EEB files

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)

1. **CHEMICAL:** Phorate Shaughnessy Code: 057201

2. **TEST MATERIAL:** Phorate Purity: 99%

3. **CITATION**

Authors: Overman, M.A. and J.D. Wisk
Title: Chronic Toxicity of Phorate to the Mysid, Mysidopsis bahia Under Flow-through Conditions
Study Completion Date: May 18,1995
Laboratory: Environmental Science & Engineering, Inc., (ESC)
Sponsor: American Cyanamid Company (954-93-206)
Laboratory Report ID: 3933052-0100-3140
MRID No.: 43730501
DP Barcode: D218100

4. **REVIEWED BY:** Dennis J. McLane, Wildlife Biologist, EEB, EFED

Signature: Dennis J. McLane **Date:** 9-1-95

5. **APPROVED BY:** Les W. Touart, Head of Section 1, EEB, EFED

Signature: L W Touart **Date:** 9-8-95

6. **STUDY PARAMETERS**

Age of Test Organism: <24 hours
Definitive Test Duration: 28 days
Study Method: Flow-through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

This study is scientifically sound but does not fulfill guideline requirements. The mysids were not paired or the pairing data was not submitted. Because the protocol attached to the study and the study both fail to mention such an important step in the study EEB feels the mysids were not paired. The lack of pairing makes it impossible to show sexual differences or compare with other studies. The test values are extremely low. For example, the test water for these tests is only tested down to 10.6 ug/L or parts/billion for organophosphate pesticides (see attached list of entitled "Filter Seawater Characterization". The MATC for growth is 7.5 ng/L or parts/trillion (In parts/billion 7.5 ppt would be 0.0075 ppb), and the LC_{50} for 28 days is 20.9 ng/L. Therefore, the MATC and LC_{50}, as well as all the test levels, fall into Brooks (1973) toxicity rating for "very highly toxic" which is for chemicals with MATC less than 100

parts/billion.

Results Synopsis
NOEC: 5.8 ng ai/L LOEC: 9.8 ng ai/L MATC: 7.5 ng ai/L
(Both total length and dry weight)

NOEC's for specific effects
Young/Female/Repro. Day: 9.8 ng ai/L
Survival: 9.8 ng ai/L
Growth 1) length: 5.8 ng ai/L
2) weight: 5.8 ng ai/L
28 Day LC_{50} 20.9 ng/L

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental

B. Rationale: The study did not provide for pairing as outlined in the ASTM protocol for mysids.

C. Repairability: This is an integral part of the study and would require that the study be repeated.

8. **MAJOR GUIDELINE DEVIATIONS:**

1) The following items were not reported.
1.1 The median time of the 1st brood release in the control treatment.
1.2 The number of brood stocks used to start the test.
1.3 Number of randomly selected pairs/treatment.
1.4 Day of the test pairing occurred.
1.5 Number of test days over which pairing occurred.
1.6 Number of brine shrimp giving per day not drops.
1.7 Number of live adult mysids at pairing.
1.8 Number of live adult mysids after pairing.
1.9 Number live young removed daily.
1.91 Number missing, if any were missing.
1.92 Number surviving between pairing and test termination.
1.93 Sterilization of the test water
1.94 Evidence of complete mixing
1.95 Evidence of accurate flow splitting
1.96 Number of times meter systems were checked daily.
1.97 Dilution water aeration
1.98 Data endpoints by sex (survival, dry weight, total length)
2) The report indicated the statistical test used was the ANOVA. However, to determine which test level was significant, multiple comparison tests such as Dunnett's,

Bonferroni's T-Test, or Tukey Test are needed.
3) EEB's calculations show that the average concentrations were not the same as those reported. The only one which appeared to make a significant change was the lowest concentration average. EEB average was 5.8 ng/L, whereas, the ESE average was 5.3 ng/L. This changed the MATC from 7.2 to 7.5 ng/L.
4) Table 2 compares the coefficient of variation (CV) for four variables: Weight, Length, Survival, and Reproduction. The CV values for reproduction are very high (79% to 200%) and indicate the ability to detect statistical differences are not as efficient as the other variables (2% to 43%) are relative low.

10. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably Americamysis bahia.	Test species is Americamysis bahia.
Source (or supplier)	Aquatic Indicators, St. Augustine, FL
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) "Mysid brood cultures were monitored at Aquatic Indicators for >10 days prior to test start" 2) No signs of disease or stress 48 hours prior to testing.
Parental Acclimation Period At least 14 days	(see above)
Chamber Location: Treatments should be randomly assigned to test chamber locations.	"indiscriminate distribution of test organisms into each of 28 retention chambers"
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.	Not reported
Brood Stock: Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtain sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	1) Not reported 2) Brood stock was only maintained for 10 days and not 14. Also the 10 day period was not at ESE, Inc but Aquatic, Indicators.

Guideline Criteria	Reported Information
Distribution: **No. of mysids before pairing:** Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/level. **No. of mysids after pairing:** ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment to replace paired males).	10 mysid per compartment, 2 compartments/level, 40 mysids/level Not reported
Pairing: 1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation). 2) Should be paired on the same day	Not reported
Feeding: 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	1) 10 drops of brine shrimp concentrate twice a day 2) The number of shrimp per drop was not provided.
Counts: Live adult mysids should be counted 1) at initiation, 2) at pairing, 3) and daily after pairing. 4) Live young must be counted and removed daily. 5) Missing or impinged animals should be recorded.	1) 40 per level 2) Not reported 3) Not reported 4) Counted but not removal not reported 5) Yes, the number impinged per Rep A and B were reported. They were not reported on a per pair basis. The number missing may be there were none missing.

Guideline Criteria	Reported Information
Controls: Survival in any control chamber (between pairing and test termination) must not be less than 70%.	Pairing was not reported. Hence, this value was not reported.
Controls: Negative control and carrier control (when applicable) are required.	Both were provided.

Comments: None

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture; 2) During the test, difference between highest and lowest measured salinities must be less than 10 g/kg. Should be measured daily. 3) Salinity should be between 15 and 30 g/kg. 4) Measured pH should be between 7.6 and 8.2. Must not deviate by more than one unit for more than 48 hours. Should be measured at the beginning, end of test and weekly. 5) Water must be free of pollutants. 6) DO must be measured @ each conc. @ least once a wk. (see details in ASTM)	1) Sand-filtered Atlantic Ocean water from Whitney Laboratory, Marineland,FL and diluted to 20% salinity with well water(no mention of sterilization or if Marineland water is treated) 2) 20 ‰ for all weekly measurements 3) (see 2 above) 4) pH measured on day 0, 7, 14, 21, 28 Total range 7.9 - 8.4; Dose level median range 8.3-8.15 5) See attached analysis 6) DO measured on day 0, 7, 14, 21, and 28.

Guideline Criteria	Reported Information
Test Temperature: 1) Mean measured temperature for each chamber at test termination should be within 1°C of selected test temperature. 2) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages. 3) For mysid shrimp, 27°C is recommended. 4) Whenever temp. is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2°C.	Temperature reported for both A and B chambers for day 0, 2, 4,7,9,11,14,16,18,21,23,25, and 28. the solvent control had the widest range 25.0-28.5°C. All other replicates ranged from 25.2-27.1°C
Photoperiod: Recommend 16L/8D.	16L/8D
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) with a dilution factor not greater than 0.5 and controls should be used.	1) Mount & Brungs proportional diluter 2) 5 Toxicant levels, Control, Solvent control 3) Dilution factor of 0.5
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 chambers was used. 2) Aeration was not used in mixing. 3) Not reported 4) Not reported

Guideline Criteria	Reported Information
Test Vessels: 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic 2) Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume acceptable 3) 90 or 140 mm inside dia. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.	1) Glass, 2) filled to 5 L. 3) 9-cm petri dishes with 14 cm high cylindrical collar of Nytex screening. Mesh size not reported
Covers 1) Flow-through: Openings in the test compartments should be covered with nylon mesh or stainless steel screen.	1) Yes
Flow Rate: 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period	1) 8.2 volume additions every 24 hours 2) At day 28 38.7 ng/L replicate A fell to 60% DO.All others were higher. 3) Calibrated but not checked twice daily during the test period.
Aeration: 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1) not reported 2)"aeration of the test chambers"

Comments: LSC method indicates Control contained 0.199 µg/L, the lowest test level was 5.285 ng/L. This is 4% of the lowest level tested.

C. **Chemical System:**

Guideline Criteria	Reported Information

Concentrations: 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. 2) Toxicant conc. must be measured in one tank at each toxicant level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage. 4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test. 5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.	1) 5 Toxicant levels, Control, Solvent control 2) Measured conc. once a week 3) Yes 4 and 5) Yes, the largest difference is 35% and this is only for one measurement (see attached table 1)
Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	1) 0.096 ml/L nominal of acetone 2) Acetone

Comments:

11. REPORTED RESULTS:

Guideline Criteria	Reported Information

Quality assurance and GLP compliance statements were included in the report?	Yes
1) At least 75% of the paired 1st generation females in the control produced young or 2) the average number of young produced by the 1st generation females in the control(s) was more than 3.	1) Pairing data was not submitted. 2) Yes (15.5 - 24.5)
Data Endpoints must include: 1) Survival of first-generation mysids Female Male 2) Number of live young produced per female 3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each 1st generation mysid alive at the end of the study Female Male 5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs.	 1) total not by sex 2) yes 3) total not by sex 4) total not by sex 5) not reported 6) not reported
Raw data included? (Y/N)	N

Effects Data:

Toxicant Conc. (µg/L)		Mean # Young/fem. /repro. day	Survival (28 days)			Mean Total Length (mm)			Mean Dry weight (mg)		
Nom.	Meas.		♂	♀	♂ & ♀	♂	♀	♂ & ♀	♂	♀	♂ & ♀
Ctrl	---	0.74	--	--	86	--	--	6.8	-	-	0.951
Sol	---	0.60	--	--	79	--	--	6.2	-	-	0.691
6.25	5.3	0.94	--	--	82	--	--	6.3	-	-	0.851
12.5	9.8	0.59	--	--	62	--	--	5.8	-	-	0.550
25.0	20.2	0.01	--	--	42	--	--	6.0	-	-	0.653
50.0	38.7	0.0	--	--	18	--	--	5.3	-	-	0.422
100	72.2	0.09	--	--	36	--	--	5.6	-	-	0.653

Toxicity Observations:

Statistical Results:

Endpoint	Method	NOEC	LOEC	MATC
Survival	ANOVA	9.8	20.2	14
Reproduction	ANOVA	9.8	20.2	14
Weight	ANOVA	20.2	38.7	27.9
Length	ANOVA	5.3	9.8	7.2
Time to Brood	ANOVA	9.8	20.2	14

Comments: The report did not indicate the statistical test used to determine which test level was significant. The ANOVA will not identify which level is significant just that there is a significant difference.

In addition to the NOEC and LOEC LC50s were determined for days 7, 14, 21, and 28. (see following list)

1. 7 day >72.2 ng/L
2. 14 day >72.2 ng/L
3. 21 day >72.2 ng/L
4. 28 day 20.9 (14.2-30.8)ng/L

12. Reviewer's Statistical Results:

Most sensitive endpoint:

Endpoint	Control	Method[1]	NOEC	LOEC
Survival	Composite	Bon[2] Tukey[3]	9.8	20.2
	Solvent	Bon Tukey	9.8	20.2
	Control	Bon Tukey	9.8	20.2
Repro-duction	Composite	--	--	>72.2
	Solvent	--	--	>72.2
	Control	--	--	>72.2
Weight	Composite	Bon Tukey	5.3	9.8
	Solvent	Bon	20.2	38.7
	Control	Bon Tukey	5.3	9.8
Length	Composite	Bon Tukey	5.3	9.8
	Solvent	Bon	20.2	38.7
	Control	Bon	5.3	9.8

[1] See attached printouts from TOXSTAT program. Notice that there are analysis using statistical approaches not reported here. These methods are appropriate for this data.
[2] Bon and Tukey are abbreviations for Bonferoni T-Test and Tukey's method of multiple comparison, respectively.

