EEB files
4.20.95

DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE 72-5

1. **CHEMICAL:** TPTH Shaughnessey #: 083601

2. **TEST MATERIAL:** Triphenyltin Hydroxide Purity: 97.9%

3. **CITATION:**

Authors:	Jon E. Rhodes
Title:	Full Life-Cycle Toxicity of Triphenyltin Hydroxide (TPTH) (HOE 029664 00 ZB99 0055 and HOE 029664 00 ZD97 0004) to the Fathead Minnow (Pimephales promelas) Under Flow-Through Conditions
Study Completion Date:	6/16/93
Laboratory:	ABC Laboratories, Inc.
Laboratory Report ID:	39443
Project ID Number:	91-0023
Sponsor:	AgrEvo USA, Company, Wilmington, Delaware
MRID No.:	43490101

4. **REVIEWED BY:**

Dennis J. McLane, Wildlife Biologist EEB, EFED, U.S.EPA

Signature: Dennis J. McLane Date: 4-19-95

5. **APPROVED BY:**

Les W. Touart, Section 1 Chief EEB, EFED, U.S.EPA

Signature: L. Touart Date: 4-20-95

6. **Study Parameters:**

Test Species: Pimephales promelas
Age or Weight: ≤24 hours
Duration of Test: 183 days
Study Method: Flow-through
Type of Concentration: measured conc.

7. **CONCLUSIONS:**

This study appears to be scientifically sound but does not fulfill guideline requirements. Growth in parental generation showed effects at all levels. Therefore, an NOEC (<0.0654 µg/L) was not derived. Survival, egg hatchabilty, and reproduction show no statistical difference from the control. The highest level tested was 0.914 µg/L.

1/20

The results of the bioaccumulation study indicate that the highest BCF was 19,700. Value of over 1,000 are of concern.

96-Hour Acute Study LC_{50}: 9.6 $\mu g/L$

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: An NOEC was not derived.

C. Reparability: None

9. SUBMISSION PURPOSE:

As per AgrEvo in their letter of December 7, 1994, "The study was required by EPA's DCI-Notice dated September 28, 1990."

10. GUIDELINE DEVIATIONS:

Items not reported:

1. Acclimation photoperiod and temperature

2. Acclimation any excess mortality

3. Schedule to show that embryos were removed at a fixed time each day so spawning activity is not disturbed unnecessarily.

4. If embryos were soaked in dilution water for at least 2 hours.

5. Time to hatch data was not reported.

6. Feeding schedule to show at that the amount of food given to the control and treated fish was kept constant between exposures.

7. Description of the dilution water aeration process.

8. Flow rate of the solvent

9. Report did not indicate that the test solution is completely mixed before introduction into the test system. All samples were composited.

10. Embryo and fry Chambers volume not reported just the diameter 9 cm

diameter 9 cm

11. Rocker arm apparatus motor rpm was not reported.

Items which differ from guidelines:

1. Survival of fry for 5 weeks rather than 4.

2. Length measured after 5 weeks rather than 4.

3. Rather than terminating the adult exposure, based on decreasing day-length photoperiod and a 1-wk period passing in which no spawning occurs. "The parental exposure was terminated on day 155 after spawning activity had tapered off in terms of frequency, number of spawns, and number of eggs/spawn."

4. All fish are transferred to the adult spawning tank 12 weeks after hatching rather than 8 weeks.

5. Flow-splitting accuracy was confirmed by volumetric method but not by the chemical analysis. All samples were composites of several replicates. Also, composites will mask replicate variation, and high and low range.

6. Flow rate did not maintain DO at above 75% of saturation. Between day 42 and day 56 the DO dropped below 75% and remained below 75% until day 70. On day 70 level 1 and 4 DO concentrations rose above 75%, but none of the others level did until day 87.(see attached graphs of DO concentrations)

7. Toxicant concentration must be measured in one tank at each toxicant level every week. However, all analyses were composites of two or more replicates.

8. One concentration must not affect any life stage. Parental growth was affected at all levels when compared to the control.

