

9-11-96



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

MEMORANDUM

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**SUBJECT:** Data review for Isoxaflutole (D222982) <sup>229696</sup>

**FROM:** Renée Costello, Biologist *Renée Costello*  
Environmental Risk Characterization Branch  
Environmental Fate and Effects Division (7507C)

**THRU:** Elizabeth Leovey, Chief *Elizabeth Leovey*  
Environmental Risk Characterization Branch  
Environmental Fate and Effects Division (7507C)

**TO:** Joanne Miller, PM 23 *Joanne Miller*  
Registration Division (7505C) *2/4/96*

The following is a summary of data reviewed for isoxaflutole, the DERs are attached to this memo:

GDLN No.	Test Material	MRID	Classification
123-2	RPA 203328	439048-26	Core
72-4	RPA 201772	439048-21	Core
72-1	RPA 202248	439048-22	Core
72-2	RPA 202248	439048-23	Core
72-3	RPA 202248	439048-24	Core
72-1	RPA 203328	439048-25	Core

If there are any questions please contact Renée Costello at 305-5294.

~~9/3/96~~

MRID No. 439048-26

**DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)**

1. **CHEMICAL:** Isoxaflutole PC Code No.: 123000

2. **TEST MATERIAL:** RPA 203328 Purity: 98.9%

3. **CITATION:**

Authors: James R. Hoberg  
Title: RPA 203328 - 5-Day Toxicity to the  
Freshwater Green Alga *Selenastrum  
capricornutum*

Study Completion Date: November 22, 1995

Laboratory: Springborn Laboratories, Inc., Wareham,  
MA

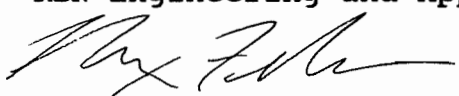
Sponsor: Rhône-Poulenc Secteur Agro, Sophia  
Antipolis, France

Laboratory Report ID: 95-11-6172

DP Barcode: D222982

MRID No.: 439048-26


4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,  
KBN Engineering and Applied Sciences, Inc.

**Signature:**  **Date:** 8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist,  
KBN Engineering and Applied Sciences, Inc.

**Signature:** P. Kosalwat **Date:** 8/8/96

5. **APPROVED BY:**

**Signature:**  **Date:** 9/3/96

6. **STUDY PARAMETERS:**

**Definitive Test Duration:** 120 hours

**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills  
the guideline requirements for an aquatic plant toxicity  
test.

**Results Synopsis**

EC<sub>50</sub>: 5.9 ppm ai 95% C.I.: 4.9 - 7.5 ppm ai

NOEC: 2.4 ppm ai Probit Slope: N/A

7.5

**8. ADEQUACY OF THE STUDY:**A. **Classification:** CoreB. **Rationale:** N/AC. **Repairability:** N/A**9. GUIDELINE DEVIATIONS: None****10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Selenastrum capricornutum</i>
<b><u>Initial Number of Cells</u></b> 3,000 - 10,000 cells/mL	3,000 cells/mL
<b><u>Nutrients</u></b> Standard formula, e.g. 20XAAP	AAP medium

**B. Test System**

Guideline Criteria	Reported Information
<b><u>Solvent</u></b>	Acetone (0.1 mL/L)
<b><u>Temperature</u></b> Skeletonema: 20°C Others: 24-25°C	24°C
<b><u>Light Intensity</u></b> Anabaena: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	4.2 - 4.5 Klux
<b><u>Photoperiod</u></b> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
<b>pH</b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 7.1 - 7.5 Final: 9.1 - 9.3

