8-8-91

MRID 418393-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Prodiamine

2. **TEST MATERIAL:** N^3, N^3-Di-n-propyl-2,4-dinitro-6-(trifluromethyl)-m-phenylenediamine; 94.3% purity; a yellow crystalline solid.

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

4. **CITATION:** Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 48-hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna). Study performed by Wildlife International Ltd., Easton, Maryland. Submitted by Sandoz Crop Protection Corporation, Des Plaines, Illinois.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
EEB/EFED

Signature: Tracy L. Perry
Date: 8/7/91

6. **APPROVED BY:**

Henry T. Craven
Head, Section IV
EEB/EFED

Signature: Henry T. Craven
Date: 8/8/91

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the data requirements for an acute toxicity test for freshwater invertebrates. The 48-hour EC_{50} and the no observed effect concentration for Daphnia magna were > 658 ppb a.i./L.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND**: Resubmission of study for registration.

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

11. **MATERIALS AND METHODS**:

A. Test Animals: Neonates were obtained from in-house cultures manintained at Wildlife International Ltd. and were less than 24-hours old at test initiation. Daphnids were cultured under test conditions and were fed a mixture of yeast, Cerophyll, and trout chow, as well as a suspension of freshwater green alga (Selenastrum capricornutum). Daphnids were not fed during the test. Daphnids in culture appeared in good health and showed no signs of disease or stress.

B. Test System: The study was conducted under flow-through conditions in 300ml glass beakers with nylon screen attached to holes in each side of the beaker. Beakers were suspended in a Teflon-lined, 8-L polyethylene aquaria filled with approximately 6.5L of test solution (test solution depth was approximately 17cm). Test chambers were randomly positioned in a temperature controlled water bath (20 C) which was enclosed in a plexiglass ventilation hood to minimixe cross-contamination. Dilution water was filtered freshwater obtained from a 45 m deep well located on the Wildlife International Ltd. site. Fluorescent lights were used to maintain a 16-hour light and 8-hour dark photoperiod. Light intensity was 20-70 footcandles at the surface of the water. A thirty minute transition period between light and dark was provided.

A proportional diluter, calibrated prior to the test and visually inspected at least twice a day, was used to deliver test media to the test vessels; 14 volume additions of test water were added to each test chamber every 24 hours. Diluter flow (non-radiolabelled test substance) was initiated approximately 19.5 hours before test initiation in order to establish equilibrium concentration of the test substance. Radio-labelled stock flow was started approximately 4 hours prior to test initiation.

C. Dosage: The test substance was dissolved in reagent grade polyethylene glycol (PEG) and sonicated to produce an initial non-radiolabelled stock solution. The initial stock was then combined with radiolabelled prodiamine stock to form a final stock solution with an active ingredient concentration of 1.25 mg/mL. Secondary stock solutions (0.75, 0.45, 0.27, and 0.16

establish equilibirum concentration of test substance. Radiolabelled stock flow was started approximately 4.5 hours prior to test initiation.

C. Dosage: The test substance was dissolved in reagent grade polyethylene glycol (PEG) and sonicated to produce an initial non-radiolabelled stock solution. The initial stock was then combined with radiolabelled prodiamine stock to form a final stock solution with an active ingredient concentration of 1.25 mg/mL. Secondary stock solutions (0.75, 0.45, 0.27, and 0.16 mg a.i./mL) were derived through further dilution with PEG. Secondary stocks were mixed with well water to produce desired test concentrations. Concentrations of PEG in the solvent control and in the treatment groups was 0.51mL/L.

Nominal test concentrations used in the study were 80, 130, 220, 360, and 600 ppb ^{14}C a.i./L. Test concentrations were chosen based on the results of an acute range finding toxicity test. One solvent control and one dilution water control were included in the study.

D. Test Design: Each test concentration contained 20 fish (2 replicates of 10 fish each). Fish were impartially removed from holding tanks in groups of 2 and distributed among the test chambers until each contained 10 organisms.

Mortalities and treatment-related effects were recorded at 7.5, 24, 48, 72, and 96 hours. The hardness, alkalinity and pH of the negative control water were measured at the beginning of the test. The pH and dissolved oxygen (DO) content of the water in alternate replicates were measured at 24-hour intervals. Temperature was measured continuously in one negative control replicate and at the beginning and end of the test in each test chamber.

