DATA EVALUATION RECORD

1. **CHEMICAL:** Carbofuran.
Shaughnessey No. 090601.

2. **TEST MATERIAL:** Carbofuran Technical, FMC #7326, M607210, Code 2843, 96.9% purity, a light brown crystalline material.

3. **STUDY TYPE:** Fish Early Life-Stage Test.
Species Tested: Sheepshead Minnow (Cyprinodon variegatus).

4. **CITATION:** Ward, G.S. 1988. Chronic Toxicity Estimate of FMC 10242 Technical (Carbofuran) to the Sheepshead Minnow (Cyprinodon variegatus) in a Flow-Through Test. FMC Corporation Study No. A87-2265. ESE No. 87332-0650-2130. Prepared by Environmental Science and Engineering, Inc., Gainesville, FL. Submitted by FMC Corporation, Philadelphia, PA. EPA Accession No. 408184-01.

5. **REVIEWED BY:**

Tom A. Bailey, Ph.D.
Fishery Biologist
EFED/EEB
U.S. EPA

Signature: Tom A. Bailey
Date: 5-30-90

6. **APPROVED BY:**

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

Signature: Henry T. Craven
Date: 6/23/90

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a fish early life-stage test. Growth (length) was the most sensitive indicator for the toxicity of Carbofuran Technical to sheepshead minnow (Cyprinodon variegatus). The MATC was determined to be less than 17.6 ug/L mean measured concentration, based on reduction in length. The NOEL could not be calculated from the data tested due to the adverse effect on growth found at all test levels.

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

Accession No. 408184-01

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5. **REVIEWED BY:**
Prapimpan Kosalwat, Ph.D.
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Signature: P. Kosalwat
Date: 11/22/88

6. **APPROVED BY:**

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Signature: Isabel C. Johnson
Date: 11/22/88

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

Signature: Henry T. Craven
Date: 4/23/90

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a fish early life-stage test. Growth (length) was the most sensitive indicator for the toxicity of Carbofuran Technical to sheepshead minnow (Cyprinodon variegatus). The MATC was determined to be less than 17.6 ug/L mean measured concentration, based on reduction in length. The NOEL could not be calculated from the data tested due to the adverse effect on growth found at all test levels.

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

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Naturally spawned sheepshead minnow (Cyprinodon variegatus) embryos less than 48 hours old were obtained from Aquatic Research Organisms, Hampton, New Hampshire. Live embryos were randomly distributed into the test chambers on the day they were received.

B. Test System: The test system was a proportional diluter with a dilution factor of 0.5. The system consisted of seven duplicate sets of glass aquaria designed to maintain approximately 6.0 L of test solution or dilution water. Two cylindrical screened retention chambers were positioned in each duplicate test aquarium. Five FMC 10242 Technical concentrations, a natural seawater control, and a dimethylformamide (DMF) solvent control were utilized. Test concentrations were prepared fresh with each diluter cycle. The solvent concentration was maintained at approximately 20 uL/L in the solvent control and all test concentrations throughout the test. Salt water used for testing was filtered (5-micrometer) natural seawater. The water was collected at Marineland, Florida, and diluted to 20 parts per thousand with ESE's well water. Prior to use, the seawater was passed through an ultraviolet sterilizer.

Flow rate to each duplicate aquarium (i.e., 7 to 8 cycles per hour) was sufficient to provide approximately 14 daily volume turnovers. Test solution delivery was directed into the retention chambers to maintain the best water quality possible throughout the test. A water bath was utilized to maintain test temperature at 27°C. A 16-hour light and 8-hour dark photoperiod with a 15-minute transition period to simulate dawn and dusk was maintained.

C. Dosage: 35-days early life-stage test.

D. Design: Embryos and juveniles were definitively tested for 35 days at nominal test concentrations of 19.75, 39.5, 79.0, 158.0, and 316.0 micrograms per liter (ug/L). The test was initiated when 25 embryos were randomly added to each retention chamber within 48 hours of fertilization. Embryos were removed daily from each retention chamber, counted, dead embryos removed, and

the chambers rinsed with freshwater to clean screens. This procedure was repeated until all living embryos had hatched. Embryo mortality and time to hatch were recorded.

After hatching, juvenile fish were fed brine shrimp (Artemia salina) nauplii at least once per day. Survival was monitored daily until test termination, and any changes in physical appearance or behavior were recorded. Growth of juveniles was determined at the end of the exposure by measuring standard length, wet weight, and dry weight of individual fish. Dry weights were obtained by drying fish overnight at 60°C and then cooling them in a desiccator prior to weighing.

