

File Copy 8-30-89

Data Evaluation Report
Ecological Effects Branch

1. Chemical Name: Cythion(Malathion)
2. Test Material: Technical grade material obtained from American Cyanamid Company, Lot No. AC-6015-136A. Percent active ingredient was 94%.
3. Study Type: 96 hour Acute Flow-through on Estuarine Fish, Cyprinodon variegatus.

4. Study Identification:

Author Bowman, Jane H.
Laboratory Analytical Biochemical Laboratories, Inc.,
Columbia, Missouri.
Study No. 37397 Study Date: 2/27/89 to 3/3/89
Submitted by American Cyanamid Company, Princeton, N.J.
Accession No. 41174301

5. Reviewed by: Brian Montague
Fishery Biologist
Ecological Effects Branch/EFED

Signature: *Brian Montague*
Date: 8/28/89

6. Approved by: Ray Matheny
Supervisory Biologist
Ecological Effects Branch/EFED

Signature: *Ray W. Matheny*
Date: 8/30/89

7. Conclusions: The study submitted by ABC Laboratories does follow most guidelines for acute testing, but has tested the fish at salinities far above normal estuarine salinity levels. This would effect pH conditions and osmotic transport rates of the fish. However, it is felt by the reviewer that this would not have changed the results significantly. At the salinity levels tested Cythion was shown to have an LC₅₀ value of ~~0.340~~ ppm placing it in a category of very highly toxic to small estuarine fish. The study is classified as core.

8. Recommendations: N/A

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9. **Study Background:** Study submitted to support re-registration action.

10. **Methods and Materials:**

A. **Test Specimens:** The test species were Sheepshead Minnows Cyprinodon variegatus obtained from Aquatic Research Organisms in Hampton, New Hampshire, Lot No. 689. They were hatched 1/23/89 and shipped 1/25/89. The quarantine period was 31 days with an acclimation period of 48 hours prior to the test. The only measurements provided were those of the control group. In this group size ranged from 7-14mm with a mean length of 11 mm. Weight range was .010-.061 gm. with mean wt. of .033 gm. Fish were fed up to 48 hours prior to test initiation on 2/27/89 and no mortality was reported for this period.

B. **Dosage:** Soft blended water was obtained by combining well water with water subjected to reverse osmosis. The hardness was 44 mg/L with T.O.C. value of under 1 ppm. This was combined with Marinemix commercial saltwater mix and trace elements were then added. The mixture was made up at an initial salinity of 28 ppt. The salt water was aerated before introduction into the test chambers. No aeration was employed in the test chambers themselves.

C. **Study Design:** The author claimed that the methods were those described by the EPA standard evaluation procedures. A diluter stock solution was prepared at 5000 mg/L (.5319 gm. of Cythion technical/100ml of dimethylformamide). A Mount and Brungs proportional diluter system provided intermittent introduction of .1 ml. of stock to 1 liter of dilution water. As the diluter system has a fixed dilution ratio of 50% the increases in test concentrations were set up with this ratio of increase. Test concentrations were based on an earlier range finding test and were set at .033, .065, .13, .25, and .50 mg/L with both dilution and vehicle controls. Later analysis of samples taken at 0 hour and at 96 hour termination yielded actual mean values of .018, .035, .074, .11, and .32 mg/L, an average deviation of 55%. A syringe dispenser delivered test solution to 28 individual test chambers. Four replicates were used per concentration and control with 5 fish per chamber. Test vessels were 1 liter beakers containing 900 ml. of solution per beaker. The beakers were covered with screen and immersed in a constant temperature water bath maintained at 22° centigrade. The test solution flow was begun 24 hours prior to the introduction of the test animals. The estimated loading factor is approximately .024 gm of fish/liter based on a mean weight of .033 gm. and a delivery rate of 250 ml. of test solution per hour for each test

vessel. Volume additions were 6.7 per day. Calibration was checked once per day. Solvent was delivered to the vehicle control test chamber at a rate of .05 ml/dose and was equivalent to that received by highest dose. A 16D/8N photoperiod was maintained. Test chambers were monitored daily for mortalities and abnormal behavior. Temperature, dissolved oxygen, pH, and salinity were monitored at 0 hour and day 4 for 14 of the chambers and at hour 48 for the other 14 chambers. Actual concentration levels were determined by gas liquid chromatography utilizing a Tracor 560 model GLS with flame photometric detector.

11. **Reported Test Results:** After 96 hours there were no mortalities in the lowest concentration or the controls. 35% mortality was recorded in the .035 concentration and 100% mortality in the 3 highest concentrations. The greatest percentage of the mortality occurred within the first 24 hours. No toxic signs were noted so it is assumed death occurs rapidly with few indicative effects. The following table is a partial summarization of data presented by ABC laboratories.