Three 5 ml samples from each replicate test chamber were collected at the beginning of the test and at 24 hour intervals 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, temperature ranged from 11.9°C to 12.5°C. Hardness was 144 mg/L, as $CaCO_3$, and alkalinity was 196 mg/L, as $CaCO_3$. The pH ranged from 8.0 to 8.1 and DO ranged from 8.8 to 9.4 mg/L.

Mean measured concentrations of the test substance ranged from

value and no mortality concentrations were >658 [ppb] a.i./L and >658 [ppb] a.i./L, respectively. "

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 with the following exception: " The empty test substance container for the radiolabelled compound was disposed of prior to the completion of the study."

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:

* Information describing range finding study procedures and results was not included in the report.

B. Statistical Analysis: N/A

C. Discussion/Results:

Information on the range finding study procedure and results should be included in the report. EEB requests that information on sample size, concentrations tested and mortality data be provided.

Was there any attempt to identify the "creamy, yellow material" that was observed at the air/water interface in the test chambers of the three highest test concentrations? EEB would like additional information on this observation.

There is a discrepancy between the EC50 value reported in the study (EC50 > 658 ppb a.i./L) and the one included in the letter (4/5/91) accompanying the study (EC50 > 675.2 ppb a.i./L). EEB requests clarification on this difference.

Although the above questions require further clarification, the study is scientifically sound and can be used to fulfill data requirements for an acute toxicity test for freshwater invertebrates.

Was there any attempt to identify the "creamy, yellow material" that was observed at the air/water interface in the test chambers of the three highest test concentrations? EEB would like additional information on this observation.

There is a discrepancy between the LC50 value reported in the study (LC50 > 829 ppb a.i./L) and the one included in the letter (4/5/91) accompanying the study (LC50 > 716.4 ppb a.i./L). EEB requests clarification on this difference.

Although the above questions require further clarification, the study is scientifically sound and fulfills the data requirements for an acute toxicity test for freshwater fish.

D. Adequacy of Study:

1) Classification: Core.

2) Rationale: N/A

3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, July 29, 1991.

Table 7.
Cumulative Percent Mortality and Observed Effects.*

Client: Sandoz Crop Protection Corporation
Test Substance: Prodiamine
Test Organism: Daphnid, Daphnia magna
Dilution Water: Well Water

Mean Measured Concentration (µg a.i./L)	Replicate	24 Hours			48 Hours		
		Immobilization	Effects	Mortality	Immobilization	Effects	Mortality
Negative Control	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
Solvent Control	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
92	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
152	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
243	A	0% (0/10)	9 AN**	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
388	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
658	A	0% (0/10)	10 AN***	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)

*Observed Effects:

A = Surfacing
B = Hyperexcitability
C = Lethargy
D = Discoloration
E = Erratic Swimming
F = Hyperventilation
G = Gulping Air
H = Hemorrhaging
I = Excessive Mucus
J = Mucus Shedding
K = Curved Spine
L = Ulcer
M = Moribund
N = Loss of Equilibrium
O = Opaque Eyes
P = Molting
AN = Appears Normal
N/A = Not Applicable

**No dead body found, but could only count 9 live, all 10 daphnids observed as normal at test termination.

***One daphnid was stuck in surface tension but was otherwise normal.

MRID 418393-03

DATA EVALUATION RECORD

1. **CHEMICAL**: Prodiamine

2. **TEST MATERIAL**: N^3, N^3-Di-n-propyl-2,4-dinitro-6-(trifluromethyl)-m-phenylenediamine; 94.3% purity; a yellow crystalline solid.

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

4. **CITATION**: Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 48-hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna). Study performed by Wildlife International Ltd., Easton, Maryland. Submitted by Sandoz Crop Protection Corporation, Des Plaines, Illinois.

5. **REVIEWED BY**:

Tracy L. Perry
Wildlife Biologist
EEB/EFED

Signature: Tracy L. Perry
Date: 8/7/91

6. **APPROVED BY**:

Henry T. Craven
Head, Section IV
EEB/EFED

Signature: Henry T. Craven
Date: 8/8/91

7. **CONCLUSIONS**: This study is scientifically sound and fulfills the data requirements for an acute toxicity test for freshwater invertebrates. The 48-hour EC_{50} and the no observed effect concentration for Daphnia magna were > 658 ppb a.i./L.

8. **RECOMMENDATIONS**: N/A

7

9. **BACKGROUND**: Resubmission of study for registration.