Diluter function was checked daily by observation and periodically by direct measurement of FMC 10242 Technical. The test concentrations were checked at test initiation, and on Days 7, 12, 16, 21, 24, 28, 31, 34, and 35 during the test. Additional samples were collected from the highest test concentration whenever events occurred which affected test concentrations. Samples were collected from all treatment duplicates at each sampling.

E. Statistics:

Hatching success and juvenile survival: Difference between the seawater control and solvent control were determined by Fishers Exact tests. Differences among the pooled controls and test concentrations were determined by analysis of variance (ANOVA), using angle (arcsine square root) transformations of the percentage survival data. If the results were statistically significant in an ANOVA test, at a probability of 0.95, statistical comparison between the pooled controls and each treatment was made using Williams' procedure for multiple comparison of several treatments to a control.

Mean standard length, wet weight, and dry weight of juvenile fish: Differences between the seawater control and solvent control were determined by a Student's t-test. Differences among the solvent control and test concentrations were determined by ANOVA. If the results were statistically significant in the ANOVA test, at a probability of 0.95, statistical comparison between the solvent control and each treatment was made using Dunnett's procedure for multiple comparison of several treatments. All differences were considered significant at the 95 percent confidence level.

12. REPORTED RESULTS: During the test, water quality remained within limits considered acceptable for sheepshead minnows. Salinity ranged from 18 to 22 parts per thousand. Temperature varied between 25 and 28°C and dissolved oxygen concentrations remained ≥ 4.3 mg/L or ≥ 61% of saturation. The pH ranged from 7.7 to 8.4.

The author reported that the concentrations of FMC 10242 Technical remained fairly stable throughout the test (Table 3-1, attached). However, on Day 3, an apparent electrical short-circuit in the Automatic Pipette pump resulted in double injections on some cycles. The pump was replaced which rectified the situation. Measurement of the highest test concentration found that the concentration increased by approximately 38% over nominal (60% increase over day-0 measurement). The system then functioned normally until Day 11 during which toxicant delivery ceased for approximately 21 hours. The actual drop in test concentrations were not measured. Based on the diluter cycling rate, the author estimated that the test concentrations could have dropped below the analytical limit of detection during the 21-hour period. On Day 34, the diluter began to cycle slower because the seawater supply was running out and then due to a malfunction in the seawater delivery system pump. The diluter was allowed to go static for the last 24 hours of exposure. The test concentrations fell by as much as 67% due presumably to physical or biological degradation processes. The mean measured concentrations of FMC 10242 Technical in seawater ranged from 76 to 110% of nominal concentrations (Table 3-2, attached).

Hatching success embryos, time to embryo hatching, survival of juvenile fish, and juvenile growth are presented in Tables 3-3, 3-5, 3-6, and 3-7, respectively. The author stated that the MATC value for sheepshead minnow was <17.6 ug/L FMC 10242 Technical based on length and wet weight. However, he did not see a clear relationship of concentration-related response in these two growth parameters. The mean dry weight also did not confirm the significance detected by length and wet weight. Since the solvent control fish were significantly larger than the seawater control fish, the author speculated that the solvent (DMF) provided an organic substrate for microorganisms which in turn provided a dietary supplement for the solvent control fish. This stimulatory growth was assumed to be present in all test concentrations in the absence of the toxicant. The author thought that the presence of toxicant in the test solutions might have affected the population of microorganisms creating an

artifact from the use of DMF. The lack of significance between the seawater control and all test concentrations except for 256 ug/L, also led the author to think the solvent-induced effect might have occurred in this case. Therefore, he believed the true MATC value for FMC 10242 Technical on sheepshead minnows was actually between 43.4 ug/L and 68.0 ug/L, based on reduction in hatching success.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
"Hatching of sheepshead minnow embryos was significantly reduced at test concentrations $\geq$ 68.0 ug/L. There was no apparent delay in hatching relative to FMC 10242 Technical exposure concentration. Mortality of juvenile fish was significantly increased at test concentrations $\geq$ 120.0 ug/L. Sheepshead minnows exposed to concentrations of FMC 10242 Technical $\geq$ 17.6 ug/L were statistically smaller than the solvent control fish on both length and wet weight bases, while only those fish exposed to 256 ug/L were significantly smaller than the solvent control fish on a dry weight basis. Differences in growth between the seawater control and the solvent control were attributed to the presence of the solvent as an organic substrate for microorganisms which were utilized by the solvent control fish as diet supplement. Based on the effects of FMC 10242 Technical on growth, estimated MATC is between 43.4 ug/L and 68.0 ug/L."