9. F1 generation - 50 embryos from each conc. level were not transferred to incubation cups for hatch. This study indicates that, "Sublots of 35 eggs, collected from spawns >50 eggs, were removed from tiles and placed into incubation cups suspended in the growth chambers."

10. A minor discrepancy makes interpreting the result difficult Table XII (hatching data) or Table XVI (growth data) for level 4. Table XII indicates that the data for level 4 are from replicate C. However, Table XVI indicates that the data is for replicate D in level 4. It appears

these should be the same replicate.

11. Hardness and pH exceeded the recommended values of hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6. The study reported: hardness:134 to 160 mg/L as $CaCO_3$ pH range: 7.74 to 8.25.

Items that were different from those recommended is EEB's protocol review in Douglas Urban's memorandum of August 25, 1992:

1. This memorandum recommended "..., 25 larval-juvenile fish should be maintained per replicate (100 per concentration) until **112 days** post-hatch when they may be thinned to 25 for retention until final selection of 8 spawning pairs." The study report indicates "...on day 90 (84 post-hatch), 12 impartially selected adults from growth replicate A and 13 from B in each treatment were transferred to spawning aquaria E." Therefore, the fish were transferred on **day 84** rather than day 112 post-hatch.

2. Similar to the above comment the memorandum indicated that, "It is recommended that overall egg hatchability be ≥80% overall, fry survival from two days post-hatch to 56 days post-hatch should be ≥80% overall, and overall survival to **day 112** post-hatch (at transfer to spawning aquaria) should be ≥70%." The date of transfer to the spawning aquaria was **84-day** post-hatch rather than **112**. At this date the lowest percent survival was 98%.

3. The study report indicated that tissue from the following levels were measured: control, low and middle. The memorandum recommended low, mid, and high treatment levels. Hence, Only two concentration levels were measured and the highest level was not measured.

4. The memorandum also indicated where to obtain the fish for analysis, "Fry to be analyzed for whole body residue can be taken from a sample at thinning, eggs from those spawned during the test, and a sample of adults can be taken at test termination. However, the following note at the bottom of Table XII indicates an entire replicate was set aside: "At least one replicate in each treatment was kept open to generate F1 tissue for determination of BCF's."

11. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: An estuarine fish species, preferably a sheepshead minnow (Cyprinodon variegatus) or fathead minnow (*Pimephales promelas*.	Pimephales promelas
Source and Acclimation of Fish 1. From wild population or Suitable laboratory culture 2.1. Sheepshead held in flowing 30°C seawater of >15% salinity for at least 2 wks. 2.2. Fathead 25°C and 16 hour/day day-light photoperiod (embryos will mature in 5 to 6 months under these conditions) 3. Neither species of fish or eggs should exhibit excess mortality.	1. Reared at ABC, Labs 2.1 N/A 2.2 Not reported 3.Not reported
Eggs from Adult Fish Artificial inducement and natural spawning are the 2 methods for obtaining a sufficient number of eggs for a chronic exposure. 1. Artificial inducement (entails the stimulation of egg production by injection of human gonadotrophic hormone. Usually 10 ♀s and 5♂s should be used.) 2. Natural spawning (is possible with a few considerations for each fish species.)	1. N/A 2. Yes
3. Adult deaths during spawning should be noted; dead animals removed but not replaced. 4. At termination of each spawning group, lengths and weights of individual fish are measured.	3. Adult deaths noted; dead animals removed 4. Yes
Feeding	

Guideline Criteria	Reported Information
1. Fry of both fish species should be fed equal portions of live brine shrimp naupii at least 2x/day about 6 hours apart for three wks (frozen naupii are not to be used).	"Parental and F1 generation fry were fed live rotifers (*Brachionus sp.*) and live brine shrimp nauplii (*Artemia*) soon after hatch began." Salmon starter was added of time. Two or three times a day."Except for open cans of *Artemia* cysts, and live cultures of *Artemia* and *Brachinous*, all food was kept frozen or refrigerated before use."
2. Juveniles (4 wks posthatch) and adults can be fed 2x/day on equal portions of dry food (e.g., Tetramin® or BiOrell) supplemented with frozen adult brine shrimp.	2. (same as above)
3. Each batch of food should be checked of pesticides and metals.	Checked but the schedule was not reported.
Embryo Removal	
1. Daily record numbers and egg fertility.	Yes
2. Examined all embryos daily with a dissecting scope or magnifying viewer to remove empty shells and opaque, or abnormal embryos.	Yes (did not indicate if magnification was used)
3. If >50% of the embryos from a spawn appear to be healthy and fertile, all embryos from that spawn should be discarded.	95% hatch was obtained day 6 post hatch
4. Embryos should be removed at a fixed time each day so spawning activity is not disturbed unnecessarily.	Removal was not reported to be at a fixed time each day.
Embryo Exposure (Four-Five Days)	

Guideline Criteria	Reported Information
1. The life-cycle chronic toxicity test must begin with embryos from at least 3 separate spawnings 2. that are ≤24 hours old 3. and have soaked in dilution water for at least 2 hours.	1. Yes 2. Yes 3. Not reported
4. Testing begins by randomly distributing 50 embryos to each of the 4 replicate larval growth chambers.	4. Yes
5. 10 embryos are transferred with a large bore eye dropper to successive incubation cups which are standing in dilution water. This is repeated until 50 embryos are in each cup. The incubation cups are then distributed to each replicate larval chamber.	5. No "Placement into incubation cups was accomplished by gently drawing 5 eggs in a small-bore glass pipet and releasing them into one impartially chosen (without regard to replicate or treatment) incubation cup."
Larval-Juvenile Exposure (Eight Weeks)	
1. After hatching, each group of larvae is randomly reduced to 25, and released in replicate larval growth chambers. 1.1 This random selection must include any fish that are lethargic or deformed.	1. Yes 1.1 Yes,"impartially selected" 1.2 Daily 1.3 Number of live fish were counted.

Guideline Criteria	Reported Information
2. At 4 and 8 wks after hatching, total lengths (mm) of all fish must be recorded. 3. The amount of food given to the control and treated fish must be kept constant between exposures.	2. 35 (5 wks) and 56 (12 wks) days after hatching total lengths (mm) of all fish were recorded. 3. Not reported
Juvenile-Adult Exposure (32-40 wks)	
1. All fish are transferred to the adult spawning tank (same concentration) 8 wks after hatching. 2. Each tank should have 25 randomly selected fish (deformed fish included).	1. No (90 days or 84 (12 wks) days post hatch) 2. Yes "impartially selected"; deformed fish not mentioned; day 90 (84 post hatch).
3.When secondary sexual characteristics are well-developed, fathead minnow (20-24 week post hatch). Mature fish should be placed in spawning tank, separate from undeveloped fish. 4. The spawning tank will be divided into 4 individual spawning chambers with appropriate spawning substrates. 5. 4 ♂s and 4 ♀s are randomly chosen and assigned to spawning chambers. 6. Substrates are examined daily and embryos removed, counted, and recorded separately for each pair.	3. Yes, but sexual characteristics not mentioned; day 92 (88 days post hatch; 4. Yes 5. 1♀ and 1♂ assigned to one of the 4 spawning chambers; 8/treatment; actually the 4 spawning chambers were only separated by stainless steel screens therefore only 4/treatment. 6. Yes

Guideline Criteria	Reported Information
7. The adult exposure (fathead minnow) should be terminated when, during the decreasing day-length photoperiod, a 1-wk period passes in which no spawning occurs. 8. Testing using sheepshead minnows should terminate after spawning is observed for 2 wks because this fish spawns readily and almost daily unless immature or affected by a pollutant.	7. No, "The parental exposure was terminated on day 155 after spawning activity had tapered off in terms of frequency, number of spawns, and number of eggs/spawn." 8. N/A
Second Generation Embryo Exposure (4-5 days)	
1. 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. 2. Those embryos not selected are discarded.	1. No, "Sublots of 35 eggs, collected from spawns >50 eggs, were removed from tiles and placed into incubation cups suspended in the growth chambers." 2. Not reported
Second Generation Larval-Juvenile Exposure (4-8 wks)	
1. 8 wk exposure begins with the release of 2 groups of 25 larvae in replicate growth chambers. 2. These larvae should have been produced from different breeding pairs in each spawning tank. 3. Selection of each group should be from early spawnings.	1. 4 replicates however because only a stainless steel screen separates the two of the 4 cells actually there were only 2 replicates; sample size ranged from 28 to 35, if 4 replicated or 56 to 70 if two 2. yes 3. Not reported

Guideline Criteria	Reported Information
4. Each group of 2nd generation fish is terminated 8 wks after hatching. 5. Fish are blotted, weighed, and measured before being discarded.	4. Not reported 5. Blotted wet weight and length measured

Comments:

B. Physical System:

Guideline Criteria	Reported Information
Test Water:	
Sheepshead Minnow 1) May be natural (sterilized and filtered) or a commercial mixture; 2) Natural seawater should have weekly range of salinity less than 6%, monthly pH range less than 0.8 pH units; 3) Salinity should be ≥ 15 parts per thousand; 4) Water must be free of pollutants.	N/A
Fathead Minnow	
1) Test water from well or spring which is not polluted 2) Sterilized and tested for pollutants 3) Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 4) Reconstituted water can be used	1) Well 2) Yes, partially sterilized 3) Hardness:134 to 160 mg/L as $CaCO_3$ pH range: 7.74 to 8.25 4) No

Guideline Criteria	Reported Information
Test Temperature: 1) For fathead minnow 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours; 2) For sheepshead minnow, 30°C is recommended.	1) Fathead range:24.2 to 25.7°C for growth chamber; For spawning 24.2 to 25.9°C 2) N/A
Photoperiod: 1) Simulate wavelength spectra of sunlight Intensity 10 to 100 lumens at water surface. 2) Sheepshead 12-hour light/12-hours dark 3) Fathead dawn-to-dusk at Evansville, IN as of Dec. 1st	1) Used wide-spectrum fluorescent bulbs and plant grow lights; 46±3.8 max. lumens at the water surface 2) N/A 3) Yes
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2. A minimum of 5 toxicant concentrations 3. with a dilution factor not greater than 0.5 and 4. 1 control should be used.	1. Intermittent flow proportional diluter 2. Yes, 5 3. Yes, 0.5 4. How many? 2 What kind? control and solvent control

Guideline Criteria	Reported Information
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10% and periodically checked.	1) No 2) Not reported 3) No 4) Yes, volumetrically but measurements not reported analytical measurements composited
Test Vessels: All glass or glass with a plastic or stainless steel frame.	Yes, glass

Guideline Criteria	Reported Information
Fathead 1. Adult spawning tanks should measure 30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm long with screened-off or separate larval tank.	1. Yes
2. Each larval section is divided in half allowing for two larval growth chambers for each adult spawning tank.	2. Yes
3. Larval chambers should be designed with glass bottoms and drains that allow water to be drawn down to 3 cm.	3. Yes
4.1. Test water must be delivered separately to each adult tank and larval section,	4.1 Yes
4.2 with one-third of the water volume going to the latter.	4.2 Not reported
5.Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	5. Yes 25 and 23 cm
Sheepshead 1. Tanks 45 x 90 x 26 cm with water depth of 19 cm recommended. 2. Larval chamber design and test water divided are the same as described for the fathead minnow.	N/A

Guideline Criteria	Reported Information
Embryo and Fry Chambers: 1. 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. 2. Chambers can be oscillated vertically (2.5 to 4.0 cm) (rocker arm apparatus, 2 rpm motor) or placed in separate chambers with self-starting siphons.	1. Yes, volume not reported just the diameter 9 cm 2. Yes, except the cups were oscillated vertically 3-6 cm; low rpm motor; rpm not reported
Flow Rate: 1. Flow rates to larval cups should provide 90% replacement in 8-12 hours. 2. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level (cannot drop below 20% with fish in the tank).	1. Yes-12.2 in a 24 hour period 2. No, First 70 days DO ranged from 63 to 100%;day 84 single replicate fell to 44%; day 85-87 DO was 62-87%; day 126 to termination 47-101%, one occasion DO below 60%; day 91 to termination of the parental generation on day 154, DO 48-97%
Aeration: 1. Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2. Test tanks and embryo chambers should not be aerated.	1. Not reported 2. Not reported

C. **Chemical System:**

Guideline Criteria	Reported Information

Concentrations: 1.1 Minimum of 5 concentrations and a control, 1.2 all replicated, plus solvent control if appropriate. 2. - Toxicant conc. must be measured in one tank at each toxicant level every week. 3. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	1. Number of conc. = 5 and replicates = 4 2. Yes, measured days -1,0 ,1 7, 14, and least weekly thereafter. 3. No, all analyses were composites of two or more replicates. 4. No, all levels were affected for the parental growth (both weight and length) when compared to the control.
Other Variables: 1. DO must be measured at each conc. at least once a week; 2. Freshwater parameters in a control and one conc. must be analyzed once a week for pH, alkalinity, hardness, and conductance 3. Natural seawater must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range < 0.8 pH units.	1. every two weeks except days 84, 85, 86, 87 additional samples were taken 2. Yes 3. N/A
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Flow rate: Not reported Solvent: acetone

12. **REPORTED RESULTS:**
Reported Statistical Results for Biological Endpoints:

Guideline Criteria	Reported Information

Data Endpoints must include:	
1. Survival of F_0 and F_1 embryos, (Examined daily and embryos removed, counted, and recorded separately for each pair)	1. Yes
2. Time required to hatch, 3. Hatching success, 4. Survival of fry for 4 wks are determined and recorded.	2. No 3. Yes 4. NO, 35 days post hatch
5. Dead embryos usually turn opaque and must be counted and 6. removed each day until hatching is complete.	5. Yes 6. Yes
7. Live fungused embryos must be must be removed daily 8. and counted as dead.	7. No 5 and 8 weeks 8. Yes
9. Survival of F_0 fish during larval-juvenile exposure period	9. Yes
10. Survival determined in each replicate growth chamber at least once a week.	10. Daily
11. Survival is determined by counting the number of live fish, because dead larvae deteriorate rapidly.	11. Yes
12. At 4 and 8 weeks after hatching, total lengths (mm) of all fish must be recorded.	12. Bioaccumulation, 96 hours LC_{50}, and observations (see below)
13. At 8 weeks after hatching of F_1 fish, weights and lengths are recorded	13. Yes
14. Incidence of pathological or histological effects	14. Yes
15. Observations of other effects or clinical signs	15. Bioaccumulation, 96 hours LC_{50}, and observations (see below)

Growth and Survival of F0 and F1 Generations at Days 35, 56, and 84					
F0	Days (post hatch)		Survival	Length	Weight
	35	NOEC/ LOEC	NSD[1]	0.469/ 0.914 µg/L	N/A
	56	NOEC/ LOEC	NSD	0.469/ 0.914 µg/L	N/A
	84	NOEC/ LOEC	NSD	0.161/ 0.231 µg/L	0.161/ 0.231 µg/L
F1					
	56	NOEC/[1] LOEC	NSD	NSD[2,3]	NSD[3]

[1] No Significant Difference
[2] Level 5 deleted from growth analysis due to statistically significant survival effect.
[3] At least one replicate in each treatment was kept open to generate F1 tissue for determination of BCF's. In level 4, there were inadequate numbers or quality of spawns to grow F1 generation fish to 56 days post-hatch.

Egg Hatchability		
Generation	Day	NOEC/LOEC
F0	---	---
---	6	>0.914/ N/A

Reproduction			
Mean Number of Days to 1st Spawn	Mean Number of Spawning Days	Mean Number of Spawns Per Pair	Mean Number of Eggs Per Spawn
NSD[1]	NSD	NSD	NSD