Nom. Concent>	Contr.	Veh. Contr.	.033	.065	.130	.250	.500mg/l
Actual Conc*.	0	0	.018	.035	.074	.110	.320mg/l
% of Nominal			55%	54%	57%	44%	64%
Total Variation			.08	.011	.022	.059	.16
Mortality 24 hr	0	0	0	25%	100%	100%	100%
96 hr	0	0	0	35%	100%	100%	100%
Water Quality							
Salinity	28-30	29	29-32	28-29	29	28	29
D.O. 96hr.	6.5	6.5	6.3	6.3			
pH (var.=.1)	8.1	8.1	8.2	8.2	8.2	8.2	8.2
Temp. avg.	22°C	23	22	22	22	23	22

* Based on mean concentration values

12. **Study Author's Conclusion:** " From the data collected during the study, the 24-, 48-, 72, and 96-hour LC₅₀ values were 0.043, 0.043, 0.040, and 0.040 mg/l, respectively, based on the average measured concentrations at 0- and 96-hours (table 5). The slope of the 96-hour dose-response line (figure 1) was 7.7 as calculated by least squares regression analysis. Mortality was the only effect noted during the study in the 0.035, 0.074, 0.11, and 0.32 mg/l test concentrations. Based on this the no-effect concentration of Cythion Technical toxicity to sheephead minnow was determined to be 0.018 mg/l."

13. Reviewer's Discussion

A. Test Procedure Though protocol does broadly follow EPA and ASTM test procedure guidelines, some deviations were noted which may have had an effect on the reported test results. These include the following criteria.

1. Testing was conducted at higher salinities than recommended. Though this may not have had adverse effect on the organisms themselves, it may have had some effect on the chemical properties of the ingredient and may not accurately reflect it's behavior at salinities equivalent to normal estuarine levels. Degradation rates of Cythion increase with increases in alkalinity, pH, temperature, and salinity. Guidelines suggest a salinity of 17 ppt and pH of 7.7 for euryhaline species.

2. Test vessels were much smaller than the recommended guidelines. However due to the use of four replicate chambers per test concentration and the small size of the test fish, loading was within acceptable levels. This is based on the size measurements provided for the controls which were the only measurements provided. It is assumed that other test animals were comparable in size as all fish were the same age and from the same lot.

3. Observations of water parameters were made on one set of test vessels at 0 and 96 hours and another set of test vessels at 48 hours. This does not allow for a continuous record in the first set of vessels. Temperature should be monitored at 6 hour intervals for continuous flow water baths instead of the 24 hour interval used in this study. This is particularly important when the test ingredient is known to be temperature sensitive. Temperature should be measured in all test vessels at initiation and termination of the test.

4. Observations of the test animals were recorded every 24 hours. No observations other than mortality were noted however. In previous studies (Murphy, 1967) a number of toxic signs were exhibited including uncoordinated movements, swimming on sides, air searching, cessation of gill movement, and finally, involuntary extension of the pectoral fins. None of these signs were noted in the A.B.C. study.

5. Variability between nominal and measured concentration levels was higher than would be expected and ranged between 32% and 80% of the nominal concentration with a mean

variability of 55%. Though they do exceed accepted levels of variability the chemical is known to demonstrate increased solubility at higher temperatures. However, excessive variability can also indicate excessive hydrolysis, oxidation, photolysis, during the experiment leading to excessive degradation of the solution in the head chamber. Excessive storage time or improper storage temperatures might also lead to premature degradation of the chemical. Laboratory records indicated that the chemical was stored 6 months prior to the test at room temperature.

B. Statistical Analysis Two runs were made on EPA's toxanol program to reflect LC₅₀ values for initial actual measured concentrations and for those at termination. The initial LC₅₀ value was determined to be .0457 ppm with a 95% confidence limits of .022 and .085ppm. Using the 96 hour measured concentration values yielded LC₅₀ value of .0332 ppm with C.L. of .014 and .063. This LC₅₀ range compares favorably with the result based on the mean average measured concentrations which was .040 mg/l.

C. Discussion of Results A 96 hour LD₅₀ value of .040 mg/L would place Cythion in the classification of very highly toxic to Cyprinodon variegatus at salinities of 28-32 ppt and a temperature of 21° C.

Adequacy of the Study

Classification: Core

Rationale: Study does provide the necessary information needed to determine an LC₅₀ value for estuarine fish.

Repairability N/A

14. Completion of One Liner: One liner completed on August 23, 1989.

Montague Cythion(Malathion) Acute Tox Estuarine Fish Cyprinodon v.

MEASURED
CONC. NUMBER NUMBER PERCENT BINOMIAL
0 hour EXPOSED DEAD DEAD PROB. (PERCENT)

.4	20	20	100	9.536742E-05
.13	20	20	100	9.536742E-05
.085	20	20	100	9.536742E-05
.04	20	7	35	13.1588
.022	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .022 AND .085 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 .0457023

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

.014	20	0	0	9.536742E-05
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THE BINOMIAL TEST SHOWS THAT .014 AND .063 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 3.326368E-02

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

meas. Conc.

96 hour

.24	20	20	100	9.536742E-05
.081	20	20	100	9.536742E-05
.063	20	20	100	9.536742E-05
.029	20	7	35	13.1588
.014	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .014 AND .063 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 3.326368E-02 = 0.033

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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