D162235
DPBARCODE (RECORD)
110201
SHAUGHNESSY NO

REVIEW NO.

EEB REVIEW

DATE IN: _____ OUT: 11/5/91

CASE # :193780 REREG CASE #: _____
SUBMISSION # :S392387 LIST A, B, C, D
ID # :055947-UR

DATE OF SUBMISSION 02/15/91

DATE RECEIVED BY EFED _____

SRRD/RD REQUESTED COMPLETION DATE 07/06/91

EEB ESTIMATED COMPLETION DATE 10/16/91

SRRD/RD **ACTION** CODE/TYPE OF REVIEW 100 NC-FOOD/FEED USE

MRID #(S) 417873-02

DP TYPE 001 SUBMISSION RELATED DATA PACKAGE

PRODUCT MANAGER, NO. JOANNE MILLER, 23

PRODUCT NAME(S) BARRICADE

TYPE PRODUCT F R I N H D HERBICIDE

COMPANY NAME SANDOZ CROP PROTECTION CORPORATION

SUBMISSION PURPOSE _____

INCLUDE USE(S) _____

COMMON CHEMICAL NAME PRODIAMINE

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of studies for the new chemical Prodiamine.

FROM: Douglas Urban, Acting Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division
(H7507C)

11/5/91

TO: Amy Rispin, Chief
Science Analysis and Coordination Staff
Environmental Fate and Effects Division

As part of the registration process for the new chemical Prodiamine, Sandoz Crop Protection Corporation has submitted the following studies:

Forbis, A., J.W. Blasberg, and L. Stuerman. 1991. 21-Day Chronic Static Renewal Toxicity of ^{14}C-Prodiamine to Daphnia magna. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. MRID No. 417873-02.

Burgess, D. and L. Stuerman. 1990. Acute Toxicity of ^{14}C-Prodiamine to Daphnia magna. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. MRID No. 417977-01.

These studies have been classified as supplemental by the Ecological Effects Branch. The attached data evaluation records will provide reasons for study classification. Although the chronic daphnia study (MRID No. 417873-02) is scientifically sound, it does not fulfill the data requirements for an aquatic invertebrate life-cycle. The acute aquatic invertebrate toxicity data requirement has been satisfied by a previously reviewed study (MRID No. 418393-03). If you have any questions, please contact Tracy Perry at 557-1451 or Henry Craven at 557-0320.

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CONCURRENCES

SYMBOL	H7507C	H7507C	H-7507C				
SURNAME	T. Perry	H. Craven	D. Urban				
DATE	11/5/91	11/5/91	11/5/91				

EPA Form 1320-1 (12-70) *U.S.GPO:1989-624-485/10186 OFFICIAL FILE COPY

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DP BARCODE: D162235

CASE: 193780 DATA PACKAGE RECORD DATE: 03/08/91
SUBMISSION: S392387 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 100 NC-FOOD/FEED USE
CHEMICAL: 110201 2,4-Dinitro-N3,N3-dipropyl-6-(trifluoromethyl)-1,3-benzened
ID#: 055947-UR N3,N3-dipropyl-2,4-dinitro-6-trifluoromethyl-m-phe
COMPANY: 055947 SANDOZ CROP PROTECTION CORPORATION
PRODUCT MANAGER: 23 ROOM:
PM TEAM REVIEWER: EUGENE WILSON 703-557-~~3943~~ 1103 ROOM: CM-2 252
RECEIVED DATE: 02/15/91 DUE OUT DATE: 08/24/91

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 162235 EXPEDITE: N DATE SENT: 03/08/91 DATE RET.: / /
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 07/06/91 CSF: Y LABEL: Y

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	/ /	/ /
BRAN: EEB	/ /	10/28/91
SECT: RS4	/ /	/ /
REVR : HCRAVEN	/ /	/ /
CONTR:	/ /	/ /

* * * DATA PACKAGE REVIEW INSTRUCTIONS * * *

please review these new aquatic toxicity data. Hoping you will get to look at these data before our meeting on March 13, 1991 which will be held in 1023 starting at 9:30, as we have discussed. I guess they expected that we would have rec. these before the meeting. There was a problem I got the packages today 03-08-91. If there is any thing we can do to help you in this regard, please call me Eugene Wilson 557-3943

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

DATA EVALUATION RECORD

1. **CHEMICAL**: Prodiamine.

