

US EPA ARCHIVE DOCUMENT

11-3-93

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Hexaflumuron. Shaughnessey Number: 118202.
- 2. **TEST MATERIAL:** XRD 473; 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl) urea; Lot No. 442-35-20i; CAS No. 86479-06-3; purity of 99.7%; water solubility of 27 µg/l; a fine white crystalline solid.
- 3. **STUDY TYPE:** 72-1. Freshwater Fish Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).
- 4. **CITATION:** Mayes, M.A. 1992. The Acute Toxicity of XRD 473 to Bluegill Sunfish: Summary Evaluation and Original Study (Conducted in 1987). Study performed by Aquatox Testing Facility, Life Science Research Ltd., Suffolk, England. Submitted by DowElanco. EPA MRID No. 426485-11.

5. **REVIEWED BY:**

Robert I. Rose  
 Ecological Effects Branch  
 Environmental Fate &  
 Effects Division (H7507C)

Signature: *[Handwritten Signature]*  
 Date: 13 NOV 1993

6. **APPROVED BY:**

Leslie W. Touart  
 Head, Section I  
 Ecological Effects Branch  
 Environmental Fate &  
 Effects Division (H7507C)

Signature: *[Handwritten Signature]*  
 Date: 3 Nov 93

~~Anthony F. Maciorowski, Chief  
 Ecological Effects Branch  
 Environmental Fate &  
 Effects Division (H7507C)~~

~~Signature:  
 Date:~~

7. **CONCLUSIONS:** This study is scientifically sound. Measured concentrations decreased substantially from test initiation to test termination; therefore, the actual concentrations to which the test organisms were exposed are unknown. Based on mean measured concentrations, the 96-hour LC<sub>50</sub> was calculated to be >255.6 µg/l which would, at worst, classify XRD 473 as highly toxic to *Lepomis macrochirus*. This study was determined to be of supplemental adequacy because of difficulty in keeping the test material in solution or suspension due to its low solubility of 27 ppb. However, the data was sufficient to indicate no acute toxicity at concentrations equal to or below solubility.

8. RECOMMENDATIONS:
9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Animals: Bluegill fry (*Lepomis macrochirus*) were obtained from Hazleton Research Products Inc., Denver, PA. During the holding period, the fry were maintained under flow-through conditions in filtered, dechlorinated tap water (hardness of 50 mg/l as CaCO<sub>3</sub>). During the fourteen days prior to test initiation, the holding water had a temperature of 16.9-20.3°C, a pH of 7.30-7.63, and a hardness of 44-47 mg/l as CaCO<sub>3</sub>. The fish were fed trout pellets daily, except on the day prior to test initiation when food was withheld.

The fish had a mean fork length of 2.77 cm and a mean wet weight of 0.27 g. Mortality during the 14-day period prior to test initiation was approximately 2%.

B. Test System: The test chambers were 14- or 25-l glass aquaria containing approximately 10 l of test solution and were held in a temperature-controlled area at 20 ±2°C. During the test, the test vessels were aerated and each vessel was placed on an air-driven magnetic stirrer and stirred with teflon-coated stir beads. The photoperiod was 16-hour light/8-hour dark with dawn/dusk simulation periods.

The dilution water was de-chlorinated tap water adjusted to a hardness of approximately 50 mg/l as CaCO<sub>3</sub> using softened, treated (reverse-osmosis) tap water. The pH of the dilution water was 8.12.

A primary stock solution (2 g/l) was prepared by dissolving the test substance in dimethylsulphoxide (DMSO). Further dilutions with DMSO and dilution water were made to prepare the test solutions.

C. Dosage: Ninety-six-hour, static test. Five nominal test concentrations (100, 200, 300, 400, and 500 µg/l) were selected for this test. In addition, a dilution water control and a solvent control were included. The solvent control contained approximately 5 ml/l of DMSO, the amount used in each exposure solution.

- D. **Design:** Ten fish were randomly added to each of two replicate vessels per treatment within 48 minutes of preparation of the test solutions. The biomass loading was 0.23 g/l. The fish were observed for signs of lethal and sublethal effects at 30 minutes, 2, 4, 20.5, 24, 48, 72, and 96 hours. The fish were not fed during the study.

Temperature, pH, and dissolved oxygen concentrations (DO) were measured in samples of each test solution at 0, 24, 48, 72, and 96 hours. Hardness of the dilution water control and the highest and lowest test concentrations was measured at test initiation and termination.

The exposure solutions and the solvent control solution were analyzed for concentrations of test material at 0 and 96 hours using high performance liquid chromatography.

- E. **Statistics:** No dose-related mortality occurred, therefore no LC<sub>50</sub> could be determined.

12. **REPORTED RESULTS:** Mean measured concentrations were 97.7, 123.5, 147.7, 219.5, and 255.6 µg/l (Table 1, attached). "All dilutions of the test material in DMSO and in water were clear and colourless."

By test termination, no dose-related mortality or adverse effects were observed. Two fish died at 100 and 500 µg/l nominal concentrations. One fish died at 300 and 400 µg/l. There was no control mortality.

During the test, the test solutions had a pH of 7.80-7.98, a DO of 92-97% of saturation, a temperature of 20.0-21.3°C, and a hardness of 50-51 mg/l as CaCO<sub>3</sub>.

A limit test (second test) was also conducted using one test level (100 mg/l). The results showed no mortality of fish exposed to 100 mg/l of XRD 473. No solvent was used in the preparation of the exposure solution. The test system and conditions of this were similar to those of the first study. However, two replicates (total of 20 fish) were used for the control and three replicates (total of 30 fish) were used for the exposure level. "At the start of the test, white particulate material was present on the base of the three test aquaria and on the surface of the controls. After 96 hours, this white material remained on the base of the test aquaria and the control water was clear and colorless." The

concentration of test material in the test solutions was not measured.

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

"The 96 H LC<sub>50</sub> was greater than the highest concentration tested -- 500 µg/l in a study which used a carrier to increase the dispersion of the chemical in water. Furthermore a limit test in which fish were exposed to 100 mg/l without a dispersant demonstrated no mortality. These data indicate that XRD 473 is 'practically non-toxic to the bluegill.'"

A GLP compliance and quality assurance statement were included in the report, indicating that the study was conducted in accordance with 40 CFR Part 160.

**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 deviations:

Measured concentrations decreased substantially from test initiation to test termination. Therefore, the actual concentrations to which the test organisms were exposed are unknown because of lack of solubility.

The test concentrations were less than 100 mg/l but not high enough to produce a precise LC<sub>50</sub>.

The concentration of test material in the dilution water control solution was not measured to determine if there was contamination. However, for this study it is probably acceptable since no mortality or sublethal effects were observed in the control.

The amount of DMSO used in the solvent control (5 ml/l) was higher than the solvent concentration recommended for a static test (0.5 ml/l).

It is unclear whether all test vessels used in each test were the same size. The report only stated that the test vessels were all-glass aquaria with a total capacity of 14 or 24 l.

The solution temperature in at least one test vessel should have been measured continuously as recommended for a system controlled by air temperature.

The dilution water was dechlorinated tap water. The SEP discourages the use of dechlorinated water because removal of chlorine is rarely complete and residual chlorine can be toxic to aquatic organisms. However, for this study it is acceptable since no mortality or sublethal effects occurred in the controls.

**B. Statistical Analysis:** No statistical analysis was necessary.

**C. Discussion/Results:** There were several minor inconsistencies between the summary report and the original report.

Final measured concentrations were 7.3-24.1% of initial measured concentrations. Therefore, the actual concentrations to which the test organisms were exposed are unknown because of lack of solubility.

The second study which exposed bluegills to the nominal of 100 mg/l was supplemental since the test solutions were aerated during the study and the concentration of test material in the test solutions was not monitored. In addition, there was evidence of control contamination.

