240712
RECORD NO.
069105, 054901
SHAUGHNESSY NO.

EEB REVIEW
DATE: IN 3-3-89 OUT:

FILE OR REG. NO. 875-RRN
PETITION OR EXP. NO.
DATE OF SUBMISSION 01-10-89
DATE RECEIVED BY HED 3-1-89
RD REQUESTED COMPLETION DATE 4-12-89
EEB ESTIMATED COMPLETION DATE 4-12-89
RD ACTION CODE/TYPE OF REVIEW 170

TYPE PRODUCT(S): I, D, H, F, N, R, S Microbiocide
DATE ACCESSION NO (S). 410089-10-14
PRODUCT MANAGER NO. J. Kempter (32)
PRODUCT NAME (S) Issue Plus II

COMPANY NAME Diversey/Wyandotte Corporation
SUBMISSION PURPOSE Proposed registration of use in laundries

SHAUGHNESSEY NO.
069105
054901

CHEMICAL AND FORMULATION % A.I.
Alkyl (50% C14, 40% C12, 10% C16) 
dimethybenzyl ammonium chloride 2.64
5-chloro-2(2,-dichloro-phenoxy phenol 1.25
phenol
Total 3.89%
MEMORANDUM

SUBJECT: Issue Plus II: 875-RRN (2407123)  

FROM: James W. Melvin, Chief  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)  

TO: W. Francis (32)  
Disinfectants Branch  
Registration Division (H7505C)  

EEB has reviewed the proposed registration of Issue Plus II for use in laundries. All of the submitted fish and wildlife studies were conducted with a 3.89% formulated product. These studies alone cannot support all formulations of these two active ingredients. EEB requires testing on the technical grade material of each active ingredient in the product to do so. However, the available acceptable data will support this formulation (3.89% a.i.) and any formulations with the a.i. < 3.89% and with no significant changes in inert ingredients.

EEB's finding of submitted fish and wildlife studies using a formulated product (3.89% a.i.):

A. Bobwhite Quail (LD$_{50}$) (410089-10)

This study indicates Issue Plus II is practically nontoxic to Bobwhite Quail with an LD$_{50}$ greater than 2000 mg/kg. This study does fulfill the requirement in support of registration for an avian acute LD$_{50}$ study for a formulated product of a 3.89% a.i. and below.
B. **Bobwhite Quail (LC$_{50}$)** (410089-11)

This study indicates Issue Plus II is practically nontoxic to bobwhite quail with an LC$_{50}$ greater than 5000 ppm. This study does fulfill the requirement in support of registration for an avian dietary LC$_{50}$ study for a formulated product of 3.89% a.i. and below.

C. **Rainbow Trout (LC$_{50}$)** (410089-12)

This study indicates Issue Plus II is slightly toxic to rainbow trout with an LC$_{50}$ of 23.4 ppm. This study does fulfill the requirement in support of registration for a coldwater fish study for a formulated product of 3.89% a.i. and below.

D. **Bluegill Sunfish (LC$_{50}$)** (410089-13)

This study indicates Issue Plus II is slightly toxic to bluegill sunfish with an LC$_{50}$ of 37.2 ppm. This study does fulfill the requirement in support of registration for a warmwater fish study for a formulated product of 3.89% a.i. and below.

E. **Daphnia magna (LC$_{50}$)** (410089-14)

This study indicates Issue Plus II is highly toxic to *Daphnia magna* with an LC$_{50}$ of 0.42 ppm. This study does not fulfill the requirement in support of registration for a freshwater invertebrate for a formulated product of 3.89% a.i. and below because the pH and dissolved oxygen was not measured.

In closing, based on a personal conversation with Walter Francis of PM (32) on May 30, 1990, he stated even though this product may be registered there are no fish and wildlife data to support each of the two active ingredients [(Alkyl 50% C$_{14}$, 40% C$_{12}$, 10% C$_{16}$ dimethylbenzyl ammonium chloride, and 5-Chloro-2(2,4-dichlorophenoxy) phenol] for use in Issue Plus II product. Therefore, to support all formulations of these two actives EEB requires the following data:

(a) The avian acute oral LD$_{50}$ for one species of waterfowl (Mallard Duck, preferable) or one species of upland game bird (Bobwhite Quail or Ring-necked Pheasant);
(b) The dietary LC\textsubscript{50} for one species of waterfowl (Mallard Duck) and one species of upland game bird (Bobwhite Quail or Ring-necked Pheasant);

(c) The 96-hour LC\textsubscript{50}'s for a coldwater species (Rainbow Trout) and a warmwater species (Bluegill Sunfish) of fish;

(d) The 48-hour LC\textsubscript{50} for an aquatic invertebrate (Daphnia sp., preferable).

The above basic studies are required on the technical grade material of each active ingredient (s) in the product.

The submitted fish and wildlife studies were conducted with a formulated product (3.89% a.i.). These studies were found acceptable and can be used to support end-products of 3.89% a.i. and below only. The Daphnia magna study, however, needs to be redone.
DATA EVALUATION RECORD

1. CHEMICAL: Issue Plus II

2. TEST MATERIAL: 3.89% (formulated)

3. TEST TYPE: Avian Acute Oral LD$_{50}$
   Test Species: Bobwhite Quail (Colinus virginianus)


5. REVIEWED BY:
   Curtis E. Laird
   Fishery Biologist
   EEB/HED
   Signature: Curtis E. Laird
   Date: 5-31-90

6. APPROVED BY:
   Norman J. Cook
   Supervisory Biologist
   EEB/HED
   Signature: Norman J. Cook
   Date: 11-22-90

7. CONCLUSIONS: This study indicates Issue Plus II is practically nontoxic to Bobwhite Quail with an LC$_{50}$ >2000 mg/kg. This does fulfill the requirement in support of registration for an Avian Acute Oral LD$_{50}$ study for a formulated product of 3.89% ai and below.

8. RECOMMENDATION: N/A

9. BACKGROUND: This study was submitted in support of Issue Plus II registration.

10. DISCUSSION OF INDIVIDUAL TEST: N/A

11. MATERIAL AND METHOD:
   A. Test Animals: Test animals were young adult Bobwhite Quail from Trace Pheasantry, Douglasville, PA 19518.
   B. Test Design: Birds were tested in wire pen (indoors), temperature was 70± 50 degrees F; photoperiod was 12L/12D.
C. **Dose:** Acute Oral Dose; Ten birds per dose level; one dose level plus negative control (0, 2000 mg/kg).

D. **Statistical Analysis:** No statistics were performed due to lack of mortality statistics.

12. **REPORTED RESULTS:** The study author found the acute oral LD$_{50}$ to be $>2000$ mg/kg.

13. **STUDY AUTHOR'S CONCLUSION/OA MEASURES:** The acute oral LC$_{50}$ was $>2000$ mg/kg. This study was conducted in accordance with the regulations set forth in the Good Laboratory practices for Nonclinical Laboratory Studies, 40 CFR 160, Federal Register, Vol. 48, November 29, 1983.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY**

   A. **TEST PROCEDURE:** The test procedure generally followed the EPA recommended protocol of October 1982.

   B. **STATISTICAL ANALYSIS:** No statistics were performed due to lack of mortality statistics.

   C. **DISCUSSION/RESULT:** Issue Plus II is practically nontoxic to Bobwhite Quail with an LD$_{50}$ $>2000$ mg/kg.

   D. **ADEQUACY OF STUDY:**
      1. Category: Core for formulated product (3.8% ai or less).
      2. Rationale: N/A
      3. Repairability: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes

16. **CBI APPENDIX:** N/A
DATA EVALUATION RECORD

1. **CHEMICAL:** Issue Plus II

2. **TEST MATERIAL:** 3.89% (formulated)

3. **TEST TYPE:** Eight-day Dietary LC$_{50}$

   **Test Species:** Bobwhite Quail (*Colinus Virginianus*)


5. **REVIEWED BY:**

   Curtis E. Laird
   Fishery Biologist
   EEB/HED

   **Signature:** Curtis E. Laird
   **Date:** 5-31-90

6. **APPROVED BY:**

   Norman J. Cook
   Supervisory Biologist
   EEB/HED

   **Signature:**
   **Date:** 6-22-90

7. **CONCLUSIONS:** This study indicates Issue Plus II is practically nontoxic to Bobwhite Quail with an LC$_{50} > 5000$ ppm. This study does fulfill the requirement in support of registration for an avian dietary study for a formulated product of 3.89% or less.