2. **TEST MATERIAL**: N^3, N^3-Di-n-propyl-2,4dinitro-6-(trifluromethyl)-m-phenylenediamine; 99.1% purity; a yellow crystalline solid.

3. **STUDY TYPE**: Acute Toxicity Test for Freshwater Invertebrates. Species tested: Daphnid (Daphnia magna).

4. **CITATION**: Burgess, D. and L. Stuerman. 1990. Acute Toxicity of ^{14}C-Prodiamine to Daphnia magna. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Submitted by Sandoz Crop Protection Corporation, Des Plaines, Illinois.

5. **REVIEWED BY**:

Tracy L. Perry
Wildlife Biologist
EEB/EFED

Signature: Tracy L. Perry
Date: 11/5/91

6. **APPROVED BY**:

Henry T. Craven
Head, Section IV
EEB/EFED

Signature: Henry T. Craven 11/5/91
Date:

7. **CONCLUSIONS**: This study is scientifically sound but does not fulfill the data requirements for an acute toxicity test with Daphnia magna. A definitive 48-hour EC_{50} and a NOEL were not determined. The 48-hour EC_{50} was reported to be >83 ppb and the NOEL was <11 ppb.

8. **RECOMMENDATIONS**: N/A

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9. **BACKGROUND**: Submission for registration.

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

11. **MATERIALS AND METHODS**:

A. Test Animals: Neonates were obtained from in-house cultures maintained at ABC Laboratory and were less than 24-hours old at test initiation. Daphnids were cultured under test conditions and were fed a suspension of at least one algae species: Selenastrum capricornutum, Ankistrodesmus falcatus and/or Chlamydomonas reinhardtii. This diet was supplemented with Rangens trout chow and Fleischmann's active dry yeast. Daphnids were not fed during the test.

B. Test System: The study was conducted under static conditions in 250 ml glass beakers containing 200 ml of test solution. Beakers were covered with petri dishes during the study to minimize evaporation and contamination. Test vessels were positioned in a temperature controlled water bath (20 ± 1.0°C) with a continuous recording of the water temperature. Dilution water was ABC's hard blended water (a blend of ABC well water and reverse osmosis water). Lighting intensity was maintained at 50-70 footcandles on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods.

C. Dosage: The Primary ^{14}C-Prodiamine Stock Solution was prepared by transferring the sample of ^{14}C-Prodiamine to 100 ml with PEG 200. The Working Stock Solution was prepared by combining 217.7 mg of non-labelled Prodiamine with the Primary Stock Solution in a 500 ml volumetric flask. " The level 5 nominal test concentration (100 ug/L) for ^{14}C-Prodiamine was prepared by making a 200 ug/ml stock solution from the ^{14}C working stock solution (550 ug/ml) in PEG 200. A 1.0 ml aliquot of this 200 ug/ml solution was diluted in a 2.0 L of testing water to yield a final concentration of 100 ug/L. Serial dilutions were used to prepare levels 1-4."

Nominal test concentrations used in the study were 13, 22, 36, 60, 100 ppb ^{14}C a.i./L. "No range-finding study was conducted with the test compound. The concentrations for the definitive study were selected by using the estimated water solubility of the compound (0.10 mg/L) as the high concentration with the rest being a logarithmic series from that concentration."

D. Test Design: Each test concentration contained 20 daphnia (2 replicates of 10 organisms each). Daphnia were impartially distributed among the test chambers.

Immobility and abnormal effects were recorded at 3-, 24-, and 48-hours. Temperature, dissolved oxygen and pH were measured in all concentrations in replicate "A" at 0-hour and in replicate "B" at 48-hours.

Samples from each replicate test chamber were collected at 0- and 48-hours during the test to verify test substance concentration. Liquid scintillation was used to measure the radioactivity of the water samples.

12. **REPORTED RESULTS:**

During the study, pH ranged from 8.2 to 8.5. and dissolved oxygen ranged from 7.9 to 8.5 mg/L. Temperature was maintained at 21°C (Table 5, attached). Hardness was 160 mg/L as $CaCO_3$, alkalinity was 184 mg/L as $CaCO_3$, and conductivity was 350 uMhos/cm (Table 1, attached).

Mean measured concentrations of the test substance ranged from 89 to 94% of nominal for the 0-hour samples and 72 to 82% of nominal for the 48-hour samples. Mean measured concentrations were 11, 19, 31, 51, and 83 ppb (Table 2, attached).

Abnormal swimming behavior was observed in all treatment levels at 48-hours (Table 4, attached). "The 3-, 24-, and 48-hour EC_{50} values were all >83 ug/L, respectively. The 48-hour dose-response slope could not be calculated. The 48-hour no-effect concentration was <11 ug/L."

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

The author presented no other conclusions than those mentioned above.

The report stated that the study was conducted in conformance with Good Laboratory Practices.

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

A. Test Procedures: The test procedures were in accordance with Subdivision E Guidelines with the following exceptions:

* The recommended test temperature for Daphnia is 20°C. This test was conducted at 21°C.

* Daphnia were "impartially," rather than randomly, distributed to test vessels.

* The author reports the estimated water solubility of prodiamine to be 100 ppb while EEB's records show a water

solubility of 13 ppb.

B. **Statistical Analysis**: N/A

C. **Discussion/Results**: This study is scientifically sound but does not fulfill the data requirements for an acute daphnia toxicity test. Since a definitive EC_{50} and NOEL were not determined, the results of this test are considered supplemental.

D. **Adequacy of Study**:

1) **Classification**: Supplemental.

2) **Rationale**: A definitive EC_{50} and NOEL were not determined.

3) **Repairability**: None.

15. **COMPLETION OF ONE-LINER**: Yes, 10/29/91

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Prodiamine

Page_____ is not included in this copy.

Pages 9 through 12 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.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

1. **CHEMICAL**: Prodiamine. Shaughnessy No. 110201.

2. **TEST MATERIAL**: Prodiamine; 99.1% active ingredient; a yellow crystalline powder.

3. **STUDY TYPE**: Daphnid Static Renewal Life-cycle Chronic Toxicity Test. Species tested: Daphnia magna.

4. **CITATION**: Forbis, A., J.W. Blasberg, and L. Stuerman. 1991. 21-Day Chronic Static Renewal Toxicity of ^{14}C-Prodiamine to Daphnia magna. Performed by Analytical Bio-Chemistry Laboratories, Inc. located in Columbia, Missouri. Study submitted by Sandoz Crop Protection Corporation, Des Plaines, Illinois.

5. **REVIEWED BY**:

Tracy L. Perry
Wildlife Biologist
EEB/EFED

Signature: Tracy L. Perry
Date: 10/15/91

6. **APPROVED BY**:

Henry T. Craven
Head, Section IV
EEB/EFED

Signature: Henry T. Craven
Date: 10/15/91

7. **CONCLUSIONS**: This study is scientifically sound but does not meet the guideline requirements for a chronic, static renewal toxicity test for the freshwater invertebrate, Daphnia magna. The dry weight of first generation daphnids was not measured at test termination and the experimental design did not follow Subdivision E Guidelines. The MATC was found to be >1.5 ppb and <2.6 ppb mean measured ^{14}C-Prodiamine concentrations.

8. **RECOMMENDATIONS**: N/A

9. **BACKGROUND:** Data submission for new chemical registration.

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Daphnia magna (<24 hours old) were obtained from in-house cultures maintained in a temperature controlled area at 20°C (±2). Cool fluorescent bulbs provided a light intensity of 40 - 80 footcandles on a 16-hour daylight and 8-hour darkness photoperiod, with 30 minute dawn and dusk transition periods. During the holding period, daphnids were fed a suspension of algae (Selenastrum capricornutum and Ankistrodesmus falcatus) supplemented with a trout chow (Rangens Salmon Starter #2) and yeast (Saccharomyces sp.) suspension.

B. **Test System:**

The test vessels were 900-ml glass jars (16.5 cm height x 8.8 cm diameter) containing 400 ml of solution (6.6 cm depth). Test chambers were immersed in a temperature controlled water bath maintained at 20°C (±2°C). Cool white fluorescent bulbs provided a 16-hour daylight, 8-hour darkness and 30-minute dusk and dawn transition periods. Light intensity ranged from 51 - 56 footcandles at the water surface during the study.

The dilution water was ABC well water which had been mixed with soft blended water (a combination of ABC well water and reverse osmosis water) to achieve a total hardness of between 160 - 180 mg/L as $CaCo_3$ (Table 1, attached).

C. **Dosage:** The Primary ^{14}C-Prodiamine Stock solution was prepared by transferring the sample of ^{14}C-Prodiamine to 100 ml with PEG 200 (Polyethylene Glycol 200). The Working Stock Solution was prepared by combining 217.7 mg of non-labelled Prodiamine with the Primary Stock Solution in a 500 ml volumetric flask. The flask was then brought to volume with PEG 200. "The level 5 nominal test concentration (100 ug/L) for the ^{14}C-Prodiamine was prepared by making a 200 ug/ml stock solution from the ^{14}C working stock solution (550 ug/ml) in PEG 200. A 1.0 ml aliquot of this 200 ug/ml solution was diluted in 2 L of testing water to yield a final concentration of 100 ug/L. Serial dilutions were used to prepare levels 1-4."

Based on results from previous testing with ^{14}C-Prodiamine, seven nominal concentrations (1.7, 3.3, 6.5, 13, 25, 50, 100 ppb), a polyethylene glycol solvent control, and a dilution water control were selected for the test.

D. **Test Design**: Four chambers were used for each concentration with 6 randomly distributed daphnids per chamber (loading rate was 1 daphnid per 67 ml dilution water). Daphnids were fed at least twice daily with a diet consisting of an algal suspension (*Selenastrum capricornutum*/ *Ankistrodesmus falcatus* - 1×10^7 cells/L for the first week and 1×10^8 cells/L for the remainder of the study). Daphnids were supplemented twice daily with a suspension of trout chow and yeast (1 ml per chamber of a 1.0 mg/ml suspension) giving a final suspended solid concentration of 5 mg/L.

Survival, abnormal effects and observance of first brood of the daphnids were recorded daily throughout the study. Counts of offspring produced were recorded every Monday, Wednesday and Friday before test solution renewal and neonates were discarded. Test chambers were cleaned with a nylon bristled brush and rinsed with ABC well water and allowed to dry until needed for the next renewal.

Temperature, dissolved oxygen and pH were measured in newly prepared parent solutions. These same parameters were measured once a week on alternating replicates of the old solutions. Temperature of the water bath was recorded continuously.

Test concentrations of ^{14}C-Prodiamine were analytically measured on days 0, 2, 4, 7, 9, 11, 14, 16, 18 and 21 (Tables 2 and 3, attached).

E. **Statistics**: For all data, the assumptions of homogeneity and normality were checked using a Shapiro-Wilk normality test and by examining the correlation between the mean and both the variance and the standard deviation. Survival data was analyzed using frequency analysis coupled with a one-tailed Fisher's exact test and the chi-square statistic. Reproduction data and adult daphnid length were analyzed by a one-way analysis of variance followed by Dunnett's one-tailed multiple means comparison procedure. If no significant difference existed between the control and the solvent control, all controls were pooled for that parameter.

12. **REPORTED RESULTS**: The twenty-one-day static renewal life-cycle chronic toxicity test was initiated on October 1, 1990 and terminated on October 22, 1990.

The mean measured concentrations of ^{14}C-Prodiamine were 1.5, 2.6, 4.9, 9.0 17, 33 and 65 ppb and ranged 65 - 88% of nominal. "Based on the analytical results between 'new' solutions and their respective 'old' solutions the test

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compound appeared to degrade slightly under static conditions in the testing water."

After 21 days of exposure to ^{14}C-Prodiamine, survival of Daphnia magna at the 65 ppb (mean measured concentration) was found to be significantly affected as compared to the pooled controls (Table 4, attached). The moving average method was used to calculate a 21-day EC_{50} of >65 ppb based on immobilization.

Adult daphnid length was significantly affected at the 33 and 65 ppb test concentrations when compared to the pooled controls (Table 5, attached).

There was no significant difference in the time to first brood between the pooled controls and the mean measured test concentrations (Table 6, attached).

Young/adult reproduction days (total number of young produced/ total number of adult reproduction days) were significantly affected at the mean measured concentrations of 2.6, 4.9, 9.0, 17, 33, 65 ppb when compared with the pooled controls (Table 7, attached).

"Based on the statistical analysis of survival, adult mean length, mean time in days to first brood and young/adult reproduction days from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be the measured ^{14}C-Prodiamine concentrations of 1.5 and 2.6 ug/L. The point estimate MATC value was calculated to be 2.0 ug/L."

Mean water quality parameters of temperature, dissolved oxygen and pH for the newly prepared parent solutions and the old solutions are presented in Table 8 (attached). Temperature was recorded continuously throughout the study and ranged from 19-21°C.

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

The author presented no conclusions other than those previously mentioned.

Quality Assurance and Good Laboratory Practices (GLP) were included in the report indicating adherence to USEPA GLP Regulations.

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

A. Test Procedure: The test procedures were in accordance with Subdivision E Guidelines with the following exceptions:

* A description (i.e. age, health) of the subculture used to produce the test organisms was not given. Daphnids which are at least 10-12 days old (those that have had at least one brood) should be used for the subculture. They should be put in a separate culture container and maintained for at least 21 days to insure that good health and conditions are present.

* The experimental design did not follow Subdivision E Guidelines. At each test concentration, 4 replicates of 6 daphnids each was used. The recommended design is 7 replicates of one daphnid each for collection of data on survival, growth, and reproduction, and 3 replicates containing 5 daphnids each at each test concentration for collection of data on survival only.

* The water quality parameters of alkalinity, hardness and conductivity were only measured in the dilution water and not in the test vessels. Guidelines state that these parameters should be measured at the beginning and end of the test and once a week in one control and one test concentration during the study.

* The dry weight of first generation daphnids was not measured as specified in the guidelines.

* When analyzing test data, the control and the solvent control should not be pooled. The solvent control alone should be used for comparison to the treatment levels.

B. **Statistical Analysis**: The MATC, calculated using EPA's SAS ANOVA program followed by Dunnett's T test, was greater than 1.5 ppb (NOEL) and less than 2.6 ppb (LOEL). The 21-day mean effective concentration (EC_{50}) was greater than 65 ppb. These values are the same as those reported by the author.

C. **Discussion**: This study is scientifically sound but does not meet the guideline requirements for a chronic, static renewal toxicity test for the freshwater invertebrate, Daphnia magna. The dry weight of the first generation daphnids was not measured at test termination and the experimental design did not follow Subdivision E Guidelines. The MATC was found to be >1.5 ppb and <2.6 ppb mean measured ^{14}C-Prodiamine concentrations.

D. **Adequacy of the Study**:

(1) **Classification:** Supplemental.

(2) **Rationale:** The dry weight of the first generation daphnids was not measured at test termination and the

experimental design did not follow Subdivision E Guidelines.

(3) **Repairability:** None.

15. **COMPLETION OF ONE-LINER**: Yes, 10-15-91.

Prodiamine

Page_____ is not included in this copy.

Pages 19 through 32 are not included.

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____ Identity of product inert ingredients.

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