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2-EEB-27-h

Reviewer: Tom A. Barlow

DRAFT

5/6/93

MRID No. 426873-01

IV-a-DAP

DATA EVALUATION RECORD

1. **CHEMICAL:** Fonofos. Shaughnessey No. 041701.

2. **TEST MATERIAL:** Technical Fonofos; *O*-ethyl *S*-phenyl (*RS*)-ethylphosphonodithioate; Batch No. P3/D7534/27; purity of 94.6% w/w.

3. **STUDY TYPE:** 72-4. *Daphnia magna* Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Farrelly, E. and M.J. Hamer. 1993. Fonofos: Chronic Toxicity to *Daphnia magna*. Report No. RJ1392B. Performed by ICI Agrochemicals, Jealotts Hill Research Station, Bracknell, Berkshire, U.K. Submitted by ICI Americas Inc., Wilmington, DE. EPA MRID No. 426873-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora

Date: 4/30/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat

Date: 4/30/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:

Date:

DRAFT

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a static-renewal, life-cycle toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC for technical fonofos, based on the most sensitive biological parameters (reproduction and length), was >0.31 and <0.64 µg/l mean measured concentrations (geometric mean MATC = 0.45 µg/l).

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

D L

11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The cultures were maintained at approximately 20°C on a 16-hour daylight. The cultures were fed an algal and yeast diet.

B. Test System: The test vessels were 250-ml glass beakers filled with 200 ml of test solution. Each vessel was covered with a watch glass to reduce evaporation. The test solutions were renewed three times weekly (days 2, 5, 7, 9, 12, 14, 16, and 19).

The test was conducted in a constant-temperature room. The photoperiod was 16 hours of fluorescent light with a light intensity of approximately 700 lux.

The dilution water was hard, blended water which was prepared by mixing "mains, dechlorinated water" (hardness of 300 mg/l as $CaCO_3$) with deionized water to obtain a hardness of 160-180 mg/l as $CaCO_3$.

The test concentrations were prepared from a primary stock solution (10 mg/ml) in methanol. A fresh aqueous solution (160 µg/l) was prepared at each renewal. Aliquots of this solution were made up to 2.18 l in dilution water to yield the desired test concentration in 0.0016% methanol.

C. Dosage: Twenty-one-day, static-renewal toxicity test. Six nominal concentrations (0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 µg ai/l) were selected for the test. A dilution water control and a solvent control were also included. The solvent control had a 0.0016% methanol concentration.

D. Design: Each treatment level consisted of 7 replicates (replicates A-G) containing one daphnid each (for monitoring survival, length, and reproduction) and 3 replicates (replicates H-J) containing five daphnids each (for monitoring survival). Test treatments were randomized within the test area. At each renewal, the adults were transferred from the old solutions to the new solutions. The daphnids were fed a yeast and algal (*Chlorella vulgaris*) suspension daily.

Immobility of the daphnids was determined daily. From day 7 onwards, the number of young produced were recorded at each renewal and at test termination. The

length of all surviving adults in replicates A-G was determined at test termination.

The pH and dissolved oxygen concentration (DO) of new and old solutions were monitored in one replicate of each treatment on days 0, 2, 5, 7, 9, 12, 14, 16, 19, and 21. The temperature was continuously monitored in the constant temperature room. The conductivity, hardness, and alkalinity of a dilution water control replicate were measured on days 6, 13, and 20.

Samples of the new and old test solutions were collected for quantitative analysis of fonofos using gas liquid chromatography.

E. **Statistics:** The LC_{50} values and 95% confidence intervals were calculated using iteratively reweighted linear regression of percentage mortality on log (concentration).

Length and reproduction of the control and solvent control organisms were compared. There was no significant difference between the controls, therefore the control data were pooled for comparison to the treatments. Length and reproduction data were analyzed using a one-way ANOVA and the least significant difference (LSD) was computed to determine if there were any significant differences between the pooled controls and the treatment groups (p=0.05).

