.7893	0.233
425	B	A	22.8379	0.172
426	B	A	23.8711	0.139
427	B	A	23.6190	0.280
428	B	A	23.8615	0.070
429	B	A	23.7303	0.222
430	B	A	25.2573	0.186
431	B	A	23.4517	0.216
432	B	A	22.6174	0.157
433	B	A	16.6194	0.168
434	B	A	22.2440	0.125
435	B	A	21.5570	0.269
436	B	A	23.9890	0.178
437	B	A	21.0747	0.160
438	B	A	25.4689	0.220
439	B	A	24.6630	0.140
440	B	A	23.8565	0.168
441	B	A	21.1191	0.190
442	B	A	21.4397	0.166
443	B	A	20.5812	0.220
444	B	A	18.9102	0.203
445	B	A	19.2617	0.125
446	B	A	22.0696	0.166
447	B	A	18.7517	0.158
448	B	A	21.2572	0.127
449	B	A	21.4264	0.173
450	B	A	19.8400	0.132
451	B	A	21.3367	0.195
452	B	A	20.2087	0.297
453	B	A	20.3459	0.160
454	B	A	23.1347	0.172
455	B	A	21.3630	0.282
456	B	A	20.8200	0.170
457	B	A	19.3893	0.154
458	B	A	24.3764	0.243
459	B	A	22.7819	0.253
460	B	A	21.0929	0.132
461	B	A	22.5638	0.161
462	B	A	22.1913	0.161
463	B	A	20.6644	0.172
464	B	A	21.5630	0.133
465	B	A	22.5742	0.164
466	B	A	22.3483	0.196
467	B	A	21.1956	0.195
468	B	A	22.1465	0.178
469	B	A	24.6989	0.187
470	B	A	22.0696	0.146
471	B	A	22.5638	0.189
472	B	A	20.0491	0.200
473	B	A	20.7899	0.181

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TRT=V

OBS	REP	AQUA	LENGTH	WEIGHT
474	A	A	17.3186	0.223
475	A	A	22.2004	0.234

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TRT=V
(continued)

OBS	REP	AQUA	LENGTH	WEIGHT
530	C	B	17.8596	0.111
531	C	B	18.3723	0.138
532	D	B	23.4878	0.167
533	D	B	24.0449	0.113
534	D	B	23.7933	0.208
535	D	B	21.2649	0.179
536	D	B	20.6624	0.080

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	0.11263924	0.00417182	1.72	0.0140
Error	523	1.26687493	0.00242232		
Corrected Total	550	1.37951417			
R-Square		C.V.	Root MSE	WEIGHT Mean	
	0.081651	26.11729	0.0492171	0.1884465	

General Linear Models Procedure

Class	Levels	Values
TRT	6	C I II III IV V
REP	8	A B C D E F G H
AQUA	4	A B C D

Number of observations in data set = 551

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General Linear Models Procedure

Dependent Variable: LENGTH					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	313.47936170	11.61034673	2.84	0.0001
Error	523	2140.21088653	4.09218143		
Corrected Total	550	2453.69024823			
R-Square		C.V.	Root MSE	LENGTH Mean	
	0.127758	9.027539	2.0229141	22.408257	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	231.43513151	46.28702630	11.31	0.0001
AQUA(TRT)	8	43.11264083	5.38908010	1.32	0.2323
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	231.22299951	46.24459990	11.30	0.0001
AQUA(TRT)	8	42.76678186	5.34609773	1.31	0.2376
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953

General Linear Models Procedure

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Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	0.11263924	0.00417182	1.72	0.0140
Error	523	1.26687493	0.00242232		
Corrected Total	550	1.37951417			
R-Square		C.V.	Root MSE	WEIGHT Mean	
	0.081651	26.11729	0.0492171	0.1884465	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.07430602	0.01486120	6.14	0.0001
AQUA(TRT)	8	0.01842720	0.00230340	0.95	0.4739
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.07412713	0.01482543	6.12	0.0001
AQUA(TRT)	8	0.01824119	0.00228015	0.94	0.4818
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761

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General Linear Models Procedure

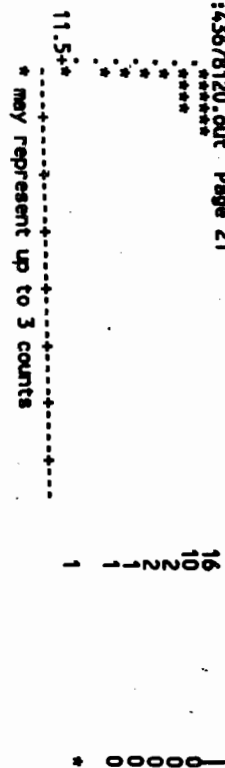
Bonferroni (Dunn) T tests for variable: LENGTH

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

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 4.092181 Critical Value of T= 2.94675

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
C - I	-0.7845	0.0401	0.8447
C - II	-0.5033	0.3213	1.1459
C - III	-0.4998	0.3213	1.1424
C - IV	-0.1431	0.6780	1.4991
C - V	1.1433	1.9679	2.7925
I - C	-0.8447	-0.0401	0.7845
I - II	-0.6740	0.2812	1.2363
I - III	-0.6710	0.2812	1.2333
I - IV	-0.3143	0.6379	1.5900
I - V	0.9726	1.9278	2.8830
II - C	-1.1459	-0.3213	0.5033
II - I	-1.2363	-0.2812	0.6740
II - II	-0.9521	0.0000	0.9522
II - III	-0.5954	0.3567	1.3089
II - IV	-0.6915	1.6467	2.6018
II - V	-1.1424	-0.3213	0.4998



