DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.

2. **TEST MATERIAL:** T-117-12 (chlorothalonil technical); 100% active ingredient; a light tan powder.

3. **STUDY TYPE:** 72-4. Saltwater Mysid Life-Cycle Toxicity Test. Species Tested: *Mysidopsis bahia*.

4. **CITATION:** Hoberg, J.R. 1991. (T-117-12) - Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*). SLI Report No. 90-05-3330. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-07.

5. **REVIEWED BY:**

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Date: 10/5/92

6. **APPROVED BY:**

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Date: 10/5/92

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Date: Tracy L. Perry 11/16/92

7. **CONCLUSIONS:** This study is not scientifically sound. Survival in the solvent control was 62% which is considered unacceptable control survival by ASTM. The concentrations of several replicates were highly variable during the test. Based on reproductive data, mysids at all chlorothalonil concentrations tested were significantly affected. The NOEC and MATC could not be determined.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: Mysids (*Mysidopsis bahia*; ≤24 hours old) were obtained from in-house cultures maintained on a 16-hour light (30-100 ft-candles) photoperiod. The culture water was from the same source as the water used in the test. The temperature during culture was 25°C and the salinity of the culture water was approximately 32 parts per thousand (ppt). The mysids were fed brine shrimp nauplii.

B. Test System: An intermittent-flow proportional diluter delivered test solution or control water to individual glass aquaria (39 x 20 x 25 cm). The aquaria were fitted with self starting siphons and the solution volume fluctuated between 4 and 7 l to ensure solution exchange. The volume of each aquarium was replaced an average of 13 times every 24 hours. The diluter was operated for approximately 30 days prior to test initiation.

The test aquaria were impartially positioned in a temperature-controlled water bath maintained at 25 ±2°C. Light was provided on a 16-hour light/8-hour dark photoperiod using fluorescent tubes with an intensity of 30-100 ft-candles.

Unpaired mysids were held in retention chambers constructed of glass petri dishes (10-cm in diameter) with 15-cm high nylon screen (363-µm mesh) collars. Pairing chambers held sexually mature male and female pairs and were constructed of cylindrical glass jars (5.1 cm diameter, 10 cm high) containing two 1.9-cm holes covered with nylon screen.

A 0.44 mg a.i./ml stock solution was prepared by dissolving 0.1108 g of test material in acetone to volume in a 250-ml volumetric flask. An appropriate volume of the stock (43.5 µl) was delivered to the diluter mixing chamber resulting in a high nominal exposure of 10 µg/l which was diluted (50%) to provide the lower nominal concentrations.

The test dilution water was filtered (20 and 5 µm) natural seawater collected from the Cape Cod Canal, Bourne, MA.

C. Dosage: Twenty-eight-day life-cycle toxicity test. Based on a preliminary testing, five nominal concentrations (0.63, 1.3, 2.5, 5.0, and 10 µg a.i./l),

a dilution water control, and a solvent control (23 µl acetone/l) were used.

D. **Design:** Mysids were impartially selected and distributed to 28 retention chambers until each contained 15 mysids. Two retention chambers were placed in each aquarium, yielding 30 mysids per replicate aquarium and 60 organisms per test level.

The mysids were fed 24 hour old brine shrimp nauplii twice daily.

To facilitate counting, the retention chambers were removed from the aquaria and placed on a black background. The number of live and dead mysids was determined daily and the chambers were gently brushed and siphoned to remove detritus. Any abnormal appearance or behavior was noted.

When the mysids reached sexual maturity (day 17), they were paired and transferred to isolation jars (10 per replicate). Mysids not used for reproduction were housed in a single retention chamber per replicate. Any paired males that died during the reproduction portion of the study were replaced. Dead females were not replaced. Reproductive output (number of offspring per female per reproductive day) was determined daily. "If the development of brood pouches used in distinguishing female organisms from males; was delayed due to toxicant exposure, those organisms were maintained in clean retention chambers until maturity was observed or until test termination."

