Data Evaluation Report
Ecological Effects Branch

1. **Chemical:** Azinphos methyl, Guthion 05-8001

2. **Test Material:** Technical grade, 88.8% purity, Lot # 79-R-225-42 received from Mobay Corp. on 4/9/87.

3. **Study Type:** Early life stage toxicity- rainbow trout

4. **Study Identification:**

   Study Author: Suprenant, Donald
   Laboratory: Springborn Life Sciences,Inc. Wareham, Mass.
   Study Date: July 9- October 2,1987
   Study No.: 274.0587.6150.121
   Submitted by: Mobay Corporation, Agricultural Chemical Div.
   Accession No. 40579-01

5. **Reviewed by:** Brian Montague, Fisheries Biologist Brian Montague
   Ecological Effects Branch
   Environmental Fate and Effects Division(H7507C)

6. **Approved by:** Ray Matheny, Supervisory Biologist Ray Matheny 1/8/90
   Ecological Effects Branch, Section I
   Environmental Fate and Effects Division

7. **Conclusions:** The study has adequately followed protocol guidelines for early life stage testing on rainbow trout. The failure of the registrant to provide the original raw data on which the laboratory has based its statistical analysis has made it impossible for the Agency to conduct independent statistical analysis of the results. MATC values reported by the laboratory are > 0.44 µg/L and < 0.98 µg/L with a NOEL estimated to be 0.23 µg/L.

8. **Recommendations:** In order to classify the study as fully acceptable the original raw data for the study must be provided to the Agency.

9. **Study Background:** Submitted to fulfill reregistration requirements dated 9/86.

10. **Methods and Materials:**

Test Specimens: Unfertilized eggs and sperm were individually packaged, refrigerated, and shipped from Mt. Lassen Trout Farm in Red Bluffs, Ca. on 7/8/87 and received by Springborn on 7/9/87. Temperature was 11°C at the time of receipt. One half the sperm were added to a stainless steel bowl and the eggs were then placed with them in a small amount of the dilution water. The mixture was swirled and the remaining sperm were then added to the bowl and allowed to stand for two minutes. Eggs were then rinsed and allowed to harden 45 minutes prior to the definitive test initiation.

Dosage: Stock solutions were prepared by dissolving 63 mg of 88.8% azinphos-methyl to 100ml of acetone. This yielded a stock solution of 0.56 mg ai/ml or 560 ppm. Ten ml. of this solution was then diluted to 100 ml with acetone resulting in a final stock solution of 56 µg/ml. Dilution water used in the test was a combination of Springborn's own wellwater combined with Wareham City's wellwater. This was held in a epoxy coated concrete reservoir. Weekly measurements yielded hardness values of 26-36 mg $CaCO_3$ /L and an alkalinity of 27-41 mg/L. pH ranged from 7.0- 7.1 and conductivity was 180 micro-ohms/cm.

Study Design: A modified proportional diluter similar to that described by Mount and Brungs was selected for introduction of test solution into the individual test aquaria during the 85 day exposure period. The solution was introduced via polyethylene tubing leading to a 50 ml. syringe equipped with a stainless steel needle. The 56 µg a.i./ml. stock solution was introduced into the mixing chamber at a ratio of .0348 ml to 1.960 L of dilution water to yield a 1 ppb solution which was also the highest test concentration utilized. This was subsequently reduced in 50% increments by the proportional diluter to yield the remaining 4 test concentrations. The test concentrations selected were based on 2 preliminary tests on juvenile trout with the same technical grade material. The first preliminary test yielded 60% mortality at 2.5 ppb and no mortality below 1.3 ppb. The second preliminary test was conducted for 17 days and yielded no mortality at nominal concentrations between .063 and 1.0 ppb. Some loss of equilibrium was observed at the 1 ppb concentration. The fertilized eggs for the definitive test were initiated 3 hours after fertilization. Fifty eggs were placed in each glass incubation jar which was covered by Nitex screen. Two incubation jars were placed in each of the 14 test aquaria and oscillated by use of a rocker arm apparatus as described by Mount, 1968. The test aquaria were maintained at 11 L of

volume and test water was introduced to the aquaria at 76 liters/day, thus providing 6.9 volume additions per 24 hour period. A lighting regime of 25-70 foot candles was provided 16 hours per day. Test aquaria were placed impartially in a recirculating water bath which maintained the temperature at 12^{0} C$\pm 1^{0}$. Dead eggs, or those displaying opaque white coloration, were removed after day 12 and placed in Stockard solution to allow later determination of the developmental stage prior to mortality.

