

## DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.  
Shaughnessey No. 121601.
2. **TEST MATERIAL:** Acetochlor technical; Lot No. QUE-9001-1482-T; 92.07% active ingredient; a dark brown liquid.
3. **STUDY TYPE:** 72-4. Freshwater Fish, Early Life-Stage Toxicity Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Rhodes, J.E. and M. Muckerman. 1992. Early Life-Stage Toxicity of Acetochlor to the Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Final Report No. 40047. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Acetochlor Registration Partnership, c/o Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 427131-04.
5. **REVIEWED BY:**  
  
Louis M. Rifici, M.S.  
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6. **APPROVED BY:**  
  
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Date: *H.T. Craven 12/2/93* *SR*
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an early life-stage toxicity test using rainbow trout. Based on the authors' analyses, the MATC was  $>0.13$  and  $<0.27$  mg/l. The geometric mean MATC was 0.19 mg/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (*Oncorhynchus mykiss*) unfertilized eggs and sperm were obtained from Mt. Lassen Trout Farms, Red Bluff, CA. The gametes were collected from adult broodstock (three males and three females) and were shipped overnight on ice. They were mixed in a plastic container, covered with dilution water (10°C), stirred, rinsed, covered with dilution water again, and allowed to water harden for approximately 1 hour.
- B. Test System: An intermittent-flow, 2-1 proportional diluter delivered test solution or control water to four replicate test chambers per concentration. The diluter stock solution was prepared in dimethylformamide (DMF). Each glass test aquarium contained two separate embryo hatching/growth chambers. The individual chambers were 15.8 x 30.5 cm with stainless-steel screen covered drains and a test solution depth of 24.5 cm. The solution volume was approximately 12 l. The aquaria were randomly positioned in a temperature-controlled water bath.

The average flow rate was 121 l/replicate/day, providing an average of 10.1 volume replacements per day. The flow rate was increased as the study progressed. During the final two weeks, the flow rate was 168 l/replicate/day (14 volume replacements/day). Flow-splitting cells divided each solution into 2 aliquots, each of which was again divided in half before being delivered to the test chambers. The accuracy of the splitting apparatus was checked before the study. Based on the final flow rate, the maximum biomass loading was 0.156 g/l/day.

Developing embryos were incubated in glass cups (9.0-cm diameter with 16-mesh Nitex® screen bottoms) suspended in the test aquaria. The cups were oscillated vertically using a rocker arm apparatus. During incubation, the embryos were kept in semi-darkness. Approximately one week after hatch was complete, the laboratory was maintained on a 16-hour daylight photoperiod under fluorescent tubes with an intensity of 52 ±3.6 foot-candles at the solution surface. Dawn and dusk simulations were used.

Hard blended well water was used as the dilution water. The water hardness ranged from 132 to 158 mg/l as  $\text{CaCO}_3$  and the pH was 7.49-8.27. The water was filtered and UV-sterilized before being delivered to the diluter.

- C. **Dosage:** Ninety-six-day flow-through test (60 days post-hatch). Based on preliminary testing, five nominal concentrations (0.031, 0.063, 0.13, 0.25, and 0.50 mg/l), a dilution water control, and a solvent control were used. The test concentrations were mg/l of whole material (i.e., not adjusted for the percentage active ingredient). The concentration of solvent was highest in the solvent control and highest test concentration (0.0125 ml DMF/l).
- D. **Design:** Forty newly-fertilized embryos (<4 hours post-fertilization) were impartially selected (in groups of 8) and impartially placed in each incubation cup (one cup per replicate, four replicates per concentration). Additionally, fifty embryos in separate incubation cups were placed in each of the four dilution water control chambers for determining fertilization success. After 11 days, the embryos reserved for fertilization determination were cleared with 10% acetic acid and observed for evidence of embryonic development.