Measured concentrations decreased substantially from test initiation to test termination. Based on mean measured concentrations, the 96-hour  $LC_{50}$  was  $>255.6 \mu\text{g/l}$  which would, at worst, classifies XRD 473 as highly toxic to *Lepomis macrochirus*. The NOEC could not be determined since mortality was noted at all exposure levels. This study was determined to be of supplemental adequacy because of difficulty in keeping the test material in solution or suspension due to its low solubility of 27 ppb. However, the data was sufficient to indicate no acute toxicity at concentrations equal to or below solubility.

**D. Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The data was sufficient to indicate no acute toxicity at concentrations equal to or below solubility.

(3) **Repairability:** No. No further testing required.

**15. COMPLETION OF ONE-LINER FOR STUDY:** No.

## DATA EVALUATION RECORD

EEB 09 NOV 1993

1. **CHEMICAL:** Hexaflumuron. Shaughnessey Number: 118202. *R. Rose*
2. **TEST MATERIAL:** XRD 473; 1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl)-3-(2,6-difluorobenzoyl) urea; Lot No. 442-35-20i; CAS No. 86479-06-3; purity of 99.7%; water solubility of 27 µg/l; a fine white crystalline solid.
3. **STUDY TYPE:** 72-1. Freshwater Fish Toxicity Test. Species Tested: Rainbow Trout (*Salmo gairdneri*).

4. **CITATION:** Mayes, M.A. 1992. The Acute Toxicity of XRD 473 to Rainbow Trout: Summary Evaluation and Original Study (Conducted in 1987). Study performed by Aquatox Testing Facility, Life Science Research Ltd., Suffolk, England. Submitted by DowElanco. EPA MRID No. 426485-12.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Rosemary Graham Mora*

Date: 17 June 93

6. **APPROVED BY:**

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

Signature: P. Kosalwat

Date: 6/17/93

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

Signature: *Henry T. Craven*

Date: 11/22/93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute toxicity study using freshwater fish. The test concentrations were less than 100 mg/l but not high enough to produce a precise LC<sub>50</sub>. Based on mean measured concentrations, the 96-hour LC<sub>50</sub> was >535.5 µg/l which, at worst, classifies XRD 473 as highly toxic to *Salmo gairdneri*. The NOEC was 183.8 µg/l.

8. **RECOMMENDATIONS:**9. **BACKGROUND:**

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

**11. MATERIALS AND METHODS:**

- A. Test Animals:** Rainbow trout fry (*Salmo gairdneri*) were obtained from West Acre Trout Farm, Kings Lynn, England. During a 21-day holding period, the fry were maintained under flow-through conditions in filtered de-chlorinated tap water and acclimated to softened water (hardness of 50 mg/l as CaCO<sub>3</sub>) over a seven day period. During the seven days prior to test initiation, the holding water had a temperature of 10.8-13.5°C, a pH of 7.27-7.70, and a hardness of 47-57 mg/l as CaCO<sub>3</sub>. The fish were fed trout pellets daily, except during the 24 hours prior to test initiation when food was withheld.

The fish had a mean fork length of 3.82 cm upon receipt from the supplier and a mean wet weight of 1.07 g at test initiation. No mortality occurred during the 14-day period prior to test initiation.

- B. Test System:** The test chambers were 14- or 25-l glass aquaria containing approximately 12 l of test solution and were held in a temperature-controlled area at 12 ±2°C. During the test, the test vessels were aerated and each vessel was placed on an air-driven magnetic stirrer and stirred with teflon-coated stir beads. The photoperiod was 16-hour light/8-hour dark with dawn/dusk simulation periods.

The dilution water was de-chlorinated tap water adjusted to a hardness of approximately 50 mg/l as CaCO<sub>3</sub> using softened, treated (reverse-osmosis) tap water. The pH of the dilution water was 8.12.

A primary stock solution (2 g/l) was prepared by dissolving the test substance in dimethylsulphoxide (DMSO). Further dilutions with DMSO and dilution water were made to prepare the test solutions.