12. **REPORTED RESULTS:** Mean measured concentrations were 0.16, 0.31, 0.64, 1.2, 2.09, and 4.90 µg/l which represents 65-80% of nominal concentrations (Table 1, attached).

The 21-day LC_{50} (95% confidence interval) for replicates H-J was 1.2 (0.9-1.4) µg/l (Table 10, attached). "In replicates A-G, at mean measured concentrations of 4.9 µg l^{-1} and 2.1 µg l^{-1}, all the *Daphnia* were dead within 2 and 4 days respectively. At 1.2 µg l^{-1}, 5 out of the 7 *Daphnia* were dead after 21 days and at 0.64 µg l^{-1}, 3 *Daphnia* died. The only other mortality was a single *Daphnia* in the control which was assessed dead on day 21" (Table 9, attached).

By test termination, length of surviving adult daphnids was reduced at 0.64 and 1.2 µg/l (Table 4, attached). The mean number of live young produced by the control and solvent control was 164 and 160, respectively (Table 5, attached). No young were produced at the two highest test concentrations. The number of young produced at 0.64 and

1.2 µg/l were significantly reduced when compared to that of the controls.

During the study, the test solutions had a pH range of 7.6-8.2 and a DO range of 7.9-9.0 mg/l. The mean temperature in the test room was 21.1 (range of 19.2-24.5°C). The total alkalinity, hardness, and conductivity of the control were 133-134 mg/l as $CaCO_3$, 172-178 mg/l as $CaCO_3$, and 366-396 µmhos/cm, respectively.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
The no-observed-effect concentration (NOEC) was 0.31 µg/l. The lowest-observed-effect concentration (LOEC) was 0.64 µg/l.

A Good Laboratory Practice Compliance Statement and a Quality Assurance Statement were included in the report indicating that this study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations for FIFRA (40 CFR 160).

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

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated as follows:

The report did not indicate the manner in which the test daphnids were assigned to the test chambers. Random assignment is required.

The temperature of the test solutions was not measured and the temperature of the test room was 19.2-24.5°C. *Daphnia magna* life-cycle tests should be conducted at 20 ±2°C.

The dilution water was a mixture of dechlorinated water and deionized water. The use of dechlorinated water is discouraged because removal of chlorine is rarely complete and residual chlorine can be toxic to aquatic organisms. The residual chlorine in the dilution water was not measured. However, for this study it is probably acceptable since control mortality, growth, and reproduction was satisfactory.

Conductivity, alkalinity, and hardness were measured only in the control; these parameters should have also been measured in one test concentration.

Light to dark and dark to light transition periods were not used as recommended in the guidelines.

B. **Statistical Analysis:** The reviewer calculated the number of young produced per female reproductive day (using the total number of young produced and the number of female reproductive days) for analysis of effects on reproduction (Tables 11-16, attached).

The reviewer used William's test to analyze the length and reproduction (number of young produced per female reproductive day) data (pages 4 and 8 of attached printouts). The results of these analyses were as the same as the authors. The survival data for replicates A-G were analyzed using Fishers Exact test (page 15 of attached printouts). Survival data for replicates H-J were analyzed as proportional survival and were arcsine squareroot transformed prior to analysis using Wilcoxons Rank Sum test. The results of this analysis showed no significant difference between the control and any treatment (page 12 of attached printouts). However, upon visual examination of the data, survival was substantially reduced at the three highest test concentrations.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a static-renewal, life-cycle toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC for technical fonofos based on the most sensitive biological parameters (length and reproduction), was >0.31 and <0.64 µg/l mean measured concentrations (geometric mean MATC = 0.45 µg/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, 14 April 1993.