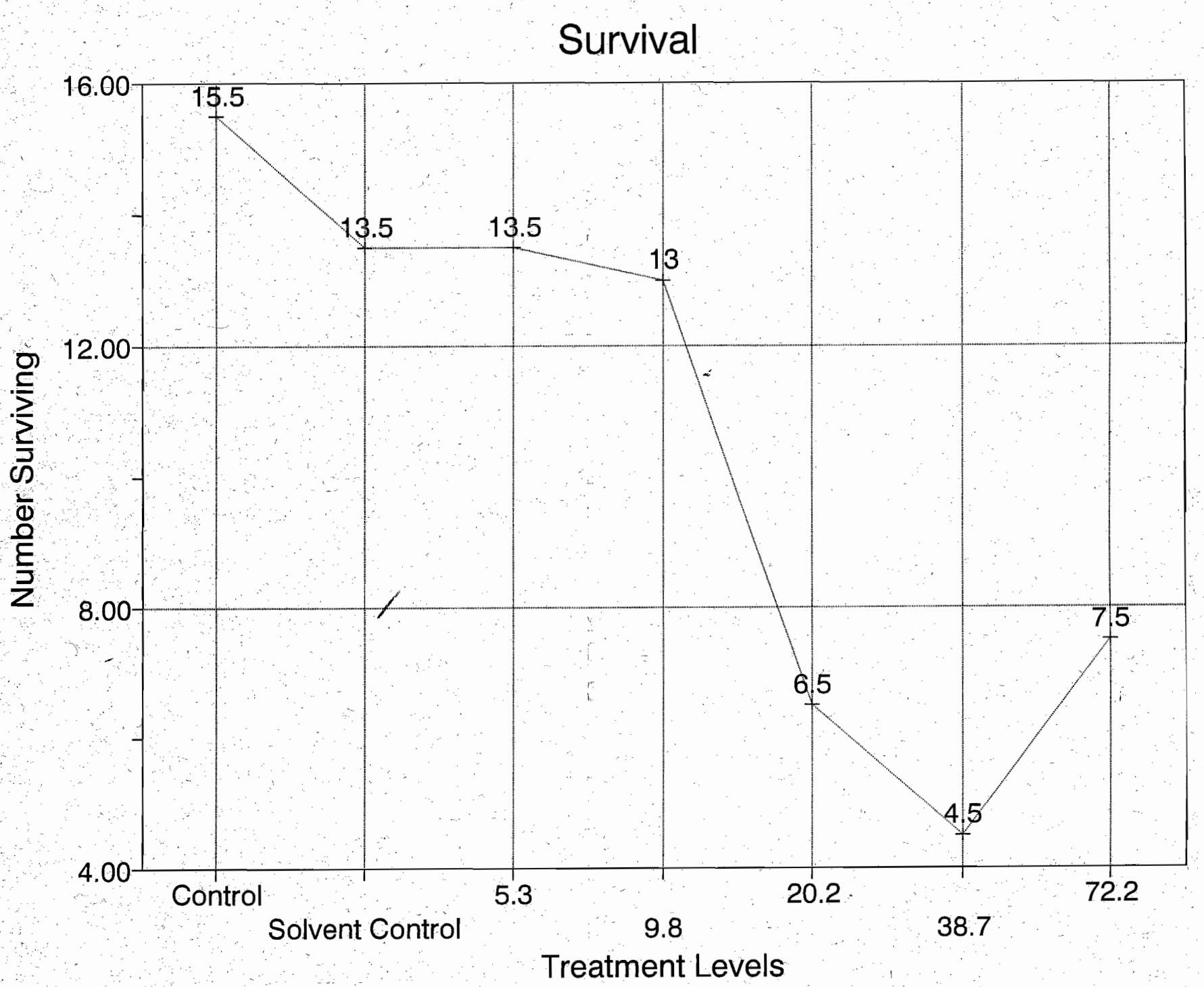
Comments: Growth (length and width) are the most sensitive parameters.