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

11. **MATERIALS AND METHODS**:

A. Test Animals: Neonates were obtained from in-house cultures manintained at Wildlife International Ltd. and were less than 24-hours old at test initiation. Daphnids were cultured under test conditions and were fed a mixture of yeast, Cerophyll, and trout chow, as well as a suspension of freshwater green alga (Selenastrum capricornutum). Daphnids were not fed during the test. Daphnids in culture appeared in good health and showed no signs of disease or stress.

B. Test System: The study was conducted under flow-through conditions in 300ml glass beakers with nylon screen attached to holes in each side of the beaker. Beakers were suspended in a Teflon-lined, 8-L polyethylene aquaria filled with approximately 6.5L of test solution (test solution depth was approximately 17cm). Test chambers were randomly positioned in a temperature controlled water bath (20 C) which was enclosed in a plexiglass ventilation hood to minimixe cross-contamination. Dilution water was filtered freshwater obtained from a 45 m deep well located on the Wildlife International Ltd. site. Fluorescent lights were used to maintain a 16-hour light and 8-hour dark photoperiod. Light intensity was 20-70 footcandles at the surface of the water. A thirty minute transition period between light and dark was provided.

A proportional diluter, calibrated prior to the test and visually inspected at least twice a day, was used to deliver test media to the test vessels; 14 volume additions of test water were added to each test chamber every 24 hours. Diluter flow (non-radiolabelled test substance) was initiated approximately 19.5 hours before test initiation in order to establish equilibrium concentration of the test substance. Radio-labelled stock flow was started approximately 4 hours prior to test initiation.

C. Dosage: The test substance was dissolved in reagent grade polyethylene glycol (PEG) and sonicated to produce an initial non-radiolabelled stock solution. The initial stock was then combined with radiolabelled prodiamine stock to form a final stock solution with an active ingredient concentration of 1.25 mg/mL. Secondary stock solutions (0.75, 0.45, 0.27, and 0.16

mg a.i./mL) were derived through further dilution with PEG. Secondary stocks were mixed with well water to produce desired test concentrations. Concentrations of PEG in the solvent control and in the treatment groups was 0.51mL/L.

Nominal test concentrations used in the study were 80, 130, 220, 360, and 600 ppb ^{14}C a.i./L. Test concentrations were chosen based on the results of an acute range finding toxicity test. One solvent control and one dilution water control were included in the study.

D. Test Design: Each test concentration contained 20 daphnia (2 replicates of 10 organisms each). Daphnia were impartially removed from holding tanks in groups of 2 and distributed among the test chambers until each contained 10 organisms.

Mortalities and treatment-related effects were recorded at 0, 24, and 48 hours. The hardness, alkalinity and pH of the negative control water were measured at the beginning of the test. The pH and dissolved oxygen (DO) content of the water in alternate replicates were measured at 24-hour intervals. Temperature was measured continuously in one negative control replicate and at the beginning and end of the test in each test chamber.

Three 5 ml samples from each replicate test chamber were collected at the beginning of the test and at 24 hour intervals 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, temperature ranged from 19.7°C to 20.0°C. Hardness was 148 mg/L, as $CaCO_3$, and alkalinity was 198 mg/L, as $CaCO_3$. The pH ranged from 7.0 to 8.3 and DO ranged from 8.8 to 9.1 mg/L.

Mean measured concentrations of the test substance ranged from 108 to 117% of nominal test concentrations: nominal (measured); 80(92), 130 (152), 220 (243), 360 (388), and 600 (658) ppb ^{14}C a.i./L. " A creamy, yellow material was observed throughout the test at the air/water interface in the mixing chambers and test chambers associated with the three highest test concentrations. "

" No sublethal effects or mortalities were observed at any concentration during the 48-hour exposure period. Based on visual interpretation of the data, the daphnid 48-hour EC_{50}

value and no mortality concentrations were >658 [ppb] a.i./L and >658 [ppb] a.i./L, respectively. "

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 with the following exception: " The empty test substance container for the radiolabelled compound was disposed of prior to the completion of the study."

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:

* Information describing range finding study procedures and results was not included in the report.

B. Statistical Analysis: N/A

C. Discussion/Results:

Information on the range finding study procedure and results should be included in the report. EEB requests that information on sample size, concentrations tested and mortality data be provided.

Was there any attempt to identify the "creamy, yellow material" that was observed at the air/water interface in the test chambers of the three highest test concentrations? EEB would like additional information on this observation.

There is a discrepancy between the EC50 value reported in the study (EC50 > 658 ppb a.i./L) and the one included in the letter (4/5/91) accompanying the study (EC50 > 675.2 ppb a.i./L). EEB requests clarification on this difference.

Although the above questions require further clarification, the study is scientifically sound and can be used to fulfill data requirements for an acute toxicity test for freshwater invertebrates.

D. Adequacy of Study:

1) Classification: Core.

2) Rationale: N/A

3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, July 29, 1991.

11

Table 7.
Cumulative Percent Mortality and Observed Effects.*

Client: Sandoz Crop Protection Corporation
Test Substance: Prodiamine
Test Organism: Daphnid, Daphnia magna
Dilution Water: Well Water

Mean Measured Concentration (µg a.i./L)	Replicate	24 Hours			48 Hours		
		Immobilization	Effects	Mortality	Immobilization	Effects	Mortality
Negative Control	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
Solvent Control	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
92	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
152	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
243	A	0% (0/10)	9 AN**	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
388	A	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
658	A	0% (0/10)	10 AN***	0% (0/10)	0% (0/10)	10 AN	0% (0/10)
	B	0% (0/10)	10 AN	0% (0/10)	0% (0/10)	10 AN	0% (0/10)

*Observed Effects:

A = Surfacing
B = Hyperexcitability
C = Lethargy
D = Discoloration
E = Erratic Swimming
F = Hyperventilation
G = Gulping Air
H = Hemorrhaging
I = Excessive Mucus
J = Mucus Shedding
K = Curved Spine
L = Ulcer
M = Moribund
N = Loss of Equilibrium
O = Opaque Eyes
P = Molting
AN = Appears Normal
N/A = Not Applicable

**No dead body found, but could only count 9 live, all 10 daphnids observed as normal at test termination.

***One daphnid was stuck in surface tension but was otherwise normal.

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Review of studies for prodiamine.
DP Barcode: D163575
ID No.: 055947-UR

FROM: Douglas Urban, Acting Branch Chief for H.T. Craven 8/6/91
Ecological Effects Branch
Environmental Fate and Effects Division
(H7507C)

TO: Joanne Miller, PM 23
Herbicide and Fungicide Branch
Registration Division (H7505C)

BACKGROUND

As part of the registration process for the chemical prodiamine, Sandoz Crop Protection Corporation has resubmitted the following studies:

Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 96-hour Flow-Through Acute Toxicity Test with the Bluegill (Lepomis macrochirus). MRID # 418393-01.

Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 96-hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss). MRID # 418393-02

Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 48-hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna). MRID # 418393-03

All of the above studies were performed by Wildlife International Ltd. located in Easton, Maryland.

REVIEW SUMMARY

These studies were reviewed and categorized by EEB as follows:

Guide. Ref. #	Test Species	% a.i.	Test Type	Test Results	Study Status
72-1	Bluegill Sunfish	94.3	96 hour Acute Tox.	96 hour LC_{50}= >0.552 ppb	core
72-1	Rainbow Trout	94.3	96 hour Acute Tox.	96 hour LC_{50}= >829 ppb	core
72-2	Daphnia	94.3	48 hour Acute Tox.	48 hour EC_{50}= >658 ppb	core

The attached data evaluation records will provide the necessary information regarding reasons for study classification. If you have any questions, please contact Tracy Perry at 557-1451 or Henry Craven at 557-0320.

DP BARCODE: D163575

CASE: 193780 DATA PACKAGE RECORD DATE: 04/15/91
SUBMISSION: S316809 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 115 NC-NON-FOOD/FEED USE
CHEMICALS: 110201 2,4-Dinitro-N3,N3-dipropyl-6-(trifluoromethyl)-1,3 93.0000%

ID#: 055947-UR N3,N3-dipropyl-2,4-dinitro-6-trifluoromethyl-m-phe
COMPANY: 055947 SANDOZ CROP PROTECTION CORPORATION
PRODUCT MANAGER: 23 JOANNE MILLER 703-557-1830 ROOM: CM-2 237
PM TEAM REVIEWER: EUGENE WILSON 703-557-3943 ROOM: CM-2 252
RECEIVED DATE: ~~12/20/85~~ DUE OUT DATE: / /

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 163575 EXPEDITE: N DATE SENT: 04/15/91 DATE RET.: / /
CHEMICAL: 110201 2,4-Dinitro-N3,N3-dipropyl-6-(trifluoromethyl)-1,3-benzened
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 08/13/91 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	04/16/91	/ /
BRAN: EEB	4/19/91	/ /
SECT: RS1	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

Please review these repeated studies of prodianine with bluegill and ranibow trout and daphnia. Solubility is yet a limiting factor in getting a study that shows a progression curve associated with dose and effect. If you need proposed labeling and a csf please call me Eugene Wilson, 557-3943, or come to room 252.