On day 19 twenty live viable embryos were randomly selected to remain in each of the incubation jars and these were placed back into their corresponding aquaria. When 5 or less viable embryos remained hatching was deemed complete(day 25). The 60 day post larval phase began with the release of the successfully hatched larvae into their respective test aquaria. After reaching swim-up stage(10 to 11 days post hatch) the larvae were fed brine shrimp larvae 2-3 times daily. Excess food and fecal matter were removed as needed. Daily observation of the larvae was made and survival estimates were made twice per week. At 60 days post hatch the larvae were anesthetized and the percent survival, mean total length, and mean weight determined. Larvae were measured and weighed individually according to the author.

Dissolved oxygen, pH, and temperature were measured daily for alternate test replicate aquaria at each test concentration. Thus, each replicate was measured every 48 hours for these parameters. Water bath temperature was continuously monitored. Hardness was measured once per week in alternating replicates of the high and low concentrations.

Initially samples were taken and analyzed to determine accuracy of the diluter apparatus and acceptability of the stock solutions. Definitive measured concentrations were removed on day 0 and every 6-8 days thereafter by volumetric pipette from mid-level in the test vessels.

Statistical Analysis: Data analysis on embryo viability, embryo survival at hatch, and larval growth and survival were statistically analyzed using mean organism response in each replicate aquarium rather than individual organism response values. Survival and viability data were transformed utilizing angular transformation before analysis. One way single classification ANOVA was used to compare controls to solvent controls. Williams test was used to compare the response of organisms at each dose level. Survival data were analyzed before larval weight and length measurements. Dose levels displaying significant mortality were excluded from analysis of larval growth. Assumption of homogeneity of variance implicit in ANOVA and Williams tests were checked by use of Bartletts test(Steel and Tory).

11. **Reported Test Results:** The data which has been submitted to the Agency concerning water quality parameters consists of mean average values derived from the raw data for D.O., pH, temperature, and hardness. Based on the data provided,

overall water quality parameters appeared to remain consistent and within acceptable guidelines for the majority of the study period. An exception to this occurred approximately 74 days into the study due to a failure of the chilling system which resulted in a 7-8°C temperature rise during an 18-24 hour period. No apparent mortality occurred as a result of this fluctuation as larval survival averaged over 80% and showed no decrease at this time. A blockage of the flow splitter leading to replicate B of the highest concentration may have been the factor leading to 100% mortality of the larvae in this test vessel. It is suspected that a combination of toxicant effect, low oxygen levels, and stress from the temperature variation all contributed to the mortality. Replicate A in the same concentration level did not suffer any unexpected mortality or loss of flow during this period. Mean measured concentrations of the test substance remained fairly consistent throughout the study. During the pre-hatch period from day 0 to day 15 concentrations measured 4-38%(based on the average of the 3 mean values for days 0, 8, and 15)above nominal concentrations. Exceptions were a 48% decrease in the .25 ppb level occurring between days 8 and 15 in one replicate and a 59% drop in measured level which occurred at the .063 ppb concentration between days 8 and 15 in one replicate. Springborn felt that this was partially due to the limitations of the gas chromatographic analysis in obtaining accurate concentration measurements and subsequently switched to a more accurate HPLC method for azinphos-methyl determination for the post hatch phase of the study. The measured concentration values obtained during the 60 day post hatch periods appeared more consistent, ranging from 12% below to 25% above nominal concentration levels. The theoretical limitation of detection for the analytical method employed was .0144 ppb. Values obtained for the control and solvent controls were less than .031 ppb with the exception of one measurement of .044 ppb on day 49 for the solvent control. Later measurement did yield a lower value.

Based on the measured concentration values the laboratory has prepared a table showing the effects on embryo viability, survival at hatch, larval survival, mean total length and mean wet weight percentages. The raw data on which these percentages are based has not been provided to the Agency. Standard deviation from the mean has been provided for weight and length measurements. Larval post hatch survival was noticeably affected at the highest measured concentration level(0.98 ppb) with 50% mortality in replicate A. A notable reduction of 29% in mean total length and 42% in mean total weight occurred in replicate A at the 0.98 ppb level in comparison to the control values. All other concentration levels compared closely to the growth and survival parameters which were obtained in the control and solvent control test vessels.

12. **Study Author's Conclusion:** "Exposure to all concentrations of azinphos-methyl tested(0.051-0.98 µg/L) did not adversely

effect embryo viability which ranged from 67 to 72%. . . Similarly, the survival of rainbow trout at the completion of the hatching period was not adversely affected and ranged from 84-96% . . . By exposure day 41(16 days post-hatch) nearly all exposed larvae in all treatment levels $\leq$ 0.44 µg/L azinphos-methyl and the controls completed their development to the swim-up stage.. . .in the high concentration of 0.98 µg/L, the ability of larvae to reach this developmental stage was retarded and larvae were beginning to exhibit a loss of equilibrium.. . . By exposure day 70 (45 days post-hatch) mean larval survival at 0.98 µg/L was 60% and statistically reduced when compared to the survival of the control larvae. . . Using the most sensitive objective criterion of effect(larval survival at test termination), the Maximum Acceptable Toxicant Concentration(MATC) of azinphos-methyl is estimated to be > 0.44 µg/L and < 0.98 µg/L(geometric mean MATC=0.66 µg/L). Based on subjective behavioral response, i.e., lethargy, the No Observed Effect Concentration(NOEC) is estimated to be the tested concentration of 0.23 µg/L."