TABLE 1 : Measured Concentrations of Fonofos (μg l^{-1})

Study Day	Age of Soln.[a]	Nominal Concentration (μg l^{-1})							
		6.4	3.2	1.6	0.8	0.4	0.2	Solvent Control	Control
0	0 hr	6.06	2.89	1.45	0.724	0.417	0.202	<0.05[b]	<0.05[b]
2	48 hr	3.74	1.71	0.95	0.505	0.299	0.144	<0.05	<0.05
	0 hr	-[c]	1.87	1.23	0.524	0.257	0.108	<0.05	<0.05
5	72 hr	-	1.87	1.01	0.525	0.248	0.123	<0.05	<0.05
	0 hr	-	-[c]	1.06	0.491	0.221	0.185	<0.05	<0.05
7	48 hr	-	-	1.06	0.539	0.263	0.140	<0.05	<0.05
	0 hr	-	-	1.19	0.632	0.265	0.148	<0.05	<0.05
9	48 hr	-	-	1.22	0.640	0.312	0.154	<0.05	<0.05
	0 hr	-	-	1.46	0.687	0.347	0.149	<0.05	<0.05
12	72 hr	-	-	1.19	0.613	0.278	0.150	<0.05	<0.05
	0 hr	-	-	1.29	0.717	0.351	0.181	<0.05	<0.05
14	48 hr	-	-	0.88	0.577	0.235	0.160	<0.05	<0.05
	48 hr[d]	-	-	1.23	0.668	0.296	0.163	<0.05	<0.05
	0 hr	-	-	1.48	0.814	0.392	0.196	<0.05	<0.05
16	48 hr	-	-	1.27	0.676	0.324	0.143	<0.05	<0.05
	0 hr	-	-	1.56	0.746	0.384	0.191	<0.05	<0.05
19	72 hr	-	-	1.38	0.641	0.331	0.170	<0.05	<0.05
	72 hr[d]	-	-	1.30	0.641	0.395	0.183	<0.05	<0.05
	0 hr	-	-	1.57	0.747	0.374	0.192	<0.05	<0.05
21	48 hr	-	-	1.30	0.658	0.336	0.149	<0.05	<0.05
	48 hr[d]	-	-	1.36	0.599	0.271	0.115	<0.05	<0.05
Mean		4.90	2.09	1.25	0.636	0.313	0.160	<0.05	<0.05

[a] 48 and 72 hour solutions are referred to in the text as "aged"
[b] approximate limit of determination under the conditions used
[c] - = solution not prepared as all P_o *Daphnia* dead
[d] samples from replicates containing a single *Daphnia* (not used to calculate mean values)

TABLE 3 : Toxicity of Fonofos to *Daphnia magna*, based on Mean Measured Concentrations (replicates H-J)

Study Day	LC_{50} ($\mu g\ l^{-1}$)	95% Confidence Limits
1	5.0	3.8 - 10.2
2	3.3	2.5 - 4.3
7	1.7	1.4 - 1.9
14	1.5	1.2 - 1.8
21	1.2	0.9 - 1.4

TABLE 4 : *Daphnia* (P_o generation) Length in mm on Day 21

Replicate	Mean Measured Concentration ($\mu g\ l^{-1}$)							
	4.9	2.1	1.2	0.64	0.31	0.16	Solvent Control	Control
A	-[a]	-	3.71	-	4.50	4.64	4.50	4.43
B	-	-	-	-	4.50	4.71	4.71	4.57
C	-	-	-	4.21	4.50	4.57	4.57	-
D	-	-	-	-	4.57	4.79	4.50	4.64
E	-	-	-	4.57	4.64	4.43	4.50	4.50
F	-	-	3.79	4.64	4.57	4.64	4.57	4.64
G	-	-	-	4.07	4.64	4.57	4.57	4.57
Mean	-	-	3.75*	4.37*	4.56	4.62	4.56	4.56

[a] - = *Daphnia* dead before day 21

* significantly different from the control at the 5% level

TABLE 5 : Total Number of Live Young (F_1 generation) Produced per *Daphnia* (P_o generation)

Replicate	Mean Measured Concentration (µg l^{-1})							
	4.9	2.1	1.2	0.64	0.31	0.16	Solvent Control	Control
A	0	0	87	84	180	166	147	167
B	0	0	29	125	183	168	168	179
C	0	0	0	147	161	148	171	136
D	0	0	0	121	171	182	152	177
E	0	0	77	170	173	172	146	147
F	0	0	88	148	191	178	148	167
G	0	0	2	51	185	169	186	178
Mean	0	0	40*	121*	178	169	160	164

* significantly different from the control (p=0.05)

APPENDIX III : Mortality Data

TABLE 9 : Study Day at which *Daphnia* was assessed dead, replicates A-G

Replicate	Mean Measured Concentration (µg l^{-1})							
	4.9	2.1	1.2	0.64	0.31	0.16	Solvent Control	Control
A	1	2	+	16	+	+	+	+
B	2	3	12	20	+	+	+	+
C	1	4	7	+	+	+	+	21
D	2	3	6	19	+	+	+	+
E	2	3	16	+	+	+	+	+
F	1	2	+	+	+	+	+	+
G	1	3	8	+	+	+	+	+

+ = Daphnia was still alive at day 21

TABLE 10 : Mortality Data, Replicates H-J

No. *Daphnia* dead, out of 5

Study Day	Mean Measured Concentration (µg l^{-1}) and Replicate																	
	4.9			2.1			1.2			0.64			0.31			0.16			Solvent Control			Control		
	H	I	J	H	I	J	H	I	J	H	I	J	H	I	J	H	I	J	H	I	J	H	I	J
1	2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	5	5	5	5	5	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	5	5	5	5	5	5	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
21	5	5	5	5	5	5	3	4	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0

APPENDIX IV : Reproduction Data

TABLE 11 : Number of Young in Mean Measured Concentration 1.2 µg l^{-1}

Replicate	Study Day							Total	# of reprod. days	# young/reprod. day
	7	9	12	14	16	19	21			
A	0	22	5	10	0	28	22	87	14	6.2
B	0	29	0	-[a]	-	-	-	29	6	4.8
C	0	-[a]	-	-	-	-	-	0	1	0
D	0	-[a]	-	-	-	-	-	0	1	0
E	0	29	27	21	0	-[a]	-	77	11	7
F	0	19	0	21	0	38	10	88	14	6.3
G	0	2	-[a]	-	-	-	-	2	4	0.5

[a] - = Adult female dead

TABLE 12 : Number of Young in Mean Measured Concentration 0.64 µg l^{-1}

Replicate	Study Day							Total		
	7	9	12	14	16	19	21			
A	0	23	22	8	31	-[a]	-	84	11	7.6
B	0	24	18	39	0	44	-[a]	125	13	9.6
C	0	25	19	31	0	33	39	147	14	10.5
D	0	23	28	37	0	33	-[a]	121	13	9.3
E	0	26	27	38	0	31	48	170	14	12.1
F	0	27	18	8	39	56	0	148	14	10.6
G	0	22	20	0	4	0	5	51	14	3.6

[a] - = Adult female dead

TABLE 13 : Number of Young in Mean Measured Concentration 0.31 µg l^{-1}

Replicate	Study Day							Total		
	7	9	12	14	16	19	21			
A	0	31	22	44	0	39	44	180	14	12.9
B	0	34	21	43	0	45	40	183	14	13.1
C	0	28	22	37	0	37	37	161	14	11.5
D	0	30	21	39	0	41	40	171	14	12.2
E	0	31	19	38	0	39	46	173	14	12.4
F	0	26	20	43	0	50	52	191	14	13.6
G	0	33	18	44	0	46	44	185	14	13.2

APPENDIX IV (continued): Reproduction Data

TABLE 14 : Number of Young in Mean Measured Concentration 0.16 µg l-1

Replicate	Study Day							Total		
	7	9	12	14	16	19	21			
A	0	22	21	0	40	45	38	166	14	11.9
B	0	34	17	0	39	44	34	168	14	12.0
C	0	28	19	0	36	47	18	148	14	10.6
D	0	27	23	36	0	49	47	182	14	13.0
E	0	26	24	35	0	43	44	172	14	12.3
F	0	29	24	0	42	45	38	178	14	12.7
G	0	26	22	0	37	50	34	169	14	12.1

TABLE 15 : Number of Young in Solvent Control

Replicate	Study Day							Total		
	7	9	12	14	16	19	21			
A	0	25	21	31	0	36	34	147	14	10.5
B	0	28	22	39	0	37	42	168	14	12.0
C	0	26	22	37	0	41	45	171	14	12.2
D	0	24	23	34	0	27	44	152	14	10.9
E	0	23	16	32	0	25	50	146	14	10.4
F	0	23	17	41	0	42	25	148	14	10.6
G	0	29	24	35	0	44	54	186	14	13.3

TABLE 16 : Number of Young in Control

Replicate	Study Day							Total		
	7	9	12	14	16	19	21			
A	0	26	23	0	36	40	42	167	14	11.9
B	0	31	25	38	0	40	45	179	14	12.8
C	0	28	29	32	0	28	19[a]	136	13	10.5
D	0	27	24	39	0	39	48	177	14	12.6
E	0	25	28	39	0	19	36	147	14	10.5
F	0	22	31	0	35	30	49	167	14	11.9
G	0	25	28	39	0	36	50	178	14	12.7

[a] - Adult female dead

Fonofos: Reproduction of Exposed Daphnia magna
File: 42687301.rep Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.814	10.164	16.044	10.164	2.814
OBSERVED	3	10	12	16	1

Calculated Chi-Square goodness of fit test statistic = 5.5546
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Fonofos: Reproduction of Exposed Daphnia magna
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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 20.58
Closest, conservative, Table H statistic = 25.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 6
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 6.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

TITLE: Fonofos: Reproduction of Exposed Daphnia magna
FILE: 42687301.rep
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	11.9000	11.9000
1	Control	2	12.8000	12.8000
1	Control	3	10.5000	10.5000
1	Control	4	12.6000	12.6000
1	Control	5	10.5000	10.5000
1	Control	6	11.9000	11.9000
1	Control	7	12.7000	12.7000
2	Solvent Control	1	10.5000	10.5000
2	Solvent Control	2	12.0000	12.0000
2	Solvent Control	3	12.2000	12.2000
2	Solvent Control	4	10.9000	10.9000
2	Solvent Control	5	10.4000	10.4000
2	Solvent Control	6	10.6000	10.6000
2	Solvent Control	7	13.3000	13.3000
3	0.16 ug/l	1	11.9000	11.9000
3	0.16 ug/l	2	12.0000	12.0000
3	0.16 ug/l	3	10.6000	10.6000
3	0.16 ug/l	4	13.0000	13.0000
3	0.16 ug/l	5	12.3000	12.3000
3	0.16 ug/l	6	12.7000	12.7000
3	0.16 ug/l	7	12.1000	12.1000
4	0.31 ug/l	1	12.9000	12.9000
4	0.31 ug/l	2	13.1000	13.1000
4	0.31 ug/l	3	11.5000	11.5000
4	0.31 ug/l	4	12.2000	12.2000
4	0.31 ug/l	5	12.4000	12.4000
4	0.31 ug/l	6	13.6000	13.6000
4	0.31 ug/l	7	13.2000	13.2000
5	0.64 ug/l	1	7.6000	7.6000
5	0.64 ug/l	2	9.6000	9.6000
5	0.64 ug/l	3	10.5000	10.5000
5	0.64 ug/l	4	9.3000	9.3000
5	0.64 ug/l	5	12.1000	12.1000
5	0.64 ug/l	6	10.6000	10.6000
5	0.64 ug/l	7	3.6000	3.6000
6	1.2 ug/l	1	6.2000	6.2000
6	1.2 ug/l	2	4.8000	4.8000
6	1.2 ug/l	3	0.0000	0.0000
6	1.2 ug/l	4	0.0000	0.0000
6	1.2 ug/l	5	7.0000	7.0000
6	1.2 ug/l	6	6.3000	6.3000
6	1.2 ug/l	7	0.5000	0.5000

