DATA EVALUATION RECORD

1. **CHEMICAL:** Cypermethrin Shaughnessey Number: 109702

2. **TEST MATERIAL:** FMC-30980 (^{14}C-labeled Cypermethrin) 98.1 % active ingredient, tan liquid.

3. **STUDY TYPE:** Early life stage toxicity test with the mysid (Mysidopsis bahia)

4. **CITATION:** Wheat, Jeff. 1992. FMC-30980 (^{14}C-labeled Cypermethrin): Chronic toxicity to the mysid, Mysidopsis bahia, under flow through test conditions. EPA Guideline 72-4. Performed by Toxikon Environmental Sciences, Jupiter, Florida. Sponsored by FMC Corporation, Princeton, New Jersey. FMC Study Number A91-3480. EPA MRID No. 427253-01.

5. **REVIEWED BY:**

Renee Lamb
Biologist
Ecological Effects Branch (H7507C)
Environmental Fate & Effects Division

Signature: Renee Lamb
Date: 5/24/93

6. **APPROVED BY:**

for Ann Stavola
Section Head Section 5
Ecological Effects Branch (H7507C)
Environmental Fate & Effects Division

Signature: Allen W. Vaughan
Date: 12-29-94

7. **CONCLUSIONS:** This study appears to be scientifically sound and fulfills the guideline requirement for a chronic toxicity study using the mysid. The MATC based upon mortality and growth was > 0.781 < 1.976 ng/L with a geometric mean of 1.242 ng/L. Therefore, cypermethrin is considered very highly toxic to developing mysid embryos.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** N/A

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

11. **MATERIALS AND METHODS:**

A. **TEST ANIMALS:** Post-larval mysids (< 24 hours old) collected from mysid cultures at Toxikon were used in the chronic tests. They were fed live brine shrimp hatched daily. No diseases were observed.

B. **TEST SYSTEM:** The definitive exposure was conducted under flow-through conditions in a modified proportional vacuum siphon diluter system based on the

original Mount and Brungs design. It was constructed of glass, silicone adhesive, and silicone tubing. The system was volumetrically calibrated to provide a test concentration series with a 50% dilution and equal solvent concentration in all test concentrations. A stock solution was mixed and the four lower test concentrations were proportionately diluted. A dilution water control and a solvent control (DMF) were maintained concurrently with the test solutions.

The dilution water was natural filtered saltwater adjusted to a salinity of 20 ppt. It was vigorously aerated prior to use.

C. **DOSAGE:** A proportional diluter was used to deliver the various test concentrations. Test concentrations were 2.0, 1.0, 0.5, and 0.25 ng ai/L. The diluter delivered approximately 1000 mL to each chamber per cycle over the duration of the test.

D. **DESIGN:** Ten mysids were impartially distributed by twos to petri dishes, for a total of 20 mysids per treatment replicate and a total of 40 mysids per treatment. The dishes were then transferred into screened retention chambers positioned within each test chamber. The treatment tanks were randomly positioned in a water bath under fluorescent lighting to an overall photoperiod of 16 hours light and 8 hours darkness. The intensity ranged from 258 to 458 lux. Test solutions were not aerated during this study.

Survival and offspring production was monitored daily and any dead were removed. After offspring release, a minimum of 20 per replicate were transferred into additional screen retention chambers within the same test camber and survival monitored for 96 hours. Mysids were fed live brine shrimp at least once daily throughout the test.

E. **STATISTICS:** Control mortality was statistically compared using Fisher's Exact Test. Student's t-test was used to evaluate control reproduction and growth of first generation mysids. If not significantly different, the control data were pooled prior to statistical evaluation with the treatments. If statistical difference was found, only the solvent control data were used for statistical evaluation with the treatments. Statistical difference in growth and reproduction were calculated using Fisher's Exact Test. Growth and reproduction was analyzed with ANOVA followed with Dunnett's at the 0.05 probability level.

12. **REPORTED RESULTS:** On day 17 of the test, the silicone stopper present in the bottom of the tank for treatment five

became dislodged allowing water from the water bath to enter the test chamber, This resulted in the death of all parental mysids in this replicate. This replicate was excluded from analysis.

Mortality of first generation mysids ranged from 8 percent (mean measured concentration of 0.411 ng/L) to 35 percent (mean measured concentration of 1.976 ng/L). Dilution and solvent control mortality was 10 and 8 percent, respectively. Mortality was statistically reduced only at 1.976 ng/L when compared to the pooled controls.

The mean number of young per female reproductive day (YPFRD) for the control and solvent control was 1.43 and 1.82, respectively. No statistical significance was found between the control, therefore, data were pooled for subsequent analysis. The YPFRD for the 0.125, 0.233, 0.411, 0.781 and 1.976 ng/L test concentrations was 1.23, 1.71, 2.35, 2.24, and 0.85, respectively.