The study was conducted in accordance with EPA Good Laboratory Practice Standards (40 CFR 160), with the exception that specified procedures for recording data were not strictly followed. A quality assurance statement was included in the report, stating that "this report has been reviewed by the Toxicology Department's Quality Assurance Unit."

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

A. Test Procedure: The test procedure and the report generally followed the SEP, except for the following deviations:

o The test material was described as "Carbofuran Technical; FMC #7326." However, FMC 10242 Technical was referred to as the test material all through the report.

o Twenty-five eggs were used per cup exposed to FMC 10242 Technical. ASTM E 1241 states that no more than 20 randomly assigned embryos should be added to each cup.

o The quality of the dilution water did not meet requirements. 1) the TOC should be less than 5 mg/L; filtered seawater used in this study had a TOC of 36.7 mg/L, 2) suspended solids at 24 mg/L exceeds the criteria level of <20 mg/L, and 3) the level of organophosphate pesticides (<0.118 g/L) exceeds the standard criteria of <0.05 g/L. Furthermore some heavy metal levels (viz. cadmium, iron, and zinc) are exceptionally high and mercury was not measured.

o The test system failed to keep the test concentrations stable due to three failures as described by the author in Section 12. At each failure, all test concentrations should have been measured and included in the mean measured concentration estimates. By ignoring the concentration drops during the first two failures, the mean measured concentrations estimated may not reflect the true values the fish were exposed to.

o Diluter malfunctions occurred for approximately 4 of the 35 days of testing (i.e., about 11% of the exposure period).

o An hourly printout of temperature was reported as being provided in Appendix F. However, Appendix F is missing from the report.

o Verification of the precise embryonic stage at the beginning of the exposure was not attempted.

o Embryos should be 2 to 24 hours old at the beginning of the test. In this study, the embryos were reported as being less than 48 hours old at test initiation. However, the age of embryos in this test is acceptable by the ASTM Standard.

o The number of males and females used to obtain the embryos was not reported.

o The test fish were fed only <u>at least once per day</u>. Test fish should be fed at least <u>twice</u> daily, according to the SEP, or "to excess" according to ASTM.

o The amount of food the fish received was not reported. Since growth parameters were used as end points of the toxic effects, each control and treatment fish should have received equal amounts of food.

o Fish should not be fed for at least 24 hours prior to test termination. This report does not specify whether

food was withheld from the test fish during the last 24 hours of the test. If not, the weight measurements at test termination would not be a meaningful parameter since they would include the weight of food in the fish's gastrointestinal tracts.

o Time to swim-up was not recorded.

o The relative standard deviation (coefficient of variation) of weights of the fish that were alive at the end of the test in any control chamber must not be greater than 40% (according to the SEP). The relative standard deviations for seawater controls were 46% and 39% for seawater control A and seawater control B, respectively.

o There is a contradiction in the report. The MATC value reported as being between 43.4 and 68.0 ug/L based on hatching success in the result and summary sections, but on growth in the conclusion section.

B. **Statistical Analysis:** The reviewer reanalyzed the data using ANOVA (Tukey's and Duncan's tests) and obtained slightly different results from the author's. Dunnett's test was also used to analyze the embryo hatching and juvenile survival data (with arcsine square root transformation on the data). The results obtained were the same as those done by Tukey's and Duncan's tests. All printouts are attached. The results are summarized below:

Concentration (ug/L)	Time to Hatch (Day)	% Embryo Hatched	% Juvenile Survival	Length (mm)	Wet Wt. (mg)	Dry Wt. (mg)
Seawater Control	6.75	81.0	89.3	10.54*	30.71*	4.89*
Solvent Control	6.50	80.0	96.5	11.97	45.28	7.35
17.6	7.50	78.0	98.8	11.08*	37.97*	7.65
43.4	6.25	80.0	88.5	10.44*	34.64*	6.75
68.0	6.75	68.0	82.8	10.04*	35.44*	6.87
120.0	7.25	68.0	62.0*	10.86*	50.01	10.13*
256.0	6.50	58.0*	52.3*	8.73*	27.19*	5.44*

* Significantly different from solvent control at $p \leq 0.05$.