Embryo mortality in all cups was recorded daily and dead embryos were removed from the cups. The number of test embryos hatched in each cup was recorded until hatch was complete. The 60-day post-hatch growth period was initiated when 95% of the embryos in the control group had hatched. On day 40, the number of fry was impartially reduced to 15 per replicate (except replicate A of the highest test level where only 11 surviving fry remained) and they were released into the chambers. Beginning on day 48 (12 days post-hatch), the fry were offered live brine shrimp nauplii three times daily. A commercial salmon starter was added to the diet on day 57. As the fry grew, the size of the salmon starter pellets was increased. Only two feedings were performed on weekends. Food was withheld 24 hours before photographic measurement (35-days post-hatch) and test termination. The aquaria were cleaned by siphoning as needed.

Behavioral or physical changes in the fry and mortality were determined daily and recorded. Dead fry were removed and discarded. Thirty-five days after hatch, the standard length of the fry was determined by the photographic method of McKim and Benoit (1971). The

fish were netted, transferred to a glass photographic chamber, and photographed. At test termination (60 days post-hatch), the standard length and blotted wet weight of the surviving fish were determined. Length was measured using a digitizing tablet.

The temperature, dissolved oxygen concentration (DO), hardness, alkalinity, conductivity, and pH were measured weekly and on days 0, 1, and 96. The temperature and DO were measured in two of four replicates for all test concentrations and controls. The hardness, alkalinity, conductivity, and pH were measured in the dilution water control, low and highest test levels. The temperature was also measured continuously with a data logger.

Concentrations of acetochlor were measured weekly and on study days 0, 1, 7, and 96. The samples were collected from two of the four replicates of each concentration and pooled before subsequent analysis by gas-liquid chromatography.

- E. **Statistics:** Comparison between the control and solvent control data to determine possible statistically significant differences were conducted using the chi-square statistic and a two-tailed Fisher's exact test for hatch and survival and t-tests for length and weight. Since no differences were noted, the control and solvent control data were pooled before subsequent analyses.

Hatchability and fry survival (days 35 and 60 post-hatch) data were analyzed using frequency analysis coupled with the chi-square statistic and a one-tailed Fisher's Exact test. Test levels with significantly reduced survival were not included in the growth analyses. Growth data were analyzed using one-way analysis of variance (ANOVA) for a nested design after McClave et al. (1981) followed by Dunnett's test. Since no departures from the normality assumption were detected, the data were not transformed prior to analysis. All conclusions of statistical significance were made at  $p \leq 0.05$ .

12. **REPORTED RESULTS:** Late on day 21, a power failure interrupted the diluter function for approximately nine hours. The flow rate during this period provided 5.6 volume additions/day.

The mean measured concentrations for the test were 0.037, 0.072, 0.13, 0.27, and 0.51 mg/l. These values represent 100-119% of nominal concentrations (Table V, attached).

Egg fertilization in the dilution water control averaged 99%. Hatch began on day 29 and was 95% complete by day 36 in the controls and test levels 1-4 (Table X, attached). Ninety-five percent hatch was never reached in the highest test concentration due to significant egg mortality. Percentage hatch was significantly reduced at mean measured concentrations of 0.27 and 0.51 mg/l when compared to the pooled control group. Normal fry swim-up began 10 days post-hatch (day 46) and was essentially complete by 19 days post-hatch (day 55). Time to hatch and time to initiation of swim-up were affected only in the 0.51 mg/l mean measured test concentration. Fish in the highest test concentration did not exhibit normal swim-up behavior.

Survival at 35 and 60 days post-hatch was significantly reduced at 0.51 mg/l when compared to the pooled control group (Table XI, attached). The 0.51 mg/l treatment was excluded from growth analyses due to significant mortality. Observations on day 95 (day 59 post-hatch) revealed that one fish in replicate B of the vehicle blank escaped from the chamber. One fish from replicate B of the 0.072 mg/l test level escaped from the chamber sometime between the 35-day post-hatch photographic measurement procedure and study termination. For statistical analysis, the initial number of fish present in each of these replicates at the beginning of this period was reduced to 14.

At 35 days post-hatch, mean standard length of fish in the 0.27 mg/l mean measured test concentration was significantly reduced compared to the pooled control group (Table XII, attached). At 60 days post-hatch, mean standard length and blotted wet weight at 0.27 mg/l were significantly lower than the pooled control.