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Univariate Procedure

Variable=WEIGHT

Moments

N	551	Sum Wgts	551
Mean	0.189446	Sum	103.834
Std Dev	0.050082	Variance	0.002508
Skewness	-0.02592	Kurtosis	0.159942
USS	20.94666	CSS	1.379514
CV	26.57625	Std Mean	0.002134
T:Mean=0	88.32467	Pr> T	0.0001
Num=>0	551	Num>0	551
M(Sign)	275.5	Pr>= M	0.0001
Sign Rank	76038	Pr>= S	0.0001

quantiles(Def=5)

100% Max	0.331	99%	0.313
75% Q3	0.221	95%	0.273
50% Med	0.188	90%	0.25
25% Q1	0.158	10%	0.125
0% Min	0.019	5%	0.105
Range	0.312	1%	0.07
Q3-Q1	0.063		
Mode	0.173		

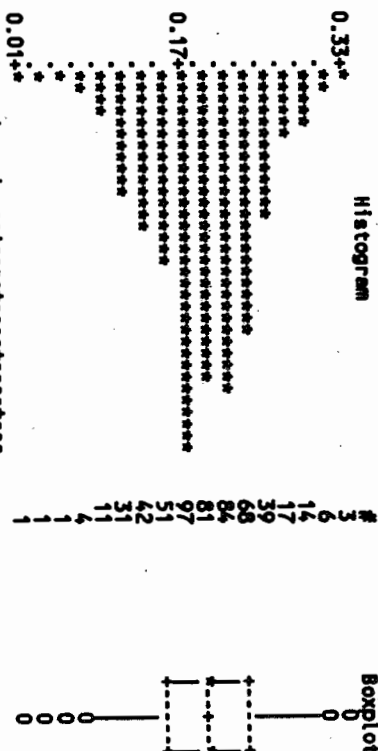
Extremes

Lowest	Obs	Highest	Obs
0.0196	303	0.316	298
0.0356	299	0.317	291
0.047	342	0.322	262
0.06	482	0.327	7
0.07	428	0.331	402

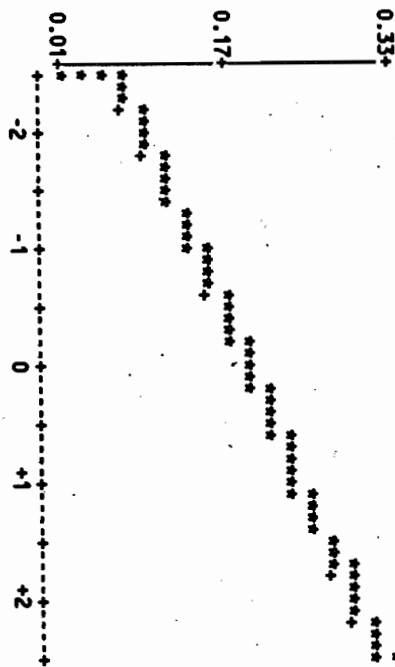
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Univariate Procedure

Variable=WEIGHT



Normal Probability Plot



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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	6	C I II III IV V
REP	8	A B C D E F G H
AQUA	4	A B C D

Number of observations in data set = 551

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General Linear Models Procedure

Dependent Variable: LENGTH	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF			
Model	27	313.47936170	11.61034673	2.84
Error	523	2140.21088653	4.09218143	0.0001
Corrected Total	550	2453.69024823		

R-Square C.V. Root MSE LENGTH Mean

0.127758 -9.027539 2.0229141 22.408257

Source	DF	Type I SS	Mean Square	F Value	Pr > F
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TRT	5	231.43513151	46.28702630	11.31	0.0001
AQUA(TRT)	8	43.11264083	5.38908010	1.32	0.2323
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	231.22299951	46.24459990	11.30	0.0001
AQUA(TRT)	8	42.76878166	5.34609773	1.31	0.2376
REP(TRT*AQUA)	14	38.93158936	2.78082781	0.68	0.7953

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General Linear Models Procedure

Dependent Variable: WEIGHT	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF			
Model	27	0.11263924	0.00417182	1.72
Error	523	1.26687493	0.00242232	0.0140
Corrected Total	550	1.37951417		
R-Square		C.V.	Root MSE	WEIGHT Mean
0.081651		26.11729	0.0492171	0.188465

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General Linear Models Procedure
Source

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.07430602	0.01486120	6.14	0.0001
AQUA(TRT)	8	0.01842720	0.00230340	0.95	0.4739
REP(TRT*AQUA)	14	0.01990603	0.00142186	0.59	0.8761

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General Linear Models Procedure

Dunnnett's One-tailed T tests for variable: LENGTH

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

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 4.092181
Critical Value of Dunnnett's T= 2.285

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

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	- C	-0.6790	-0.0401	0.5989
III	- C	-0.9602	-0.3213	0.3177
II	- C	-0.9575	-0.3213	0.3150
IV	- C	-1.3142	-0.6780	-0.0417
V	- C	-2.6069	-1.9679	-1.3290

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General Linear Models Procedure

Dunnnett's One-tailed T tests for variable: WEIGHT

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

Alpha= 0.05 Confidence= 0.95 df= 523 MSE= 0.002422
Critical Value of Dunnnett's T= 2.285

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

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	- C	-0.017761	-0.002216	0.013330
III	- C	-0.020351	-0.004806	0.010740
IV	- C	-0.022179	-0.006700	0.008760
II	- C	-0.023255	-0.006776	0.008704
V	- C	-0.051389	-0.035844	-0.020298