13. **Reviewers Discussion:** The study has followed ASTM and EPA recommended guidelines for early life stage testing on freshwater fish in most instances. Some deviations from recommended procedures include:

1. Optimal suggested temperature for conductance of salmonid early life stage testing is 10°C not 12°C as used by Springborn.

2. Developing salmonid embryos should be kept in darkness or very dim lighting below 20 foot candles in intensity. The laboratory maintained a 16 hour lighting regime using 25-70 foot candle intensity.

3. The 7-8°C temperature increase experienced near the end of the testing period is a clear departure from acceptable protocol.

4. Failure of one of the dilution splitters near the end of the test nullifies the usefulness of any of the data concerning this replicate chamber collected after the splitter failure. Data collected prior to the failure, however, is useable. The use of the mortality for this vessel in determining mean values for survival is not recommended.

5. Though several instances of excessive variability in measured concentrations occurred during the study it is felt that these may have been errors in sampling or measurement as the subsequent measurements failed to show significant variation from the mean values for the corresponding concentration level.

Statistical Analysis: The Agency was not provided with the necessary raw data to perform comparative statistical analysis. Specifically the actual laboratory records

providing length and weight measurements on each larvae are not provided. Raw data listing daily temperature,D.O.,pH and hardness levels has not been provided. Actual embryo and larval observation records and mortality counts are not provided. Percentages representing this data do not represent adequate information nor do mean averages which do not provide the reviewer with data to assess extremes of variability or their duration which may have occurred during the study.

Adequacy of the Study:

Classification: Supplemental

Rationale: The registrant has not provided the Agency with the raw data generated during the study needed to make a complete assessment of the reported results.

Recommendations: Mobay must submit the raw data mentioned under the Reviewers Discussion above.

14. One Liner: N/A

Table 1. Water quality determinations made during the early life exposure of rainbow trout (Salmo gairdneri) to technical grade azinphos-methyl.

Nominal Conc. (µg/L)	Mean Dissolved Oxygen (mg/L)	Mean Temperature (°C)	Mean Total Hardness (mg/L as $CaCO_3$)	pH Range
1.0	8.3 (1.1)[a]	13 (0.91)	32 (1.3)	6.6-7.7
0.50	8.1 (1.3)	13 (0.93)	----	6.7-7.7
0.25	8.3 (1.2)	13 (0.94)	----	6.7-7.7
0.12	8.5 (1.0)	13 (0.95)	----	6.7-7.7
0.062	8.6 (0.91)	13 (0.90)	32 (2.9)	6.7-7.7
Solvent Control	8.1 (1.4)	13 (0.90)	32 (1.9)	6.6-7.7
Control	8.9 (0.75)	13 (0.89)	32 (1.9)	6.7-7.8

[a]Values presented in () = standard deviation.

Table 2. Results of the measurement of azinphos-methyl by a gas chromatographic procedure in the test aquaria for the rainbow trout (Salmo gairdneri) early life stage exposure.

Nominal Concentration (µg/L)	Pretest (µg/L)	Day 0 (µg/L)	Day 8 (µg/L)	Day 15[a] (µg/L)
1.0	0.93 0.96	1.1 1.1	1.1 1.4	1.4 1.3
0.50		0.53 0.51	0.54 0.66	0.49 0.70
0.25	0.20 0.36	0.42 0.48	0.43 0.48	0.3[illegible] 0.25
0.12		0.12 0.16	0.17 0.23	0.097 0.18
0.063	0.056 0.026	0.094 0.12	0.064 0.11	0.057 0.068
Solvent Control	<0.016 <0.016	<0.016 <0.016	<0.016 <0.016	<0.016 <0.016
Control	<0.016 <0.016	<0.016 <0.016	<0.016 <0.016	<0.016 <0.016

[a]Measurements incorporated into calculation of mean measured test concentrations.

Table 3. Measured concentrations of azinphos-methyl during the 85 day exposure (60 days post hatch exposure) of rainbow trout (Salmo gairdneri) early life stage study.

