DATA EVALUATION RECORD

1. **CHEMICAL:** Atrazine. Shaughnessey No. 080803.

2. **TEST MATERIAL:** 1) Nonradiolabeled Atrazine Technical; Lot No. SG8029BA10; CAS No. 1912-24-9; 97.1% active ingredient; a white powder. 2) Radiolabeled Atrazine; Lot No. CL-XXVI-I; 96.3% purity; 37.8 μCi/mg specific activity; a white powder.

3. **STUDY TYPE:** 72-5. Fish Life-Cycle Toxicity Test. Species Tested: Fathead Minnow (*Pimephales promelas*).

4. **CITATION:** Dionne, E. 1992. Atrazine Technical - Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. SLI Report No. 92-7-4324. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 425471-03.

5. **REVIEWED BY:**

Alvaro A. Yamhure
Aquatic Biologist, EEB/EFED
USEPA

Signature: Alvaro A. Yamhure
Date: 1/4/94

6. **APPROVED BY:**

Daniel Rieder,
Head Section 3
EEB/EFED

Signature: Daniel Rieder
Date: 1-6-94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a life-cycle toxicity test using freshwater fish and we have therefore rated this study as **supplementary**.

Barring any proof to the contrary, EEB has based its rating on the following observations: 1) It is unclear to what extent, if any, the observed adverse effects on the length and weight of the F_1 fathead minnows may have been due to the solvent (DMF), to Atrazine or a combination thereof; 2) An MATC could not be determined because the adverse length and weight effects occurred at all treatment levels of the F_1; 3) There was no mortality in the solvent control and 4) the test appears to be otherwise scientifically sound. The LOEC was 0.15 mg a.i./l (ppm) but effects may have been observed at even lower concentrations if these had been tested.

DMF is an EPA/EEB-approved solvent for this type of testing. Further, past experience indicates that DMF when used in concentrations under 0.1 ml/l (the performing laboratory claims to have used a concentration of 0.0211 ml/l), does not produce adverse effects on the test organisms or their progeny. Given the specific circumstances of this test, the possibility of contamination of the solvent control must be considered.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Fathead minnow (*Pimephales promelas*) embryos were obtained from in-house cultures. The culture water used was from the same source and of similar quality as the test dilution water. Embryos from 12 separate spawns were obtained for the test. The embryos were less than 24 hours old at test initiation.

B. **Test System:** An intermittent flow proportional diluter system with a 50% dilution factor was used. During the pre-spawning phase of the study, the flow rate provided 7.7 volume turnovers per day. During the spawning phase, flow was reduced to 500 ml at a rate of $\geq$270 cycles per day. The exposure system was two-tiered consisting of an upper and lower level water bath, each containing 14 aquaria. The glass aquaria measured 60 x 30 x 30 cm and were randomly positioned in the water baths. A 15-cm high end-drain maintained a solution volume of 27 l. A 7.5 x 16 x 7.5 cm incubation chamber was positioned at the inflow end of each aquarium and contained two embryo incubation cups. Test solution flowed into and passed through the incubation chamber by means of a self-starting cap-siphon. The embryo incubation cups were 5-cm diameter glass jars with nylon screen bottoms (40 mesh). Two larval growth chambers measuring 30 x 13 x 25 cm were placed in each aquarium on the upper level of the diluter system. Additional F_1 larval growth chambers constructed of petri dishes (10-cm diameter) and 28-cm high sides of 40-mesh nylon screening were used to rear extra fish for residue analysis at the highest concentration.

The aquaria on the lower level were separated into two spawning compartments using a nylon mesh screen divider. Spawning tiles constructed of halved 10-cm lengths of PVC pipe (4-cm diameter) were placed in each compartment.

The test temperature was 25 ±1°C. The test system was maintained under a graduated photoperiod (varied from 10.5 to 15.75 hours of light/day) depending on the developmental stages (Benoit, 1981). Light intensity varied from 40 to 90 footcandles. The entire test system was enclosed in black plastic curtains to prevent disturbance and minimize the influence of laboratory lighting on the intended photoperiod.

The dilution water was well water supplemented with Town of Wareham well water. The water had a total hardness range of 20-40 mg/l as $CaCO_3$, a pH of 6.9-7.5, and a conductivity of 80-150 μmhos/cm.

During the exposure, several radiolabeled stock solutions (94.63 mg a.i./ml) were prepared. The stocks were prepared by dissolving an appropriate amount of nonradiolabeled test material and an appropriate amount of ^{14}C-Atrazine primary stock solution in dimethyl formamide (DMF). With each diluter cycle, 0.082 ml of the radiolabeled stock solution was pumped into the diluter mixing chamber which also received 3.88 l of dilution water. The stock and dilution water were mixed using a magnetic stirrer and a Teflon-coated stir bar. The concentration in the mixing chamber was equivalent to the highest test concentration (20 mg a.i./l) and was subsequently diluted to provide the four remaining treatment levels.

C. **Dosage:** Two-hundred and seventy-four-day, flow-through toxicity test. Based on the results of preliminary testing, five nominal concentrations 0.13, 0.25, 0.50, 1.0, and 2.0 mg a.i./l), a solvent control (0.0211 ml DMF/l), and a dilution water control were used. The solvent control contained the same amount of solvent used in the highest test concentration.

D. **Design:** Each treatment level and control were replicated two times. Fathead minnow embryos were randomly assigned, five at a time, to each of the 28 incubation cups until each cup contained 35 embryos. Two cups were placed in each replicate in the upper level of the test system.