Mean length of mysids in the pooled controls was 7.0 mm. Mean length of mysids were 6.9, 7.1, 7.3, 7.1 and 6.8 mm at each respective test concentration. Mean dry weight of the pooled control mysids was 0.97 mg. Mean dry weights of mysids were 0.97, 1.04, 1.11, 1.10 and 0.82 mg at the respective test concentrations. Total length of mysids was significantly reduced at the 1.976 ng/J while dry weight of mysids was not significantly reduced at any test concentration.

Growth data was analyzed to determine if there were increased differences in growth when sex was taken into account. Both length and dry weight of male mysids in the 1.976 ng/L group was statistically reduced from the pooled controls while only female dry weight in the 1.976 group was statistically reduced.

Second generation mysid survival was not adversely affected. Survival was 98 and 86 percent in the dilution water and solvent control, respectively. Survival was not less than 95% in any of the treatment chambers.

Test temperature during the study ranged form 21.8 to 29.3°C with a mean and standard deviation of 26.1 ± 1.1°C. The salinity remained between 19 and 22 ppt during the test with the t=exception of one day when it rose to 25 ppt due to a malfunction of the salinity control system. DO concentrations remained ≥ 4.4 mg/L (≥ 62% saturation) in the controls, and ranged from 4.4 to 7.0 mg/L (61 to 95% saturation) in the test chambers. The pH ranged form 8.1 to 8.5 in all test chambers.

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

"The MATC based upon mortality and growth was > 0.781 < 1.976 ng/L with a geometric mean of 1.242 ng/L."

The report has a quality assurance statement signed by a quality assurance officer.

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

A. **TEST PROCEDURE:** There is no official EPA SEP protocol for this study, therefore the "Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids", ASTM E 1191-90, was used to evaluate the scientific validity of this study. The test was in accordance with the ASTM, with the exception of the following deviations which did not affect the scientific validity of the study:

- Silicone stopper became dislodged allowing water form the bath to enter the tank. As a result, all parental mysids died. This replicate was excluded from any analyses;

- Temperature deviated from the recommended 27°C. The mean temperature for the study was 26°C.

B. **STATISTICAL ANALYSIS:** The data was analyzed using EEB's Toxanal and Toxstat programs (see attached).

C. **DISCUSSION/RESULTS:** This study appears to be scientifically sound and fulfills the guideline requirement for a chronic toxicity study using the mysid. The MATC based upon mortality and growth was > 0.781 < 1.976 ng/L with a geometric mean of 1.242 ng/L. Therefore, cypermethrin is considered very highly toxic to developing mysid embryos.

D. **ADEQUACY OF STUDY:**
   (1) **CLASSIFICATION:** core
   (2) **RATIONALE:** N/A
   (3) **REPAIRABILITY:** N/A

15. **COMPLETION OF ONE-LINER:** May 24, 1993.

Cypermethrin # of offspring per female reproductive day
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	1.160	1.670	1.430
2	solvent control	4	1.570	2.210	1.815
3	0.125	4	1.180	1.300	1.233
4	0.233	4	1.580	1.880	1.710
5	0.411	4	1.690	3.250	2.350
6	0.781	4	1.740	2.890	2.235
7	1.976	2	0.670	1.030	0.850

Cypermethrin # of offspring per female reproductive day
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.069	0.263	0.131
2	solvent control	0.084	0.290	0.145
3	0.125	0.003	0.057	0.029
4	0.233	0.018	0.134	0.067
5	0.411	0.438	0.662	0.331
6	0.781	0.257	0.507	0.253
7	1.976	0.065	0.255	0.180

Cypermethrin # of offspring per female reproductive day
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	5.486	0.914	6.482
Within (Error)	19	2.671	0.141	
Total	25	8.157		

Critical F value = 2.63 (0.05,6,19)
Since F > Critical F REJECT Ho:All groups equal

Cypermethrin # of offspring per female reproductive day

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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.430	1.430		
2	solvent control	1.815	1.815	-1.450	
3	0.125	1.233	1.233	0.744	
4	0.233	1.710	1.710	-1.055	
5	0.411	2.350	2.350	-3.465	
6	0.781	2.235	2.235	-3.032	
7	1.976	0.850	0.850	1.784	

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=19,6)

Cypermethrin # of offspring per female reproductive day
File: d:femrep.dat Transform: NO TRANSFORM