Fonofos: Reproduction of Exposed Daphnia magna
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	7	11.843	11.843	12.011
2	Solvent Control	7	11.414	11.414	12.011
3	0.16 ug/l	7	12.086	12.086	12.011
4	0.31 ug/l	7	12.700	12.700	12.011
5	0.64 ug/l	7	9.043	9.043	9.043
6	1.2 ug/l	7	3.543	3.543	3.543

Fonofos: Reproduction of Exposed Daphnia magna
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	12.011				
Solvent Control	12.011	0.166		1.69	k= 1, v=36
0.16 ug/l	12.011	0.166		1.77	k= 2, v=36
0.31 ug/l	12.011	0.166		1.79	k= 3, v=36
0.64 ug/l	9.043	2.776	*	1.80	k= 4, v=36
1.2 ug/l	3.543	8.228	*	1.81	k= 5, v=36

s = 1.887
Note: df used for table values are approximate when v > 20.

Fonofos: Length of Exposed Daphnia magna
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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.211	7.986	12.606	7.986	2.211
OBSERVED	2	10	11	9	1

Calculated Chi-Square goodness of fit test statistic = 1.5247
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Fonofos: Length of Exposed Daphnia magna
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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 23.80
Closest, conservative, Table H statistic = 38.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 5
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 4.50
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

TITLE: Fonofos: Length of Exposed Daphnia magna
FILE: 42687301.len
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	4.4300	4.4300
1	Control	2	4.5700	4.5700
1	Control	3	4.6400	4.6400
1	Control	4	4.5000	4.5000
1	Control	5	4.6400	4.6400
1	Control	6	4.5700	4.5700
2	Solvent Control	1	4.5000	4.5000
2	Solvent Control	2	4.7100	4.7100
2	Solvent Control	3	4.5700	4.5700
2	Solvent Control	4	4.5000	4.5000
2	Solvent Control	5	4.5000	4.5000
2	Solvent Control	6	4.5700	4.5700
2	Solvent Control	7	4.5700	4.5700
3	0.16 ug/l	1	4.6400	4.6400
3	0.16 ug/l	2	4.7100	4.7100
3	0.16 ug/l	3	4.5700	4.5700
3	0.16 ug/l	4	4.7900	4.7900
3	0.16 ug/l	5	4.4300	4.4300
3	0.16 ug/l	6	4.6400	4.6400
3	0.16 ug/l	7	4.5700	4.5700
4	0.31 ug/l	1	4.5000	4.5000
4	0.31 ug/l	2	4.5000	4.5000
4	0.31 ug/l	3	4.5000	4.5000
4	0.31 ug/l	4	4.5700	4.5700
4	0.31 ug/l	5	4.6400	4.6400
4	0.31 ug/l	6	4.5700	4.5700
4	0.31 ug/l	7	4.6400	4.6400
5	0.64 ug/l	1	4.2100	4.2100
5	0.64 ug/l	2	4.5700	4.5700
5	0.64 ug/l	3	4.6400	4.6400
5	0.64 ug/l	4	4.0700	4.0700
6	1.2 ug/l	1	3.7100	3.7100
6	1.2 ug/l	2	3.7900	3.7900

Fonofos: Length of Exposed Daphnia magna
File: 42687301.len Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	4.558	4.558	4.581
2	Solvent Control	7	4.560	4.560	4.581
3	0.16 ug/l	7	4.621	4.621	4.581
4	0.31 ug/l	7	4.560	4.560	4.560
5	0.64 ug/l	4	4.372	4.372	4.372
6	1.2 ug/l	2	3.750	3.750	3.750

Fonofos: Length of Exposed Daphnia magna
File: 42687301.len Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	4.581				
Solvent Control	4.581	0.334		1.71	k= 1, v=27
0.16 ug/l	4.581	0.334		1.79	k= 2, v=27
0.31 ug/l	4.560	0.025		1.81	k= 3, v=27
0.64 ug/l	4.372	2.361	*	1.82	k= 4, v=27
1.2 ug/l	3.750	8.119	*	1.83	k= 5, v=27

s = 0.122
Note: df used for table values are approximate when v > 20.

