**SHOAUGHNESSEY NO.** 125401

**REVIEW NO.**

---

**EEB REVIEW**

**DATE:** **IN** 9-30-85 **OUT** 02 DEC 1985

**FILE OR REG. NO.** 279-GNLE, 279-GNLG, 279-GNLU

**PETITION OR EXP. NO.**

**DATE OF SUBMISSION** 9-16-85

**DATE RECEIVED BY HED** 9-26-85

**RD REQUESTED COMPLETION DATE** 12-2-85

**EEB ESTIMATED COMPLETION DATE** 11-25-85

**RD ACTION CODE/TYPE OF REVIEW** 106

---

**TYPE PRODUCT(S):** I, D, H, F, N, R, S Herbicide

**DATA ACCESSION NO(S).**

**PRODUCT MANAGER NO.** R. Taylor (25)

**PRODUCT NAME(S)** Command Products

**COMPANY NAME** FMC Corporation

**SUBMISSION PURPOSE** Submission of data in response to EEB's review of fish early life stage toxicity test using rainbow trout

**SHAUGHNESSEY NO.** 125401

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**CHEMICAL, & FORMULATION**

2-(2-chlorophenyl)-methyl-4-4-

dimethyl-3-isoaxazolidinone

---

**% A.I.**

---

**X** 641
EEB REVIEW

Command Herbicide

100 Submission Purpose and Label Information

100.1 Submission Purpose and Pesticide Use

The registrant (FMC Corp.) submitted data from a freshwater fish early life-stage test in support of registration of Command for use on soybeans.

101 Hazard Assessment

101.2 Likelihood of Adverse Effects on Nontarget Organisms

**Freshwater fish**

On the basis of data reviewed previously (LC$_{50}$ = 19 mg/l for rainbow trout; LC$_{50}$ = 34 mg/l for bluegill sunfish), EEB did not anticipate a hazard to freshwater fish from the proposed use of Command on soybeans. For this reason, a freshwater fish early life-stage test was not required. FMC Corp. did conduct the test, however, and data from that test indicate the MATC for Command technical on rainbow trout is between 2.29 mg/l and 4.35 mg/l. The MATC would be approximately 50 to 90 times the value for the aquatic estimated environmental concentration (0.05 ppm) calculated by the Exposure Assessment Branch for use of Command on soybeans. These figures support EEB's original conclusion, that use of Command on soybeans at proposed rates will not result in hazard to freshwater fish.

101.4 Adequacy of Toxicity Data

The registrant submitted a fish early life-stage test on rainbow trout. The study was determined to be supplemental, due to significant deviations from the recommended protocol.
Conclusions

EEB has reviewed the submitted fish early life-stage test on rainbow trout. As noted above (Sec. 101.4), the study was determined to be supplemental. The data will be placed in the EEB file for future reference.

Allen W. Vaughan, Entomologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Norman Cook, Section Head
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Michael Slimak, Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769)
1. **Chemical**: Command (FMC 57020)

2. **Test Material**: Technical, 95.6% a.i.

3. **Study Type**: Chronic fish early life-stage toxicity test (freshwater)
   
   **Species Tested**: Rainbow trout (*Salmo gairdneri*)


5. **Reviewed By:**
   
   Allen W. Vaughan  
   Entomologist  
   EEB/HED  
   
   **Signature**: Allen W. Vaughan  
   **Date**: 11/26/85

6. **Approved By:**
   
   Norman J. Cook  
   Supervisory Biologist  
   EEB/HED  
   
   **Signature**: Norman J. Cook  
   **Date**: 11/26/85

7. **Conclusions:**

   This study is scientifically sound, and determines that the maximum acceptable toxicant concentration (MATC) for Command technical to rainbow trout is between 2.29 mg/l and 4.35 mg/l.

   The study does not fulfill the guideline requirement for a fish early life-stage toxicity test, for the following reasons:

   1. Test was initiated with "eyed" eggs which were exposed to the toxicant for only 6-8 days prior to hatch. 10 days would be the minimum acceptable period of exposure in this test.

