

MRID No.: 445051-20

DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 123-2 (TIER II)

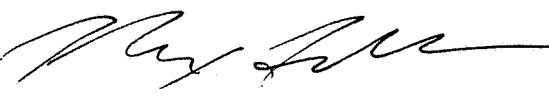
1. CHEMICAL: Mesotrione PC Code No.: 129990

2. TEST MATERIAL: ZA1296 Purity: 97.6%

3. CITATION

Authors: D.V. Smyth, S.J. Kent, and N. Shillabeer
Title: ZA1296: Toxicity to the Marine Alga
Skeletonema costatum
Study Completion Date: September 28, 1996
Laboratory: Bixham Environmental Laboratory, Brixham,
Devon, UK
Sponsor: ZENECA Ag Products, Wilmington, DE
Laboratory Report ID: BL5779/B
DP Barcode: D245475
MRID No.: 445051-20

4. REVIEWED BY: Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.

Signature:  Date: 8/25/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.

Signature: P. Kosalwat Date: 8/25/98

5. APPROVED BY:

Signature:  Date: 6/12/00

6. STUDY PARAMETERS

Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills
the guideline requirements for an algal toxicity test.

Results Synopsis - Based on mean measured concentrations

EC ₅₀ : 20 ppm	95% C.I.: 17 - 23 ppm
NOEC: 0.5 ppm	Probit Slope: N/A

8. ADEQUACY OF THE STUDY**A. Classification:** Core**B. Rationale:** N/A**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS:** No significant deviations.**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Skeletonema costatum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	10,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Marine culture medium by Walsh and Alexander (1980)

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	20.1 - 20.2°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.48 KLux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	16 h light, 8 h dark

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 7.8 - 8.2 Final: 8.2 - 8.7

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 65 mg/L
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	3 (6 in the control)
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Algal particle density
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq$ 2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Concentration (mg/L)		Mean Particle Density ($\times 10^4$ cells/mL)	% reduction	120-Hour pH
Nominal	Mean Measured			
Control	<0.013	64.5	--	8.6
0.50	0.50	60.9	6	8.6
1.0	1.0	56.5	12	8.6
2.0	2.1	54.5	16	8.6
4.0	4.1	58.5	9	8.6
8.0	8.6	54.0	16	8.7
16	16	47.3	27	8.5
32	32	5.66	91	8.3
65	64	1.50	98	8.2

Other Significant Results: Area under the growth curve was the most sensitive end-point measured in this study.

Statistical Results for Area Under the Growth Curve

Statistical Method: Probit method was used for calculating the EC_{50} and Dunnett's test for mean separation. Results based on nominal concentrations.

EC_{50} : 7.7 mg/L

95% C.I.: 3.1 - 24 mg/L

Probit Slope: Not reported

NOEC: 0.50 mg/L

Statistical Results for Growth Rate

Statistical Method: Probit method was used for calculating the EC_{50} and Dunnett's test for mean separation. Results based on nominal concentrations.

EC_{50} : 52 mg/L

95% C.I.: 13 - >65 mg/L

Probit Slope: Not reported

NOEC: 8.0 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Nonlinear model for EC₅₀ and Williams' test for mean comparisons. Results based on mean measured concentrations.

EC₅₀: 20 ppm

95% C.I.: 17 - 23 ppm

Probit Slope: N/A

NOEC: 0.5 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on mean measured concentrations, the 120-hour EC₅₀ and NOEC for *Skeletonema costatum* exposed to ZA1296 were 20 and 0.5 ppm, respectively. This study is classified as **Core**.