At termination, the F_0 mysids (males and females were recorded separately) were blotted dry, dried at 60°C for 24 hours, cooled in a desiccator, and weighed to the nearest 0.01 mg. Before drying, brine shrimp nauplii were removed from the female brood sacs when observed, but eggs and juveniles were not removed.

The dissolved oxygen concentration (DO) and pH were measured daily in each aquarium. The temperature and salinity in both replicates of the dilution water control were measured daily. Temperature of a solvent control chamber was continuously monitored using a minimum/maximum thermometer.

Water samples were collected from each replicate aquarium on days 0, 7, 14, 23, and 28 for chemical analysis. The highest test concentration was also

sampled on day 3 (but the results were not reported). The concentration of T-117-12 was determined using gas chromatography.

E. **Statistics:** The endpoints analyzed were survival, dry body weight by sex, and reproduction. The responses of the dilution water control and solvent control mysids were compared using t-tests. The survival, reproduction, and growth of the solvent and dilution water controls were not significantly different. All statistical comparisons of treatment response were made to the pooled control data. The survival data were arcsine square root transformed prior to analysis. Homogeneity of variance and normality for each data set were checked using Bartlett's test and the chi-square test, respectively. All data sets were analyzed using William's test and a 95% level of certainty.

12. **REPORTED RESULTS:** No undissolved test material was observed in the exposure solutions. The mean measured concentrations were 0.65, 0.83, 1.2, 3.0, and 5.7 µg a.i./l (Table 2, attached).

The survival of adult mysids was reported in Table 3 (attached). After 28 days, there was no significant difference between pooled control and exposed mysid survival.

The number of offspring/female/reproductive day at concentrations ≥1.2 µg a.i./l was significantly reduced when compared to the pooled control (Table 3, attached).

Mean body weight at test termination (day 28) was not significantly affected by exposure to T-117-12 at the concentrations tested (Table 4, attached).

During the test, the DO was maintained between 79 and 117% of saturation. The pH was 7.7-8.0 and the temperature was 23-26°C. The salinity ranged from 31 to 33 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The maximum acceptable toxicant concentration (MATC) was <1.2 µg a.i./l and >0.83 µg a.i./l (geometric mean MATC = 1.0 µg a.i./l), based on the most sensitive parameter, mysid reproduction.

Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The stability, characterization,

and verification of the test substance identity was the responsibility of the test sponsor. The dates of quality assurance inspections were included in the report.

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

A. Test Procedure: ASTM guidelines (1990) were used to evaluate this study. The test was not scientifically sound. Deviations from the ASTM were the following:

On test days 0, 7, and 23, several replicates had measured concentrations which were more than 30% higher than the time-weighted average concentrations (TWAC) for those replicates (Table 2, attached). Replicate A of the 2.5 µg/l level (1.2 µg/l mean measured concentration) was more than 30% higher than the TWAC on days 0 and 7 and less than 50% of the TWAC on day 23.

Survival in the solvent control replicate A was 47% (Table 3, attached). Survival in replicate B was 77% giving a combined survival for the solvent control of 62%. Control survival of at least 70% is required.

The test material was not identified by a batch or lot number.

Mysids were dried for only 24 hours; 72-96 hours or to a constant weight is recommended. In addition, the mysids were weighed to the nearest 0.01 mg; 0.001 mg is recommended.

The method used for transferring mysids to the test vessels was not described in the report or the study protocol. Mysids must be handled gently using nylon screen or wide-bore glass pipettes.

The temperature during the test (23-26°C) was lower than recommended (27°C).

No raw water quality values and survival, reproduction, or individual weight measurements were presented in the report.

B. Statistical Analysis: Survival data did not meet the assumption of homogeneity of variances due to zero variance in the dilution water control data. The data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's and Kruskal-Wallis tests (Toxstat Version 3.3). Survival of the mysids was not

significantly affected by exposure to the test material (see attached printout 1-3).