Each day until hatching began (day 3), embryos in each cup were removed and counted. Dead embryos were discarded. On day 3, live embryos and hatched larvae were observed and counted but not removed from the incubation cups. When hatching was complete (day 4), percentage hatching success was calculated.

Newly-hatched fry were fed live brine shrimp nauplii three times daily (twice on weekends) for the first 25 days and fed live brine shrimp nauplii and frozen brine shrimp for the next four days. Following this period, juvenile and adult fish were fed frozen brine shrimp once daily and Zeigler® Brothers Prime flakes once daily.

Twenty-five newly-hatched larvae were impartially selected from each embryo incubation group and placed into a larval growth chamber in the corresponding exposure aquarium providing four groups of larvae per treatment level (two groups per replicate). The growth chambers were examined daily for dead larvae. After 30 and 60 days of exposure, each group was photographed over a grid for length determinations. Percentage survival was also determined at these intervals. On day 60, the two larval groups in each aquarium were combined and randomly thinned to 25 larvae per aquarium. Four 60-day old larvae from the highest treatment level were radiometrically analyzed for whole-body ^{14}C residues.

After approximately 140 days of exposure, two spawning groups consisting of 1 male and 2 females each were transferred to the corresponding lower level spawning aquarium. During spawning activity, dead males were replaced with fish from the appropriate upper level aquaria. Dead females were not replaced since reproduction was assessed on a per female basis. Spawning substrates were checked daily for the presence of eggs. For each spawning group, the number of eggs spawned and the number of eggs incubated were recorded. Fifty embryos from the first 10 spawns consisting of $\geq$50 eggs in each aquarium were incubated and the percentage hatch determined. Subsequently, every third spawn of $\geq$50 eggs in each aquarium was incubated for percentage hatch determination. "Eggs not used for F_1 exposure were collected from the highest treatment level with surviving and reproducing F_0 adults and were incubated to produce two additional life stages of fathead minnows for tissue analysis of total ^{14}C content."

As F_1 embryo groups hatched, groups of 25 newly-hatched fry were established in each aquarium. Two larval groups from separate spawning groups could be reared in each aquarium at any one time. After 30 days post-hatch, each larval group was terminated and individual total length and wet weight were determined. Percentage survival was also determined for each group. The whole body of several F_1 larvae exposed to the highest test level was analyzed for total ^{14}C content.

Exposure of F_0 parental spawners was terminated when no spawning had occurred in any aquarium for 14 consecutive days (day 274). At test termination, each fish was individually measured and weighed and internally examined to verify sex and gonadal condition. Two mature male and two mature female fish from the highest concentration were sampled to determine ^{14}C tissue content.

The test aquaria were scraped and siphoned at least three times weekly. All diluter cells were brushed and siphoned weekly and the glass delivery tubes were brushed monthly. The calibration of the diluter was checked weekly.

The dissolved oxygen concentration (DO), temperature, and pH were measured daily in an alternating replicate of each treatment. In addition, the temperature of one replicate aquarium on each level of the system was recorded continuously using minimum/maximum thermometers. Hardness, alkalinity, and conductivity were measured weekly in one control aquarium and one treatment aquarium on a rotating basis.

Prior to the initiation of spawning, the concentration of Atrazine in each replicate was determined at least weekly. After the initiation of spawning, solution samples were taken weekly from an alternating replicate of each test level in each level of the test system. All samples were analyzed using a radiometric procedure (liquid scintillation method). In addition, both replicate solutions of the high, middle, and low test concentrations, and the dilution water control were analyzed every third week for Atrazine using gas chromatography with nitrogen-phosphorus detection (GC-NPD).

E. **Statistics:** Endpoints statistically analyzed were listed in Table 1 (attached). Hatch and survival data for the dilution water control and solvent control were

compared using Fisher's Exact test. The responses of the treatments were compared to either the pooled control or the solvent control using the Cochran-Armitage Trend test. Length, weight, and reproduction data for the control and solvent control were compared using Student's t-test. All data were tested for normality and homogeneity of variance using Shapiro Wilks test and Bartlett's test, respectively. The responses of the exposed fish were compared to either the pooled control or the solvent control using William's test. Test levels which were significantly different from the control(s) for survival were excluded from subsequent analyses.

12. **REPORTED RESULTS:** Mean measured concentrations for the exposure period using radiometric analysis were 0.15, 0.25, 0.46, 0.99, and 2.0 mg a.i./l (Table 5, attached). Measured concentrations were generally consistent between replicate vessels and sampling intervals. The results of GC-NPD analyses of the high, middle, and low test level solutions compared favorably to the radiometric results.

Mean hatching success (%) and larval survival of F_0 embryos at all exposure levels were comparable to those of the pooled control (Table 8, attached). Statistical comparison of F_0 total length after 30-days post-hatch determined that at 0.99 and 2.0 mg a.i./l, both parameters were significantly lower than those of the pooled control (Table 8, attached).

Following 60 days of exposure, larval survival at all test levels was comparable to that of the pooled controls (Table 8, attached). Length at 0.46, 0.99, and 2.0 mg a.i./l and weight at 0.99 and 2.0 mg a.i./l were statistically different from those of the pooled controls.

At test termination, survival among fish exposed to the 0.99 and 2.0 mg a.i./l concentrations was significantly reduced when compared to the pooled control data (Table 9, attached). Since survival effects were observed, the growth data for the 0.99 and 2.0 mg a.i./l were not statistically analyzed. Analysis of male and female growth at exposure levels $\leq$0.46 mg a.i./l showed no significant difference when compared to the pooled controls.