   2. Test duration was only 50 days post-hatch. Sixty days would be the appropriate minimum duration.

8. **Recommendations**: N/A
9. **Background:** This submission, containing raw data from a fish early life-stage toxicity test with the technical pesticide, was submitted by the registrant in response to a request from EEB. EEB had reviewed the study previously and had determined the need for raw data to validate the study.

10. **Discussion of Individual Test:** N/A

11. **Materials and Methods**

   A. **Test Animals** were rainbow trout embryos obtained from Mount Lassen Trout Farm, Red Bluffs, CA.

   B. **Test System:** glass aquaria with constant test volume of 11 l well water; flow-through exposure using proportional diluter; test duration = 57 days.

   C. **Dose:** Flow-through bioassay using measured concentrations; dimethyl formamide solvent.

   D. **Design:** Five concentrations (nominal = 1.27, 2.66, 5.31, 10.62, 21.24 mg/l; measured = 1.12, 2.29, 4.35, 8.45, 18.18 mg/l) plus control and solvent control (29 ul/l DMF); 2 reps. of each, 40 larvae/rep.

   E. **Statistics:**

   Percentage hatchability of embryos and survival, length, and weight of larvae after 49 days post-hatch exposure, were subjected to analysis of variance (Steel and Torrie, 1960). Data for percentage hatchability and survival were transformed to arc sin percentage prior to analysis. If treatment effects were indicated, the means of these parameters were compared to those from the control and solvent control using Dunnett's procedure (Steel and Torrie, 1960). When a treatment mean was significantly different from the control means (P=0.05), that treatment was considered to be a toxicant effect level.

12. **Reported Results:**

   All rainbow trout exposed to 18.18 mg/l died as embryos (prior to hatching) or as larvae within 24 hours of hatching. Hatchability of embryos exposed to 8.45 mg/l or less was unaffected. No rainbow trout larvae survived 49 days of post-hatch exposure to 8.45 mg/l FMC 57020. Exposure to 4.35 mg/l of FMC 57020 for 57 days resulted in significantly decreased survival, mean total length and average wet weight in comparison to the controls.
Rainbow trout larvae exposed to measured FMC 57020 concentrations of 2.29 mg/l or less suffered no apparent adverse effects. The maximum acceptable toxicant concentration (MATC) was, therefore, determined to be between 2.29 mg/l and 4.35 mg/l. Data on embryo hatchability, survival, mean total length, and average wet weight are attached.

13. **Study Author's Conclusions/Q.A. Measures:**

   Maximum acceptable toxicant concentration (MATC): 2.29 mg/l < MATC < 4.35 mg/l.

   Test was conducted according to the Springborn Bionomics protocol entitled "Methods for conducting early life-stage toxicity tests with rainbow trout (Salmo gairdneri)," 1983. The data and report were conducted in accordance with all pertinent EPA Good Laboratory Practice regulations. Also, the report was reviewed by FMC Corp., Toxicology Department's Quality Assurance Unit.

14. **Reviewer's Discussion and Interpretation of the Study**

   **A. Test Procedures:** Following were the major deviations from recommended procedure:

   1. test was initiated with "eyed" eggs (18 days post-fertilization). Standard procedure indicates that fertilized eggs may be used within 48 hours of spawning;

   2. range of time-to-hatch was only 6-8 days, as opposed to 10 days minimum;

   3. test duration was only 50 days post-hatch, instead of 60 days.

   **B. Statistical Analysis:**

   EEB's independent validation of the analyses performed by the authors indicates that the procedures used were appropriate. Results of EEB's validation support the reported results of the study. Copies of EEB's validation are attached to this review.
C. Discussion/Results:

This study is scientifically sound, and determines that the MATC for Command technical to rainbow trout is between 2.29 and 4.35 mg/l. These figures would probably be slightly lower if the methodology had adhered more closely to the recommended protocol (i.e., longer period of exposure prior to hatch, and longer period of exposure overall). It is because of these deviations that the study is considered supplemental.

