

US EPA ARCHIVE DOCUMENT

MRID No. 406501-02

**DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4**

1. **CHEMICAL:** Ethoprop PC Code No.: 041101

2. **TEST MATERIAL:** Ethoprop technical Purity: >95.5%

3. **CITATION:**

Author: D.C. Surprenant
Title: The Toxicity of Ethoprop Technical to
Fathead Minnow (*Pimephales promelas*)
Embryos and Larvae

Study Completion Date: December 3, 1987

Laboratory: Springborn Life Sciences, Inc., Wareham,
MA

Sponsor: Rhone-Poulenc Ag Company, Research
Triangle Park, NC

Laboratory Report ID: BW-87-6-2408

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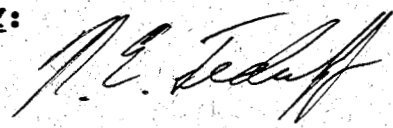

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist
KBN Engineering and Applied Sciences, Inc.

Signature:  Date: 7/8/97

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat Date: 7/8/97

5. **APPROVED BY:**

Signature:  Date: 7/30/97 
10/31/97

6. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a fish early life-stage toxicity test. Based on mean measured concentrations, the MATC for fathead minnows exposed to ethoprop technical was between 26 and 54 ppb. The geometric mean MATC was determined to be 37 ppb.

7. **ADEQUACY OF THE STUDY:**

A. **Classification:** Supplemental.

B. **Rationale:** Raw data for the chemical analyses and the length and weight measurements were not included in the report.



- C. **Repairability:** Yes, if the individual dry weights and lengths for the fish exist, then proper analysis and incorporation into the study report may upgrade the study to "Core."

8. **MAJOR GUIDELINE DEVIATIONS:**

1. Only two replicates per group were used for both the hatching and post-hatch exposure periods.
2. Morphological and behavioral observations were not reported in the results.

9. **MATERIALS AND METHODS:**

A. **Biological System**

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	<i>Pimephales promelas</i>
Source: Commercial fishery, wild, or brood stock.	In-house brood stock
Age at beginning of test: Embryos 2 to 24 hours old.	<24 hours old at initiation
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	60 eggs/cup, 2 cups per treatment or control 40 fish/replicate, 2 replicates per treatment or control
Posthatch: % of embryos that produce live fry must be $\geq 50\%$ in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	83% egg hatch in dilution water and 84% in solvent control. 1.1 times
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish fed two or three times a day. Food was not withheld before study termination.

Guideline Criteria	Reported Information
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Larval survival was estimated at least twice weekly.
Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $< 70\%$.	Average of 94% and 91% survival in the dilution water and solvent control groups, respectively. Survival in each control group replicate was $\geq 90\%$.
Controls: Negative control and carrier control (when applicable) are required.	Dilution water and solvent controls.

Comments: Only two replicates were used for each treatment level and control. Larval survival was estimated twice weekly, but only data for the termination of the study (31 days post-hatch) were reported.

B. Physical System

Guideline Criteria	Reported Information
Test Water: 1) May be natural (well or spring) or reconstituted water. 2) Water should be sterilized with UV radiation and screened for contaminants. 3) Hardness of 40-48 mg/L as CaCO_3 , pH of 7.2-7.6	1) Natural well water. 2) Water was aerated and screened for contaminants, but not sterilized. 3) Mean hardness of 29 to 31 mg/L as CaCO_3 , pH range of 6.6 to 7.5.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For fathead minnow, 25°C is recommended.	Average of 24°C .
Photoperiod: Recommend 16L/8D.	16 h light, 8 h dark

Guideline Criteria	Reported Information
<p>Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</p>	<p>Intermittent-flow proportional diluter.</p> <p>Treatment concentrations of 31, 63, 130, 250, and 500 µg/L, corrected for purity.</p>
<p>Toxicant Mixing:</p> <ol style="list-style-type: none"> 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%. 	<ol style="list-style-type: none"> 1) Mixing chamber used. 2) No aeration of exposure solutions. 3) Mixing confirmed by analysis. 4) Not reported.
<p>Test Vessels: All glass or glass with stainless steel frame.</p>	<p>39 x 20 x 25 cm glass aquaria.</p>
<p>Embryo Cups: 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p>	<p>150-ml glass jars with 40-mesh Nitex-screen bottoms.</p>
<p>Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.</p>	<p>7 volume replacements/24 h.</p> <p>90% replacement every 7-8 h.</p> <p>DO and chemical concentrations confirmed by analysis.</p>
<p>Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	<p>DO ≥86% (7.2 mg/L) of saturation at all times.</p>

Comments: Dilution water was not sterilized.

C. Chemical System

Guideline Criteria	Reported Information
<p>Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <ul style="list-style-type: none"> - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage. 	<p>-Yes; control, solvent control and five concentrations.</p> <p>-Yes, measured on day 0, 4, and weekly thereafter in each replicate chamber</p> <p>-Yes</p>
<p>Other Variables: DO must be measured at each conc. at least once a week.</p>	<p>Yes, DO \geq86% at all times.</p>
<p>Solvents: Should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>Acetone (18 μL/L).</p>

Comments: None.

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Data Endpoints must include:</p> <ul style="list-style-type: none"> - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs. 	<p>Data include:</p> <ul style="list-style-type: none"> - Hatching survival; - Time to hatch (4 days); - 31-day post-hatch survival; - 31-day post-hatch length; - 31-day post-hatch wet weight.
<p>Raw data included? (Y/N)</p>	<p>No</p>

Effects Data

Toxicant Concentration (ppb)		Survival at Hatch (%)	Percent Post-Hatch Survival (31 days)	Total Length (mm)	Wet Weight (mg)
Nominal	Measured				
Control	<3.4	83	94	32	348
S. Con.	<3.4	84	91	33	351
31	26	90	91	32	348
63	54	89	94	30	298
130	96	92	51	20	142
250	210	84	10	17	78
500	420	92	6	12	24

Toxicity Observations: None reported.

Statistical Results:

Statistical Method: Williams' test

NOEC: 26 ppb LOEC: 54 ppb MATC: 37 ppb

Most sensitive endpoint: larval growth (total length and wet weight)

Comments: Data from the treatment groups were compared to data from the pooled control. Statistical analysis of growth data used mean replicate values.

11. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Williams' test

NOEC: 26 ppb LOEC: 54 ppb MATC: 37 ppb

Most sensitive endpoint: larval growth (wet weight)

Comments: Data from the treatment groups were compared to data from the solvent control group.

12. **REVIEWER'S COMMENTS:** This study is scientifically sound but does not fulfill the guideline requirements for a fish early life-stage toxicity test using the fathead minnow. Based on mean measured concentrations, the MATC for fathead minnows exposed to ethoprop technical was between 26 and 54 ppb. The geometric mean MATC was determined to be 37 ppb. This study is classified as **Supplemental**.