Compound-related morphological and behavioral effects (fish resting on the bottom, loss of equilibrium, and erratic swimming patterns were noted at 0.27 and 0.51 mg/l.

The dissolved oxygen concentration ranged from 7.9 to 10.8 mg/l or 75 to 100% of saturation at 10.0-11.0°C. The pH values ranged from 7.49 to 8.35 and the conductivity was 305-382  $\mu$ S/cm. The hardness and alkalinity ranged from 132 to 158 mg/l as  $\text{CaCO}_3$  and 148 to 170 mg/l as  $\text{CaCO}_3$ , respectively. With the exception of days 21, 47, and 58, the temperature ranged from 9.8 to 11.0°C. On day 21, a high temperature of 12.8°C resulted from the nine-hour power

failure. On days 47 and 58, the temperature was approximately 11.6°C.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Based on the most sensitive endpoint (i.e., embryo hatch and growth), the maximum acceptable toxicant concentration (MATC) limits were estimated to be 0.13 mg/l and 0.27 mg/l (geometric mean MATC = 0.19 mg/l).

Quality Assurance and Study Compliance Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

**14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

**A. Test Procedure:** The test procedures were generally in accordance with the SEP or ASTM (1992), except for the following:

The raw data for fish length and weight were not provided in the report. These data must be provided to allow the reviewer to independently verify the MATC.

**B. Statistical Analysis:** The reviewer used one-way ANOVA and Dunnett's test (Toxstat version 3.3) to analyze percentage hatch and survival (days 35 and 60 post-hatch) data. For each parameter analyzed, the responses in the highest test level (0.51 mg/l) were significantly lower than those of the solvent control (printouts 1-3, attached). The raw data for fish length and weight were not provided in the report.

**C. Discussion/Results:** Mean length and wet weight data for each replicate were reported in Table XII (attached). After 35 days (post-hatch) exposure, the authors determined that the standard length of larvae in the two highest test levels (33.1 and 23.2 mm) was significantly lower than pooled control length. In the remaining three levels, mean lengths (34.7, 34.4, and 34.2 mm) were similar to those of the dilution water control and solvent control (34.4 and 34.5 mm, respectively). At test termination, mean standard length and wet weight in the two highest concentrations were again significantly lower than those of the pooled control. Mean length and weight in the remaining three test levels were actually higher than the same parameters in the dilution water control and the solvent control.

Raw growth data were not provided in the report. These data must be provided to allow the reviewer to independently verify the MATC. However, through careful review of the mean growth data it is highly unlikely that an independent analysis of the raw data would change the MATC presented in the report. Therefore, the authors' estimate of the MATC will be accepted.

This study is scientifically sound and meets the guideline requirements for an early life-stage toxicity test using rainbow trout. Based on the authors' analyses, the MATC was  $>0.13$  and  $<0.27$  mg/l. The geometric mean MATC was 0.19 mg/l.

**D. Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 05-13-93.

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ACETOCHLOR

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Pages 8 through 11 are not included.

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427131-04, acetochlor, percentage hatch  
 File: a:42713104.dtl Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies  
 Data PASS normality test. Continue analysis.

Bartlett's test for homogeneity of variance  
 Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

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GRP1 (SOLVENT CTRL) MEAN =	1.4185	CALCULATED t VALUE =	-0.6936
GRP2 (BLANK CTRL) MEAN =	1.4518	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0334		

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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

ANOVA TABLE

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SOURCE	DF	SS	MS	F
Between	6	1.022	0.170	18.540
Within (Error)	21	0.193	0.009	
Total	27	1.216		

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Critical F value = 2.57 (0.05,6,21)  
 Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

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GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.418	0.975		
2	dilution contrl	1.452	0.988	-0.492	
3	0.037	1.402	0.969	0.239	
4	0.072	1.402	0.969	0.239	
5	0.13	1.415	0.975	0.048	
6	0.27	1.328	0.938	1.335	
7	0.51	0.866	0.575	8.150 *	

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Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

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GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	4			
2	dilution contrl	4	0.075	7.7	-0.012
3	0.037	4	0.075	7.7	0.006
4	0.072	4	0.075	7.7	0.006
5	0.13	4	0.075	7.7	0.000
6	0.27	4	0.075	7.7	0.037
7	0.51	4	0.075	7.7	0.400

