DATA EVALUATION RECORD

1. **CHEMICAL:** Malathion.
   Shaughnessey Number: 057701.

2. **TEST MATERIAL:** Cythion® Technical (Malathion); Lot No. AC-6015-136A; 94% active ingredient; a yellow liquid.

3. **STUDY TYPE:** Fish early life stage toxicity test.
   Species Tested: Rainbow trout (Oncorhynchus mykiss, previously Salmo gairdneri).

4. **CITATION:** Cohle, P. 1989. Early Life Stage Toxicity of Cythion® to Rainbow Trout (Oncorhynchus mykiss) in a Flow-Through System. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Report No. 37400. Submitted by American Cyanamid Company, Princeton, New Jersey. MRID No. 414224-01.

5. **REVIEWED BY:**

   Kimberly Rhodes
   Associate Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Kimberly Rhodes
   Date: June 19, 1990

6. **APPROVED BY:**

   Pim Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: 6/19/90

   Henry T. Craven
   Supervisor, EEB/HED
   USEPA

   Signature: 1/30/91
   Date: Henry T. Craven 1/30/91

7. **CONCLUSIONS:** This study appears to be scientifically sound and fulfills the guideline requirements for a fish early life stage toxicity test. The maximum acceptable toxicant concentration (MATC) of Cythion for rainbow trout (Oncorhynchus mykiss) embryos and larvae was determined to be between 21 µg/L and 44 µg/L mean measured concentration (geometric mean MATC = 30.4 µg/L).

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Unfertilized rainbow trout (Oncorhynchus mykiss, previously Salmo gairdneri) eggs and milt were obtained from a commercial supplier in California. Upon receipt, the eggs were slowly acclimated from approximately 7°C to 8°C without allowing contact with water. After the unfertilized eggs and milt reached 8°C, the eggs were gently poured into a dry plastic pan and the milt was thoroughly mixed with the eggs. After the addition of the milt, enough control water to cover the eggs was added and gently stirred to ensure maximum fertilization. Approximately 60 seconds after mixing, the eggs were rinsed with control water several times then covered again with water and allowed to water harden for 1.5 hours before distribution to the test system incubation cups.

B. **Test System:** A two-liter proportional diluter system described by Mount and Brungs (1967), utilizing a syringe dispenser, was used for the intermittent introduction of an aqueous solution of Cythion to four replicate test chambers per concentration. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. For the first 90 days of the 97 day study, test solution was delivered to the 12 liter replicate chambers at an average rate of approximately 103 L/replicate/day. The flow rate was increased during the last week to approximately 137 L/replicate/day as a precaution against the increased oxygen demand that larger fry place on the test water.

The inside dimensions of the glass test aquaria measured approximately 15.6 x 30.7 centimeters (cm) with a water depth of approximately 25 cm, yielding an approximate 12-liter replicate-chamber volume. Each replicate test aquarium drain was covered with a 16-mesh stainless steel screen to prevent escape of the rainbow trout fry. The rainbow trout eggs were incubated in cups suspended in the treatment and control water. These egg incubation cups were made from 9.0-cm diameter x 14-cm high glass tubing with 16-mesh Nytex® screening attached to the bottom with silicone sealant.

The test chambers were immersed in a water bath held at approximately 10°C by refrigeration units. After the embryos had hatched into fry, the aquaria were illuminated by incandescent and wide spectrum fluorescent bulbs during a 16-hour daylight photoperiod. The average light intensity was 131 ± 26 foot-candles at the water surface. The lights were located approximately one meter above the surface of the water in the test chambers.

The dilution water was obtained from uncontaminated deep well water, part of which was passed through a reverse osmosis system, and then blended back with well water to obtain a total hardness of approximately 40 to 50 mg/L as $CaCO_3$ and a pH of 7.8.

C. **Dosage:** 97-day flow-through early life stage test.

D. **Design:** Based on the results of two preliminary range-finding tests a control, solvent control and five nominal Cythion concentrations of 5, 10, 20, 40, and 80 µg/L based on active ingredient were chosen for testing.

The definitive study was initiated by impartially selecting and distributing 35 newly fertilized rainbow trout eggs per incubator cup for a total of 140 eggs per toxicant concentration, control, and solvent control. The eggs were fertilized less than eight hours before test initiation. In addition, 50 eggs were placed in separate incubator cups in each of 4 replicates of the control chambers for determining viability. Egg mortality, as discerned by a distinct change in coloration, was recorded daily and dead eggs were removed to prevent fungal growth.

After 11-days of exposure, the eggs reserved for viability (fertilization success) determination were removed and placed in a 10% glacial acetic acid solution. After several minutes in the solution, the embryos became clear. Fertilization and embryo development were indicated by the presence of a neural keel, which was visible as a white line.