D. Adequacy of Study:

1. Classification: Supplemental
2. Rationale: Exposure period prior to hatch is too brief; overall test duration is too short.
3. Reparability: None


16. CBI Appendix: N/A
Table 4. Hatchability of embryos and survival, total length and wet weight of rainbow trout (Salmo gairdneri) larvae exposed to FMC 57020 for 57 days.

<table>
<thead>
<tr>
<th>Mean measured concentrations (mg/L)</th>
<th>Embryo hatchability (%)</th>
<th>Larvae (30 days post-swim-up)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
<td>Mean total length (S.D) (mm)</td>
</tr>
<tr>
<td>18.18 A</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.45 A</td>
<td>72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 (2)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.35 A</td>
<td>81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.29 A</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 (2)</td>
</tr>
<tr>
<td>1.12 A</td>
<td>68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 (2)</td>
</tr>
<tr>
<td>control A</td>
<td>83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 (2)</td>
</tr>
<tr>
<td>control (DMF) B</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 (2)</td>
</tr>
<tr>
<td>solvent A</td>
<td>82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 (3)</td>
</tr>
<tr>
<td>control (DMF) B</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41 (2)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significantly (P=0.05) different from control.
SAS

10:03 WEDNESDAY, NOVEMBER 20, 1985

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS LEVELS VALUES

TRT 7 A B C D E F G

NUMBER OF OBSERVATIONS IN DATA SET = 28

SAS

10:03 WEDNESDAY, NOVEMBER 20, 1985

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE | PR > F | R-SQUARE | C.V.
MODEL   | 6  | 13803.27073720 | 2300.54512287 | 111.34  | 0.0001 | 0.969523 | 8.4520
ERROR   | 21 | 433.89964876   | 20.66188804   |        |        |          |        
CORRECTED TOTAL | 27 | 14237.17038595 | 4.54553496    | 53.7808833

SOURCE | DF | TYPE I SS | F VALUE | PR > F | DF | TYPE III SS | F VALUE | PR > F
TRT     | 6  | 13803.27073720 | 111.34  | 0.0001 | 6  | 13803.27073720 | 111.34  | 0.0001

SAS

10:03 WEDNESDAY, NOVEMBER 20, 1985

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: RESPONSE
NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE,
NOT THE EXPERIMENTWISE ERROR RATE

ALPHA=0.05 DF=21 MSE=20.6619

NUMBER OF MEANS
CRITICAL RANGE  6.6759  7.01172  7.24379  7.38102  7.49028  7.5756

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN GROUPING | MEAN | N | TRT
A                | 68.369 | 4 | E
A                | 66.034 | 4 | A
B                | 63.498 | 4 | B
B                | 60.390 | 4 | D
B                | 59.825 | 4 | C
C                | 58.349 | 4 | F
C                | 0.000  | 4 | G

Command herbicide
Rainbow trout
% embryo hatch

1

SAS

10:03 WEDNESDAY, NOVEMBER 20, 1985
SAS

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS     LEVELS   VALUES
TRT       6        A B C D E F

NUMBER OF OBSERVATIONS IN DATA SET = 12

SAS

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE  DF  SUM OF SQUARES  MEAN SQUARE  F VALUE  PR > F  R-SQUARE  C.V.
MODEL   5   9526.51157352  1905.30231470  523.10  0.0001  0.997711  3.1019
ERROR   6   21.85391044   3.64231841           1.90848589 61.52642561
CORRECTED TOTAL 11   9548.36548397

SAS

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: RESPONSE
NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE, NOT THE EXPERIMENTWISE ERROR RATE

ALPHA=0.05  DF=6  MSE=3.64232

NUMBER OF MEANS 2  3  4  5  6
CRITICAL RANGE  4.66906  4.84023  4.91923  4.95925  4.97758

Means with the same letter are not significantly different.