BONFERRONI T-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	control	4			
2	solvent control	4	0.697	48.8	-0.385
3	0.125	4	0.697	48.8	0.198
4	0.233	4	0.697	48.8	-0.280
5	0.411	4	0.697	48.8	-0.920
6	0.781	4	0.697	48.8	-0.805
7	1.976	2	0.854	59.7	0.580

growth as total length of mysids
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	6.900	7.200	7.025
2	solvent control	4	6.900	7.200	7.025
3	0.125	4	6.800	7.000	6.925
4	0.233	4	7.000	7.100	7.050
5	0.411	4	7.100	7.500	7.350
6	0.781	4	6.900	7.300	7.100
7	1.976	2	6.700	6.900	6.800

growth as total length of mysids
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.016	0.126	0.063
2	solvent control	0.016	0.126	0.063
3	0.125	0.009	0.096	0.048
4	0.233	0.003	0.058	0.029
5	0.411	0.030	0.173	0.087
6	0.781	0.027	0.163	0.082
7	1.976	0.020	0.141	0.100

growth as total length of mysids
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.561	0.093	5.471
Within (Error)	19	0.322	0.017	
Total	25	0.883		

Critical F value = 2.63 (0.05,6,19)
Since F > Critical F REJECT Ho:All groups equal

growth as total length of mysids

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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	7.025	7.025		
2	solvent control	7.025	7.025	0.000	
3	0.125	6.925	6.925	1.085	
4	0.233	7.050	7.050	-0.271	
5	0.411	7.350	7.350	-3.525	
6	0.781	7.100	7.100	-0.813	
7	1.976	6.800	6.800	1.993	

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=19,6)

growth as total length of mysids
File: d:lencyp.dat Transform: NO TRANSFORM

BONFERRONI T-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	control	4			
2	solvent control	4	0.242	3.4	0.000
3	0.125	4	0.242	3.4	0.100
4	0.233	4	0.242	3.4	-0.025
5	0.411	4	0.242	3.4	-0.325
6	0.781	4	0.242	3.4	-0.075
7	1.976	2	0.297	4.2	0.225

Growth as dry weight of mysids
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.930	1.070	0.985
2	solvent control	4	0.900	1.010	0.958
3	0.125	4	0.880	1.030	0.958
4	0.233	4	0.990	1.130	1.035
5	0.411	4	0.990	1.170	1.118
6	0.781	4	1.030	1.190	1.095
7	1.976	2	0.740	0.930	0.835

Growth as dry weight of mysids
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.004	0.065	0.032
2	solvent control	0.002	0.048	0.024
3	0.125	0.004	0.062	0.031
4	0.233	0.004	0.065	0.032
5	0.411	0.007	0.085	0.043
6	0.781	0.005	0.070	0.035
7	1.976	0.018	0.134	0.095

Growth as dry weight of mysids
File: d:weicyp.dat Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.163	0.027	5.400
Within (Error)	19	0.098	0.005	
Total	25	0.261		

Critical F value = 2.63 (0.05,6,19)
Since F > Critical F REJECT Ho:All groups equal

Growth as dry weight of mysids

File: d:weicyp.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.985	0.985		
2	solvent control	0.958	0.958	0.550	
3	0.125	0.958	0.958	0.550	
4	0.233	1.035	1.035	-1.000	
5	0.411	1.118	1.118	-2.650	
6	0.781	1.095	1.095	-2.200	
7	1.976	0.835	0.835	2.449	

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=19,6)

Growth as dry weight of mysids
File: d:weicyp.dat Transform: NO TRANSFORM

BONFERRONI T-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	control	4			
2	solvent control	4	0.131	13.3	0.028
3	0.125	4	0.131	13.3	0.028
4	0.233	4	0.131	13.3	-0.050
5	0.411	4	0.131	13.3	-0.132
6	0.781	4	0.131	13.3	-0.110
7	1.976	2	0.161	16.3	0.150

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

lamb cypermethrin CYPERMETHRIN CUMUL. MORTALITY

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1.976	16.5	3.5	21.2121	0
.781	40	6	15	0
.411	40	3	7.500001	0
.233	33	1	3.0303	0
.125	33	1	3.0303	0

THE BINOMIAL TEST SHOWS THAT 1.976 AND +INFINITY CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.976

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.5743692	1	.927063

SLOPE = 1.045869
95 PERCENT CONFIDENCE LIMITS = .2532346 AND 1.838502

LC50 = 9.471048
95 PERCENT CONFIDENCE LIMITS = 2.547858 AND 74494.04

LC10 = .5782243
95 PERCENT CONFIDENCE LIMITS = .2341407 AND 1.595613

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

lamb cypermethrin CYPERMETHRIN SECOND GEN SURVIVAL

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1.976	20	0	0	0
.781	40	1	2.5	0
.411	40	0	0	0
.233	40	0	0	0
.125	40	2	5	0

THE BINOMIAL TEST SHOWS THAT 1.976 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.976

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	4.211926	1	.3370469

SLOPE = -.7010078

95 PERCENT CONFIDENCE LIMITS =-2.139684 AND .7376688

LC50 = 2.806691E-04

95 PERCENT CONFIDENCE LIMITS = 0 AND 3.031336E-02

LC10 = 1.819165E-02

95 PERCENT CONFIDENCE LIMITS = 0 AND .137051

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