Results of statistical analyses were in general agreement with those done by the author, except for the embryo hatching success. Three methods (Tukey's, Duncan's, and Dunnett's) were used by the reviewer to analyze the hatching data. The results were all the same (see attached printouts), i.e., only embryo hatching at 256 ug/L was significantly lower than that in the solvent control ($p \leq 0.05$). The difference between the reviewer's analyses and the author's was probably because the author pooled the seawater control and solvent control data before comparing to those in the treatments. In the reviewer's opinion, this practice should not have been done since it is obvious from the test data that the solvent used in this study may have exerted some effects on sheepshead minnow's early life stages. ASTM specifies that "if a statistically significant difference in either survival or growth is detected between the two controls, only the solvent control may be used.....as the basis for calculation of results."

C. Discussion/Results: Since the solvent (DMF) used in this study had an apparent effect on sheepshead minnow, the comparisons should be made among the results obtained from the solutions that contained solvent (i.e., all treatments versus solvent control). Growth was the most sensitive indicator of the toxicity of Carbofuran Technical in this study. Except for the data obtained from the 120-ug/L test level, there were negative trends on growth with increasing concentrations of Carbofuran Technical. The issue whether food supply limited the growth in the seawater control could gave been avoided if the guidelines of feeding all test concentrations "to excess" had been followed (as opposed to feeding once a day).

Based on length data, all concentrations tested significantly reduced the growth of sheepshead minnow juveniles as compared to the solvent control. Therefore, the MATC value of Carbofuran Technical for sheepshead minnow was less than 17.6 ug/L mean measured concentration. The no-observed-effect level (NOEL) could not be calculated due to the adverse effect on growth (length) at all test levels.

D. Adequacy of the Study:

(1) **Classification:** Supplemental.

(2) **Rationale:** The range of concentrations tested did not include a no-observed-effect level. Therefore, a more precise MATC could not be estimated. In addition, the quality of dilution water did not meet requirements.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, November 9, 1988.

Table 3-1. Nominal and Measured FMC 10242 Technical Concentrations During an Early Life Stage Test with Sheepshead Minnow (Cyprinodon variegatus)

Nominal Concentration (ug/L; ppb)	Rep	Measured Concentration (ug/L; ppb)											
		0	3	5	7	12	16	21	24	28	31	34	35
Seawater Control	A	<10.0	--	--	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
	B	<10.0	--	--	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Solvent Control	A	<10.0	--	--	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
	B	<10.0	--	--	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
19.75	A	17.8	--	--	13.5	20.1	19.8	20.0	19.6	19.0	21.2	14.2	11.9
	B	15.7	--	--	13.8	19.6	19.5	19.2	18.6	17.6	20.6	17.3	13.7
39.5	A	41.5	--	--	34.3	47.3	47.9	48.9	47.8	47.5	53.0	42.2	30.2
	B	37.3	--	--	33.4	46.5	44.8	45.0	45.8	44.2	53.3	44.0	32.2
79	A	66.0	--	--	56.0	81.7	62.5	74.5	69.0	77.8	79.8	68.6	47.5
	B	62.4	--	--	52.1	71.0	74.7	77.4	77.1	71.0	82.7	63.2	45.4
158	A	128	--	--	102	127	126	132	125	121	135	116	81.3
	B	131	--	--	98.2	126	129	129	123	120	135	122	87.8
316	A	258	414	245	227	294	293	266	295	294	314	140	100
	B	259	--	--	218	286	293	263	295	290	300	158	122

Source: ESE, 1988.

Table 3-2. Nominal and Mean Measured FMC 10242 Technical Concentrations During an Early Life Stage Test with Sheepshead Minnow (Cyprinodon variegatus)

Nominal Concentrations (ug/L; ppb)	Rep	Measured Concentrations (ug/L) Replicate Mean (±SD*)	Measured Concentrations (ug/L) Treatment Mean (±SD)	Percent of Nominal
Seawater Control	A	ND+	ND	---
	B	ND		
Solvent Control	A	ND	ND	---
	B	ND		
19.75	A	17.7 (3.3)	17.6 (2.8)	89
	B	17.6 (2.4)		
39.5	A	43.9 (7.2)	43.4 (6.6)	110
	B	42.8 (6.4)		
79	A	68.3 (10.9)	68.0 (11.1)	86
	B	67.7 (11.9)		
158	A	119 (16.2)	120 (15.3)	76
	B	120 (15.2)		
316	A	262 (81.2)	256 (72.0)	81
	B	248 (62.7)		

SD = Standard Deviation.
ND = Not Detected; <10.0 ug/L.

Source: ESE, 1988.