The reproduction data are summarized in Table 10 (attached). No significant differences were established for any of the reproduction parameters analyzed. "The variability in the number of eggs/female observed between replicate treatment levels was considered a typical response."

Hatching success of F_1 embryos was evaluated on an average of 20 groups of 50 eggs per replicate for each treatment and control. Utilizing the Cochran-Armitage Trend Test to statistically analyze hatching success indicated that hatchability of embryos exposed to concentrations $\geq$0.25 mg a.i./l was significantly reduced when compared to the pooled controls. Statistical analysis using Williams' Test showed no significant difference in hatching success of any exposure levels when compared to the pooled controls (Table 11, attached). Results obtained from these two statistical methods were not in agreement. "Evaluation of the results suggests that the Williams' test may provide a more biologically realistic analysis because the Cochran-Armitage Trend Test does not take into account inter-replicate variance in determination of significant differences."

Following 30 days of post-hatch exposure, larval survival in all exposure groups was statistically comparable to those of the pooled controls. Total length and wet weight of F_1 larvae exposed to concentrations $\geq$0.46 mg a.i./l were significantly lower than those of the solvent control and pooled control, respectively (Table 11, attached). "Larval survival and growth (total length and wet weight) were based on the performance of an average of four larval groups per treatment level (2 groups per replicate exposure aquarium). The larval groups in each replicate aquarium were the progeny of two different spawning groups."

The results of analyses for total ^{14}C-residues in tissue samples are presented in Table 12 (attached). F_1 embryos had the lowest bioconcentration factor (BCF) and F_0 adults had the highest BCF.

Mean water quality values and ranges for each concentration are presented in Tables 3a and 3b (attached). Continuous temperature monitoring established the temperature range as 23-26°C. During the study, DO ranged from 4.6 to 8.5 mg/l (55-103% of saturation) and pH ranged from 6.8 to 7.5. The conductivity was 120-180 µmhos/cm. The alkalinity and hardness were 12-26 mg/l as $CaCO_3$ and 22-36 mg/l as $CaCO_3$, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Based on the adverse effects on F_0 and F_1 growth at the 0.46 mg a.i./l test concentration, the maximum acceptable toxicant concentration (MATC) for Atrazine to fathead minnow was estimated to be >0.25 and <0.46 mg a.i./l. The geometric mean MATC was 0.34 mg a.i./l.

Quality Assurance and Good Laboratory Practice Compliance Statements were included in the report, indicating that the study was conducted in accordance with USEPA Good Laboratory Practice Standards (40 CFR Part 160). The dates and types of quality assurance audits performed were also included in the report.

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

A. Test Procedure: The test procedures were generally in accordance with the SEP except for the following:

Raw water quality data were not included in the report. However, for each parameter measured, the range of measured values was reported and indicated that adequate water quality was maintained.

The recommended study design is 50 embryos/incubation cup, one cup/growth chamber and four replicate growth chambers/treatment. In this test, only two replicate growth chambers were used per treatment level, with two incubation cups/chamber and 35 embryos/cup.

The hardness of the dilution water (22-36 mg/l as $CaCO_3$) was less than recommended (40-48 mg/l).

The light intensity used during the test (430-969 lux) was greater than recommended by the SEP (10-100 lux).

B. Statistical Analysis: The reviewer used two computer programs (Toxstat version 3.3 and Systat 5.0), depending on the type of data, to analyze embryo, juvenile, and adult survival, and juvenile and adult growth. Some proportional data were arcsine square root transformed prior to analysis but, in most cases, transformation did not improve the homogeneity of variance of the data. Two-way ANOVA was used when appropriate.

For all parameters measured, hatching success, survival, and reproduction in the treatment groups were statistically comparable to the solvent control data (printouts, attached).

Control F_0 lengths at 30 days were significantly less than those of the solvent control. F_0 lengths at 30 days were significantly reduced at 0.99 mg a.i./l when compared to the dilution water control and at 0.25, 0.99, 2.0 mg a.i./l when compared to the solvent control(printouts, attached). F_0 lengths at 60 days

were significantly reduced at concentration ≥0.99 mg a.i./l when compared to the solvent control (printouts, attached). No significant difference in F_0 lengths at 60 days was noted between any treatment and the dilution water control (printouts, attached). In addition, no significant difference in F_0 growth (length and weight) at test termination was noted between any treatment and either the solvent control or the dilution water control (printouts, attached).

The analysis of F_1 larval weights determined that all test levels were significantly lower than the dilution water control weights (printouts, attached). Concentrations ≥0.46 mg a.i./l were significantly lower than the solvent control (printouts, attached). Mean weights were 0.239, 0.186, 0.198, 0.174, 0.157, 0.134, and 0.110 g for the dilution water control, solvent control and the five treatment levels, respectively. F_1 lengths were significantly reduced at concentrations ≥0.25 mg a.i./l when compared to the solvent control (printouts, attached). F_1 lengths at all test concentrations were significantly reduced when compared to the dilution water control (printouts, attached). Mean lengths were 29.8, 28.2, 28.5, 27.1, 26.7, 24.5, and 23.3 mm for the dilution water control, solvent control and the five treatment levels, respectively.

C. **Discussion/Results:** The analytical results indicate that the concentration of the test material was generally stable throughout the test period. Percentage relative standard deviations by concentration ranged from 4.5 to 23.3%.