	NOMINAL CONCENTRATIONS (µg/L)							
	1.0	0.50	0.25	0.12	0.062	CONTROL	SOLVENT CONTROL	QA
Day 22	0.88[a]	0.14	0.21	0.089	0.061	<0.030	<0.030	0.225 (0.200)[b]
	0.81	0.37	0.25	0.12	0.043	<0.030	<0.030	0.334 (0.400)
Day 29/4[c]	1.0	0.46	0.26	0.11	0.050	<0.029	<0.029	0.193 (0.200)
	0.99	0.48	0.23	0.12	<0.029[d]	<0.029	<0.029	0.824 (0.800)
Day 36/11	1.4	0.56	0.29	0.17	0.058	<0.019	<0.019	0.629 (0.800)
	1.3	0.60	0.25	0.15	0.055	<0.019	<0.019	0.289 (0.300)
Day 42/17	0.93	0.43	0.19	0.21	0.045	<0.030	<0.030	0.381 (0.900)
	0.67	0.29	0.26	0.19	0.039	lost	0.031	0.445 (0.500)
Day 49/24	1.0	0.50	0.25	0.16	0.067	<0.031	0.044	0.598 (0.600)
	1.0	0.39	0.26	0.16	0.061	<0.031	<0.031	0.221 (0.300)
Day 56/31[e]	1.1	0.55	0.21	0.81	0.072	<0.031	<0.031	0.676 (1.00)
	1.1	0.53	0.27	0.16	0.074	<0.031	<0.031	0.173 (0.250)
Day 63/38	0.92	0.42	0.21	<0.050[d]	0.067	<0.030	<0.030	1.68 (2.00)
	0.93	0.43	0.20	0.15	0.051	<0.030	<0.030	0.460 (0.500)
Day 71/46	0.91	0.43	0.20	0.12	0.037	<0.030	<0.030	0.896 (1.00)
	0.91	0.45	0.20	0.11	0.046	<0.030	<0.030	0.370 (0.400)
Day 77/52	0.87	0.47	0.22	0.11	0.044	<0.030	<0.030	1.72 (2.00)
	0.93	0.44	0.22	0.11	0.043	<0.030	<0.030	0.506 (0.500)
Day 85/60	0.84	0.42	0.17	0.11	0.033	<0.030	<0.030	1.32 (1.60)
	0.60	0.42	0.15	0.10	0.048	<0.030	<0.030	0.274 (0.400)

[a]Values presented for each interval represent the individual measurement of the A and B replicate exposure solutions.

[b]Values presented in parentheses = the nominal fortified concentration.

[c]Test day/# days of post-hatch exposure.

[d]These values are excluded from the mean measured concentration.

[e]The results of the analyses of the QA sample for this sampling interval did not meet the standard acceptable criteria established at SLS. Therefore, all measured concentrations established for this time interval were reported but not used to calculate the mean measured concentration.

Table 5. Embryo viability, survival of embryos at hatch and survival, total length, and wet weight of rainbow trout (Salmo gairdneri) exposed to azinphos-methyl (A.I.) for 85 days (60 days post-hatch).

Mean Measured Concentration (µg/L)		Embryo Viability (%)	Survival of Organisms at Hatch (%)	Larvae (60 days post-hatch) Larvae Survival (%)	Mean Total Length (cm)	Mean Wet Weight (g)
0.98	A	76	96	50	40 (5.4)[b]	0.9422 (0.3130)
	B	65	95	0	0 (-)	0.0000 (-)
	Mean	71	96	25[a]	40 (5.4)	0.9422 (0.3130)
0.44	A	73	96	85	56 (3.3)	1.6548 (0.3330)
	B	71	79	75	56 (5.5)	1.7837 (0.3655)
	Mean	72	87	80	56 (4.4)	1.7152 (0.3490)
0.23	A	73	90	95	57 (2.1)	1.6999 (0.2365)
	B	71	79	90	56 (3.1)	1.6839 (0.2722)
	Mean	72	85	93	56 (2.6)	1.6922 (0.2510)
0.14	A	74	92	90	56 (4.9)	1.6221 (0.3590)
	B	63	84	70	57 (2.6)	1.8150 (0.3068)
	Mean	69	88	80	56 (4.1)	1.7185 (0.3458)
0.051	A	67	88	95	55 (3.5)	1.5318 (0.3204)
	B	72	94	80	56 (2.8)	1.5783 (0.2700)
	Mean	70	91	88	55 (3.2)	1.5531 (0.2950)
Solvent Control	A	75	92	90	56 (3.0)	1.6068 (0.3106)
	B	68	75	90	57 (3.6)	1.8466 (0.3583)
	Mean	72	84	90	56 (3.3)	1.7267 (0.3521)
Control	A	67	90	90	56 (2.9)	1.6341 (0.2728)
	B	67	91	90	55 (3.4)	1.5493 (0.2822)
	Mean	67	90	90	56 (3.2)	1.5917 (0.2769)
Pooled Controls		69	84	90	56 (3.2)	1.6592 (0.32)

[a]Statistically ($P \leq 0.05$) and biologically significantly different from the pooled control data.

[b]Values in parentheses are standard deviations.