ZA1296 - SKELETONEMA

File: 44505120

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	6	64.467	64.467	64.467
2	0.50	3	60.900	60.900	60.900
3	1.0	3	56.533	56.533	56.533
4	2.1	3	54.500	54.500	56.517
5	4.1	3	58.533	58.533	56.517
6	8.6	3	53.967	53.967	53.967
7	16	3	47.267	47.267	47.267
8	32	3	5.657	5.657	5.657
9	64	3	1.497	1.497	1.497

ZA1296 - SKELETONEMA

File: 44505120

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	64.467				
0.50	60.900	1.421		1.72	k= 1, v=21
1.0	56.533	3.162	*	1.80	k= 2, v=21
2.1	56.517	3.168	*	1.83	k= 3, v=21
4.1	56.517	3.168	*	1.84	k= 4, v=21
8.6	53.967	4.185	*	1.85	k= 5, v=21
16	47.267	6.855	*	1.85	k= 6, v=21
32	5.657	23.438	*	1.85	k= 7, v=21
64	1.497	25.096	*	1.86	k= 8, v=21

s = 3.548

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

NOEC = 0.50 mg/L

MAX FEKEN ZA1296 SKELETONEMA 08-03-98

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
64	100	98	98	0
32	100	91	91	0
16	100	27	27	0
8.600001		100	16	16
4.1	100	9	9	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 20.15297

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	1.573863E-02	16.91032	15.08152 18.98162

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.6854881	12.75882	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.111818
95 PERCENT CONFIDENCE LIMITS = .5354128 AND 5.688223

LC50 = 16.83761
95 PERCENT CONFIDENCE LIMITS = 5.786504 AND 50.62808

LC10 = 6.578984
95 PERCENT CONFIDENCE LIMITS = 5.896659E-02 AND 12.61631

ZA1296 - SKELETONEMA

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0		70.30	67.00	61.60	58	65.4	64.5
2	0.5	-0.30103	62.40	56.80	63.50	.	.	.
3	1.0	0.00000	50.60	61.20	57.80	.	.	.
4	2.0	0.30103	55.20	50.80	57.50	.	.	.
5	4.0	0.60206	57.20	58.20	60.20	.	.	.
6	8.0	0.90309	52.80	52.90	56.20	.	.	.
7	16.0	1.20412	42.60	46.50	52.70	.	.	.
8	32.0	1.50515	5.16	6.59	5.22	.	.	.
9	65.0	1.81291	1.80	1.36	1.33	.	.	.

MODEL: COUNT = CO * PROB NORM (LOG_EC50 - LOG_CONC) / SIGMA)

WEIGHTED REGRESSION 11:46 Monday, August 3, 1998

Non-Linear Least Squares Iterative Phase			
Dependent Variable COUNT		Method: Gauss-Newton	
Iter	LOG_EC50	SIGMA	Weighted SS
0	1.219000	64.500000	43.220073
1	1.312065	0.212593	59.292044
2	1.299527	0.210601	59.845224
3	1.300421	0.209347	59.825364
4	1.300229	0.209515	59.830289
5	1.300260	0.209487	59.829496
6	1.300255	0.209491	59.829626
7	1.300255	0.209490	59.829605
8	1.300255	0.209491	59.829608
9	1.300255	0.209491	59.829608
10	1.300255	0.209491	59.829608
11	1.300255	0.209491	59.829608

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable COUNT
Regression	3	1403.360000	
Residual	27	27.0045421	467.7866667
Uncorrected Total	30	1430.3645421	1.0001682
(Corrected Total)	29	1257.6213891	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval
LOG_EC50	1.3002527	0.0267507585	Lower 1.243367677 Upper 1.355142870
SIGMA	0.20949052	0.0232282029	0.161830559 0.257150489
CO	59.82960791	1.7361879487	56.267272172 63.391943639

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.62570634	1	
CO	-0.391996134	0.2466397647	1

MODEL: COUNT = CO * PROB NORM (LOG_EC50 - LOG_CONC) / SIGMA)

SUMMARY OF NONLINEAR REGRESSION

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
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ZA1296 - SKELETONEMA