The solvent (DMF) used in this test appeared to have adverse effects on both F_1 weight and length. Since only the highest test concentrations and solvent control solutions contained the maximum amount of DMF used in the test, comparing the growth data from the lower four treatment levels to those of the solvent control would underestimate the toxicity of the test material. To be conservative and better protect non-target organisms, the F_1 growth data at each treatment level should be compared to the dilution water control data. Therefore, the lowest-observed-effect concentration (LOEC) in this test was 0.15 mg a.i./l, the lowest concentration tested. The no-observed-effect concentration (NOEC) could not be determined.

This study is scientifically sound but does not meet the guideline requirements for a life-cycle toxicity

test using freshwater fish. The maximum acceptable toxicant concentration (MATC) for fathead minnows exposed to Atrazine could not be determined from the test due to adverse effects on F_1 growth at all treatment levels.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The MATC for fathead minnows exposed to Atrazine could not be determined from the test due to adverse effects on F_1 growth at all treatment levels.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 29 January 1993.

Table 1. Schematic summary of the experimental design for the life cycle study with fathead minnow (*Pimephales promelas*) and Atrazine Technical.

Phase	Test Days	# Organisms	Endpoint
I. F_0 Hatching Success	0 - 4	140 embryos per concentration 70 per replicate 35 per incubation cup	% Live Hatch
II. F_0 Larval Exposure	4 - 64	100 larvae per concentration 50 per replicate 25 per growth chamber	% Survival Total Length
	64 - 274	50 larvae per concentration 25 per replicate	% Survival Total Length Wet Weight
III. F_0 Reproduction	140 - 274	4 groups of 1 male/ 2 females per concentration; 2 groups per replicate	# egg/female # spawns/female # eggs/spawn
IV. F_1 Hatching Success	141 - 247	1000 embryos - average per concentration	% Live Hatch
V. F_1 Larval Exposure	144 - 255	4 larval groups (25 individuals/group) average per concentration	% Survival Total Length Wet Weight
VI. F_0 Termination	274	All surviving F_0 fish	% Survival Male Total Length Maie Wet Weight Female Total Length Female Wet Weight

Table 3a. Results of dissolved oxygen concentration, temperature and pH measurements recorded during the chronic exposure of fathead minnow (*Pimephales promelas*) to Atrazine Technical.

Nominal Concentration (mg A.I./L)		Dissolved Oxygen[a] (mg/L)		Temperature (°C)		pH	
	Replicate	A	B	A	B	A	B
2.0	Mean (SD)[b]	7.0 (0.50)	7.0 (0.51)	25 (0.47)	25 (0.47)	NA[c]	NA
	Range	4.6 - 8.2	5.1 - 8.3	23 - 25	24 - 25	6.9 - 7.5	6.9 - 7.5
1.0	Mean (SD)	7.1 (0.52)	7.4 (0.43)	25 (0.45)	25 (0.46)	NA	NA
	Range	5.6 - 8.3	5.2 - 8.2	24 - 25	24 - 25	6.9 - 7.5	6.8 - 7.5
0.50	Mean (SD)	7.3 (0.72)	7.4 (0.44)	25 (0.46)	25 (0.44)	NA	NA
	Range	5.3 - 8.4	5.6 - 8.2	24 - 25	24 - 25	6.8 - 7.5	6.9 - 7.5
0.25	Mean (SD)	7.6 (0.42)	7.4 (0.43)	25 (0.48)	25 (0.45)	NA	NA
	Range	6.1 - 8.5	5.2 - 8.4	23 - 25	24 - 25	6.9 - 7.5	6.9 - 7.5
0.13	Mean (SD)	7.6 (0.39)	7.5 (0.36)	25 (0.46)	25 (0.46)	NA	NA
	Range	6.4 - 8.4	6.4 - 8.4	24 - 25	24 - 26	6.9 - 7.5	6.9 - 7.5
Solvent Control	Mean (SD)	7.1 (0.49)	6.9 (0.72)	25 (0.45)	25 (0.44)	NA	NA
	Range	5.6 - 8.4	4.8 - 8.4	23 - 25	24 - 25	6.9 - 7.5	6.9 - 7.5
Control	Mean (SD)	7.8 (0.37)	7.7 (0.35)	25 (0.47)	25 (0.44)	NA	NA
	Range	6.4 - 8.5	6.6 - 8.5	23 - 25	24 - 26	6.9 - 7.5	6.9 - 7.5

[a] 100% of saturation at 25 °C (mean test solution temperature) = 8.3 mg/L. Mean dissolved oxygen concentrations during this exposure ranged from 55 to 103% of saturation.
[b] SD = Standard Deviation
[c] NA = Not Applicable

Table 3b. **Results of total hardness, total alkalinity and specific conductance measurements recorded during the chronic exposure of fathead minnow (*Pimephales promelas*) to Atrazine Technical.**