**C. Test Design**

Guideline Criteria	Reported Information
<b><u>Dose range</u></b> 2X or 3X progression	2X
<b><u>Doses</u></b> at least 5	Definitive test: 0.63, 1.3, 2.5, 5.0, and 10 mg ai/L
<b><u>Controls</u></b> negative and/or solvent	Negative and solvent control
<b><u>Replicates per dose</u></b> 3 or more	3
<b><u>Duration of test</u></b> 120 hours	120 hours
<b>Daily observations were made?</b>	Yes
<b><u>Method of Observations</u></b>	Cellular counts
<b><u>Maximum Labeled Rate</u></b>	Maximum labeled rate for parent compound isoxaflutole (RPA 201772) is 0.1888 lb ai/A

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Initial and 120 h cell densities were measured?</b>	Yes
<b>Control cell count at 120 hr <math>\geq</math>2X initial count?</b>	Yes
<b>Initial chemical concentrations measured? (Optional)</b>	Yes
<b>Raw data included?</b>	Yes

**Dose Response**

Mean Measured Concentration (mg ai/L)	Avg. Cell Density ( $\times 10^4$ cells/ml)	% reduction*	120-Hour pH
Control	208	--	9.3
Solvent Cont.	279	--	9.3
0.61	227	19	9.3
1.3	246	12	9.3
2.4	242	13	9.2
4.6	125	55	9.2
9.4	134	52	9.1

\*Compared to the solvent control

**Other Significant Results:** Bloated algal cells and cell fragments were observed in all treatment concentrations and solvent controls.

**Statistical Results**

Statistical Method: The "best fit" linear regression based on the highest coefficient of determination ( $r^2$ ) was used for estimating the  $EC_{50}$  and Williams' test was used for mean comparisons. Results were based on the mean measured concentrations.

$EC_{50}$ : >9.4 mg ai/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 2.4 mg ai/L

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Moving average method for  $EC_{50}$  and Williams' test for mean comparisons. Results based on the mean measured concentrations. All comparisons were made versus the solvent control.

$EC_{50}$ : 5.9 ppm ai

95% C.I.: 4.9 - 7.5 ppm ai

Probit Slope: N/A

NOEC: 2.4 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on mean measured concentrations, the 120-hour EC<sub>50</sub> and NOEC for *Selenastrum capricornutum* exposed to RPA 203328 was 5.9 and 2.4 ppm ai, respectively. This study is categorized as **Core**.

MAX FEKEN RPA 203328 SELENASTRUM CAPRICORNUTUM 08-06-96

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.399999		100	52	52
4.6	100	55	55	0
2.4	100	13	13	0
1.3	100	12	12	0
.61	100	19	19	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.288935

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.102894	5.891247	4.871376	7.513031

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.09465	10.50964	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 1.103937  
95 PERCENT CONFIDENCE LIMITS = -.493781 AND 2.701656

LC50 = 7.94624  
95 PERCENT CONFIDENCE LIMITS = 1.801426 AND +INFINITY

LC10 = .5619971  
95 PERCENT CONFIDENCE LIMITS = 0 AND 2.218191

\*\*\*\*\*

RPA 203328 - SELENASTRUM CAPRICORNUTUM  
 File: 43904826 Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

-----  
 GRP1 (SOLVENT CRTL) MEAN = 278.6667 CALCULATED t VALUE = 2.3342  
 GRP2 (BLANK CRTL) MEAN = 208.3333 DEGREES OF FREEDOM = 4  
 DIFFERENCE IN MEANS = 70.3333  
 -----

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

RPA 203328 - SELENASTRUM CAPRICORNUTUM  
 File: 43904826 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

-----  

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	243.500	243.500	243.500
2	0.61	3	227.000	227.000	238.333
3	1.3	3	246.000	246.000	238.333
4	2.4	3	242.000	242.000	238.333
5	4.6	3	125.667	125.667	129.667
6	9.4	3	133.667	133.667	129.667

 -----

RPA 203328 - SELENASTRUM CAPRICORNUTUM  
 File: 43904826 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

-----  

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	243.500				
0.61	238.333	0.196		1.75	k= 1, v=15
1.3	238.333	0.196		1.84	k= 2, v=15
2.4	238.333	0.196		1.87	k= 3, v=15
4.6	129.667	4.308	*	1.88	k= 4, v=15
9.4	129.667	4.308	*	1.89	k= 5, v=15

 -----

s = 37.373

Note: df used for table values are approximate when v > 20.