8. **RECOMMENDATION:** N/A

9. **BACKGROUND:** This study was submitted in support of Issue Plus II registration.

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

11. **MATERIAL and METHOD:**

    A. **Test Animals:** Test animals were 7-10 day old Bobwhite Quail from Trace Pheasantry, Douglasville, PA.

    B. **Test Design:** Birds were tested in brooder units; size units = 35 x 100 x 24 CM.
C. **Dose:** Nominal dietary concentration; ten birds per dose level; one dose level plus control (0,5000 ppm).

D. **Statistical Analysis:** No statistics were performed due to lack of mortality data.

12. **REPORTED RESULTS:** The study author found the eight-day dietary LC$_{50}$ to be >5000 ppm.

13. **STUDY AUTHOR'S CONCLUSION/QA MEASURES:** Eight-day dietary LC$_{50}$ was >5000 ppm. This study was conducted in accordance with the regulations set forth in the Good Laboratory Practices for Nonclinical Laboratory Studies, 40 CFR 160, Federal Register, Vol. 48, November 29, 1982.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY**

A. **TEST PROCEDURE:** The test procedure generally followed the recommended EPA Protocol of October 1982 except the temperature, humidity and photo period was not reported.

B. **STATISTICAL ANALYSIS:** No statistics were performed due to lack of mortality.

C. **DISCUSSION/RESULT:** Issue Plus II is practically nontoxic to Bobwhite Quail with an LC$_{50}$ >5000 ppm. The temperature, humidity and photo period were not given and did not appear to have a bearing on the test outcome since there were no mortalities.

D. **ADEQUACY OF STUDY:**

1. Category: Core for a formulated product
2. Rationale: N/A
3. Repairability: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY**

16. **CBI APPENDIX:** N/A
DATA EVALUATION RECORD

1. CHEMICAL: Issue Plus II

2. TEST MATERIAL: 3.89% a.i. (formulated)

3. TEST TYPE: 96-hour LC₅₀
   Test Species: Rainbow Trout (Salmo gairdneri)


5. REVIEWED BY:
   Curtis E. Laird
   Fishery Biologist
   EEB/HED
   Signature: Curtis E. Laird
   Date: 5-31-90

6. APPROVED BY:
   Norman J. Cook
   Supervisory Biologist
   EEB/HED
   Signature: Norman J. Cook
   Date: 6-22-90

7. CONCLUSIONS: This study indicates Issue Plus II is slightly toxic to rainbow trout with an LC₅₀ of 23.4 ppm. This study does fulfill the requirement in support of registration for a coldwater fish study for a formulated product 3.89% and below.

8. RECOMMENDATION:

9. BACKGROUND: This study was submitted in support of Issue Plus II registration.

10. DISCUSSION OF INDIVIDUAL TEST: N/A

11. MATERIAL AND METHOD:
    A. Test Animals: Test animals were rainbow trout (Salmo gairdneri) from Limestone Springs, Richmond, PA. Size = ≈ 2.0g
B. Test Design: Fish were tested in 20 liter glass aquarium with 10 liters of test solution; temperature was 17±2°C; photoperiod was 14 L/10 D; D. O. ranged from 7.4 mg/l to 8.6 mg/l; PH ranged from 6.3 to 6.8.

C. Dose: Statis bisosassay using nominal concentrations; ten fish per does level; five dose levels plus control (0, 18, 24, 32, 42, and 56 ppm).

D. Statistical Analysis: Litchfield and Wilcoxon

12. REPORTED RESULTS: The study author found the 96-hour LC₅₀ to be 23.4 ppm.

13. STUDY AUTHOR'S CONCLUSION/QA MEASURES: The 96-hour LC₅₀ was 23.4 ppm. This study was conducted in accordance with the regulations set forth in the Good Laboratory Practice for Nonclinical Laboratory Studies, 40 CFR 160, Federal Register, Vol. 48, November 29, 1983.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY

A. Test Procedure: The test procedures complied with the recommended EPA Protocol of October 1982 except food was withheld for 24 hours instead of 96 hours.