When hatching commenced, the number of eggs hatched in each incubation cup was recorded daily until day 39. Hatch was determined to be complete on this day since no viable embryos (capable of hatch) remained in any test chamber. The number of larval fry was reduced to 15 per replicate on day 39. Since ≥95% hatch had

occurred by day 37 of the study, this day became day 0 for the 60 day post-hatch growth period.

The fry were released from the incubation cups into the growth chambers on study day 46 (9 days post-hatch). Abnormal (sublethal) behavioral or physical changes and mortality were monitored by visually inspecting each growth chamber daily and recording the data. Survival data were collected for statistical analysis on both growth measurement days (days 37 and 60 post-hatch). Feeding also began on study day 46. Initially, the fry were fed live brine shrimp nauplii. Ground salmon starter was added to their diet on day 54. The fish were generally fed 3 times per day.

Growth, as determined by standard length of the fry, was determined by the photographic method of McKim and Benoit (1971) on study day 74. At test termination, study day 97 (60 days post-hatch), all surviving fish were measured for standard length and wet weight.

Water quality parameters of temperature, dissolved oxygen concentration, conductivity, and pH were measured on days 0, 1, 7 and on every 7th day thereafter until test termination. Temperature and dissolved oxygen concentration were measured in one replicate of each concentration on the designated sample days. Temperature was also monitored continuously with a data logger. Conductivity and pH were measured in a single replicate of the control, low, and high test concentration. Hardness and alkalinity were measured from 50 mL water samples taken from the control, low and highest level according to the same schedule as the other parameters with the exception of day 1. The measured concentrations of Cythion in test water were determined on days 0, 1, 7, 14 and on every 7th day thereafter up to and including day 97, which was study termination day. Concentrations of Cythion Technical were measured by use of gas liquid chromatography.

E. **Statistics:** Comparison analyses between the control, solvent control, and five test levels were carried out using the measured parameters of hatchability, survival, standard length and wet weight. The statistical data were analyzed by a Systat® computer program (1985) and by a Toxstat program (1988).

Comparison analyses between the control and solvent control were conducted to determine possible statistical differences. If no significant differences were noted, only the control was used for statistical comparisons.

Dichotomous data (e.g., hatch or survival) were analyzed by using 2 X 2 contingency tables (pairing control to each exposure level). The data then underwent arcsine transformation prior to an ANOVA. Dunnett's, Williams and Tukey's mean comparison tests were performed to determine if significant differences between control and treatment levels existed. All differences were considered significant at the 95% confidence level.

Continuous data (e.g., length and weight) were also analyzed by ANOVA. Tukey's HSD mean comparison test was used to determine which exposure levels differed from the control values. Homogeneity of group variance was analyzed using Bartlett's test. All differences were considered significant at the 95% confidence level.

12. **REPORTED RESULTS:** The mean measured concentrations of Cythion were 5.1, 9.9, 21, 44, and 84 µg/L (Table 4, attached). The mean measured concentrations ranged from 99 to 110% of the nominal test concentrations.

Hatch began on day 29 of the study and continued until day 39 (Table 9, attached). Hatch was ≥99% complete by day 37 in all test concentrations and controls. A dose-response relationship with regard to time to hatch was not indicated.

Newly hatched fry began swimming up from the bottom of the test chambers at 8 days post-hatch (day 45). The number of fry swimming up in each chamber was recorded for days 45-56. All fish in the highest mean measured concentration (84 µg/L) and several of the fish in the 44 µg/L mean measured test level never swam up during this period.

Sublethal physical and behavioral effects noted in the fish during the study included fish on the bottom of the test chamber, quiescence, abnormal discoloration, exophthalmia, spinal curvature, distended abdomen, loss of equilibrium, erratic swimming, swimming vertically, surfacing and irregular respiration (usually consisting of exaggerated opercular movement during respiration). One fish was also noted as having a white substance protruding from the gills. This appeared to be a transient effect noted on two days

during the study. Sublethal effects noted in the fish were persistent and appeared to be part of a dose-response pattern in the two highest test concentrations. The most notable effects in these two treatment levels were fish resting on the bottom of the chambers, discoloration, spinal curvature and quiescence.

Egg viability was determined from a viability test that indicated mean viability to be 99%. Therefore, hatch calculations were based upon 35 viable eggs per replicate or 140 viable eggs per test level at initiation. Percent hatch in the control, solvent control and the five test concentrations was 94, 94, 86, 86, 85, 89, and 86%, respectively (Table 10, attached). The contingency table analysis indicated that hatch in the 5.1, 9.9, 21, and 84 µg/L test levels were significantly reduced when compared to the control. Hatch in the 44 µg/L test concentration was not significantly reduced. A dose response to the test compound had not been demonstrated since a pattern of decreasing hatch with increasing concentration was not indicated. The data were also analyzed using Dunnett's, William's, and Tukey's mean comparison tests. According to these tests, hatch was not significantly reduced in any test level.