RPA 203328 - SELENASTRUM CAPRICORNUTUM

File: 43904826

Transform: NO TRANSFORMATION

ANOVA TABLE

---

SOURCE	DF	SS	MS	F
Between	6	60765.143	10127.524	10.479
Within (Error)	14	13530.667	966.476	
Total	20	74295.810		

---

Critical F value = 2.85 (0.05,6,14)

Since  $F > \text{Critical F}$  REJECT  $H_0$ : All equal

RPA 203328 - SELENASTRUM CAPRICORNUTUM

File: 43904826

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	SOLVENT	278.667	278.667		
2	CONTROL	208.333	208.333	2.771	*
3	0.61	227.000	227.000	2.035	
4	1.3	246.000	246.000	1.287	
5	2.4	242.000	242.000	1.445	
6	4.6	125.667	125.667	6.028	*
7	9.4	133.667	133.667	5.712	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

RPA 203328 - SELENASTRUM CAPRICORNUTUM

I a: 43904826

Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	SOLVENT	3			
2	CONTROL	3	64.220	23.0	70.333
3	0.61	3	64.220	23.0	51.667
4	1.3	3	64.220	23.0	32.667
5	2.4	3	64.220	23.0	36.667
6	4.6	3	64.220	23.0	153.000
7	9.4	3	64.220	23.0	145.000

NOEL = 2.4 mg a1 / L

RPA 203328 - SELENASTRUM CAPRICORNUTUM

File: 43904826

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	3	278.667	278.667	278.667
2	CONTROL	3	208.333	208.333	230.833
3	0.61	3	227.000	227.000	230.833
4	1.3	3	246.000	246.000	230.833
5	2.4	3	242.000	242.000	230.833
6	4.6	3	125.667	125.667	129.667
7	9.4	3	133.667	133.667	129.667

RPA 203328 - SELENASTRUM CAPRICORNUTUM

File: 43904826

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	278.667				
CONTROL	230.833	1.884	*	1.76	k= 1, v=14
0.61	230.833	1.884	*	1.85	k= 2, v=14
1.3	230.833	1.884	*	1.88	k= 3, v=14
2.4	230.833	1.884		1.89	k= 4, v=14
4.6	129.667	5.870	*	1.90	k= 5, v=14
9.4	129.667	5.870	*	1.91	k= 6, v=14

s = 31.088

Note: df used for table values are approximate when v > 20.

9-3-96

MRID No. 439048-21

**DATA EVALUATION RECORD  
AQUATIC INVERTEBRATE LIFE CYCLE TEST  
GUIDELINE 72-4**

1. **CHEMICAL:** Isoxaflutole **PC Code No.:** 123000

2. **TEST MATERIAL:** Isoxaflutole **Purity:** 96.8%  
(RPA 201772 Technical)

3. **CITATION:**

**Author:** Joseph V. Sousa  
**Title:** Isoxaflutole - Chronic Toxicity to Mysids  
(*Mysidopsis bahia*) Under Flow-Through  
Conditions

**Study Completion Date:** December 1, 1995

**Laboratory:** Springborn Laboratories, Inc., Wareham,  
MA

**Sponsor:** Rhône-Poulenc Ag Company, Research  
Triangle Park, NC

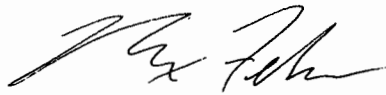
**Laboratory Report ID:** 95-8-6017

**MRID No.:** 439048-21

**DP Barcode:** D222982

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,  
KBN Engineering and Applied Sciences, Inc.