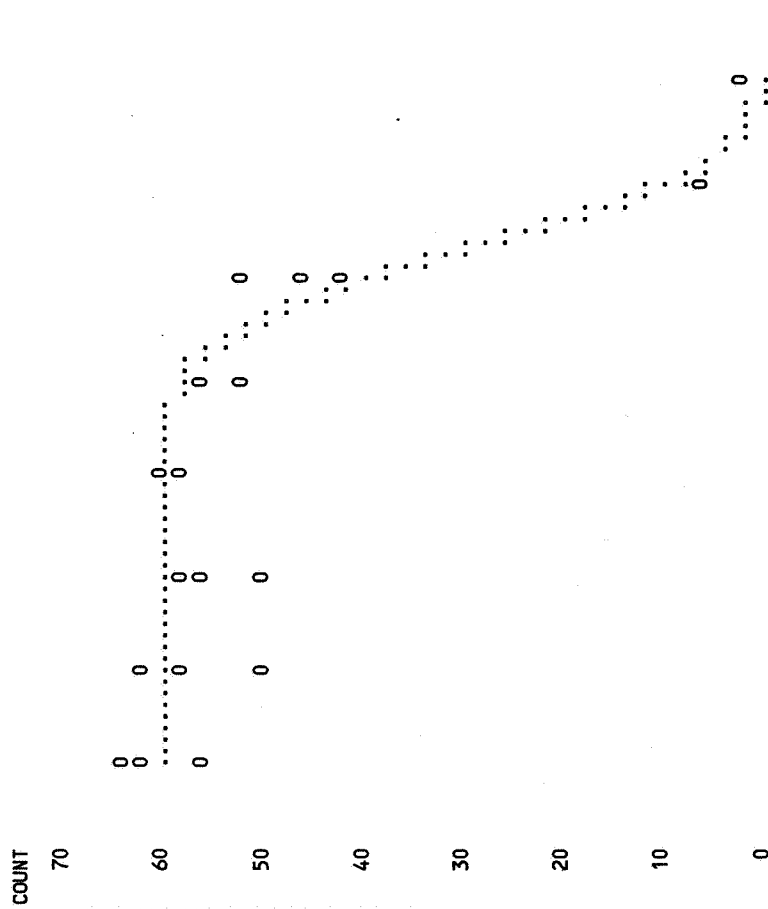
11:46 Monday, August 3, 1998

MODEL: COUNT = CO * PROB NORM (LOG_EC50 - LOG_CONC) / SIGMA)

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Plot of COUNT*LOG_CONC. Symbol used is 'O'.

Plot of PRED*LOG_CONC. Symbol used is 'O'.



NOTE: 2151 obs had missing values. 2083 obs hidden.

COMPARISON OF MEANS FOR NOEL DETERMINATION

TEST IF TREATMENT IS LESS THAN CONTROL

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General Linear Models Procedure

Class Level Information

Class	Levels	Values
DOSE	9	0 1 2 4 8 16 32 65 0.5

Number of observations in data set = 54

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NOTE: Due to missing values, only 30 observations can be used in this analysis.

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	14734.41401	1841.80175	146.27	0.0001
Error	21	264.42693	12.59176		
Corrected Total	29	14998.84095			

R-Square 0.982370
C.V. 7.585695
Root MSE 3.548487
RESPONSE Mean 46.77867

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	14734.41401	1841.80175	146.27	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	14734.41401	1841.80175	146.27	0.0001

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	64	46.66667	4.27442004
1	56	53.33333	5.41233160
2	54	50.00000	3.40440891
4	58	53.33333	1.52752523
8	53	96.66667	1.93476958
16	47	26.66667	5.09345986
32	5	65.66667	0.80884692
65	3	1.4966667	0.26312228
0.5	3	60.9000000	3.59304884

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:46 Monday, August 3, 1998

General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 21 MSE= 12.59176
Critical Value of Dunnett's T= 2.625

File:44505120.sas Page 4
Comparisons significant at the 0.05 level are indicated by ****.