The reproduction data (except for the highest concentration where there was no reproduction) were analyzed using one-way ANOVA and various parametric multiple comparisons. Compared to the solvent control, there was no effect on reproduction (see attached printout 4). However, compared to the dilution water control, all exposed mysids had significantly reduced reproductive output. In this test, the solvent appears to adversely affect the mysids. Since the solvent concentration was not the same in all test concentrations (and the solvent control contained the highest solvent concentration used in the test), it would be best to compare the treatments to the dilution water control data.

Growth data were not analyzed since only the average growth by replicate data were included in the report.

C. **Discussion/Results:** This study is not scientifically sound. Survival in the solvent control was 62% which is considered unacceptable control survival by ASTM. The concentrations of several replicates were highly variable during the test. The NOEC and MATC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The test concentrations were variable and did not meet ASTM requirements. In addition, the average solvent control survival was only 62%.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 09-30-92.

REFERENCES:

ASTM. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. E1191 - 90.

Table 2. **Measured concentrations of T-117-12 in the exposure solutions during the 28 day life cycle test with mysid shrimp (*Mysidopsis bahia*).**

Nominal Concentration (µg/L)	Measured Concentrations (µg/L)											
	Day 0		Day 7		Day 14		Day 23		Day 28		Mean[a] (SD)[b]	% of nominal
	A	B	A	B	A	B	A	B	A	B		
10	5.4	5.8	5.5	5.6	5.5	3.1	6.2	7.1*	6.4	6.1	5.7 (1.02)	57
5.0	3.5	3.6	3.0	3.1	2.0	2.2	3.0	3.5	2.9	3.2	3.0 (0.53)	60
2.5	1.7*	1.1	1.5*	1.4	1.2	0.86	0.27•	1.7*	1.4	1.4	1.2 (0.43)	48
1.3	1.0*	1.0	0.78	0.88	0.70	0.82	0.74	0.78	0.67	0.88	0.83 (0.12)	64
0.63	0.83	0.75	0.67	0.60	0.64	0.62	0.70	0.66	0.55	0.52	0.65 (0.093)	103
Solvent Control	<0.061	<0.061	<0.58	<0.58	<0.071	<0.071	<0.067	<0.067	<0.073	<0.073		
Control	<0.061	<0.061	<0.58	<0.58	<0.071	<0.071	<0.067	<0.067	<0.073	<0.073		
QC #1[c]	0.446 (0.64)[d]		0.727 (0.64)		0.662 (0.61)		0.715 (0.61)		0.587 (0.61)			
QC #2	3.75 (4.9)		4.85 (4.9)		5.06 (5.1)		4.91 (5.1)		4.61 (5.1)			
QC #3	7.58 (10.2)		10.2 (10.2)		8.60 (10.2)		10.3 (10.2)		9.32 (10.2)			

TWA conc Rep A / Rep B

5.74 / 5.33
2.77 / 3.01
1.12 / 1.29
0.76 / 0.85
0.67 / 0.64

* 30% greater than TWA conc.
• 50% lower than TWA conc.

[a] Values presented are based on the analytical (unrounded) results rather than the rounded values (2 significant figures) presented in this table.
[b] Standard deviation, N=10.
[c] QC = Quality Control Sample
[d] Value in parentheses represents the nominal fortified concentration.

average % of nominal 66.4%

Table 3. Summary of survival and reproductive success (offspring/female/reproductive day) for the 28-day life cycle test exposing mysid shrimp (*Mysidopsis bahia*) to T-117-12.