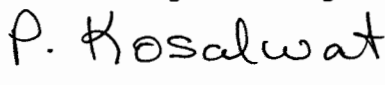
**Signature:**



**Date:** 8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist,  
KBN Engineering and Applied Sciences, Inc.

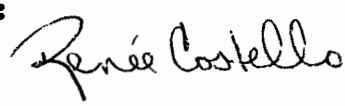
**Signature:**



**Date:** 8/8/96

5. **APPROVED BY:**

**Signature:**



**Date:** 9/3/96

6. **STUDY PARAMETERS:**

**Age of Test Organism:** ≤24 hours  
**Definitive Test Duration:** 28 days  
**Study Method:** Flow-Through  
**Type of Concentrations:** Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills  
the guideline requirements for a saltwater invertebrate life-  
cycle test.

**Results Synopsis:** Most sensitive endpoint: survival

**NOEC:** 1.0 ppb ai **LOEC:** 1.9 ppb ai **MATC:** 1.4 ppb ai

LOEC's for specific endpoints:

Neonates Produced: >3.8 ppb ai  
 Mysid Survival: 1.9 ppb ai  
 Growth (length): >1.9 ppb ai  
 Growth (weight): >1.9 ppb ai

**8. ADEQUACY OF THE STUDY:**

- A. **Classification:** Core
- B. **Rationale:** N/A
- C. **Repairability:** N/A

**9. GUIDELINE DEVIATIONS:** Since an EPA SEP for the mysid life cycle test does not exist, ASTM's Standard Guide for Conducting Life-Cycle Toxicity Tests With Saltwater Mysids (E1191-90) was used as a guidance in this evaluation. Deviations from the ASTM's guidelines are noted as follows:

- 1. The source of the fresh water used to prepare the artificial seawater was not reported. However, the dilution water was apparently adequate for satisfactory survival, growth, and reproduction of the control mysids.

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS**

**A. Test Organisms/Acclimation**

Guideline Criteria	Reported Information
<b><u>Species</u></b> <i>Mysidopsis</i> spp.	<i>Mysidopsis bahia</i>
<b><u>Source</u></b> Laboratory, commercial, or wild stock.	In-house cultures
<b><u>Parental Acclimation Conditions</u></b> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Held under test conditions at 25-26°C in dilution water
<b><u>Parental Acclimation Period</u></b> At least 14 days.	Continuous

Guideline Criteria	Reported Information
<b><u>Age of Parental Stock</u></b> At least 10-12 days old at the beginning of the acclimation period.	Not reported
<b><u>Food</u></b> Brine shrimp nauplii in possible combination with rotifers and/or algae.	Mysids were fed freshly hatched brine shrimp nauplii twice daily, with one feeding supplemented with Selco®.
<b><u>Food Concentration</u></b> 150 brine shrimp nauplii per mysid per day.	Not reported
<b>Were mysids in good health during acclimation period?</b>	Yes

#### B. Test System

Guideline Criteria	Reported Information
<b><u>Test Water</u></b> Unpolluted saltwater that has been tested for contaminants, or appropriate reconstituted water.	Artificial seawater prepared from filtered freshwater to a salinity of 25 ± 3%.
<b><u>Water Temperature</u></b> 27 ± 2°C.	26-28°C
<b><u>pH</u></b>	8.0 to 8.3
<b><u>Dissolved Oxygen</u></b> ≥60% throughout test.	≥78% of saturation during the test
<b><u>Test Vessels or Compartments</u></b> 1. <b><u>Material</u></b> : Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <b><u>Size</u></b> : 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.	1. Glass 2. Each aquaria (39 x 20 x 25 cm) contained two retention chambers consisting of glass Petri dishes (10 cm diameter) with 15-cm high Nitex® screen collars.