Table 3-3. Hatching Success of Sheepshead Minnow (Cyprinodon variegatus) Embryos Exposed to FMC 10242 Technical

Mean Measured Concentration (ug/L; ppb)	Percentage Hatch*				
	Replicate A		Replicate B		Treatment Mean
	1	2	1	2	
Seawater Control	76	92	80	76	81
Solvent Control	80	68	84	88	80
17.6	72	84	88	68	78
43.4	76	76	88	80	80
68.0	76	64	68	64	68+
120	64	64	64	80	68+
256	60	48	72	52	58+

*25 embryos tested per screened chamber; 50 per replicate.

+Significantly ($P \geq 0.95$) reduced hatching success compared to the pooled controls.

Source: ESE, 1988.

Table 3-5. Test Day on which Hatching of Sheepshead Minnow (Cyprinodon variegatus) Embryos Exposed to FMC 10242 Technical Met or Exceeded 90 Percent Completion

Mean Measured Concentration (ug/L; ppb)	Time to Hatch (days)				
	Replicate A		Replicate B		Treatment Mean
	1	2	1	2	
Seawater Control	7	7	6	7	6.75
Solvent Control	7	7	6	6	6.5
17.6	7	7	8	8	7.5
43.4	7	6	6	6	6.25
68.0	6	5	8	8	6.75
120	8	7	7	7	7.25
256	7	6	6	7	6.5

Source: ESE, 1988.

Table 3-6. Survival of Sheepshead Minnows (Cyprinodon variegatus) Exposed to FMC 10242 Technical in a 35-Day Early Life Stage Test

Mean Measured Concentration (ug/L; ppb)	Percent Survival*				Treatment Mean
	Replicate A		Replicate B		
	1	2	1	2	
Seawater Control	95	87	80	95	89
Solvent Control	95	100	100	91	96
17.6	100	100	95	100	99
43.4	95	100	59	100	91
68.0	84	94	53	100	82
120	50	44	94	60	62+
256	47	50	50	62	52+

*Survival is calculated by dividing the number of surviving juveniles on Day 35 by the total number of embryos which hatched.

+Significantly ($P \geq 0.95$) reduced survival compared to the pooled controls.

Source: ESE, 1988.

Table 3-7. Growth, as Standard Length and Dry Weight, of Sheepshead Minnows (Cyprinodon variegatus) Exposed to FMC 10242 Technical

Mean Measured Concentration (ug/L; ppb)	N	Treatment Mean Std Length in mm (±S.D.)		Wet Weight in mg (±S.D.)		Dry Weight in mg (±S.D.)	
Seawater Control	72	10.5+	1.2	30.7+	11.9	4.9	2.2
Solvent Control	77	12.0	1.2	45.3	14.3	7.3	2.5
17.6	78	11.1+	1.3	38.0+	14.2	7.6	2.9
43.4	70	10.4+	1.3	34.6+	14.3	6.7	3.0
68.0	56	10.0+	1.5	35.6+	16.5	6.8	3.4
120	42	10.8+	1.8	50.0	27.4	10.1	6.4
256	30	8.7+	2.1	27.2+	22.1	5.4+	5.4

+Significant ($P \geq 0.95$) reduction in growth of fish when compared to the solvent control.

Source: ESE, 1988.

Carbofuran Technical

say No. _____ Chemical Name _____ Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Succession | Chemical % Active | Results | Reviewer/ Date | Validat Status

Avian Reproduction,

Species: _____

Lab:

Acc*;

Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Chg Inh.		
Control						
Treatment I						
Treatment II						
Treatment III						

Study Duration:

Comments:

Field Study(Simulated/Actual)

Species: _____

Lab:

Acc.*;

Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
Control						
Treatment I						
Treatment II						
Treatment III						

Crop/Site: Study Duration:

Comments:

Chronic fish,

Species Cyprinodon variegatus 96.9

Lab: Environmental Science & Engineering, Inc.

Acc.*; 408184-01

Concentrations Tested (ppb*)= 17.6, 43.4, 68.0, 120.0, 256.0

MATC = > — <17.6 ppb*. Effected Parameter = Growth (length)

Contr. Mort.(%)= 10.7 Sol. Contr. Mort.(%)= 3.5 PK/11-9-88 Supp

Comments:

* Mean measured Concentration

Chronic invertebrate

Species _____

Lab

Acc.*

Concentrations Tested (pp__)= _____

MATC =>____< ____pp__. Effected Parameter(s) _____

Contr. Mort.(%)=_____ Sol. Contr. Mort.(%)=_____

Comments:
