2/22/95

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

MAR 23 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: Review of fish early life stage study for Ethyl Parathion Technical (057501-9)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 2/22/95

To: Tom Moriarty
Special Review Branch
Special Review and Reregistration Division (7508W)

The Ecological Effects Branch (EEB) has completed its review of the fish early life stage study submitted by A/S Cheminova for Ethyl Parathion Technical. The following is a brief summary of the data reviewed:

CITATION: Suprenant, Donald C. 1988. The toxicity of Ethyl parathion Technical to Sheepshead Minnow (Cyprinodon variegatus) embryos and larvae. Laboratory project ID 88-5-2657. Study sponsor A/S Cheminova. Performed by Springborn Laboratories, Inc., Wareham, MA. MRID No 415431-01.

CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirement for a fish early life stage study. In these studies, the dilution water is periodically scanned for the presence of pesticides. Because ethyl parathion residues were detected in both the solvent and the dilution water controls, EEB requires a chemical screen completed at the time of the study to verify ethyl parathion was not contaminating the water source. This study can be upgraded with the submission of the chemical screen. The MATC value based on statistically significant differences for both weight and length is > .19 and < .37 µg a.i./L. The NOEC and LOEC are .19 and .37 µg a.i./L, respectively. Therefore, Ethyl Parathion Technical is considered highly toxic to developing fish embryos.

If you have any questions contact Renée Costello at 305-5294.

MRID No. 415431-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Parathion Shaughnessey Number: 057501-9

2. **TEST MATERIAL:** Technical Parathion, 98.0% active ingredient, a brown liquid.

3. **STUDY TYPE:** Ethyl Parathion Technical - Sheepshead Minnow (Cyprinodon variegatus) early life stage toxicity test

4. **CITATION:** Suprenant, Donald C. 1988. The toxicity of Ethyl parathion Technical to Sheepshead Minnow (Cyprinodon variegatus) embryos and larvae. Laboratory project ID 88-5-2657. Study sponsor A/S Cheminova. Performed by Springborn Laboratories, Inc., Wareham, MA. MRID No 415431-01.

5. **REVIEWED BY:**

Renée Costello
Biologist
Ecological Effects Branch (7507C)
Environmental Fate & Effects Division

Signature: Renée Costello
Date: 12/11/91

6. **APPROVED BY:**

Allen Vaughan
Acting Chief, Section 5
Ecological Effects Branch (7507C)
Environmental Fate & Effects Division

Signature: Allen W. Vaughan
Date: 2.22-95

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirement for a fish early life stage study. In these studies, the dilution water is periodically scanned for the presence of pesticides. Because ethyl parathion residues were detected in both the solvent and the dilution water controls, EEB requires a chemical screen completed at the time of the study to verify ethyl parathion was not contaminating the water source. This study can be upgraded with the submission of the chemical screen. The MATC value based on statistically significant differences for both weight and length is > .19 and < .37 µg a.i./L. The NOEC and LOEC are .19 and .37 µg a.i./L, respectively. Therefore, Ethyl Parathion Technical is considered highly toxic to developing fish embryos.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** N/A

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

11. **MATERIALS AND METHODS:**

A. **TEST ANIMALS:** Fertilized sheepshead minnow embryos obtained from a commercial supplier were less than 24 hours old at test initiation.

B. **TEST SYSTEM:** Embryo incubation cups were 8 cm high glass jars with 40 mesh Nitex screen bottoms. The cups were oscillated in the test solutions with a Mount rocker arm apparatus.

A modified proportional diluter (.50 dilution factor) was used to prepare and deliver the test solution to the aquaria during the 35 day test. A 50 Ml gas tight syringe was mechanically activated during each diluter cycle to deliver .0174 mL of the 166 µg a.i./mL stock solution into the mixing chamber containing 1.93 L of water. This stock solution was proportionally diluted to deliver the selected range of nominal exposure concentrations. Each of the glass test aquaria measured 39 x 20 x 25 cm with a 14.5 cm high side drain that maintained a constant exposure volume of 11 L. The diluter delivered .5 L of solution to each aquarium at an average rate of 143 times per day. This was an approximate flow rate of 605 aquarium volumes per 24 hour period, with a 90% replacement time of 7 hours.

16 hours of light was provided at 10 - 100 foot candles at the surface each day. The aquaria were randomly positioned in a circulating water bath to maintain the test solution temperatures at 25 ± 1°C.

The dilution and control water was natural seawater pumped from Cape Cod Canal in Bourne, Massachusetts, about 4 meters offshore at a depth of approximately 05 meters. The water was passed through a series of polypropylene core filters (20 and 5 microns) and an activated charcoal filter. It had a salinity range of 30 - 32°/oo and a pH range of 7.7 - 8.0.

C. **DOSAGE:** Nominal test concentrations were 0, .093, .19, .37, .75, and 1.5 mg/L. There was a dilution and a solvent control.

D. **DESIGN:** 75 embryos per incubation cup, 1 cup per test aquarium, 2 aquaria per test concentration. After hatching, 25 live larvae were randomly selected and placed in their respective aquaria upon initiation of the 28 day post-hatch larval exposure test. The larvae were fed live brine shrimp 3 times daily and twice daily on weekends and holidays. Larval survival was estimated twice weekly. At 28 days post-hatch, the percentage larval survival was determined. At

termination, the larvae were weighed and measured individually.

E. **STATISTICS:** Data was analyzed using various statistical tests.

12. **REPORTED RESULTS:** The test concentrations based on mean measured concentrations were 1.3, .72, .36, .21, and .10 µg a.i./L (range from 87% to 111% of the nominal).

During the latter half of the early life stage exposure, measurable quantities of Ethyl Parathion Technical were detected in both the dilution water and solvent control solutions (see Table 2). This is attributed to contamination during the handling and analysis of these samples and not indicative of the exposure conditions for either controls. Samples of laboratory dilution water are examined periodically for the presence of pesticides. Results of these analyses demonstrate that Ethyl Parathion has never been detected in any of these samples.

Parathion Technical appeared soluble at all concentrations.

No mortality was observed at any test concentration after 144 hours of exposure. The larvae in solutions ≥ 1.5 µg a.i./L exhibited abnormal behavior. Larvae exposed to concentrations ≤ .75 µg a.i./L appeared normal throughout the exposure period.

See Table 1 for water quality measurements.

See Table 4 for biological data, including # larvae hatched and larval survival, collected during the test. Larval growth was the only indicator of the toxicity of parathion to the sheepshead minnow. Mean total length at the highest concentration (1.3 µg a.i./L) was 21 mm and significantly less than the length of control larvae (23 mm) (see Figure 4). Mean wet weight ranged from 167 to 202 mg and was unaffected when compared to the control data (199 mg).

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

The MATC of Ethyl Parathion Technical to Sheepshead Minnow embryos-larvae was > .72 and < 1.3 µg a.i./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:** This test is in accordance with EPA's SEP protocol.

B. **STATISTICAL ANALYSIS:** Data was analyzed using EEB's

Toxstat. (See attached tables.) The MATC is > .19 and < .37 µg a.i./L. This value differs from the reported MATC. It is based on statistically significant differences from the controls for both length and weight data which were found at the .37 µg a.i./L concentration.

C. **DISCUSSION/RESULTS:** This study appears to be scientifically sound but does not fulfill the guideline requirement. The MATC value based on statistically significant differences for both weight and length is > .19 and < .37 µg a.i./L. The NOEC and LOEC are .19 and .37 µg a.i./L, respectively. Therefore, Ethyl Parathion Technical is considered highly toxic to developing fish embryos. This study can be upgraded with submission of chemical screen (see section 14D).

D. **ADEQUACY OF STUDY:**

(1) **CLASSIFICATION:** Supplemental

(2) **RATIONALE:** Dilution water is periodically scanned for presence of pesticides. Since ethyl parathion residues were detected in both the solvent and the dilution water controls, EEB requires a chemical screen completed at the time of this study to verify ethyl parathion was not contaminating the water source.

(3) **REPAIRABILITY:** Upgradable to core with submission of chemical screen mentioned above.

Table 1. Water quality determinations made during the 28-day exposure (28 days post-hatch [illegible] embryos and larvae to Ethyl Parathion Technical

Nominal Concentration (µg A.I./L)	Mean Dissolved Oxygen (mg/L)	Mean Temperature (°C)	pH
1.5	[illegible] (0.8)[1]	25 (0.5)[1]	7.5
0.75	[illegible] (0.3)[1]	25 (0.5)[1]	7.5
0.37	[illegible] (0.0)[1]	25 (0.5)[1]	7.5
0.19	[illegible] (0.0)[1]	25 (0.5)[1]	7.5
0.093	[illegible] (0.2)[1]	25 (0.5)[1]	7.5
Solvent Control	[illegible] (0.0)[1]	25 (0.5)[1]	7.5
Control	[illegible] (0.5)[1]	25 (0.5)[1]	7.5

Values presented in ( ) = standard deviation.