Guideline Criteria	Reported Information
<p><b><u>Type of Dilution System</u></b>                      Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>Intermittent-flow proportional diluter.</p>
<p><b><u>Flow Rate</u></b>                      At least 5 volume additions per 24 hours.</p>	<p>12 volume additions per 24 hours providing 90% replacement in approximately 5 hours.</p>
<p><b><u>Aeration</u></b>                      Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>Dilution water was aerated prior to use.</p>
<p><b><u>Photoperiod</u></b>                      16 hours light, 8 hours dark</p>	<p>16 hours light, 8 hours dark</p>
<p><b><u>Solvents</u></b>                      Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	<p>Solvent: acetone                      Maximum conc.: 0.0065 mL/L</p>

**C. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Duration</u></b></p>	<p>28 days</p>
<p><b><u>Nominal Concentrations</u></b>                      Control(s) and at least 5 test concentrations; dilution factor not less than 50%.</p>	<p>Dilution water control, solvent control, and five treatment concentrations: 0.31, 0.62, 1.2, 2.5, and 5.0 µg ai/L.</p>

8 15

Guideline Criteria	Reported Information
<p><b><u>Number of Test Organisms</u></b>  60 mysids/level;  At least two test replicate vessels, each containing two chambers, with each chamber containing 15 mysids until 10 or 14 days after initiation. After sexing, at least 10 mated pairs per replicate.</p>	60 mysids/level; 2 replicate vessels each containing 2 retention chambers with 15 mysids each for the first 14 days; 10 pairing jars with mated pairs and the remaining males and females separated in single retention chambers from day 15 to test termination.
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	Impartially distributed
<p><b><u>Renewal</u></b>  Parent mysids in all beakers must be transferred to containers with fresh test solution (&lt; 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).</p>	N/A
<p><b><u>Water Parameter Measurements</u></b></p> <ol style="list-style-type: none"> <li>1. Dissolved oxygen must be measured at each concentration at least once a week.</li> <li>2. pH must be measured once a week in one test concentration and in one control.</li> <li>3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.</li> </ol>	<ol style="list-style-type: none"> <li>1. Dissolved oxygen was measured daily in each vessel.</li> <li>2. The pH was measured daily in each test vessel.</li> <li>3. Temperature was measured daily in each vessel and continuously in one replicate vessel of the dilution water control.</li> </ol>
<p><b><u>Chemical Analysis</u></b>  Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.</p>	Samples removed and analyzed on days 0, 2, 7, 14, 21, and 28. Results based on the mean measured concentrations.



**12. REPORTED RESULTS****A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<b>Control Mortality</b> ≤30% between pairing and test termination.	18% and 23% for control and solvent control, respectively.
Did at least 75% of the paired female mysids in each control produce at least 3 young by test termination?	Yes
<b>Data Endpoints</b> - Survival of first-generation mysids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation mysid alive at the end of the test, - Observations of other effects or clinical signs.	- Survival of parental mysids, - Number of offspring per female per reproductive day, - Male and female dry weight and length of surviving first generation mysids.
Raw data included?	Yes

**Effects Data**

Toxicant Concentration (µg ai/L)		Percent Dead or Immobile (28 Days)	Number of Young per Female per Reprod. Day	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Measured				
Control	<0.1	18	0.44	M 7.1 F 7.0	M 0.84 F 0.85
Solvent	<0.1	23	0.53	M 7.1 F 7.0	M 0.85 F 0.97
0.31	0.30	23	0.73	M 7.1 F 7.1	M 0.76 F 1.0

Toxicant Concentration ( $\mu\text{g ai/L}$ )		Percent Dead or Immobile (28 Days)	Number of Young per Female per Reprod. Day	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Measured				
0.62	0.52	37	0.67	M 7.3 F 7.2	M 0.84 F 0.96
1.2	1.0	30	0.58	M 7.1 F 7.0	M 0.89 F 1.0
2.5	1.9	52	0.40	M 6.9 F 7.1	M 0.84 F 0.99
5.0	3.8	87	0.53	M 7.1 F 7.2	M 0.84 F 0.85

Toxicity Observations: None.