DOSE Comparison	Simultaneous Lower Confidence Limit		Difference Between Means	Simultaneous Upper Confidence Limit	
	Lower Confidence Limit	Upper Confidence Limit			
0.5 - 0	-10.154	3.020	-3.567	3.020	
4 - 0	-12.520	0.654	-5.933	0.654	
1 - 0	-14.520	-1.346	-7.933	-1.346	
2 - 0	-16.554	-3.380	-9.967	-3.380	
8 - 0	-17.087	-3.913	-10.500	-3.913	
16 - 0	-23.787	-10.613	-17.200	-10.613	
32 - 0	-65.397	-52.223	-58.810	-52.223	
65 - 0	-69.557	-56.383	-62.970	-56.383	

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0		70.30	67.00	61.60	58	65.4	64.5
2	0.5	-0.30103	62.40	56.80	63.50	.	.	.
3	1.0	0.00000	50.60	61.20	57.80	.	.	.
4	2.1	0.32222	55.20	50.80	57.50	.	.	.
5	4.1	0.61278	57.20	58.20	60.20	.	.	.
6	8.6	0.93450	52.80	52.90	56.20	.	.	.
7	16.0	1.20412	42.60	46.50	52.70	.	.	.
8	32.0	1.50515	5.16	6.59	5.22	.	.	.
9	64.0	1.80618	1.80	1.36	1.33	.	.	.

MODEL: COUNT = CO * PROBNOEM (LOG_EC50 - LOG_CONC) / SIGMA) 2
 WEIGHTED REGRESSION 13:25 Monday, August 3, 1998

Non-Linear Least Squares Iterative Phase				
Dependent Variable		COUNT		
LOG_EC50		Method: Gauss-Newton		
Iter	LOG_EC50	CO	Weighted SS	Weighted SS
0	1.226000	0.321000	64.500000	41.304067
1	1.312567	0.208271	59.416533	24.680636
2	1.299107	0.208952	59.938739	25.763653
3	1.300210	0.207571	59.91837	25.988118
4	1.299995	0.207758	59.917702	25.965277
5	1.300029	0.207728	59.916783	25.969106
6	1.300023	0.207732	59.916930	25.968499
7	1.300024	0.207732	59.916906	25.968596
8	1.300024	0.207732	59.916910	25.968580
9	1.300024	0.207732	59.916909	25.968583
10	1.300024	0.207732	59.916909	25.968582
11	1.300024	0.207732	59.916909	25.968582

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable	Weighted MS
Regression	3	1403.3600000		467.7866667
Residual	27	25.9685824		0.9617993
Uncorrected Total	30	1429.3285824		
(Corrected Total)	29	1254.7565749		

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval
LOG_EC50	1.30002388	0.0259227423	Lower 1.246835215 Upper 1.353212536
SIGMA	0.20773172	0.0222309245	0.162117984 0.253345453
CO	59.91690942	1.7158146632	56.396375889 63.437442948