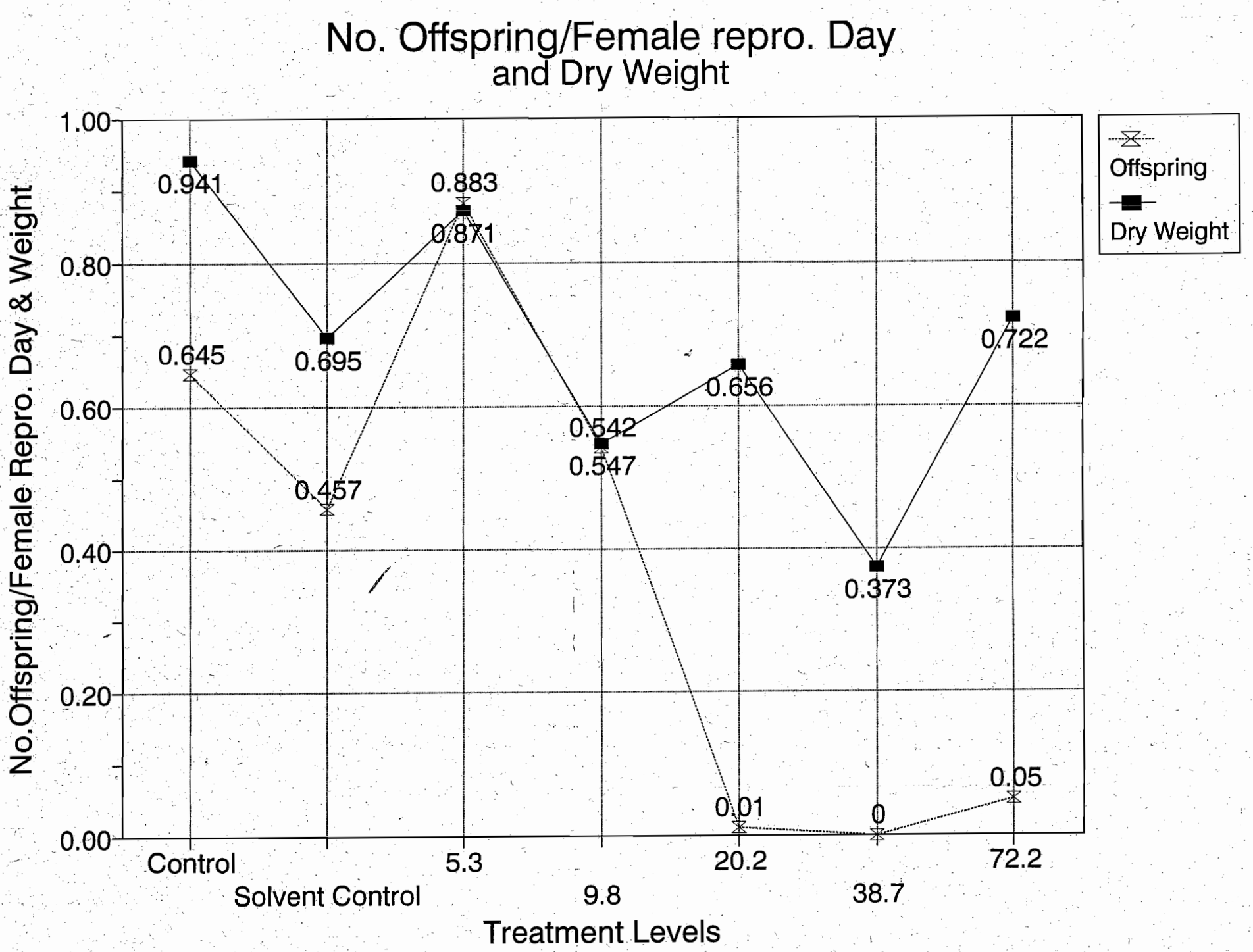
The results are very erratic. The attached graphs (Total length, Survival, and No. Offspring/Female Reproductive Day) show that all but survival the solvent control was performed worst than the 5.3 level. Also the highest performed better than the 9.8, 20.2, and 38.7 ng/L levels. In addition the means EEB derived are slightly different than those reported in the study (see the following table).