Nominal Concentration (mg A.I./L)		Total Hardness (mg/L as $CaCO_3$)		Total Alkalinity (mg/L as $CaCO_3$)		Specific Conductance (μmhos/cm)	
		A	B	A	B	A	B
2.0	Mean (SD)[a]	28 (4.0)	28 (2.0)	20 (6.0)	23 (3.1)	150 (15)	150 (12)
	Range	24 - 32	26 - 30	14 - 26	20 - 26	130 - 160	140 - 160
1.0	Mean (SD)	30 (3.3)	28 (2.6)	23 (3.0)	21 (2.3)	140 (17)	150 (8.9)
	Range	26 - 34	24 - 30	18 - 26	18 - 24	120 - 160	130 - 150
0.50	Mean (SD)	30 (2.6)	28 (3.8)	20 (1.7)	20 (2.6)	150 (19)	140 (8.4)
	Range	28 - 34	24 - 34	18 - 22	18 - 24	130 - 180	130 - 150
0.25	Mean (SD)	30 (3.6)	32 (3.7)	20 (1.7)	21 (4.1)	150 (15)	150 (17)
	Range	24 - 32	28 - 36	18 - 22	16 - 24	130 - 160	130 - 160
0.13	Mean (SD)	30 (5.3)	29 (3.1)	21 (4.2)	20 (3.5)	150 (12)	150 (10)
	Range	26 - 36	26 - 32	18 - 26	18 - 24	140 - 160	140 - 160
Solvent Control	Mean (SD)	29 (3.8)	28 (3.5)	21 (4.3)	22 (3.2)	150 (21)	150 (12)
	Range	24 - 34	24 - 34	14 - 26	18 - 26	120 - 180	130 - 170
Control	Mean (SD)	30 (2.8)	26 (9.6)	18 (2.9)	19 (2.5)	150 (11)	140 (11)
	Range	24 - 34	22 - 34	12 - 22	14 - 22	130 - 160	130 - 160

[a] SD = Standard Deviation

Table 5. **Concentrations of Atrazine measured (radiometric analysis) in the exposure solutions during the full life cycle exposure of fathead minnow (*Pimephales promelas*).**

Day		Nominal Concentration (mg A.I./L)						
		2.0	1.0	0.50	0.25	0.13	Solvent Control	Control
		Measured Concentration (mg A.I./L)						
0	A	2.1	0.93	0.47	0.27	0.14	<0.013	<0.012
	B	2.0	0.96	0.47	0.26	0.13	<0.012	<0.012
4	A	1.9	0.97	0.48	0.26	0.13	<0.012	<0.012
	B	1.9	0.90	0.45	0.25	0.12	<0.012	<0.012
9	A	1.9	0.94	0.46	0.27	0.13	<0.012	<0.012
	B	2.0	0.95	0.47	0.26	0.12	<0.012	<0.012
17	A	2.0	0.94	0.45	0.24	0.14	<0.012	<0.012
	B	1.9	0.95	0.45	0.25	0.14	<0.012	<0.012
23	A	1.9	0.95	0.47	0.26	0.13	<0.012	<0.012
	B	1.9	0.88	0.46	0.26	0.13	<0.012	<0.012
30	A	2.0	0.96	0.48	0.26	0.13	<0.013	<0.013
	B	2.0	0.98	0.45	0.25	0.13	<0.013	<0.013
37	A	1.9	1.0	0.48	0.26	0.13	<0.013	<0.013
	B	1.9	1.0	0.50	0.26	0.14	<0.013	<0.013
44	A	1.9	1.0	0.50	0.26	0.13	<0.012	<0.012
	B	2.0	1.1	0.50	0.27	0.14	<0.012	<0.012
51	A	1.9	1.1	0.48	0.25	0.16	<0.012	<0.012
	B	2.0	1.1	0.45	0.27	0.15	<0.012	<0.012
58	A	2.0	0.99	0.48	0.28	0.14	<0.012	<0.012
	B	2.0	0.96	0.47	0.26	0.14	<0.012	<0.012
65	A	2.0	1.0	0.48	0.27	0.13	<0.011	<0.011
	B	1.9	0.98	0.48	0.27	0.13	<0.011	<0.011
71	A	2.0	0.95	0.47	0.24	0.13	<0.011	<0.011
	B	2.0	1.0	0.45	0.25	0.13	<0.011	<0.011
79	A	2.0	0.99	0.46	0.25	0.13	<0.011	<0.011
	B	1.9	0.98	0.48	0.26	0.13	<0.011	<0.011

Table 5. Continued

Day		Nominal Concentration (mg A.I./L) 2.0	1.0	0.50	0.25	0.13	Solvent Control	Control
		Measured Concentration (mg A.I./L)						
86	A	2.0	0.95	0.46	0.27	0.14	<0.011	<0.011
	B	2.0	0.94	0.46	0.27	0.13	<0.011	<0.011
93	A	1.9	0.95	0.47	0.25	0.14	<0.011	<0.011
	B	2.0	0.97	0.46	0.26	0.14	<0.011	<0.011
100	A	2.0	0.98	0.47	0.26	0.26	<0.011	<0.011
	B	2.0	0.97	0.48	0.26	0.13	<0.011	<0.011
107	A	2.0	0.97	0.49	0.27	0.14	<0.012	<0.012
	B	2.0	0.97	0.47	0.25	0.13	<0.012	<0.012
114	A	1.8	0.92	0.45	0.25	0.13	<0.012	<0.012
	B	1.8	0.91	0.45	0.25	0.12	<0.012	<0.012
121	A	1.9	0.91	0.45	0.26	0.13	<0.012	<0.012
	B	1.9	0.96	0.46	0.25	0.13	<0.012	<0.012
128	A	2.0	0.99	0.46	0.26	0.14	<0.012	<0.012
	B	2.0	0.98	0.48	0.26	0.13	<0.012	<0.012
135	UA[a]	2.0	1.0	0.44	0.26	0.13	<0.012	<0.012
	LA	2.0	0.95	0.49	0.26	0.14	<0.012	<0.012
142	UB	2.0	1.0	0.47	0.25	0.13	<0.012	<0.012
	LB	1.9	0.98	0.49	0.26	0.14	<0.012	<0.012
149	UA	2.0	1.0	0.46	0.26	0.12	<0.012	<0.012
	LA	2.0	0.94	0.47	0.26	0.15	<0.012	<0.012
156	UB	1.9	0.96	0.45	0.23	0.11	<0.012	0.017[b]
	LB	1.9	0.86	0.45	0.25	0.13	<0.012	<0.012
163	UA	2.1	1.0	0.50	0.28	0.17	<0.012	<0.012
	LA	2.3	1.0	0.53	0.29	0.15	<0.012	<0.012
170	UB	1.9	1.1	0.44	0.23	0.15	<0.012	<0.012
	LB	1.9	0.97	0.45	0.23	0.16	<0.012	<0.012
177	UA	2.1	1.0	0.45	0.24	0.24	<0.012	<0.012
	LA	2.1	1.0	0.47	0.25	0.25	<0.012	<0.012
178	UA	NA[c]	NA	NA	NA	0.19	NA	NA
	LA	NA	NA	NA	NA	0.18	NA	NA
184	UB	2.0	1.0	0.43	0.25	0.15	<0.012	<0.012
	LB	2.0	0.97	0.45	0.23	0.15	<0.012	<0.012

Table 5. Continued

Day		Nominal Concentration (mg A.I./L) 2.0	1.0	0.50	0.25	0.13	Solvent Control	Control
		Measured Concentration (mg A.I./L)						
191	UA	1.9	0.99	0.44	0.23	0.14	<0.012	<0.012
	LA	1.9	0.93	0.44	0.23	0.13	<0.012	<0.012
198	UB	2.0	0.93	0.44	0.24	0.16	<0.012	<0.012
	LB	2.0	0.90	0.50	0.25	0.17	<0.012	<0.012
205	UA	2.0	0.92	0.42	0.23	0.14	<0.012	<0.012
	LA	1.9	0.90	0.45	0.25	0.14	<0.012	<0.012
212	UB	2.0	0.99	0.46	0.25	0.16	<0.013	<0.013
	LB	2.0	0.89	0.46	0.26	0.18	<0.013	<0.013
219	UA	1.9	1.1	0.44	0.23	0.20	<0.013	<0.013
	LA	2.2	1.1	0.43	0.26	0.21	<0.013	<0.013
226	UB	2.0	1.1	0.43	0.25	0.20	<0.013	<0.013
	LB	2.1	1.1	0.41	0.26	0.19	<0.013	<0.013
233	UA	2.1	1.0	0.44	0.23	0.12	<0.013	<0.013
	LA	2.0	1.0	0.46	0.25	0.12	<0.013	<0.013
240	UB	2.0	1.0	0.41	0.24	0.23	<0.013	<0.013
	LB	2.0	0.95	0.43	0.24	0.24	<0.013	<0.013
247	UA	1.9	0.96	0.45	0.24	0.13	<0.013	<0.013
	LA	2.1	0.94	0.49	0.24	0.12	<0.013	<0.013
254	UB	2.1	1.2	0.48	0.24	0.15	<0.013	<0.013
	LB	2.0	1.1	0.49	0.27	0.15	<0.013	<0.013
261	UA	1.7	0.99	0.47	0.25	0.10	<0.013	<0.013
	LA	1.7	1.0	0.50	0.26	0.12	<0.013	<0.013
268	UB	2.1	1.1	0.44	0.24	0.25	<0.013	<0.013
	LB	2.0	1.1	0.47	0.24	0.24	<0.013	<0.013
274	UA	2.0	1.0	0.46	0.25	0.10	<0.013	<0.013
	LA	2.1	1.0	0.51	0.28	0.15	<0.013	<0.013
Mean[d]		2.0 (0.089)	0.99 (0.061)	0.46 (0.022)	0.25 (0.013)	0.15 (0.035)	NA	NA

[a] UA and UB = Upper Level, Replicate A and Upper Level, Replicate B; LA and LB = Lower Level, Replicate A and Lower Level, Replicate B.

[b] Concentration recovered for this control sample is believed due to contamination during the analytical process and therefore is not representative of the exposure conditions.

[c] NA = Not Applicable

[d] Mean measured concentrations are presented with the standard deviation in parentheses and were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.

Table 8. Embryo hatching success, larval survival and growth (total length and weight) of the F_0 fathead minnow (*Pimephales promelas*) after 30 and 60 days post-hatch exposure to Atrazine Technical.

Mean Measured Concentration (mg A.I./L)	Replicate	Embryo Hatching Success (%)	Day 30 Larval Survival (%)	Day 30 Total Length[a] (mm)	Day 60 Larval Survival (%)	Day 60 Total Length[a] (mm)	Day 60 Wet Weight[a] (g)
2.0	A	84	90	28 (3.0)	90	40 (3.9)	0.5341 (0.19)
	B	79	98	28 (3.3)	98	39 (4.6)	0.5208 (0.20)
	Mean	**82**	**94**	**28 (3.1)[b]**	**94**	**39 (4.3)[b]**	**0.5270 (0.19)[b]**
0.99	A	83	92	28 (4.0)	90	40 (5.2)	0.5052 (0.17)
	B	81	90	27 (3.7)	90	38 (4.9)	0.5386 (0.27)
	Mean	**82**	**91**	**27 (3.9)[b]**	**90**	**39 (5.0)[b]**	**0.5219 (0.22)[b]**
0.46	A	76	96	30 (3.6)	96	40 (5.6)	0.5881 (0.20)
	B	83	92	30 (4.0)	92	39 (6.1)	0.6256 (0.18)
	Mean	**79**	**94**	**30 (3.8)**	**94**	**39 (5.9)[b]**	**0.6060 (0.19)**
0.25	A	81	90	29 (3.8)	90	40 (4.2)	0.5484 (0.23)
	B	83	96	28 (4.2)	96	40 (5.3)	0.5599 (0.18)
	Mean	**82**	**93**	**29 (4.0)**	**93**	**40 (4.8)**	**0.5547 (0.20)**
0.15	A	84	96	30 (3.5)	96	40 (5.0)	0.5052 (0.18)
	B	83	96	29 (3.9)	96	40 (4.7)	0.6267 (0.20)
	Mean	**84**	**96**	**30 (3.7)**	**96**	**40 (4.8)**	**0.5660 (0.20)**
Solvent Control	A	84	96	31 (2.5)	96	42 (4.4)	0.7176 (0.25)
	B	83	94	32 (3.3)	94	42 (4.3)	0.6547 (0.19)
	Mean	**84**	**95**	**31 (3.0)**	**95**	**42 (4.3)**	**0.6875 (0.22)**
Control	A	81	94	29 (3.7)	94	40 (4.4)	0.6178 (0.20)
	B	84	94	29 (4.8)	94	41 (5.2)	0.5980 (0.23)
	Mean	**83**	**94**	**29 (4.3)**	**94**	**41 (4.8)**	**0.6079 (0.21)**
Pooled Control		83	95	30	95	41	0.6470

[a] Measurement presented is the mean with the corresponding standard deviation in parentheses.
[b] Significantly different ($p \leq 0.05$) as compared to the pooled control data, based on Williams' Test.

Table 9. Survival and growth of F_0 fathead minnow (*Pimephales promelas*) at the termination (274 days) of the chronic exposure to Atrazine Technical.

Mean Measured Concentration (mg A.I./L)	Replicate	Number of Mortalities Between Days 66 and 274[a]	Survival at Test Termination (%)	Mean Total Length[b] (mm)		Mean Wet Weight[b] (grams)	
				Male	Female	Male	Female
2.0	A	4 (2)	84	79 (8)	65 (2)	6.453 (1.36)	2.588 (0.397)
	B	4 (1)[c]	88	74 (6)	65 (3)	5.475 (1.11)	2.769 (0.657)
	Mean	**4**	**86[e]**	**76[f]**	**65[f]**	**5.946[f]**	**2.694[f]**
0.99	A	1[d]	100	78 (5)	66 (4)	5.620 (1.29)	2.509 (0.467)
	B	9 (3)	64	73 (9)	65 (5)	5.468 (1.71)	2.880 (0.839)
	Mean	**5**	**82[e]**	**76[f]**	**65[f]**	**5.600[f]**	**2.658[f]**
0.46	A	1 (1)	96	82 (6)	65 (6)	6.259 (1.31)	2.766 (0.591)
	B	3	88	78 (6)	64 (4)	5.931 (1.34)	2.420 (0.460)
	Mean	**2**	**92**	**80**	**64**	**6.095**	**2.593**
0.25	A	2[d]	100	81 (7)	67 (4)	6.211 (1.40)	2.930 (0.548)
	B	2 (2)	92	78 (5)	60 (5)	5.670 (1.073)	2.063 (0.238)
	Mean	**2**	**96**	**79**	**64**	**5.941**	**2.496**
0.15	A	3 (3)	88	81 (9)	65 (3)	6.606 (1.93)	2.688 (0.415)
	B	1 (1)	96	77 (5)	61 (4)	5.280 (1.16)	2.239 (0.300)
	Mean	**2**	**92**	**79**	**63**	**5.943**	**2.464**
Solvent Control	A	0	100	81 (6)	63 (2)	6.521 (1.70)	2.666 (0.392)
	B	2 (2)	92	80 (7)	65 (3)	6.047 (1.43)	2.767 (0.331)
	Mean	**1**	**96**	**81**	**64**	**6.237**	**2.701**
Control	A	3 (2)	88	80 (8)	64 (3)	6.743 (1.62)	2.773 (0.366)
	B	1[d]	100	77 (7)	64 (6)	5.754 (1.15)	2.714 (0.682)
	Mean	**2**	**94**	**79**	**64**	**6.249**	**2.744**
Pooled Control		1	95	80	64	6.266	2.730

[a] The number of mortalities in each aquaria was established by subtracting the number of fish surviving in each aquaria at test termination from 25, the number of 60 day old juveniles placed in each aquaria. The number in parentheses represents the mortality occurring in the spawning chamber and which is attributed to spawning behavior.

[b] Standard deviation is presented in parentheses.

[c] One of the mortalities recorded in this replicate is believed due to an injury and is therefore not related to exposure to the test material.

[d] Mortality/mortalities recorded in this replicate is believed due to an injury and is therefore not related to exposure to the test material.

[e] Significantly different ($p \leq 0.05$) as compared to the pooled control data, based on Cochran-Armitage Trend Test.

[f] Data not statistically analyzed due to a survival effect at this treatment level.

Table 10. The reproductive success of F_0 fathead minnows (*Pimephales promelas*) during the chronic exposure to Atrazine Technical.

Mean Measured Concentration (mg A.I./L)	Replicate	Total Number Spawns	Number Eggs/ Spawn	Total Number Eggs	Number Spawns/ Female	Number Eggs/ Female	Number Females
2.0	A	59	60	3555	10	593	6
	B	102	98	9972	17	1662	6
	Mean	**81**	**79**	**6764**	**14**	**1128**	**6**
0.99	A	55	94	5149	9	858	6
	B	80	73	5803	13	967	6
	Mean	**68**	**84**	**5476**	**11**	**913**	**6**
0.46	A	63	97	6113	11	1019	6
	B	49	81	3981	8	664	6
	Mean	**56**	**89**	**5047**	**9.5**	**842**	**6**
0.25	A	32	113	3609	6	722	5
	B	59	80	4702	12	940	5
	Mean	**46**	**97**	**4156**	**9.0**	**831**	**5**
0.15	A	60	99	5912	10	985	6
	B	61	120	7295	10	1216	6
	Mean	**61**	**110**	**6604**	**10**	**1101**	**6**
Solvent Control	A	88	140	12314	15	2052	6
	B	67	95	6379	13	1276	5
	Mean	**78**	**118**	**9347**	**14**	**1664**	**6**
Control	A	75	87	6493	13	1082	6
	B	35	90	3160	6	527	6
	Mean	**55**	**89**	**4827**	**10**	**805**	**6**
Pooled Control		66	103	7087	12	1234	6

Table 11. **Survival and growth (total length and wet weight) of F_1 fathead minnow (*Pimephales promelas*) exposed for 30 days post-hatch to Atrazine Technical.**

Mean Measured Concentration (mg A.I./L)		Hatching Success (%)	Survival N[a]	30 Day Post-Hatch Larvae Survival (%)	Total Length[b] (mm)	Wet Weight (g)	N[c]
Replicate							
2.0	A	76	22	78	23	0.106	4
	B	76	30	92	24	0.112	5
	Mean	**76[d]**	**26**	**85**	**23[e] fg**	**0.109[e] hi**	**5**
0.99	A	91	19	89	24	0.112	4
	B	56	21	79	26	0.159	4
	Mean	**74[d]**	**20**	**84**	**25[e] fg**	**0.135[e] hi**	**4**
0.46	A	80	20	82	27	0.159	4
	B	83	15	92	27	0.155	4
	Mean	**81[d]**	**18**	**87**	**27[e] fg**	**0.157[e] hi**	**4**
0.25	A	76	18	88	27	0.177	3
	B	88	17	85	27	0.171	4
	Mean	**82[d]**	**18**	**87**	**27 fg**	**0.174 h**	**4**
0.15	A	85	18	85	28	0.189	4
	B	87	21	87	29	0.206	4
	Mean	**86**	**20**	**86**	**28 f**	**0.198 h**	**4**
Solvent Control	A	83	26	87	28	0.188	4
	B	85	21	90	28	0.184	4
	Mean	**84**	**24**	**89**	**28 f**	**0.186 h**	**4**
Control	A	83	21	88	30	0.245	4
	B	92	12	89	30	0.234	4
	Mean	**88**	**17**	**89**	**30**	**0.239**	**4**
Pooled Control		86	20	89	NA	0.213	4

[a] N = Number of egg groups (50 eggs/group) incubated and evaluated for hatching success.
[b] Due to a significant difference between the two control groups, statistical comparisons for total length data were conducted with the solvent control data.
[c] N = Number of larval groups (25 larvae/group) reared and evaluated for survival and growth.
[d] Significantly different ($p \leq 0.05$) as compared to the pooled control data, based on Cochran-Armitage Trend Test, however, no statistical difference was established using Williams' test.
[e] Significantly different ($p \leq 0.05$) as compared to the solvent control/pooled control data, based on Williams' test.

Reviewer's analyses
f = significantly different ($p \leq 0.05$) from control data
g = " " " " Solvent control data

h = significantly different ($p \leq 0.05$) from control data
i = " " " " Solvent control data

Table 12. **^{14}C-residue concentrations in tissue samples of fathead minnows (*Pimephales promelas*) exposed during the full-life cycle exposure to Atrazine Technical.**

Generation and Life-Stage[a]	Tissue Type	Measured ^{14}C-Residue Concentration (mg/kg)
F_0 Larvae 60 days old (post-hatch)	whole organism	12 13 14 13 **Mean 13 (6.5x)[b]**
F_0 Parental Adults at Test Termination	whole organism	**Male** 18 15 **Mean 17 (8.5x)[b]** **Female** 18 16 **17 (8.5x)[b]**
F_1 Embryos (≤ 24 hours old)	whole organism - composite sample	6.9 7.7 **Mean 7.3 (3.7x)[b]**
F_1 Embryos (approximately 3 days old)	whole organism - composite sample	9.2 9.0 **Mean 9.1 (4.6x)[b]**
F_1 Larvae (14 days old)	whole organism -	6.6 6.6 **Mean 6.6 (3.3x)[b]**
F_1 Larvae (30 days old)	whole organism -	10 11 11 14 **Mean 12 (6.0x)[b]**

[a] Organisms used for analysis were exposed to 2.0 mg A.I./L.
[b] Bioconcentration factor (BCF) is presented in parentheses.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Atrazine Technical Shaughnessy No. 080803 Pesticide Use _____

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/ REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	**% AI**	**MATC LC_{50}**	**DAYS**	**AFFECTED PARA.**	**STUDY/ REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
1. *Pimephales promelas*	97.1	CND	274	F_1 length & weight	1992 /1993	425471-03 Supplemental	SLI	RGM
2.								
3.								

COMMENTS: Results based on mean measured concentrations; SLI=Springborn Laboratories Inc. CND=could not be determined.

LOEC = 0.15 mg a.i./L (mean measured conc.), the lowest concentration tested