B. Statistical Analysis: Probit analysis method

C. Discussion/Result: Issue Plus II is slightly toxic to rainbow trout with LC₅₀ of 23.4 ppm.

D. Adequacy of Study:

1. Category: Core for a formulated product (3.89% and below)

2. Rationale: N/A

3. Reparability: N/A

15. Completion of one-liner for study: Yes

16. CBI Appendix N/A
Laird  Issue Plus II  Rainbow Trout  5-1-89

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<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
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The binomial test shows that 18 and 32 can be used as statistically sound conservative 95 percent confidence limits, because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 for this set of data is 22.94587

Results calculated using the moving average method

<table>
<thead>
<tr>
<th>SPAAN</th>
<th>G</th>
<th>LC50</th>
<th>95 percent confidence limits</th>
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</thead>
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25.18742

Results calculated using the probit method

<table>
<thead>
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<th>H</th>
<th>Goodness of fit probability</th>
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<tbody>
<tr>
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</table>

Slope = 13.30338

95 percent confidence limits = 8.097988 and 16.50877

LC50 = 23.38573

95 percent confidence limits = 21.58145 and 25.29662

LC10 = 18.77095

95 percent confidence limits = 15.6161 and 20.53991

***************************************************************************************
DATA EVALUATION RECORD

1. CHEMICAL: Issue Plus II

2. TEST MATERIAL: 3.89% (formulated)

3. TEST TYPE: 96-hour LC₅₀ for fish

   Test Species: Bluegill sunfish (Lepomis macrochirus)


5. REVIEWED BY:
   Curtis E. Laird
   Fishery Biologist
   EEB/HED

   Signature: [Signature]
   Date: 5-31-90

6. APPROVED BY:
   Norman J. Cook
   Supervisory Biologist
   EEB/HED

   Signature: [Signature]
   Date: 6-22-90

7. CONCLUSIONS: This study indicates Issue Plus II is slightly toxic to bluegill sunfish with an LC₅₀ of 37.2 ppm. This study does fulfill the requirement in support of registration for a formulated product of 3.89% and below.

8. RECOMMENDATION: N/A

9. BACKGROUND: This study was submitted in support of Issue Plus II registration.

10. DISCUSSION OF INDIVIDUAL TEST: N/A

11. MATERIAL AND METHOD:

   A. Test Animals: Test animals were bluegill sunfish (Lepomis Macrochirus) from S. P. Engineering, Salem, PA; Size = ~ 2.0g.

   B. Test Design: Fish were tested in 20 liter glass aquarir with 10 liters of test solution; temperature was 22 ± 1°C; photoperiod was 14L/10D.
C. **Dose**: Static bioassay using nominal concentrations; ten fish per dose level; five dose levels plus negative control (0, 18, 24, 32, 42 and 56 ppm).

D. **Statistical Analysis**: Litchfield & Wilson

12. **REPORTED RESULTS**: The study author found the 96-hour LC$_{50}$ to be 34.0 (31.5 - 38.9) ppm.

13. **STUDY AUTHOR'S CONCLUSION/OA MEASURES**: The 96-hour LC$_{50}$ was 35 ppm. This study was conducted in accordance with the regulations set forth in the Good Laboratory Practice for Nonclinical Laboratory Studies, 40 CFR 160, Federal Register, Vol. 48, November 29, 1983.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY**

   A. **Test Procedure**: The test procedure complied with the recommended EPA protocol of Oct. 1982 except the food was withheld for 24 hours instead of 96 hours.

   B. **Statistical Analysis**: Probit Analysis showed the 96-hour LC$_{50}$ to be 37.2 (0 to infinity) ppm.

   C. **Discussion/Result**: Issue Plus II is slightly toxic to rainbow trout with an LC$_{50}$ of 37.2 ppm.

   D. **Adequacy of Study**:

      1. **Category**: Core for a formulated product
      2. **Rationale**: N/A
      3. **Reparability**: N/A

15. Completion of one-liner for study: Yes

16. **CBI Appendix**: N/A
THE BINOMIAL TEST SHOWS THAT 24 AND 56 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 43.53002

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN  G  LC50  95 PERCENT CONFIDENCE LIMITS
4  5.135013E-02  36.19442  33.22418
39.98501

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS  G  H  GOODNESS OF FIT PROBABILITY
3  1.119012  3.883748  8.678317E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE =  7.794325.
95 PERCENT CONFIDENCE LIMITS = -.4507752 AND 16.03943