Command herbicide
Rainbow trout
% survival

1
GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS LEVELS VALUES

TRT  5  A  B  C  D  E

NUMBER OF OBSERVATIONS IN DATA SET = 10

SAS

DEPENDENT VARIABLE: RESPONSE

SOURCE   DF  SUM OF SQUARES  MEAN SQUARE  F VALUE  PR > F  R-SQUARE  C.V.

MODEL   4  10.40000000  2.60000000  5.20  0.0499  0.806202  1.8085

ERROR  5  2.50000000  0.50000000

CORRECTED TOTAL  9  12.90000000

SAS

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: RESPONSE

NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE, NOT THE EXPERIMENTWISE ERROR RATE

ALPHA=0.05  DF=5  MSE=0.5

NUMBER OF MEANS

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

Command herbicide

Rainbow trout

Mean total length (mm)

SAS

VARIABLE  N  MEAN  STANDARD  MINIMUM  MAXIMUM  STD ERROR  SUM  VARIANCE  C.V.

RESPONSE  2  40.50000000  0.70710678  40.00000000  41.00000000  0.50000000  81.00000000  0.50000000  1.746
### General Linear Models Procedure

**Class Level Information**

<table>
<thead>
<tr>
<th>CLASS</th>
<th>LEVELS</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td>5</td>
<td>A B C D E</td>
</tr>
</tbody>
</table>

**Number of Observations in Data Set = 10**

**SAS**

9:57 Wednesday, November 20, 1985

### General Linear Models Procedure

**Dependent Variable: Response**

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARE</th>
<th>F VALUE</th>
<th>PR &gt; F</th>
<th>R-SQUARE</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODEL</td>
<td>4</td>
<td>19895.40000000</td>
<td>4973.85000000</td>
<td>5.71</td>
<td>0.0417</td>
<td>0.820331</td>
<td>5.4277</td>
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<tr>
<td>ERROR</td>
<td>5</td>
<td>4357.50000000</td>
<td>871.50000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORRECTED TOTAL</td>
<td>9</td>
<td>24252.90000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SAS**

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### General Linear Models Procedure

**Duncan's Multiple Range Test for Variable: Response**

**Note:** This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

**Alpha=0.05**  **DF=5**  **MSE=871.5**

**Number of Means**  2  3  4  5

**Critical Range**  75.9669  78.2996  79.1161  79.5543

**Means with the same letter are not significantly different.**

**Duncan Grouping**

<table>
<thead>
<tr>
<th>MEAN</th>
<th>N</th>
<th>TRT</th>
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<tbody>
<tr>
<td>A</td>
<td>600.00</td>
<td>2 A</td>
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<tr>
<td>A</td>
<td>557.50</td>
<td>2 B</td>
</tr>
<tr>
<td>A</td>
<td>551.50</td>
<td>2 D</td>
</tr>
<tr>
<td>A</td>
<td>547.50</td>
<td>2 C</td>
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<tr>
<td>B</td>
<td>463.00</td>
<td>2 E</td>
</tr>
</tbody>
</table>

**Command Herbicide**

**Rainbow trout**

**Avg. wet weight (mg)**

**SAS**

9:57 Wednesday, November 20, 1985

### Variable Summary

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>N</th>
<th>MEAN</th>
<th>STANDARD DEVIATION</th>
<th>MINIMUM</th>
<th>MAXIMUM</th>
<th>STD ERROR OF MEAN</th>
<th>SUM</th>
<th>VARIANCE</th>
<th>C.V.</th>
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</thead>
<tbody>
<tr>
<td>TRT=A</td>
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<tr>
<td>RESPONSE</td>
<td>2</td>
<td>600.00</td>
<td>35.35533906</td>
<td>575.00000000</td>
<td>625.00000000</td>
<td>25.00000000</td>
<td>1200.00000000</td>
<td>1250.00000000</td>
<td>5.893</td>
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