



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 10 1993

MEMORANDUM

Subject: Trifluralin Vertebral Lesion Study Review and Update of Data Requirements (D182361).

To: Walter Waldrop, PM 71
Special Review and Reregistration Divisions, H7508W

From: Anthony F. Maciorowski, Chief *Anthony F. Maciorowski*
Ecological Effects Branch
Environmental Fate and Effects Division, H7507C

EEB has completed the review of the Vertebral Lesion Study on Fathead minnows submitted by DowElanco for Trifluralin. The following is a summary of that review:

Meyerhoff, R.D. and M.D. Gunnoe. 1992. The Toxicity of Trifluralin to Fathead Minnow (Pimephales promelas) in a 35-Day Vertebral Lesion Study. Laboratory Project ID: F00890. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424396-01.

This study does not satisfy the requirement for a fish vertebral lesion study with trifluralin. There was contamination in both the acetone and water controls that led to detectable residues in the fish at termination; this may have led to unreliable statistical analysis. The stock fish that were used as a negative control were three to four weeks older than the test organisms at the time of radiographic exams. Also the stock fish had high incidence of wavy ribs (27.5%) and vertebral anomalies (23.8%). Based on the discrepancies found in this study and that the stock fish were not suitable for negative controls, the data generated cannot be used in an ecological risk assessment for trifluralin.

The Registration Division has requested an indication if Guideline #72-7, Aquatic Field Study, can be waived with this submittal. Even though this study is invalid, the Aquatic Field Study is no longer required. With the previously submitted in-house field data (Accession Nos. 26013, 260214) and the newly published results of the National Study of Chemical Residues in Fish, Office of Science and Technology/EPA - 9/92, EEB has ample data to perform a risk assessment regarding the hazards to non-target fish.

There is an ecological 'Level of Concern' for fish that are exposed to low levels of trifluralin. The National Study of

Chemical Residues in Fish reports that residue levels of trifluralin are present in fish that were sampled throughout the United States at and above levels that have been found to produce vertebral lesions in field studies. [NSCRF reported residues of 0-458 ng/g, 1985 Field Study reported vertebral anomalies in fish with residues of 2.0 to 290.0 ng/g].

The following data requirements are outstanding:

#123-1 Tier 2 Seed Germination (cabbage and onion only)

Tier 2 Seedling Emergence

#123-2 Tier 2 Aquatic Plant Growth and Reproduction

The following species:

-Lemna gibba

-Skeletonema costatum

-Anabaena flos-aquae

- a freshwater diatom

#201-1 Droplet Size Spectrum

#201-2 Drift Field Evaluation

Questions regarding this review, please contact Dana Lateulere, 308-2856.

Quad III/2
August 10, 1992



Office of Pesticide Programs (H7504C)
Document Processing Desk (RS 179)
U.S. Environmental Protection Agency
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

Attention: Ms. Terri Stowe (H7508W)

RE: Trifluralin Data Development Consortium 59011
NOEC Level for Trifluralin to Fish
Submission of Study Under Guideline 72-4 to Satisfy Needs for Risk Assessment

In a protocol approved by EPA, DowElanco conducted a modified early life stage fish study to establish a definitive NOEC for trifluralin. This NOEC is to be used as the basis to conduct a risk assessment to determine the necessity of conducting an additional field study.

This submission contains:

<u>Volume & Guideline Ref. No.</u>	<u>MRID</u>	<u>Study/Contents</u>
Volume 1 (Administrative)		Transmittal Document (this letter) General Summary for Public Release (F T2E TWFISH AM 127-128)
Volume 2 (72-4)	42439601	Study entitled: The Toxicity of Trifluralin to Fathead Minnow (<i>Pimephales promelas</i>) in a 35-Day Vertebral Lesion Study, Laboratory I.D. F00890 (3 copies) (T2E TWFISH AM 129-277)

The submitted study demonstrated that a no-observed-effect concentration does exist for trifluralin. Trifluralin was bioconcentrated by fathead minnows and residues in fish tissue increased as exposure concentrations increased. Bioconcentration factors ranged from 1750 to 8870 in whole fish. No significant reduction in survival or growth was observed during the test at analyzed trifluralin concentrations $\leq 3.2 \mu\text{g/L}$. A similar no-effect concentration ($1.9 \mu\text{g/L}$) for survival and growth was found in a fathead minnow life-cycle study reported in 1976. Exposure to trifluralin concentrations of 0.7 to $30 \mu\text{g/L}$ resulted in a concentration-related increase in the incidence of vertebral lesions, but no statistically significant effect was found at $0.3 \mu\text{g/L}$. The severity of the vertebral lesions only appeared to increase at trifluralin concentrations of 8.6 and $30 \mu\text{g/L}$. Histopathologic findings were consistent with these radiographic findings. Survival, size, appearance, lesion frequency, and lesion severity in fathead minnows were not affected by exposure to an average trifluralin concentration of $0.3 \mu\text{g/L}$ for 35 days. The average trifluralin residue level in fathead minnows was 2.66 mg/kg when exposed to this trifluralin concentration in water. Based on the results of this study, the chronic no-observed effect concentration in water for fathead minnows was $0.3 \mu\text{g/L}$.

The safety of trifluralin to fish can be assessed by comparing measured concentrations of trifluralin in water and duration of exposure in the field to the results from this laboratory toxicity study with fathead minnows. Francis *et al.* (1985) (MRID # 00155978) collected samples from a 2.1-acre farm pond that received runoff from a 39-acre watershed treated with trifluralin and never detected the compound in pond water (detection limit 0.3 $\mu\text{g/L}$). Sheets *et al.* (1972) were, however, able to find a trifluralin concentration of 1.6 $\mu\text{g/L}$ in a pond, but this concentration declined to 0.29 $\mu\text{g/L}$ 6 days later. Trifluralin rapidly dissipates from surface waters, with a calculated half-life between 3 and 7 days (Francis *et al.*, 1985). Analysis of 1072 samples of surface water collected from community water systems throughout major soybean growing regions resulted in detection of trifluralin in only three samples. These three samples had trifluralin concentrations of 0.20, 0.21, and 0.32 $\mu\text{g/L}$ (Meyerhoff and Francis, 1988) (MRID # 40809601). The EPA STORET Water Quality File contained results of trifluralin analyses for at least 2168 water samples collected from 246 sites in 14 midwestern, western, and southern states and Manitoba. Most of these sites are in major agricultural areas. Trifluralin was either not detected or was at concentrations less than 1 $\mu\text{g/L}$ in over 99% of the samples (Meyerhoff and Francis, 1988). Based on information from all of these sources, over 98% of the surface water samples (>3300) collected from rivers, streams, and ponds for trifluralin analysis had concentrations < 0.2 $\mu\text{g/L}$.

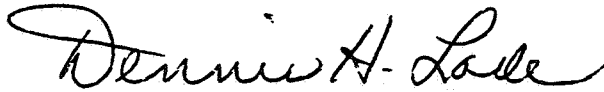
Since the no-observed effect concentration of trifluralin for all effects, including vertebral lesions, in fathead minnows is 0.3 $\mu\text{g/L}$, it is unlikely that populations of fish in agricultural regions will be significantly affected from exposure to the low levels of trifluralin that rapidly dissipate from surface waters.

There appears to be no need to conduct a survey of fish in the field looking for tissue residues of trifluralin. Chronic tissue levels would have to be higher than 2.66 mg/kg to be near levels found in fathead minnows with increased incidence of vertebral lesions. These levels are substantially higher than any previously found in the field (Francis *et al.*, 1985)

If you have questions, please do not hesitate to call the study director, Dr. Roger Meyerhoff, at (317)277-4748 or myself at (317)870-7269.

Sincerely,

DowElanco



Dennis H. Lade, Ph.D.
Product Registration Manager

DHL:mt
DHL10

cc: Joanne I. Miller (PM-23)

DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin.
Shaughnessey No. 036101.
2. **TEST MATERIAL:** Trifluralin (compound 036352); α, α, α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Lot No. 326EF8; 99.86% active ingredient.
3. **STUDY TYPE:** 72-4. Freshwater Fish, Early Life-Stage Flow-Through Toxicity Test. Species Tested: Fathead Minnow (*Pimephales promelas*).
4. **CITATION:** Meyerhoff, R.D. and M.D. Gunnoe. 1992. The Toxicity of Trifluralin to Fathead Minnow (*Pimephales promelas*) in a 35-Day Vertebral Lesion Study. Laboratory Project ID: F00890. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424396-01.
5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M Rifici*
Date: 10/27/92
6. **APPROVED BY:**

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

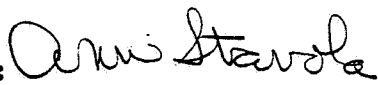
Signature: P. Kosalwat
Date: 10/27/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: *Henry T Craven*
Date: 3/12/93
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an early life-stage toxicity test using fathead minnows. The test results provide valuable supplemental information on the pathological effects of trifluralin in juvenile fathead minnows. However, trifluralin effects on the early life-stages of the fathead minnow (i.e., embryos and larvae) have not been determined since this test was performed using 30-day old juvenile fish. Based on pathology observations at the end of the test, the MATC of trifluralin for juvenile fathead minnows was >0.3 and $<0.7 \mu\text{g/l}$ mean measured concentration.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin.
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3. **STUDY TYPE:** 72-7a Special Circumstance. Freshwater Fish, 35 Day Vertebral Lesion Study. Species Tested: Fathead Minnow (Pimephales promelas).
4. **CITATION:** Meyerhoff, R.D. and M.D. Gunnoe. 1992. The Toxicity of Trifluralin to Fathead Minnow (Pimephales promelas) in a 35-Day Vertebral Lesion Study. Laboratory Project ID: F00890. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424396-01.
5. **REVIEWED BY:**
Dana Lateulere, Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division
(Edited KBN submittal,
L. Rifici, 10/27/92)
Signature: 
Date: 3/3/93
6. **APPROVED BY:**
Ann Stavola, Section Head
Ecological Effects Branch
Environmental Fate and
Effects Division
Signature: 
Date: 3/10/93
7. **CONCLUSIONS:** This study does not satisfy the requirement for a fish vertebral lesion study with trifluralin. There was contamination in both the acetone and water controls that led to detectable residues in the fish at termination; this may have led to unreliable statistical analysis. The stock fish that were used as a negative control were three to four weeks older than the test organisms at the time of radiographic exams. Also the stock fish had high incidence of wavy ribs (27.5%) and vertebral anomalies (23.8%). Based on the discrepancies found in this study and that the stock fish were not suitable for negative controls, the data generated cannot be used in an ecological risk assessment for trifluralin.
8. **RECOMMENDATIONS:**

9. **BACKGROUND:** This study was required based on a level of concern from pre-existing data that indicated low levels of trifluralin may cause vertebral lesions in fish.

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 embryos were held in test dilution water for 4 days prior to hatching and remained in the brood unit for an additional 30 days before initiating the study.

B. **Test System:** A proportional diluter delivered test solution or control water to each of four replicate test chambers per concentration. The test chambers were 10-gallon glass aquaria containing approximately 18 l of test solution (solution depth of 15 cm). The test solution overflowed from each aquarium through a hole drilled in the side. The aquaria were arranged in a randomized block design on the test rack. The laboratory was maintained on a 16-hour daylight photoperiod during acclimation and testing.

A primary stock solution (0.51 mg/ml) of the test material was prepared in acetone. The diluter stock solution, a nominal concentration of 50 µg/l, was prepared using 20 ml of the acetone stock and 205 l of dilution water in a stainless steel barrel. Three barrels were prepared daily. An automated injector delivered acetone to the solvent control solution to provide a concentration of 0.1 ml acetone/l. The diluter cycling rate provided 4.4 volume replacements per day.

The dilution water was well water obtained on-site. The water was treated to remove iron, 50% of the mineral content (using electrodialysis), and excess CO₂ (to adjust pH). The water was stored in underground tanks and warmed or cooled to test temperature before delivery to the diluter system. During the 5-week pretest period, the water characteristics averaged a hardness of 131 mg/l as CaCO₃, an alkalinity of 138 mg/l as CaCO₃, and a conductivity of 234 µS/cm.

C. **Dosage:** Thirty-five-day flow-through test. Five nominal concentrations (0.6, 1.9, 5.6, 16.7, and 50.0 µg/l), a dilution water control, and a solvent control were used.

- D. **Design:** Thirty fish were placed in each aquarium. The biomass of fish at test termination did not exceed 0.1 g/l/day or 0.53 g/l at any time.

The aquaria were observed three times per week for dead and deformed fish and any dead fish were removed. Behavioral effects were noted. The minnows were fed live brine shrimp and salmon starter mash 2-3 times daily throughout the test. The aquaria were cleaned of excess food and fecal material at least 3 times per week.

At test initiation, all fish in each replicate were transferred to a tared water-filled container. Initial group weights in each replicate were then determined to the nearest 0.01 g. Initial lengths were determined photographically. At test termination, the total length and body depth of fish from each replicate were determined photographically. The fish were also weighed (blotted dry) individually to the nearest 0.0001 g.

Ten fish from each replicate were collected, if possible, for whole body residue analysis. In addition, fish collected from the stock population of minnows used for the study were sampled for residue analysis and recovery validation. Approximately 20 fish per replicate were radiographically examined to determine the incidence of vertebral lesions. Histologic examination was conducted on selected fish to confirm radiologic findings. In addition, two fish from each group were prepared for histopathological examinations.

The temperature, dissolved oxygen concentration, and pH were measured daily in one replicate of the controls and each treatment level. The temperature was also measured continuously in one control aquarium with a 7-day temperature recorder. The hardness, alkalinity, total ammonia, and conductivity were measured in one replicate of the solvent control and highest test level at test initiation and at least weekly thereafter.

Concentrations of Trifluralin were measured on study days 6, 9, 13, 16, 20, 23, 27, 30, and 35. The samples were collected from the four replicates of each concentration and pooled before analysis by gas chromatography.

- E. **Statistics:** Analysis of variance (ANOVA) and Dunnett's test were used to analyze fish survival, length, depth, and weight data. Survival data were arcsine square root transformed prior to analysis. Dilution water control and solvent control responses were compared using F-tests and pooled prior to subsequent analyses.

Results from radiographic analyses were analyzed using a sequential trend test. All hypothesis testing was performed at $\alpha=0.05$.

12. **REPORTED RESULTS:** No trifluralin was detected in the controls. The mean measured concentrations were 0.3, 0.7, 3.2, 8.6, and 30 $\mu\text{g/l}$. These values represented 37 to 61% of nominal concentrations (Table 1, attached). The analytical limit of detection was 0.5 $\mu\text{g/l}$. No trifluralin was detected in the lowest test level (0.3 $\mu\text{g/l}$ mean measured concentration) on days 13, 27, 30 and 35, these values were averaged in as 0 to determine the mean measured concentration.

"The concentration of trifluralin in fish tissue was directly related to trifluralin concentrations in test solutions. Mean analyzed concentrations of trifluralin in fish tissue were 2.66, 4.23, 17.0, 24.7, and 52.6 mg/kg for test solutions containing average trifluralin concentrations of 0.3, 0.7, 3.2, 8.6, and 30 $\mu\text{g/l}$, respectively" (Table 2, attached). The bioconcentration factors were 8870, 6040, 5310, 2870, and 1750. All acetone and dilution water control fish contained detectable levels of trifluralin, but most were below the limit of quantitation (0.05 mg/kg). No residues (detection limit = 0.02 mg/kg) were found in stock fish from the same lot as the test fish.

At test termination, the survival of fathead minnows at 8.6 and 30 $\mu\text{g/l}$ was significantly reduced compared to the pooled controls (Table 5, attached).

No treatment-related behavioral effects were observed in fish exposed to test concentrations $\leq 0.7 \mu\text{g/l}$. The average size of the treatment and control fish at the beginning of the study were similar (Table 4, attached). Since the growth of the dilution water control fish and solvent control fish were not significantly different, the two groups were pooled prior to analysis. Total length at concentrations $\geq 8.6 \mu\text{g/l}$ was significantly lower than that of the pooled control (Table 5, attached). Body weight and depth were not significantly effected by exposure to trifluralin (Table 5, attached).

There was a concentration-dependent increase in spinal column compression/deviation (Table 6, attached). An increased incidence of wavy rib bones was also noted in trifluralin-exposed fish. Many of the fish with remarkably wavy ribs had little or no spinal column abnormalities and many fish with prominent spinal column abnormalities had minimal or no rib abnormalities. Wavy ribs were found more often in fish exposed to acetone than in fish from the water control or stock culture. No increase in the incidence or severity of spinal column or rib abnormalities was noted in the fish exposed to 0.3 $\mu\text{g/l}$ trifluralin.

The temperature of the test solutions ranged from 23.6 to 25.8°C. Dissolved oxygen concentrations averaged 6.5 ± 0.9 (2.5-9.1) mg/l. The pH values ranged from 7.7 to 8.2. The conductivity, hardness, and alkalinity in the solvent control and 30 $\mu\text{g/l}$ test solution averaged 261 $\mu\text{S/cm}$, 117 mg/l as CaCO_3 , and 148 mg/l as CaCO_3 , respectively. Un-ionized ammonia levels were <0.01 mg/l during the study.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No significant reduction in fathead minnow survival or growth relative to controls was observed during the test at concentrations ≤ 3.2 $\mu\text{g/l}$. Exposure to 0.7-30 $\mu\text{g/l}$ resulted in a concentration-related increase in the incidence of vertebral lesions, but no statistically significant effect was found at 0.3 $\mu\text{g/l}$. The histopathologic findings were consistent with the radiographic findings. The incidence of abnormal ribs was significantly increased in the three highest concentrations, but did not follow a concentration-response. The presence of acetone appeared to strongly influence the occurrence of wavy ribs.

Over 98% of water samples collected from rivers, streams, and ponds for analysis of trifluralin have concentrations <0.2 $\mu\text{g/L}$. Trifluralin rapidly dissipates from surface water, with a half-life between 3 and 7 days. Since the no-observed-effect concentration of trifluralin is for all effects, including vertebral lesions, in fathead minnows is 0.3 $\mu\text{g/L}$, it is unlikely that populations for fish in agricultural regions are significantly affected from exposure to the low levels of trifluralin that rapidly dissipate from surface waters.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in compliance with EPA Good Laboratory Practice Standards (40 CFR Part 160). The dates and types of quality assurance inspections were also reported.

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

- A. Test Procedure: The study authors cited U.S. EPA SEP (1982) and ASTM (1988) [early life stage test guidance] as procedures similar to those used during this study [EPA has no specific vertebral lesion SEP). The following are discrepancies found with the study:

The physical characteristics of the test material (i.e., physical state, appearance) were not described.

The report did not state whether the fish were randomly or impartially placed in the test containers.

The fish were fed live brine shrimp and salmon starter 2-3 times daily. The ASTM recommends feeding four times daily (two times daily on weekends).

A 30-minute transition period between light and dark is recommended in the SEP. A transition period was not used in the study. The light intensity was not reported.

All acetone and water control fish had detectable trifluralin residues at test termination, the author states that the "control contamination was somehow related to the exposure system". Benefin was also found in three of four replicates of the acetone controls.

Stock fish that were used as a negative control for the comparison of vertebral lesion occurrences were three to four weeks older than the test organisms. ASTM requires a negative control; based on the age difference the stock fish cannot be used for comparisons.

- B. Statistical Analysis: The reviewer has determined the data to be unreliable; therefore, statistical analysis was not performed.

- C. Discussion/Results: Based on the control contamination that occurred, the results from this test are not reliable. The trifluralin contamination led to detectable residue levels in all of the acetone and water control fish that were analyzed at the termination of the test. The percentage of vertebral lesion incidence found in the controls may be attributed to the contamination; therefore, statistical

analysis using the controls may give misleading results.

The occurrence of wavy ribs was significantly increased in the acetone controls when compared to the dilution water controls; however both had a high occurrence of the abnormality, 22.8% and 15% respectively. The combination of trifluralin and acetone evidently heightened the chances of wavy ribs in the fathead minnows. Therefore, statistically analyzing the treatment with these control data are unreliable.

The stock fish were utilized (somewhat) as a negative control; a negative control is required by ASTM standards. The comparison of stock fish vertebral lesion incidences with those in the test fish is not valid based on the age difference of the fish. The fish in the test were 65 days old when terminated for radiographic exams, the stock fish were not terminated for another three to four weeks. Because of the age difference, the stock fish radiographic exams cannot be used as a negative control. Also, the occurrence of vertebral lesions (23.8%) in the stock fish was abnormally high (Couch, et. al. 1979, Wells & Cowan, 1982). Therefore, based on ASTM guidelines, the study is deficient without a negative control.

Based on the afore mentioned discrepancies, the data generated from this study cannot be used in an Ecological Risk Assessment for trifluralin.

D. Adequacy of the Study:

- (1) **Classification:** Invalid.
- (2) **Rationale:** Contamination of controls, control effects, negative controls were not the same age as test organisms.
- (3) **Repairability:** No.

References:

Wells, D.E. and A. A. Cowan. "Vertebral Dysplasia in Salmonids caused by the Herbicide Trifluralin". Environmental Pollutions (Series A) 29 (1982) 249-260.

Couch, J.A. et al. "Vertebral Dysplasia in young fish exposed to the Herbicide Trifluralin". Journal of Fish Diseases, 1979, 2, 35-42.

Page _____ is not included in this copy.

Pages 14 through 20 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
 - ☐ Identity of product impurities.
 - ☐ Description of the product manufacturing process.
 - ☐ Description of quality control procedures.
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