Table 2. Measured concentrations of Ethyl Parathion Technical during the early life-stage exposure of the sheepshead minnow (Cyprinodon variegatus).

Nominal Concentration (µg A.I./L)	Measured Concentration (µg A.I./L)					
	Day 0	Day 7	Day 15	Day 21	Day 28	Day 35
1.5	0.43 1.4	1.4 1.9	1.9 1.7	1.5 1.6	1.1 1.2	1.1 1.0
0.75	0.48 0.76	0.78 0.95	0.84 —	0.93 0.86	0.61 0.69	0.41 0.58
0.37	0.36 0.34	0.46 0.32	0.42 0.40	0.45 0.43	0.35 0.31	0.28 0.22
0.19	0.21 0.20	0.19 0.17	0.28 0.27	0.23 0.28	0.21 0.21	0.17 0.13
0.093	0.066 0.086	0.086 0.097	0.12 0.13	0.13 0.14	0.081 0.10	0.087 0.089
Solvent Control	< 0.013 < 0.013	< 0.013 < 0.013	0.017 0.025	0.694 0.035	0.047 0.032	0.028 0.028
Control	< 0.013 < 0.013	< 0.013 < 0.013	0.039 < 0.013	0.087 0.031	0.064 0.034	0.029 0.014
0.200 (QA)[a]	0.178 (89)[b]	0.25 (125)	0.206 (103)	0.204 (102)	—	0.168 (84)
0.800 (QA)	0.772 (97)	1.131 (142)	0.206 (186)	—	—	—
1.00 (QA)	—	—	—	0.965 (105)	1.02 (102)	0.779 (78)
1.50 (QA)	1.49 (99)	1.29 (86)	1.40 (93)	1.52 (101)	—	0.924 (62)

[a] QA = Quality Assurance sample.
[b] Value in parentheses is the percent of the nominal fortified concentration.

Table 4. Number of hatched larvae (test day 7) and survival, total length and wet weight of sheepshead minnow (Cyprinodon variegatus) larvae after 28 days post-hatch exposure to Ethyl Parathion.

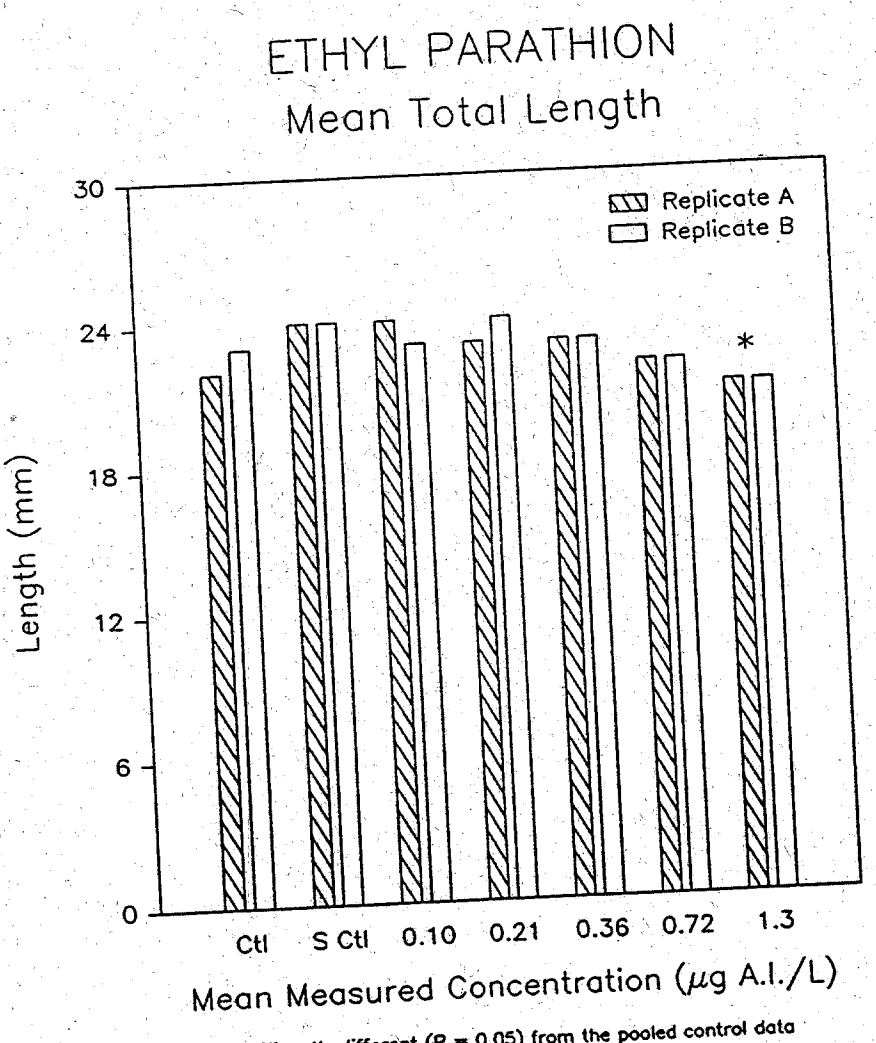
Mean Measured Concentration (µg A.I./L)		# Larvae Hatched[c] at Day 7	Larval Survival (%)	Mean Total Length (S.D.) (mm)	Mean Wet Weight (S.D.) (mg)
1.3	A	31	100	21 (1.7)	156 (40)
	B	32	100	21 (1.3)	177 (37)
	Mean	32	100	21 (1.6)[a]	167 (39)
0.72	A	25	92	22 (1.2)	170 (35)
	B	25	100	22 (2.1)	179 (42)
	Mean	25	96	22 (1.7)	175 (39)
0.36	A	33	84	23 (0.86)	180 (27)
	B	38	100	23 (1.3)	198 (41)
	Mean	36	92	23 (1.2)	190 (36)
0.21	A	25	92	23 (1.4)	184 (37)
	B	18	100	24 (1.7)	226 (54)
	Mean	22	96	24 (1.6)	202 (49)
0.10	A	33	96	24 (0.88)	225 (32)
	B	31	100	23 (0.84)	180 (25)
	Mean	32	98	24 (0.97)	202 (37)
Solvent Control	A	34	88	24 (1.0)	195 (27)
	B	31	88	24 (1.2)	238 (44)
	Mean	33	88	24 (1.1)	217 (42)
Control	A	28	96	22 (1.1)	169 (26)
	B	25	100	23 (2.4)	195 (53)
	Mean	27	98	23 (2.0)	183 (44)
Pooled[b] Controls (Mean)		30	93	23	199