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427131-04, acetochlor, 35-day post-hatch survival  
 File: a:42713104.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality  
 Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and  
 should not be performed.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1.105	0.184	38.707
Within (Error)	21	0.100	0.005	
Total	27	1.205		

Critical F value = 2.57 (0.05,6,21)  
 Since  $F > \text{Critical } F$  REJECT  $H_0$ : All groups equal

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.408	0.983		
2	dilution contrl	1.408	0.983	0.000	
3	0.037	1.380	0.967	0.571	
4	0.072	1.441	1.000	-0.678	
5	0.13	1.441	1.000	-0.678	
6	0.27	1.408	0.983	0.000	
7	0.51	0.850	0.564	11.451	*

Dunnett table value = 2.46 (1 Tailed Value,  $P=0.05$ ,  $df=20,6$ )

DUNNETTS 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	solvent control	4			
2	dilution contrl	4	0.052	5.2	0.000
3	0.037	4	0.052	5.2	0.016
4	0.072	4	0.052	5.2	-0.017
5	0.13	4	0.052	5.2	-0.017
6	0.27	4	0.052	5.2	0.000
7	0.51	4	0.052	5.2	0.419

427131-04, acetochlor, 60-day post-hatch survival  
 File: a:42713104.dt3 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality  
 Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1.811	0.302	44.419
Within (Error)	21	0.143	0.007	
Total	27	1.953		

Critical F value = 2.57 (0.05,6,21)  
 Since  $F > \text{Critical } F$  REJECT  $H_0$ : All groups equal

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.408	0.983		
2	dilution contrl	1.408	0.983	0.000	
3	0.037	1.380	0.967	0.478	
4	0.072	1.441	1.000	-0.568	
5	0.13	1.408	0.983	0.000	
6	0.27	1.408	0.983	0.000	
7	0.51	0.684	0.402	12.428	*

Dunnett table value = 2.46 (1 Tailed Value,  $P=0.05$ ,  $df=20,6$ )

DUNNETTS 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	solvent control	4			
2	dilution contrl	4	0.065	6.6	0.000
3	0.037	4	0.065	6.6	0.016
4	0.072	4	0.065	6.6	-0.017
5	0.13	4	0.065	6.6	0.000
6	0.27	4	0.065	6.6	0.000
7	0.51	4	0.065	6.6	0.582

TITLE: 427131-04, acetochlor, percentage hatch

FILE: a:42713104.dtl

TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	1.0000	1.4917
1	solvent control	2	1.0000	1.4917
1	solvent control	3	0.9500	1.3453
1	solvent control	4	0.9500	1.3453
2	dilution contrl	1	1.0000	1.4917
2	dilution contrl	2	0.9750	1.4120
2	dilution contrl	3	0.9750	1.4120
2	dilution contrl	4	1.0000	1.4917
3	0.037	1	0.9250	1.2934
3	0.037	2	0.9750	1.4120
3	0.037	3	0.9750	1.4120
3	0.037	4	1.0000	1.4917
4	0.072	1	0.9250	1.2934
4	0.072	2	0.9750	1.4120
4	0.072	3	0.9750	1.4120
4	0.072	4	1.0000	1.4917
5	0.13	1	1.0000	1.4917
5	0.13	2	0.9750	1.4120
5	0.13	3	0.9500	1.3453
5	0.13	4	0.9750	1.4120
6	0.27	1	0.8750	1.2094
6	0.27	2	0.9500	1.3453
6	0.27	3	0.9500	1.3453
6	0.27	4	0.9750	1.4120
7	0.51	1	0.4500	0.7353
7	0.51	2	0.4500	0.7353
7	0.51	3	0.8000	1.1071
7	0.51	4	0.6000	0.8861