- C. Dosage:** Ninety-six-hour, static test. Five nominal test concentrations (100, 200, 300, 400, and 500 µg/l) were selected for this test. In addition, a dilution water control and a solvent control were included. The solvent control contained approximately 5 ml/l of DMSO, the amount used in each exposure solution.
- D. Design:** Ten fish were randomly added to each of two replicate vessels per treatment within 39 minutes of preparation of the test solutions. Biomass loading was 0.89 g/l. The fish were observed for signs of lethal

and sublethal effects at 30 minutes, 2, 4, 5.5, 24, 48, 72, and 96 hours. The fish were not fed during the study.

Temperature, pH, and dissolved oxygen concentrations (DO) were measured in samples of each test solution at 0, 24, 48, 72, and 96 hours. Hardness of the dilution water control and the highest and lowest test concentrations was measured at test initiation and termination.

The exposure solutions and the solvent control solution were analyzed for concentrations of test material at 0 and 96 hours using high performance liquid chromatography.

E. **Statistics:** No dose-related mortality occurred, therefore no  $LC_{50}$  could be determined.

12. **REPORTED RESULTS:** Mean measured concentrations were 98.4, 183.8, 273.3, 360.0, and 535.5  $\mu\text{g}/\text{l}$  (Table 1, attached). "All dilutions of the test material in DMSO and in water were clear and colourless."

By test termination, no dose-related mortality or adverse effects were observed. One fish died at 300 and 500  $\mu\text{g}/\text{l}$  nominal concentrations. One fish at 400  $\mu\text{g}/\text{l}$  was subdued and resting upright on the bottom of the test vessel. This fish was dark compared to control fish at test termination. There was no control mortality.

During the test, the test solutions had a pH of 7.56-7.69, a DO of 93-99% of saturation, a temperature of 12.0-12.8°C, and a hardness of 50-53 mg/l as  $\text{CaCO}_3$ .

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "The 96 H  $LC_{50}$  is greater than the highest concentration tested -- 500  $\mu\text{g}/\text{l}$ . This concentration is approximately 19 times the water solubility of XRD 473 (27  $\mu\text{g}/\text{l}$ ). Under the proposed use conditions these data indicate that XRD 473 does not represent a significant risk to rainbow trout."

A GLP compliance and quality assurance statement were included in the report, indicating that the study was conducted in accordance with 40 CFR Part 160.

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 deviations:

The concentration of test material in the dilution water control solution was not measured to determine if there was any contamination. However, for this study it is probably acceptable since no mortality or sublethal effects were observed in the control.

The amount of DMSO used in the solvent control (5 ml/l) was higher than the solvent concentration recommended for a static test (0.5 ml/l).

The solution temperature in at least one test vessel should have been measured continuously as recommended for a system controlled by air temperature.

It is unclear whether all test vessels used in each test were the same size. The report only stated that the test vessels were all-glass aquaria with a total capacity of 14 or 24 l.

The dilution water was dechlorinated tap water. The SEP discourages the use of dechlorinated water because removal of chlorine is rarely complete and residual chlorine can be toxic to aquatic organisms. However, for this study it is acceptable since no mortality or sublethal effects occurred in the controls.

Biomass loading during this study (0.89 g/l) was slightly higher than recommended (0.8 g/l).

- B. **Statistical Analysis:** No statistical analysis was necessary.

- C. **Discussion/Results:** There were several minor inconsistencies between the summary report and the original report. These discrepancies did not affect the validity of the study.

This study is scientifically sound but does not meet the guideline requirements for an acute toxicity study using freshwater fish. The test concentrations were less than 100 mg/l but not high enough to produce a precise LC<sub>50</sub>. Based on mean measured concentrations, the 96-hour LC<sub>50</sub> was >535.5 µg/l which, at worst, classifies XRD 473 as highly toxic to *Salmo gairdneri*. The NOEC was 183.8 µg/l since no mortality or sublethal effects occurred at and below this concentration.

**D. Adequacy of the Study:**

- (1) **Classification:** Supplemental.
- (2) **Rationale:** The test concentrations were less than 100 mg/l but not high enough to produce a precise LC<sub>50</sub>.
- (3) **Repairability:** No.

**15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 4 June 1993.**