The survival of trout fry continuously exposed to Cythion during the 60-day post-hatch growth period is shown in Table 10 (attached). Analysis of the 37-day post-hatch data indicated that fry survival in the exposure aquaria was significantly reduced in the two highest test concentrations (44 and 84 µg/L) when compared to the control. At 60 days post-hatch, survival was still reduced in the two highest test concentrations with no additional concentrations showing significant effects. Control fry survival was 83% on day 37 and 82% on day 60. Survival in the highest test concentration (84 µg/L) was 0% on both days 37 and 60 days post-hatch, while survival in the 44 µg/L concentration was 50% at 37 days post-hatch, and 43% at day 60 post-hatch.

Results for the effect of Cythion on length of rainbow trout are shown in Table 10 (attached). Statistical analysis for day 37 shows that only fish in the 44 µg/L experienced a significant reduction ($P \leq 0.05$) in length when compared to the control. However, by day 60 post-hatch, analysis indicated no growth effects (length or weight) in any of the test concentrations still containing fish.

During the test, the water quality parameters were characterized as having a dissolved oxygen concentration range of 7.1 to 10.2 mg/L (66 and 90% of saturation at 10°C and 12°C, respectively), a temperature range of 9.7 to 13.2°C, a pH range of 7.4 to 8.1, a conductivity range of 66 to 122 µmhos, an alkalinity range of 32 to 60 mg/L as $CaCO_3$, and a total hardness range of 22 to 52 mg/L as $CaCO_3$.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The Maximum Acceptable Toxicant Concentration (MATC) limits for Cythion based on the results of this study are estimated to be the mean concentrations of 21 µg/L (no-observed-effect concentration or NOEC) and 44 µg/L (lowest observed effect concentration or LOEC). The point estimate MATC value (defined as the geometric mean of the LOEC and the NOEC) is 30.4 µg/L Cythion.

A GLP compliance statement was included in the report and the study was audited by a QA unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (40 CFR 160).

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP recommends that well water with a hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 be used as the dilution water. The dilution water used for the toxicity test was well water with a total hardness range of 22 to 52 mg/L as $CaCO_3$ and a pH range of 7.4 to 8.1.

o The SEP recommends a light intensity of 37-74 footcandles. This test used 131 footcandles.

o The SEP states that embryo incubation cups can be oscillated in the test water or placed in separate chambers with self-starting siphons to insure adequate exchange of water and test material. During this test, the rocker arm motor was not turned on at any time during the incubation period.

o The concentration of solvent in the solvent control was not reported.

o The SEP recommends a temperature of 10 ± 2°C. With the exception of day 84, all temperature deviations were higher than the test range, the highest being 13.6°C. On day 84, a temperature of 7.8°C was recorded.

B. **Statistical Analysis:** The reviewer evaluated embryo hatchability and larval survival following an arcsine square-root transformation of the data. The growth data, standard length and wet weight, were statistically evaluated by ANOVA without any transformations. All printouts are attached.

The reviewer confirmed that hatchability of rainbow trout embryos was not significantly reduced in any test concentration when compared to the control or solvent control.

All fish were dead in the highest test concentration at test termination (60 days post-hatch). Therefore, the reviewer transformed 0% survival in the highest test concentration by the method recommended by EPA (1988). The reviewer confirmed that survival was significantly affected in the two highest mean measured test concentrations (44 and 84 µg/L) when compared to the control or solvent control using ANOVA followed by Tukey's multiple comparison test.

The author found no significant difference in length and weight of rainbow trout fry when compared to the control at 60 days post-hatch. The reviewer analyzed the growth data by comparing the treated fish to the solvent control fish, by using ANOVA followed by Tukey's and Bonferroni's multiple comparison tests. The highest test concentration was excluded from analysis since all fish were dead at test termination.

The reviewer found a significant difference (P=0.01) in length of rainbow trout fry in the 44 µg/L mean measured concentration when compared to the solvent control. The reviewer found a significant difference in weight of rainbow trout fry in the 9.9 µg/L and the 44 µg/L mean measured test concentrations. The reduction of weight in the 9.9 µg/L test concentration is probably not dose related since the next highest test concentration (21 µg/L) did not show a significant reduction in weight.

C. **Discussion/Results**: The study results appear scientifically valid. Minor deviations from the recommended protocols probably did not significantly affect the toxicity results. The maximum acceptable toxicant concentration (MATC) of Cythion for rainbow trout (Oncorhynchus mykiss) embryos and larvae was determined to be between 21 µg/L and 44 µg/L mean measured concentration.

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 06-06-90.

16. **REFERENCES:**

U.S. Environmental Protection Agency. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA/600/4-87/028. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, May 1988.

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