TITLE: 427131-04, acetochlor, 35-day post-hatch survival

FILE: a:42713104.dtl2

TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	1.0000	1.4413
1	solvent control	2	1.0000	1.4413
1	solvent control	3	0.9330	1.3090
1	solvent control	4	1.0000	1.4413
2	dilution contrl	1	1.0000	1.4413
2	dilution contrl	2	0.9330	1.3090
2	dilution contrl	3	1.0000	1.4413
2	dilution contrl	4	1.0000	1.4413
3	0.037	1	0.8670	1.1975
3	0.037	2	1.0000	1.4413
3	0.037	3	1.0000	1.4413
3	0.037	4	1.0000	1.4413
4	0.072	1	1.0000	1.4413
4	0.072	2	1.0000	1.4413
4	0.072	3	1.0000	1.4413
4	0.072	4	1.0000	1.4413
5	0.13	1	1.0000	1.4413
5	0.13	2	1.0000	1.4413
5	0.13	3	1.0000	1.4413
5	0.13	4	1.0000	1.4413
6	0.27	1	1.0000	1.4413
6	0.27	2	1.0000	1.4413
6	0.27	3	1.0000	1.4413
6	0.27	4	0.9330	1.3090
7	0.51	1	0.4550	0.7403

7	0.51	2	0.6000	0.8861
7	0.51	3	0.6000	0.8861
7	0.51	4	0.6000	0.8861

TITLE: 427131-04, acetochlor, 60-day post-hatch survival  
 FILE: a:42713104.dt3  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	1.0000	1.4413
1	solvent control	2	1.0000	1.4413
1	solvent control	3	0.9330	1.3090
1	solvent control	4	1.0000	1.4413
2	dilution contrl	1	1.0000	1.4413
2	dilution contrl	2	0.9330	1.3090
2	dilution contrl	3	1.0000	1.4413
2	dilution contrl	4	1.0000	1.4413
3	0.037	1	0.8670	1.1975
3	0.037	2	1.0000	1.4413
3	0.037	3	1.0000	1.4413
3	0.037	4	1.0000	1.4413
4	0.072	1	1.0000	1.4413
4	0.072	2	1.0000	1.4413
4	0.072	3	1.0000	1.4413
4	0.072	4	1.0000	1.4413
5	0.13	1	0.9330	1.3090
5	0.13	2	1.0000	1.4413
5	0.13	3	1.0000	1.4413
5	0.13	4	1.0000	1.4413
6	0.27	1	1.0000	1.4413
6	0.27	2	1.0000	1.4413
6	0.27	3	1.0000	1.4413
6	0.27	4	0.9330	1.3090
7	0.51	1	0.2730	0.5498
7	0.51	2	0.5330	0.8184
7	0.51	3	0.3330	0.6151
7	0.51	4	0.4670	0.7524

## CONCENTRATION DATA

ROW	min	twa	max
1	0.018495	0.036990	0.048086
2	0.035872	0.071745	0.093268
3	0.067135	0.134271	0.174552
4	0.131901	0.263802	0.342943
5	0.254193	0.508385	0.660901

## DISSOLVED OXYGEN DATA

ROW	min	twa	max
1	4.67240	9.3448	12.1482
2	4.67448	9.3490	12.1536
3	4.59141	9.1828	11.9377
4	4.60417	9.2083	11.9708
5	4.75755	9.5151	12.3696
6	4.71120	9.4224	12.7491
7	4.66823	9.3365	12.1374
8	4.68438	9.3688	12.1794
9	4.63177	9.2635	12.0426
10	4.62240	9.2448	12.0182
11	4.66250	9.3250	12.1225
12	4.61172	9.2234	11.9905
13	4.79193	9.5839	12.4590
14	4.71693	9.4339	12.2640

## TEMPERATURE DATA

ROW	min	twa	max
16	5.17948	10.3589	13.4665
17	5.18864	10.3771	13.4902
18	5.17318	10.3464	13.4303
19	5.21354	10.4271	13.5552
20	5.27370	10.5474	13.7116
21	5.21536	10.4307	13.5599
22	5.19401	10.3880	13.5044
23	5.16302	10.3260	13.4239
24	5.15885	10.3177	13.4130
25	5.17422	10.3484	13.4530
26	5.16745	10.3349	13.4354
27	5.30339	10.6068	13.7888
28	5.24089	10.4818	13.6263
29	5.25443	10.5089	13.6615