Mean Measured Concentration (µg/L)		First Generation (F^0) Survival (%) Day 28	Reproductive Success	(N)[a]
5.7	A	80	0.00	8
	B	73	0.00	9
	Mean	77	0.00[c]	
3.0	A	77	0.06	9
	B	53	0.09	6
	Mean	65	0.075[c]	
1.2	A	80	0.17	8
	B	90	0.05	14
	Mean	85	0.11[c]	
0.83	A	80	0.38	13
	B	73	0.24	11
	Mean	77	0.31	
0.65	A	70	0.31	11
	B	87	0.23	11
	Mean	79	0.27	
Solvent Control	A	47	0.30	4
	B	77	0.31	9
	Mean	62	0.31	
Control	A	77	0.96	11
	B	77	0.51	9
	Mean	77	0.74	
Pooled Control[b]	Mean	70	0.52	33

[a] N = denotes the number of female organisms surviving at test termination.
[b] Control and solvent data were not significantly (P = 0.05) different from one another; therefore, data from each control group were pooled.
[c] Indicates a significant difference (P = 0.05) from the pooled control data (based on Williams' Test).

Table 4. **Summary of growth (total dry body weight) measurements of first generation male and female mysid shrimp (*Mysidopsis bahia*) recorded at termination of the life cycle test with T-117-12.**

Mean Measured Concentration (µg/L)		Total Dry Body Weight (mg) Males Mean[a]	SD	(N)	Females Mean[a]	SD	(N)
5.7	A	0.74 ±	0.12	16	0.85 ±	0.16	8
	B	0.68 ±	0.13	13	0.83 ±	0.23	9
	Mean	0.71 ±	0.13		0.84 ±	0.19	
3.0	A	0.74 ±	0.077	14	0.90 ±	0.16	9
	B	0.74 ±	0.25	10	0.96 ±	0.14	6
	Mean	0.74 ±	0.16		0.93 ±	0.15	
1.2	A	0.65 ±	0.11	16	0.77 ±	0.11	8
	B	0.58 ±	0.093	13	0.74 ±	0.18	14
	Mean	0.62 ±	0.11		0.75 ±	0.15	
0.83	A	0.79 ±	0.11	11	1.1 ±	0.18	13
	B	0.60 ±	0.081	11	0.90 ±	0.27	11
	Mean	0.69 ±	0.14		1.0 ±	0.24	
0.65	A	0.73 ±	0.15	10	0.98 ±	0.31	11
	B	0.67 ±	0.10	15	0.85 ±	0.19	11
	Mean	0.69 ±	0.12		0.92 ±	0.26	
Solvent Control	A	0.70 ±	0.26	10	1.1 ±	0.23	4
	B	0.60 ±	0.21	14	0.75 ±	0.13	9
	Mean	0.64 ±	0.23		0.87 ±	0.25	
Control	A	0.69 ±	0.95	12	0.76 ±	0.11	11
	B	0.95 ±	0.21	14	1.00 ±	0.19	9
	Mean	0.83 ±	0.23		0.88 ±	0.20	
Pooled Control	Mean[b]	0.74 ±	0.25		0.88 ±	0.21	

[a] Each mean value was calculated from the original raw data and not the values presented in this table.
[b] Control and solvent control data were not significantly ($P \leq 0.05$) different from one another; therefore the treatment data were compared to the pooled control data.

Shaughnessey # 081901 Chemical Name chlorothalonil Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish Species: Lab: MRID #	_____	Concentrations Tested (pp___) - _____ MATC - >_____<_____ pp___. Effected Parameters - _____ Control Mortality (%) - Solvent Control Mortality (%) - Comments:	_____	_____
Chronic Invertebrate Species: Mysidopsis bahia Lab: Springborn Labs. Inc. MRID # 424338-07	100	Concentrations Tested (pp b*) - 0.65, 0.83, 1.2, 3.0, 5.7 MATC - > 0.83 < 1.2 pp b*. Effected Parameters - Reproductive output Control Mortality (%) - 23 Solvent Control Mortality (%) - 38 Comments: * mean measured concentrations	LAR 9/30/92	Invalid

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt1 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

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.