**B. Statistical Results:** The results are based on mean measured concentrations.

Endpoint	Method	NOEC ( $\mu\text{g ai/L}$ )	LOEC ( $\mu\text{g ai/L}$ )
Survival	Williams' test	1.0	1.9
Reproduction <sup>a</sup>	Williams' test	1.0	ND <sup>b</sup>
Length <sup>a</sup>	Williams' test	1.0	ND
Dry weight <sup>a</sup>	Williams' test	1.0	ND

<sup>a</sup>Since survival was significantly affected at the two highest treatment levels (1.9 and 3.8  $\mu\text{g ai/L}$ ), reproductive and growth data for these treatment levels were not included in the statistical analysis.

<sup>b</sup>ND - Not determined

**13. VERIFICATION OF STATISTICAL RESULTS**

Endpoint	Method	NOEC (ppb ai)	LOEC (ppb ai)
Survival	Williams' test	1.0	1.9
Reproduction	Visual inspection	3.8	>3.8
Length <sup>a</sup>	Bonferroni's test	1.9	>1.9
Dry weight <sup>a</sup>	Bonferroni's test	1.9	>1.9

<sup>a</sup>Growth data from the highest test concentration were not included in the analysis due to complete mortality in some replicates.

**14. REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements for a mysid life-cycle test, and can be classified as **Core**. Based on the most sensitive endpoint (survival), the NOEC and LOEC are 1.0 and 1.9 ppb ai, respectively. The geometric mean MATC is 1.4 ppb ai.

ISOXAFLUTOLE - MYSID SURVIVAL

File: 43904821

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	2	0.765	1.068	1.102
2	CONTROL	2	0.820	1.136	1.102
3	0.30	2	0.770	1.071	1.071
4	0.52	2	0.635	0.923	0.958
5	1.0	2	0.700	0.992	0.958
6	1.9	2	0.485	0.770	0.770
7	3.8	2	0.134	0.363	0.363

ISOXAFLUTOLE - MYSID SURVIVAL

File: 43904821

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	1.102				
CONTROL	1.102	0.347		1.89	k= 1, v= 7
0.30	1.071	0.022		2.00	k= 2, v= 7
0.52	0.958	1.136		2.04	k= 3, v= 7
1.0	0.958	1.136		2.06	k= 4, v= 7
1.9	0.770	3.053	*	2.07	k= 5, v= 7
3.8	0.363	7.222	*	2.08	k= 6, v= 7

s = 0.098

Note: df used for table values are approximate when v > 20.

NOEL = 1.0  $\mu$ g ai/L

ISOXAFLUTOLE - MYSID SURVIVAL

File: 43904821

Transform: ARC SINE(SQUARE ROOT(Y))

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

-----  
 GRP1 (SOLVENT CRTL) MEAN = 1.0685      CALCULATED t VALUE = -0.6683  
 GRP2 (BLANK CRTL) MEAN = 1.1363      DEGREES OF FREEDOM = 2  
 DIFFERENCE IN MEANS = -0.0678  
 -----

TABLE t VALUE (0.05 (2), 2) = 4.303      NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2), 2) = 9.925      NO significant difference at alpha=0.01

ISOXAFLUTOLE - MYSID SURVIVAL

File: 43904821

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

-----  
 G. JP      IDENTIFICATION      N      ORIGINAL MEAN      TRANSFORMED MEAN      ISOTONIZED MEAN  
 -----  
 1      GRPS 1&2 POOLED      4      0.793      1.102      1.102  
 2      0.30      2      0.770      1.071      1.071  
 3      0.52      2      0.635      0.923      0.958  
 4      1.0      2      0.700      0.992      0.958  
 5      1.9      2      0.485      0.770      0.770  
 6      3.8      2      0.134      0.363      0.363  
 -----