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

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
147807	EFGB	01/26/90	04/26/90	Y		
147809	EEB	09/20/89	12/20/89	Y		
150542	TB/HFAS	02/13/90	03/01/90	Y		

MRID 418393-01

DATA EVALUATION RECORD

1. **CHEMICAL**: Prodiamine

2. **TEST MATERIAL**: N^3, N^3-Di-n-propyl-2,4-dinitro-6-(trifluromethyl)-m-phenylenediamine; 94.3% purity; a yellow crystalline solid.

3. **STUDY TYPE**: Acute Toxicity Test for Freshwater Fish. Species tested: Bluegill sunfish (Lepomis macrochirus).

4. **CITATION**: Holmes, C.M. and G.T. Peters. 1991. Prodiamine: A 96-hour Flow-Through Acute Toxicity Test with the Bluegill (Lepomis macrochirus). Study performed by Wildlife International Ltd., Easton, Maryland. Submitted by Sandoz Crop Protection Corporation, Des Plaines, Illinois.

5. **REVIEWED BY**:

Tracy L. Perry
Wildlife Biologist
EEB/EFED

Signature: Tracy L. Perry
Date: 8/7/91

6. **APPROVED BY**:

Henry T. Craven
Head, Section IV
EEB/EFED

Signature: Henry T. Craven
Date: 8/8/91

7. **CONCLUSIONS**: This study is scientifically sound and fulfills the data requirements for an acute toxicity study for freshwater fish. The 96-hour LC_{50} and no mortality concentration for the bluegill sunfish were > 552 ppb a.i./L.

8. **RECOMMENDATIONS**: N/A

9. **BACKGROUND**: Resubmission of study for registration.

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

11. **MATERIALS AND METHODS**:

A. Test Animals: Juvenile bluegill sunfish (Lepomis macrochirus) were obtained from Northeastern Biological located in Rhinebeck, New York. All fish were from the same source and year class and the standard length of the largest fish was no more than twice that of the shortest fish. The average length of 10 control organisms was 29mm (range = 26mm to 32mm length). The average wet weight of the same fish was 0.72g (range = 0.58g to 0.93g). Loading (total wet weight of fish per liter of test solution) was 0.08g of fish per liter of solution that passed through the test chambers in 24 hours. Instantaneous loading was 0.48 g/L at any given time.

Test organisms were acclimated to test conditions for approximately 2.5 days before the study began. During the acclimation period, fish were fed salmon mash and/or flake food, supplied by Ziegler Brothers, Inc., Garderners, PA. Fish were not fed 48 hours prior to or during the test. Test organisms showed no signs of disease or stress and no mortality occurred during the acclimation period.

B. Test System: The study was conducted under flow-through conditions in Teflon-lined 25-L polyethylene aquaria filled with 15 L of test solution (water depth was approximately 17 cm). Test chambers were randomly positioned in a temperature controlled water bath (22 C) which was enclosed in a plexiglass ventilation hood to minimize cross-contamination. Dilution water was filtered freshwater obtained from a 45 m deep well located on the Wildlife International Ltd. site. Fluorescent lights were used to maintain a 16-hour light and 8-hour dark photoperiod. Light intensity was 20-50 footcandles at the surface of the water. A thirty minute transition period between light and dark was provided.

A proportional diluter, calibrated prior to the test and visually inspected at least twice a day, was used to deliver test media to the test vessels; six volume additions of test water were added to each test chamber every 24 hours. Diluter flow (non-radiolabelled test substance) was initiated approximately 66 hours before test initiation in order to establish equilibrium concentration of test substance. Radio-labelled stock flow was started approximately 7 hours prior to

test initiation.

C. Dosage: The test substance was dissolved in reagent grade polyethylene glycol (PEG) and sonicated to produce an initial non-radiolabelled stock solution. The initial stock was then combined with radiolabelled prodiamine stock to form a final stock solution with an active ingredient concentration of 1.25 mg/mL. Secondary stock solutions (0.75, 0.45, 0.27, and 0.16 mg a.i./mL) were derived through further dilution with PEG. Secondary stocks were mixed with well water to produce desired test concentrations. Concentrations of PEG in the solvent control and in the treatment groups was 0.51mL/L.