Fonofos: Survival of Exposed Daphnia magna
File: 42687301.sur Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	3	16	5	0

Calculated Chi-Square goodness of fit test statistic = 9.7772
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Fonofos: Survival of Exposed Daphnia magna
File: 42687301.sur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: Fonofos: Survival of Exposed Daphnia magna
FILE: 42687301.sur
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.3453
1	Control	2	1.0000	1.3453
1	Control	3	1.0000	1.3453
2	Solvent Control	1	0.8000	1.1071
2	Solvent Control	2	1.0000	1.3453
2	Solvent Control	3	1.0000	1.3453
3	0.16 ug/l	1	1.0000	1.3453
3	0.16 ug/l	2	1.0000	1.3453
3	0.16 ug/l	3	1.0000	1.3453
4	0.31 ug/l	1	0.8000	1.1071
4	0.31 ug/l	2	1.0000	1.3453
4	0.31 ug/l	3	1.0000	1.3453
5	0.64 ug/l	1	1.0000	1.3453
5	0.64 ug/l	2	1.0000	1.3453
5	0.64 ug/l	3	1.0000	1.3453
6	1.2 ug/l	1	0.4000	0.6847
6	1.2 ug/l	2	0.2000	0.4636
6	1.2 ug/l	3	0.8000	1.1071
7	2.1 ug/l	1	0.0000	0.2255
7	2.1 ug/l	2	0.0000	0.2255
7	2.1 ug/l	3	0.0000	0.2255
8	4.9 ug/l	1	0.0000	0.2255
8	4.9 ug/l	2	0.0000	0.2255
8	4.9 ug/l	3	0.0000	0.2255

Fonofos: Survival of Exposed Daphnia magna
File: 42687301.sur Transform: ARC SINE(SQUARE ROOT(Y))

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	Control	1.345				
2	Solvent Control	1.266	9.00	None	3	
3	0.16 ug/l	1.345	10.50	None	3	
4	0.31 ug/l	1.266	9.00	None	3	
5	0.64 ug/l	1.345	10.50	None	3	
6	1.2 ug/l	0.752	6.00	None	3	
7	2.1 ug/l	0.226	6.00	None	3	
8	4.9 ug/l	0.226	6.00	None	3	

Critical values use k = 7, are 1 tailed, and alpha = 0.05

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	1	6	7
0.16	0	7	7
TOTAL	1	13	14

CRITICAL FISHERS VALUE (7,7,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	1	6	7
0.31	0	7	7
TOTAL	1	13	14

CRITICAL FISHERS VALUE (7,7,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	6	1	7
0.64	4	3	7

TOTAL	10	4	14

CRITICAL FISHERS VALUE (7,7,6) (p=0.05) IS 1. b VALUE IS 4.
Since b is greater than 1 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	6	1	7
1.2	2	5	7
TOTAL	8	6	14

CRITICAL FISHERS VALUE (7,7,6) (p=0.05) IS 1. b VALUE IS 2.
Since b is greater than 1 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	6	1	7
2.1	0	7	7
TOTAL	6	8	14

CRITICAL FISHERS VALUE (7,7,6) (p=0.05) IS 1. b VALUE IS 0.
Since b is less than or equal to 1 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

	NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	6	1	7
4.9	0	7	7
TOTAL	6	8	14

CRITICAL FISHERS VALUE (7,7,6) (p=0.05) IS 1. b VALUE IS 0.
Since b is less than or equal to 1 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	7	1	
1	0.16	7	0	
2	0.31	7	0	
3	0.64	7	3	
4	1.2	7	5	
5	2.1	7	7	*
6	4.9	7	7	*

Ecological Effects Branch One-Liner Data Entry Form

Chemical Fonofos Shaughnessy No. 041701 Pesticide Use Insecticide

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Daphnia magna*	94.6	0.45 µg/l	21	reprod. & length	1993/1993	426873-01 Core	ICI	RGM
2.								
3.								

COMMENTS: Results based on mean measured concentrations. ICI=ICI Agrochemicals,Jealotts Hill Research Station.