ISOXAFLUTOLE - MYSID SURVIVAL

File: 43904821

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

-----  
 IDENTIFICATION      ISOTONIZED MEAN      CALC. WILLIAMS      SIG P=.05      TABLE WILLIAMS      DEGREES OF FREEDOM  
 -----  
 GRPS 1&2 POOLED      1.102  
 0.30      1.071      0.388      1.86      k= 1, v= 8  
 0.52      0.958      1.770      1.96      k= 2, v= 8  
 1.0      0.958      1.770      2.00      k= 3, v= 8  
 1.9      0.770      4.059      \*      2.01      k= 4, v= 8  
 3.8      0.363      9.038      \*      2.02      k= 5, v= 8  
 -----

s = 0.094

Note: df used for table values are approximate when v > 20.

21  
to

Variable N Mean Std Dev Minimum Maximum

MLNGTH	19	7.1052632	0.5016348	5.8000000	8.0000000
FLNGTH	27	7.1370370	0.3212334	6.5000000	7.7000000
MWGT	19	0.7621053	0.1660515	0.3400000	1.0500000
FWGT	27	1.0022222	0.1868429	0.6200000	1.4100000

TRT=I

Variable N Mean Std Dev Minimum Maximum

MLNGTH	15	7.2600000	0.2501428	6.7000000	7.6000000
FLNGTH	23	7.1956522	0.3067102	6.5000000	8.0000000
MWGT	15	0.8380000	0.0970420	0.6500000	1.0200000
FWGT	23	0.9630435	0.2244677	0.4500000	1.3800000

TRT=I

Variable N Mean Std Dev Minimum Maximum

MLNGTH	20	7.1200000	0.3607011	6.5000000	7.7000000
FLNGTH	22	7.0222723	0.3421665	6.5000000	7.9000000
MWGT	20	0.8940000	0.1284482	0.6400000	1.1400000
FWGT	22	1.0000000	0.2065361	0.5900000	1.4900000

TRT=IV

Variable N Mean Std Dev Minimum Maximum

MLNGTH	21	6.9000000	0.3301515	6.2000000	7.4000000
FLNGTH	8	7.0500000	0.4105745	6.4000000	7.7000000
MWGT	21	0.8357143	0.0861145	0.6900000	0.9700000
FWGT	8	0.9862500	0.2597217	0.6100000	1.3800000

TRT=cont

Variable N Mean Std Dev Minimum Maximum

MLNGTH	22	7.0681818	0.2643705	6.5000000	7.4000000
FLNGTH	27	7.0185185	0.3088901	6.5000000	7.6000000
MWGT	22	0.8418182	0.1332608	0.5400000	1.0800000
FWGT	27	0.8548148	0.2063500	0.3600000	1.2200000

TRT=solv

Variable N Mean Std Dev Minimum Maximum

MLNGTH	24	7.1041667	0.2726348	6.5000000	7.7000000
FLNGTH	22	7.0181818	0.3775061	6.2000000	7.9000000
MWGT	24	0.8483333	0.1053634	0.6600000	1.0500000
FWGT	22	0.9745455	0.2261842	0.6000000	1.5300000

ISOXAFUTOLE - MYSID CHRONIC TEST

General Linear Models Procedure  
 Class Level Information

Class	Levels	Values
TRT	6	I II III IV cont solv
REP	2	a b

Number of observations in data set = 148

Group Obs Dependent Variables

1	121	MLNGTH MWGT
2	129	FLNGTH FWGT

NOTE: Variables in each group are consistent with respect to the presence or absence of missing values.