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

GRP1 (SOLVENT CRTL) MEAN =	0.9130	CALCULATED t VALUE =	-1.0000
GRP2 (BLANK CRTL) MEAN =	1.0706	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-0.1576		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.099	0.017	0.955
Within (Error)	7	0.121	0.017	
Total	13	0.221		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	0.913	0.620		
2	water control	1.071	0.770	-1.197	
3	0.65	1.097	0.785	-1.394	
4	0.83	1.066	0.765	-1.160	
5	1.2	1.178	0.850	-2.013	
6	3.0	0.943	0.650	-0.228	
7	5.7	1.066	0.765	-1.160	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	water control	2	0.360	58.1	-0.150
3	0.65	2	0.360	58.1	-0.165
4	0.83	2	0.360	58.1	-0.145
5	1.2	2	0.360	58.1	-0.230
6	3.0	2	0.360	58.1	-0.030
7	5.7	2	0.360	58.1	-0.145

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent control	0.913	0.620	8.500
2	water control	1.071	0.770	15.000
3	0.65	1.097	0.785	16.000
4	0.83	1.066	0.765	15.500
5	1.2	1.178	0.850	25.000
6	3.0	0.943	0.650	9.500
7	5.7	1.066	0.765	15.500

Calculated H Value = 5.141 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 1	0 6	0 4	0 7	0 2	0 3	0 5
1	solvent control	0.913	0.620	\						
6	3.0	0.943	0.650	.	\					
4	0.83	1.066	0.765	.	.	\				
7	5.7	1.066	0.765	.	.	.	\			
2	water control	1.071	0.770	.	.	.	.	\		
3	0.65	1.097	0.785	.	.	.	.	.	\	
5	1.2	1.178	0.850	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.114

data compared to dilution water control data only

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.057	0.011	0.956
Within (Error)	6	0.072	0.012	
Total	11	0.129		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	water control	1.071	0.770		
2	5.7	1.066	0.765	0.044	
3	0.65	1.097	0.785	-0.237	
4	0.83	1.066	0.765	0.044	
5	1.2	1.178	0.850	-0.983	
6	3.0	0.943	0.650	1.167	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	water control	2			
2	5.7	2	0.294	38.2	0.005
3	0.65	2	0.294	38.2	-0.015
4	0.83	2	0.294	38.2	0.005
5	1.2	2	0.294	38.2	-0.080
6	3.0	2	0.294	38.2	0.120

424338-07, chlorothalonil, young/reproductive day
File: a:42433807.dt3 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts 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

GRP1 (SOLVENT CRTL) MEAN =	0.3050	CALCULATED t VALUE =	-1.9196
GRP2 (BLANK CRTL) MEAN =	0.7350	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-0.4300		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.554	0.111	5.451
Within (Error)	6	0.122	0.020	
Total	11	0.676		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 3 1 4 2
6	3.0	0.075	0.075	\
5	1.2	0.110	0.110	. \
3	0.65	0.270	0.270	. . \
1	solvent control	0.305	0.305	. . . \
4	0.83	0.310	0.310	. . . . \
2	water control	0.735	0.735	* * . . . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (6,6) = 5.63 s = 0.020

data compared to dilution water control only
WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	water control	2	0.735	0.735	0.735
2	0.65	2	0.270	0.270	0.290
3	0.83	2	0.310	0.310	0.290
4	1.2	2	0.110	0.110	0.110
5	3.0	2	0.075	0.075	0.075

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
water control	0.735				
0.65	0.290	2.850	*	2.02	k= 1, v= 5
0.83	0.290	2.850	*	2.14	k= 2, v= 5
1.2	0.110	4.003	*	2.19	k= 3, v= 5
3.0	0.075	4.227	*	2.21	k= 4, v= 5

s = 0.156

TITLE: 424338-07, chlorothalonil, 28-day survival
FILE: a:42433807.dt1
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.4700	0.7554
1	solvent control	2	0.7700	1.0706
2	water control	1	0.7700	1.0706
2	water control	2	0.7700	1.0706
3	0.65	1	0.7000	0.9912
3	0.65	2	0.8700	1.2019
4	0.83	1	0.8000	1.1071
4	0.83	2	0.7300	1.0244
5	1.2	1	0.8000	1.1071
5	1.2	2	0.9000	1.2490
6	3.0	1	0.7700	1.0706
6	3.0	2	0.5300	0.8154
7	5.7	1	0.8000	1.1071
7	5.7	2	0.7300	1.0244