Nominal test concentrations used in the study were 80, 130, 220, 360, and 600 ppb ^{14}C a.i./L. Test concentrations were chosen based on the results of an acute range finding toxicity test. One solvent control and one dilution water control were included in the study.

D. Test Design: Each test concentration contained 20 fish (2 replicates of 10 fish each). Fish were impartially removed from holding tanks in groups of 2 and distributed among the test chambers until each contained 10 organisms.

Mortalities and treatment-related effects were recorded at 17, 24, 48, 72, and 96 hours. The hardness, alkalinity and pH of the negative control water were measured at the beginning of the test. The pH and dissolved oxygen (DO) content of the water in alternate replicates were measured at 0, 24, 48, and 96 hours. Temperature was measured continuously in one negative control replicate and at the beginning and end of the test in each test chamber.

Three 5 ml samples from each replicate test chamber were collected at the beginning of the test and at 24 hour intervals 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, temperature ranged from 21.7°C to 22.1°C. Hardness was 144 mg/L, as $CaCO_3$, and alkalinity was 192 mg/L, as $CaCO_3$. The pH ranged from 8.1 to 8.2 and DO ranged from 8.6 to 9.2 mg/L. DO and pH readings were inadvertently not recorded at 72 hours.

Mean measured concentrations of the test substance ranged from 92 to 118% of nominal test concentrations: nominal

(measured); 80(94), 130 (144), 220 (213), 360 (354), and 660 (552) ppb ^{14}C a.i./L. " A creamy, yellow material was observed throughout the test at the air/water interface in the mixing chambers and test chambers associated with the three highest test concentrations. "

" No sublethal effects or mortalities were observed at any concentration during the 96-hour exposure period. Based on visual interpretation of the mortality data, the bluegill 96-hour LC_{50} value and no mortality concentrations were >552 [ppb] a.i./L and >552 [ppb] a.i./L, respectively. "

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 with the following exception: " The empty test substance container for the radiolabelled compound was disposed of prior to the completion of the study."

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:

* Test fish were acclimated to test conditions for only 2.5 days prior to testing vs. the recommended 2 weeks.

* Information describing range finding study procedures and results was not included in the report.

* Dimensions of the test vessels were not provided.

B. Statistical Analysis: N/A

C. Discussion/Results:

Information on the range finding study procedures and results should be included in the report. EEB requests that information on sample size, concentrations tested and mortality data be provided.

Was there any attempt to identify the "creamy, yellow material" that was observed at the air/water interface in the test chambers of the three highest test concentrations? EEB would like additional information on this observation.

Although the above questions require further clarification, the study is scientifically sound and fulfills the data requirements for an acute toxicity test for freshwater fish.

D. Adequacy of Study:

1) Classification: Core.

2) Rationale: N/A

3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, July 29, 1991.

20

Table 7.
Cumulative Percent Mortality and Treatment-Related Effects.*

Client: Sandoz Crop Protection Corporation
Test Substance: Prodiamine
Test Organism: Bluegill, Lepomis macrochirus
Dilution Water: Well Water

Mean Measured Concentration (µg a.i./L)	Replicate	17 Hours		24 Hours		48 Hours		72 Hours		96 Hours	
		Mortality	Effects	Mortality	Effects	Mortality	Effects	Mortality	Effects	Mortality	Effects
Negative Control	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
Solvent Control	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
94	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
144	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
213	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
354	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
552	A	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN	0% (0/10)	10 AN
	B	0% (0/9)**	9 AN	0% (0/9)	9 AN	0% (0/9)	9 AN	0% (0/9)	9 AN	0% (0/9)	9 AN

*Observed Effects:

A = Surfacing
B = Hyperexcitability
C = Lethargy
D = Discoloration
E = Erratic Swimming
F = Hyperventilation
G = Gulping Air
H = Hemorrhaging
I = Excessive Mucus
J = Mucus Shedding
K = Curved Spine
L = Ulcer
M = Moribund
N = Loss of Equilibrium
O = Opaque Eyes
P = Molting
AN = Appears Normal
N/A = Not Applicable

**Only nine fish exposed to 552 µg a.i./L concentration.