Toxicant Conc. (µg/L)[1]		Mean Young/fem./ repro. day		Survival (28 days)		Mean Total Length (mm)		Mean Dry weight (mg)	
Nom.	Meas.	EEB	ESE	EEB	ESE	EEB	ESE	EEB	ESE
trl	---	0.645	0.74	91	86	6.725	6.8	0.941	0.951
ol	---	0.457	0.60	81.8	79	6.25	6.2	0.695	0.691
.25	5.3	0.883	0.94	84.4	82	6.375	6.3	0.871	0.851
2.5	9.8	0.542	0.59	70.3	62	5.825	5.8	0.547	0.550
5.0	20.2	0.01	0.01	41.9	42	6.0	6.0	0.656	0.653
0.0	38.7	0	0.0	25	18	5.1	5.3	0.373	0.422
00	72.2	0.05	0.09	38.9	36	5.675	5.6	0.722	0.653

Notice that several of the reported means are different from those determined EEB's Toxstat program.

In addition to the MATC ESE provided a 28 day LC_{50}. EEB verify the $_{50}$ calculations used Toxanal. Toxanal indicates that the probit method did not t. The binomial test and moving average angle method provided the following timates, respectively, 19.6 and 23.2 ng/L. Based on the ESE value of 20.9 /L is acceptable.


Survival
16.00
12.00
8.00
4.00
Number Surviving
15.5
13.5
13.5
13
6.5
4.5
7.5
Control
Solvent Control
5.3
9.8
20.2
38.7
72.2
Treatment Levels


No. Offspring/Female repro. Day
and Dry Weight
No.Offspring/Female Repro. Day & Weight
1.00
0.80
0.60
0.40
0.20
0.00
0.941
0.695
0.883
0.871
0.542
0.547
0.656
0.373
0.722
0.645
0.457
0.01
0
0.05
Control
Solvent Control
5.3
9.8
20.2
38.7
72.2
Treatment Levels
Offspring
Dry Weight