TITLE: 424338-07, chlorothalonil, young/reproductive day
FILE: a:42433807.dt3
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.3000	0.3000
1	solvent control	2	0.3100	0.3100
2	water control	1	0.9600	0.9600
2	water control	2	0.5100	0.5100
3	0.65	1	0.3100	0.3100
3	0.65	2	0.2300	0.2300
4	0.83	1	0.3800	0.3800
4	0.83	2	0.2400	0.2400
5	1.2	1	0.1700	0.1700
5	1.2	2	0.0500	0.0500
6	3.0	1	0.0600	0.0600
6	3.0	2	0.0900	0.0900

concentration data

ROW	day0	day7	day14	day23	day28	min	twa	max
1	5.40	5.50	5.50	6.20	6.40	2.87143	5.74286	7.46571
2	5.80	5.60	3.10	7.10*	6.10	2.66518	5.33036	6.92946
3	3.50	3.00	2.00	3.00	2.90	1.38393	2.76786	3.59821
4	3.60	3.10	2.20	3.50	3.20	1.50714	3.01429	3.91857
5	1.70*	1.50*	1.20	0.27°	1.40	0.56143	1.12286	1.45971
6	1.10	1.40	0.86	1.70*	1.40	0.64161	1.28321	1.66818
7	1.00*	0.78	0.70	0.74	0.67	0.38241	0.76482	0.99427
8	1.00	0.88	0.82	0.78	0.88	0.42643	0.85286	1.10871
9	0.83	0.67	0.60	0.70	0.55	0.33339	0.66679	0.86682
10	0.75	0.60	0.64	0.66	0.52	0.31902	0.63804	0.82945

* higher

° Lower

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 8 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil: submission of individual growth data for mysid shrimp life-cycle study (MRID No. 424338-07).

FROM: Anthony Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Walter Waldrop, PM 71
Reregistration Branch
Special Review and Reregistration Division (7508W)

In EEB's review of July 22, 1993, the mysid shrimp life-cycle study (MRID No. 424338-07) conducted with chlorothalonil was upgraded from invalid to supplemental. The study had the potential to be upgraded to core upon submission of individual growth data for both male and female mysid shrimp. ISK Biotech Corporation has provided these data with the current submission.

Individual growth data were analyzed using one-way analysis of variance (ANOVA) and William's test (Toxstat Version 3.3). Results show that female body weight at test termination was not significantly affected by exposure to chlorothalonil technical at the concentrations tested. Male body weight, however, was significantly increased at the top two test concentrations (see attached). Therefore, the NOEL/LOEL for male mysid shrimp weight were found to be 1.2 ug/L and 3.0 ug/L, respectively. The reproductive NOEL/LOEL, however, were 0.83 ug/L and 1.2 ug/L, respectively (see DER).

This study may now be upgraded to core and will fulfill guideline requirements for the mysid shrimp life-cycle study 72-4(b) with technical chlorothalonil (T-117-12). If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