[1] No Significant Difference

Tissue Bioconcentration							
	Level 1		Level 3		Level 1 BCF		Level 3	
Newly Fertilized Embryos	93.1 to 141 µg/kg		348 to 504 µg/kg		1420-2160X		1510-2180X	
72-96 Hour Old Embryos	161 to 207 µg/kg		511 µg/kg		2460-3170X		2210X	
10-14 day Post-Hatch Larvae	414 to 429 µg/kg		No Samples		6330-6560X		No Samples	
Pre-Spawn Adults	601 to 793 µg/kg		1600 to 2030 µg/kg		9190-12100X		6930-8790X	
Post-Spawn Adults	♂ 1100 to 1290 µg/kg	♀ 876 to 902 µg/kg	♂ 3300 to 4270 µg/kg	♀ 2820 to 3370 µg/kg	♂ 16800-19700X	♀ 13400-13800X	♂ 14300-18500X	♀ 12200-14600X

96-Hour Acute Study LC_{50}:

9.6 µg/L

Morphological and Behavioral Observations

Parental Generation
1) Spinal curvature
2) Erratic swimming behavior
3) Malformed tail fin
4) Minor hemorrhaging
5) Bloating of females during latter stages of spawning phase
6) Quiescence
7) Fish resting on the bottom of the test chamber
8) Eye malformation

ABC's Conclusion: "These physical phenomena, with the exception of the eye malformations, were noted in 1-2 individuals in the dilution water control and in levels 1,2,4, and 5. The eye malformations appeared only in level 4

spawning adults. It is unlikely that these responses were the result of exposure to TPTH and they are not biologically significant."

F1 Generation
1) Spinal curvature
2) Erratic swimming behavior
3) Quiescence
4) Surfacing

ABC's Conclusion: "These physical phenomena were noted in 1-2 indivisuals in the dilution water control, vehicle blank, and levels 2,4,and5. It is unlikely that these responses were the result of exposure to TPTH and they are not biologically significant. "

Other Observations

These two statements would appear to show that only one replicate was available for the 0.469 µg/L level, Level 4. Also, Table XII and Table XVI only report data for one replicate for Level 4. "Adults in the 0.469 µg/L mean measured test concentration produced fewer eggs, resulting in fewer available data sets for evaluation.""In level 4, there were inadequate numbers or quality of spawns to grow F1 generation fish to 56 days post-hatch."

The number of embryos killed by fungus was very high in the vehicle coontrol. The following table shows the high mortality in vehicle control.

Hours	Control	Vehicle Control	0.0654	0.161	0.231	0.454	0.916
24	4	5	0	0	0	3	7
48	1	27	1	0	0	2	1

The time to hatch data was not presented in a table. However, the following statements was made: "Time to hatch proceeded at approximately the same rate in all treatments and did not appear to be concentration dependent." Also, ABC found no statistical differences.

Raw data included? (Y)

Statistical Results:

Statistical Method: ANOVA

NOEL: 0.161 µg/L LEL: 0.231 µg/L

MATC: 0.191 µg/L

Most sensitive endpoint: Parental generation growth

Comments:

13. Reviewer's Discussion:

Statistical Results

Statistical Method: both Williams and ANOVA

NOEC:<0.0654 µg/L LOEC: N/A MATC: N/A

Most Sensitive Endpoint: Parental generation growth
Attached are the EEB printout from Toxstat showing that both the ANOVA and Williams indicate that when compared to the control rather than the pooled control the lowest concentration cause effects on growth to the parental generation. Based on this an NOEC and LOEC were not derived by the test.

Significant Items

The failed to produce an NOEC for growth of parental generation.

The DO in the growth chambers was below guideline recommended level of 75% from day 56 to day 86 for all but level 1 and level 3 concentrations. Also, the DO fell below 75% on day 154 for all but the control and levels 4 and the control. When, where and how the dilution water was aerated was not reported. It appears flow rate has an influence the flow of 12.2 volumes /24 hour period in the growth chamber provided higher concentrations than the 10.8 in the spawning chamber. (See attached graphs of growth and spawning chambers)

Flow-splitting accuracy was not verified by chemical analysis.

Several items mentioned in EEB's protocol review (Doug Urban's memorandum of August 25, 1992) were not addressed most importantly: tissue for the bioaccumulation study should have been from the low, mid, and high levels rather than the control, low, and mid levels.