Asymptotic Correlation Matrix

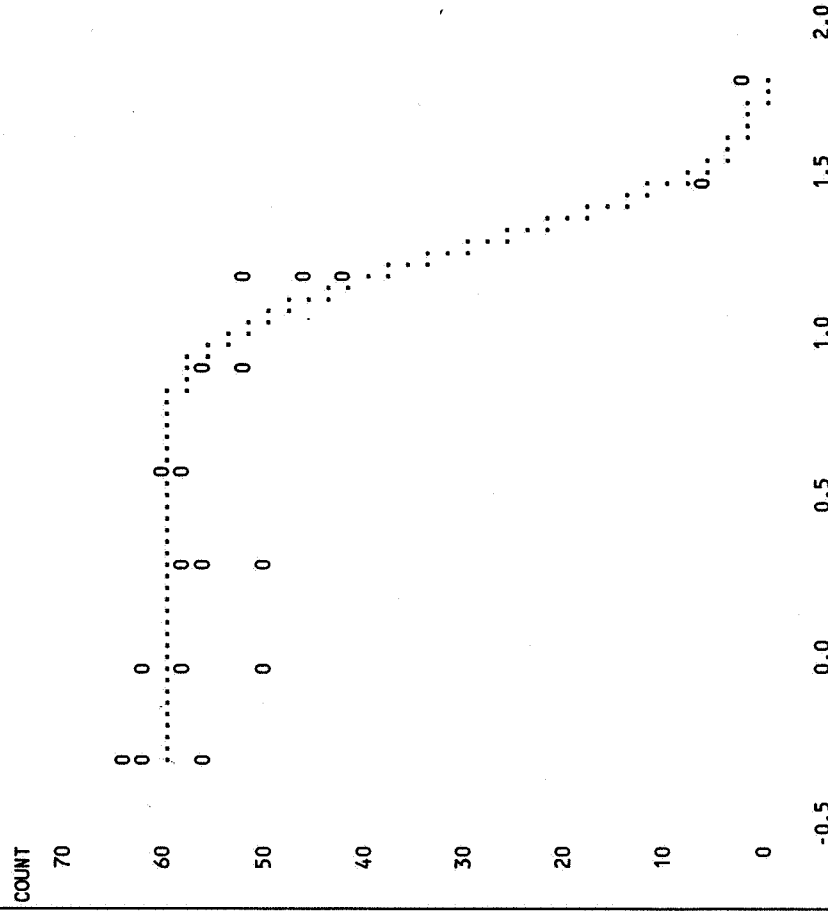
Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.621999958	1	
CO	-0.405143537	0.2603734174	1

MODEL: COUNT = CO * PROBNOEM (LOG_EC50 - LOG_CONC) / SIGMA) 3
 SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
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1 0 0.20773 59.9169 25.9686 19.9537 4
 ZA1296 - SKELETONEMA
 MODEL: COUNT = CO * PROBNOEM (LOG_EC50 - LOG_CONC) / SIGMA)
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Plot of COUNT*LOG_CONC. Symbol used is 'O'.
 Plot of PRED*LOG_CONC. Symbol used is 'O'.



NOTE: 2144 obs had missing values. 2074 obs hidden.

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 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure
 Class Level Information

Class	Levels	Values
DOSE	9	0 1 16 32 64 0.5 2.1 4.1 8.6

Number of observations in data set = 54

File:44505120.sas Page 3
NOTE: Due to missing values, only 30 observations can be used in this analysis.

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE				
Source	DF	Sum of Squares	Mean Square	F Value Pr > F
Model	8	14734.41401	1841.80175	146.27 0.0001
Error	21	264.42693	12.59176	
Corrected Total	29	14998.84095		
R-Square C.V. Root MSE RESPONSE Mean				
		0.982370	7.585695	3.548487 46.77867

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	14734.41401	1841.80175	146.27	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	14734.41401	1841.80175	146.27	0.0001

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	6	64.466667	4.27442004
1	3	56.533333	5.41233160
16	3	47.266667	5.09345986
32	3	5.656667	0.80884692
64	3	1.496667	0.26312228
0.5	3	60.900000	3.59304884
2.1	3	54.500000	3.40440891
4.1	3	58.533333	1.52752523
8.6	3	53.966667	1.93476958

ZA1296 - SKELETONEMA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
13:25 Monday, August 3, 1998

General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 21 MSE= 12.59176
Critical Value of Dunnnett's T= 2.625

(1)

Simultaneous				
DOSE Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
0.5 - 0	-10.154	-3.567	3.020	
4.1 - 0	-12.520	-5.933	0.654	***
1 - 0	-14.520	-7.933	-1.346	***
2.1 - 0	-16.554	-9.967	-3.380	***
8.6 - 0	-17.087	-10.500	-3.913	***
16 - 0	-23.787	-17.200	-10.613	***
32 - 0	-65.397	-58.810	-52.223	***
64 - 0	-69.557	-62.970	-56.383	***