LC50 =  37.16107
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 =  25.53578
95 PERCENT CONFIDENCE LIMITS = 0 AND 34.83336
DATA EVALUATION RECORD

1. CHEMICAL: Issue Plus II

2. TEST MATERIAL: 3.89% (formulated)

3. TEST TYPE: 48-hour LC50

   Test Species: Daphnia magna

4. STUDY IDENTIFICATION: Terrell, Y. (1988) Acute Toxicity of
   Issue Plus on Daphnia Magna; Project No. 88-475; Prepared by
   American Standards Biosciences Corp. for Diversey/Wyandotte
   Corporation, 1532 Biddle Avenue, Wyandotte, Michigan 48192;
   ACC. No. 410089:14.

5. REVIEWED BY:
   Curtis E. Laird
   Fishery Biologist
   EEB/HED
   Signature: Curtis E. Laird
   Date: 5-31-90

6. APPROVED BY:
   Norman J. Cook
   Supervisory Biologist
   EEB/HED
   Signature: Norman J. Cook
   Date: 6-22-90

7. CONCLUSIONS: This study indicates Issue Plus is highly toxic
   to Daphnia Magna with an LC50 of 0.42 ppm. This study does not
   fulfill the requirement in support of registration for a freshwater
   invertebrate for a 3.89% formulation and below.

8. RECOMMENDATION: N/A

9. BACKGROUND: This study was submitted in support of Issue Plus
   registration.

10. DISCUSSION OF INDIVIDUAL TEST: N/A

11. MATERIAL and METHOD:

    A. Test Animals: Test animals were Daphnia magna from
       S. P. Engineering, Salem, PA; Age = <24 hours old.

    B. Test Design: Daphnia were tested in 500 ml glass beakers
       with 400 ml of test solution; temperature was 19°C; photoperiod
       was 14L/10D.
C. **Dose:** Static bioassay using nominal concentration; 20 daphnid per dose level; 7 dose levels plus negative control (0, 0.24, 0.32, 0.42, 0.56, 0.75, 1.0, and 1.35 ppm).

D. **Statistical Analysis:** Litchfield and Wilcoxon.

12. **REPORTED RESULTS:** The study author found the 48-hour LC$_{50}$ to be 0.43 (0.37 - 0.50) ppm.

13. **STUDY AUTHOR'S CONCLUSION/OA MEASURES:** The 48-hour LC$_{50}$ was 0.43 ppm. This study was conducted in accordance with the regulations set forth in the Good Laboratory Practices for Nonclinical Laboratory Studies, 40 CFR 160, Federal Register, Vol. 48, November 29, 1983.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY**

A. **Test Procedure:** The test procedure complied with the recommended EPA protocol of Oct. 1982, except the PH and D.O. was not measured and a formulated product was used.

B. **Statistical Analysis:** Probit analysis.

C. **Discussion/Result:** Issue Plus is highly toxic to *Daphnia magna* with an LC$_{50}$ of 0.42 (0.36 - 0.47) ppm.

D. **Adequacy of Study:**

1. **Category:** Supplemental
2. **Rationale:** Due to lack of PH and dissolved oxygen measurements, and a formulated product was used.
3. **Reparability:** Not reparable

15. **Completion of one-liner for study.** Yes

16. **CBI Appendix:** N/A
Laird Issue Plus II  Daphnia magna  5-3-89

<table>
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<tr>
<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
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</tr>
</tbody>
</table>

The binomial test shows that .32 and .56 can be used as statistically sound conservative 95 percent confidence limits, because the actual confidence level associated with these limits is greater than 95 percent.

An approximate LC50 for this set of data is .3799496

Results calculated using the moving average method:

<table>
<thead>
<tr>
<th>SPAN</th>
<th>LC50</th>
<th>95 percent confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
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<td>.400597 .340912 .462681</td>
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</table>

Results calculated using the probit method:

<table>
<thead>
<tr>
<th>ITERATIONS</th>
<th>5</th>
<th>H</th>
<th>GOODNESS OF FIT PROBABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
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</tbody>
</table>

Slope = 5.002136

95 percent confidence limits = 3.548555 and 6.455716

LC50 = .4162147

95 percent confidence limits = .3623608 and .471393

LD10 = .2319668

95 percent confidence limits = .1705862 and .2792307