Fermenta T-117-12 study # 10273.0289.6100.530 Dry weights of mysids

Male Weights

	Solvent Control		Control		0.65 ug/L		0.83 ug/L		1.2 ug/L		3.0 ug/L		5.7 ug/L	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	0.96	0.62	0.62	0.95	0.7	0.63	0.71	0.58	0.58	0.55	0.67	0.44	0.7	0.87
	0.86	0.6	0.95	0.91	0.79	0.55	0.71	0.56	0.6	0.57	0.72	0.86	0.6	0.83
	0.63	0.56	0.53	0.87	0.6	0.75	0.95	0.58	0.69	0.45	0.8	0.6	0.97	0.63
	1.13	0.42	0.7	1.24	0.98	0.56	0.68	0.46	0.8	0.59	0.69	0.62	0.64	0.65
	0.63	0.33	0.54	0.86	0.74	0.56	0.74	0.61	0.56	0.43	0.74	1.3	0.68	0.63
	0.67	1.02	0.98	0.58	0.91	0.72	0.71	0.68	0.43	0.65	0.8	0.6	0.67	0.84
	0.58	0.79	0.65	0.72	0.46	0.7	0.88	0.47	0.64	0.62	0.56	0.76	0.66	0.66
	0.85	0.23	0.53	1.09	0.69	0.8	0.69	0.64	0.66	0.69	0.69	0.8	0.94	0.73
	0.48	0.61	0.77	0.67	0.76	0.67	0.74	0.66	0.64	0.61	0.82	0.51	0.9	0.83
	0.24	0.56	0.88	1.27	0.65	0.51	0.97	0.72	0.66	0.67	0.78	0.86	0.71	0.62
		0.76	0.61	1.16		0.83	0.89	0.63	0.61	0.59	0.87		0.67	0.48
		0.45	0.46	0.97		0.55			0.67	0.41	0.72		0.67	0.58
		0.86		0.9		0.8			0.63	0.66	0.75		0.64	0.5
		0.56		1.08		0.68			0.83		0.71		0.9	
	24		26		25	0.69	22		0.52		24		0.62	
									0.88				0.8	
									29					29
Mean	0.703	0.597857	0.685	0.947857	0.728	0.666666	0.788181	0.599090	0.65	0.576153	0.737142	0.735	0.735625	0.680769
std	0.255083	0.210427	0.174121	0.206478	0.148383	0.103417	0.110618	0.081173	0.113431	0.092784	0.076503	0.245549	0.123448	0.129580
n	10	14	12	14	10	15	11	11	16	13	14	10	16	13
minimum	0.24	0.23	0.46	0.58	0.46	0.51	0.68	0.46	0.43	0.41	0.56	0.44	0.6	0.48
maximum	1.13	1.02	0.98	1.27	0.98	0.83	0.97	0.72	0.88	0.69	0.87	1.3	0.97	0.87

Mean(A&B)	0.641666	0.826538	0.6912	0.693636	0.616896	0.73625	0.711034
std	0.230852	0.231014	0.124241	0.135384	0.109450	0.164020	0.127008
n	24	26	25	22	29	24	29
min	0.23	0.46	0.46	0.46	0.41	0.44	0.48
max	1.13	1.27	0.98	0.97	0.88	1.3	0.97

Mean(pooled controls)	0.7378
std	0.246876
n	50
min	0.23
max	1.27

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Fermenta T-117-12 study # 10273.0289.6100.530 Dry weights of mysids

Female Weights

Solvent Control		Control		0.65 ug/L		0.83 ug/L		1.2 ug/L		3.0 ug/L		5.7 ug/L	
A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	0.63	0.71	0.99	1.05	1.1	0.89	0.77	0.73	0.94	0.81	0.84	0.86	0.98
1.09	0.83	0.73	0.78	1.05	0.63	1.03	0.8	0.68	0.72	0.89	1.06	0.85	0.76
1.49	0.82	0.95	1.12	0.93	0.88	1.13	0.91	0.7	0.6	1.19	1.1	0.93	1.08
1.01	0.56	0.7	0.78	0.55	0.88	1.4	0.87	0.94	0.73	0.8	0.76	0.94	0.65
	0.93	0.88	1.3	0.93	0.66	1.4	1.22	0.87	0.78	1	0.94	0.61	1.27
	0.88	0.73	0.89	1.67	0.63	0.85	0.73	0.86	0.78	0.87	1.05	0.75	0.77
	0.64	0.57	1.13	1.2	0.98	0.93	1.13	0.66	0.76	0.63		1.13	0.67
	0.75	0.81	0.98	1.07	1.24	1.11	1.43	0.7	0.92	0.92		0.75	0.53
	0.67	0.7	1.26	0.87	0.77	1.14	0.97		0.57	1.03			0.8
13		0.91		0.49	0.84	1.02	0.45		0.98		15		17
		0.67		1.01	0.77	1.12	0.67		1.01				
		20				0.87			0.39				
						1.08			0.67				
						24			0.55				
								22					