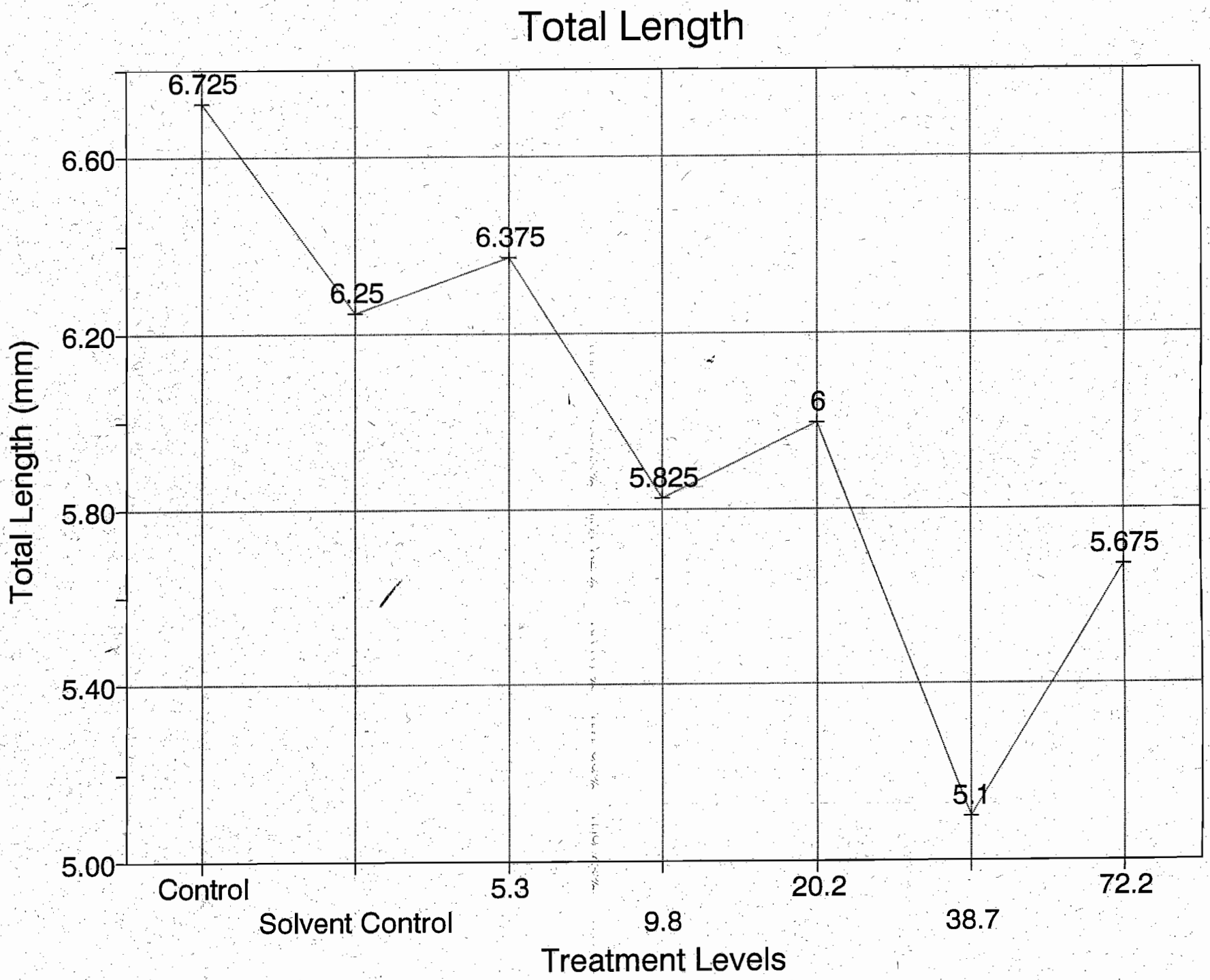

Total Length
6.725
6.25
6.375
5.825
6
5.1
5.675
6.60
6.20
5.80
5.40
5.00
Total Length (mm)
Control
Solvent Control
5.3
9.8
20.2
38.7
72.2
Treatment Levels

Table 1

comparison of the individual measurement to the mean

Conc.	0	7	14	21	28	ESE avg	EEB avg
6.25	4.83	5.187	5.181	6.073	7.856	5.285	5.8254
12.5	8.13	9.564	9.709	9.333	12.066	9.796	9.7604
25	17.73	19.056	21.079	19.421	23.534	20.164	20.164
50	33.69	36.483	39.727	38.737	44.957	38.646	38.7188
100	72.03	73.475	62.489	73.588	79.172	72.151	72.1508
		with ESE Average					
6.25	91.39%	98.15%	98.03%	114.91%	148.65%		
12.5	82.99%	97.63%	99.11%	95.27%	123.17%		
25	87.93%	94.51%	104.54%	96.32%	116.71%		
50	87.18%	94.40%	102.80%	100.24%	116.33%		
100	99.83%	101.84%	86.61%	101.99%	109.73%		
		with EEB average					
6.25	82.91%	89.04%	88.94%	104.25%	134.86%		
12.5	83.30%	97.99%	99.47%	95.62%	123.62%		
25	87.93%	94.51%	104.54%	96.32%	116.71%		
50	87.01%	94.23%	102.60%	100.05%	116.11%		
100	99.83%	101.84%	86.61%	101.99%	109.73%		

Table 2
Comparison of CV for Mysid Phorate Stud

	Weight Mean	SD	CV
Control	0.941	0.147	15.62%
Solvent Control	0.695	0.124	17.84%
5.8	0.871	0.211	24.23%
9.8	0.547	0.074	13.53%
38.7	0.656	0.033	5.03%
20.2	0.373	0.159	42.63%
72.2	0.722	0.15	20.78%
	Survival		
	Mean	**SD**	**CV**
Control	15.5	0.707	4.56%
Solvent Control	13.5	0.707	5.24%
5.8	13.5	0.707	5.24%
9.8	13	2.828	21.75%
20.2	6.5	0.707	10.88%
38.7	4.5	0.707	15.71%
72.2	7.5	0.707	
	Offspring		
	Mean	**SD**	**CV**
Control	0.645	0.508	78.76%
Solvent Control	0.457	0.411	89.93%
5.8	0.883	0.757	85.73%
9.8	0.542	0.444	81.92%
20.2	0.01	0.02	200.00%
38.7	0	0	
72.2	0.05	0.1	200.00%
	Length		
	Mean	**SD**	**CV**
Control	6.725	0.206	3.06%
Solvent Control	6.25	0.289	4.62%
5.8	6.375	0.32	5.02%
9.8	5.825	0.171	2.94%
20.2	6	0.141	2.35%
38.7	5.1	0.557	10.92%
72.2	5.675	0.222	3.91%