[a] Indicates significantly different (P = 0.05) from the control (pooled control and solvent control) data.

[b] Pooled mean control and solvent control data.

[c] Number of hatched larvae following 7 days exposure (completion of the hatching period). Seventy-five embryos were exposed to each replicate solution. Non-viable eggs were not removed from the exposure vessels.

Figure 4 **Mean total length of Sheepshead minnow (Cyprinodon variegatus) exposed to Ethyl Parathion Technical for 35 days (28 days post-hatch).**



* Significantly different (P = 0.05) from the pooled control data

parathion early - weight
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.085	0.014	7.000
Within (Error)	320	0.535	0.002	
Total	326	0.620		

Critical F value = 2.18 (0.05,6,120)
Since F > Critical F REJECT Ho:All groups equal

parathion early - weight
File: a:paraweig.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	solvent	0.217	0.217		
2	control	0.183	0.183	3.647	*
3	.093	0.202	0.202	1.552	
4	.19 (NOEC)	0.202	0.202	1.460	
5	.37 (LOEC)	0.190	0.190	2.869	*
6	.75	0.175	0.175	4.497	*
7	1.5	0.167	0.167	5.382	*

MATC

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=120,6)

parathion early - weight
File: a:paraweig.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	solvent	44			
2	control	49	0.023	10.4	0.034
3	.093	49	0.023	10.4	0.014
4	.19	41	0.024	10.9	0.014
5	.37	46	0.023	10.6	0.027
6	.75	48	0.023	10.5	0.042
7	1.5	50	0.022	10.4	0.050

Parathion early - length
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	294.382	49.064	24.643
Within (Error)	320	637.239	1.991	
Total	326	931.621		

Critical F value = 2.18 (0.05,6,120)
Since F > Critical F REJECT Ho:All groups equal

Parathion early - length
File: a:paraleng.dat Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	23.955	23.955		
2	control	22.531	22.531	4.859	*
3	.093	23.735	23.735	0.750	
4	.19	23.585	23.585	1.205	
5	.37	22.891	22.891	3.573	*
6	.75	22.167	22.167	6.071	*
7	1.5	21.100	21.100	9.787	*

NOEC .19
MATC
LOEC .37

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=120,6)

Parathion early - length
File: a:paraleng.dat Transform: NO TRANSFORMATION

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	solvent control	44			
2	control	49	0.712	3.0	1.424
3	.093	49	0.712	3.0	0.220
4	.19	41	0.744	3.1	0.369
5	.37	46	0.723	3.0	1.063
6	.75	48	0.715	3.0	1.788
7	1.5	50	0.708	3.0	2.855