Mean	1.1475	0.745555	0.76	1.025555	0.983636	0.852727	1.074615	0.904545	0.7675	0.742857	0.904444	0.958333	0.8525	0.834444
std	0.231858	0.127191	0.114542	0.191187	0.313887	0.194683	0.176711	0.274421	0.105931	0.179504	0.159382	0.136002	0.156090	0.233404
n	4	9	11	9	11	11	13	11	8	14	9	6	8	9
minimum	1	0.56	0.57	0.78	0.49	0.63	0.85	0.45	0.66	0.39	0.63	0.76	0.61	0.53
maximum	1.49	0.93	0.95	1.3	1.67	1.24	1.4	1.43	0.94	1.01	1.19	1.1	1.13	1.27

Mean(A&B)		0.869230		0.8795		0.918181		0.996666		0.751818		0.926		0.842941
std		0.248007		0.201663		0.263540		0.237755		0.154384		0.147880		0.194896
n		13		20		22		24		22		15		17
min		0.56		0.57		0.49		0.45		0.39		0.63		0.53
max		1.49		1.3		1.67		1.43		1.01		1.19		1.27

Mean (Pooled Controls)	0.875454
std	0.217343
n	33
min	0.56
max	1.49

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Chlorothalonil: mysid shrimp - male weight
File: chloroth.mys Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	0.6417	CALCULATED t VALUE =	-3.1618
GRP2 (BLANK CRTL) MEAN =	0.8265	DEGREES OF FREEDOM =	48
DIFFERENCE IN MEANS =	-0.1849		

TABLE t VALUE (0.05 (2),60) =	2.000**	SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),60) =	2.660**	SIGNIFICANT DIFFERENCE at alpha=0.01

Chlorothalonil: mysid shrimp - male weight
File: chloroth.mys Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	solvent control	24	0.230	1.130	0.642
2	0.65 ug/L	25	0.460	0.980	0.691
3	0.83	22	0.460	0.970	0.694
4	1.2	29	0.410	0.880	0.617
5	3.0	24	0.440	1.300	0.736
6	5.7	29	0.480	0.970	0.711

Chlorothalonil: mysid shrimp - male weight
File: chloroth.mys Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	solvent control	0.053	0.231	0.047
2	0.65 ug/L	0.015	0.124	0.025
3	0.83	0.018	0.135	0.029
4	1.2	0.012	0.109	0.020
5	3.0	0.027	0.164	0.033
6	5.7	0.016	0.127	0.024

Chlorothalonil: mysid shrimp - male weight
File: chloroth.mys Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	24	0.642	0.642	0.642
2	0.65 ug/L	25	0.691	0.691	0.664
3	0.83	22	0.694	0.694	0.664
4	1.2	29	0.617	0.617	0.664
5	3.0	24	0.736	0.736	0.722
6	5.7	29	0.711	0.711	0.722

Chlorothalonil: mysid shrimp - male weight
File: chloroth.mys 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
solvent control	0.642				
0.65 ug/L	0.664	0.505		1.66	k= 1, v=147
0.83	0.664	0.488		1.73	k= 2, v=147
1.2	0.664	0.523		1.75	k= 3, v=147
3.0	0.722	1.844	*	1.77	k= 4, v=147
5.7	0.722	1.929	*	1.77	k= 5, v=147

s = 0.152

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

NOEC = 1.2 µg/L

LOEC = 3.0 µg/L