ISOXAFUTOLE - MYSID CHRONIC TEST  
 13:38 Tuesday, August 6, 1996  
 General Linear Models Procedure

Dependent Variable: MLNGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.2291015	0.2048502	1.77	0.1125
Error	114	13.2310638	0.1160620		
Corrected Total	120	14.4601653			

Source DF Type I SS Mean Square F Value Pr > F

TRT	5	1.2253810	0.2450762	2.11	0.0690
REP	1	0.0037205	0.0037205	0.03	0.8582

Source DF Type III SS Mean Square F Value Pr > F

TRT	5	1.2288377	0.2457675	2.12	0.0683
REP	1	0.0037205	0.0037205	0.03	0.8582

ISOXAFUTOLE - MYSID CHRONIC TEST  
 13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dependent Variable: MWGT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.1921510	0.0320252	2.15	0.0534
Error	114	1.7012011	0.0149228		
Corrected Total	120	1.8933521			

22

R-Square	C.V.	Root MSE	MMGHT Mean
0.101487	14.58291	0.1222	0.8377

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.1751414	0.0350283	2.35	0.0455
REP	1	0.0170096	0.0170096	1.14	0.2879
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.1743000	0.0348600	2.34	0.0464
REP	1	0.0170096	0.0170096	1.14	0.2879

ISOXAFUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure  
Least Squares Means

TRT	MLNGTH	PR >  T	H0: LSMEAN(I)=LSMEAN(J)	MMGHT	Mean
	LSMEAN	1/J	2	3	4
I	7.10555750	1	0.1962	0.8909	0.0585
II	7.25888151	2	0.2385	0.0022	0.0978
III	7.12055925	3	0.8909	0.0022	0.6199
IV	6.89866846	4	0.0585	0.0022	0.0403
cont	7.06818182	5	0.7268	0.0978	0.8741
solv	7.10416667	6	0.7268	0.0978	0.1065
			0.9894	0.1710	0.7211
			0.8741	0.0464	0.7211

TRT	MMGHT	PR >  T	H0: LSMEAN(I)=LSMEAN(J)	MMGHT	Mean
	LSMEAN	1/J	2	3	4
I	0.76147590	1	0.0646	0.0011	0.0494
II	0.84039157	2	0.0646	0.0011	0.9647
III	0.89280422	3	0.0011	0.2131	0.1600
IV	0.83856139	4	0.0494	0.9647	0.1796
cont	0.84181818	5	0.0380	0.9723	0.9307
solv	0.84833333	6	0.0224	0.8440	0.7900
			0.8440	0.2319	0.8569
			0.2319	0.7900	0.8569

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

ISOXAFUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: MLNGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.116062  
Critical Value of T= 2.99831

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	I - II	-0.06523	0.04567	0.15656
I	I - III	-0.06098	0.05600	0.18111
I	I - IV	-0.06911	0.05829	0.17272
I	I - cont	-0.05615	0.13189	0.24923
I	I - solv	-0.15656	-0.04567	0.06523
II	II - I	-0.10159	0.08652	0.11462
II	II - III	-0.11022	0.01033	0.13089
II	II - IV	-0.09683	0.01262	0.12206
II	II - cont	-0.02625	0.08623	0.19870
II	II - solv	-0.02625	0.08623	0.19870

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	I - II	-0.20890	0.14000	0.48890
I	I - III	-0.19807	0.15474	0.50735
I	I - solv	-0.18037	0.15583	0.49204
II	II - I	-0.15021	0.19182	0.53385
II	II - cont	0.01468	0.36000	0.70532
II	II - IV	-0.48890	-0.14000	0.20890
II	II - solv	-0.31250	0.01474	0.34197
III	III - I	-0.29343	0.01583	0.32510
III	III - cont	-0.26377	0.05182	0.34741
III	III - IV	-0.09915	0.22000	0.35915
I	I - II	-0.50755	-0.15474	0.19807
I	I - III	-0.34197	0.01474	0.31250
I	I - solv	-0.31257	0.00110	0.31477
II	II - I	-0.28283	0.03708	0.35699
II	II - cont	-0.11816	0.20526	0.52868
I	I - II	-0.49204	-0.15583	0.18037
I	I - III	-0.32510	0.01583	0.29343
I	I - solv	-0.31477	-0.00110	0.31257
II	II - I	-0.26551	0.03598	0.33748
II	II - cont	-0.10105	0.20417	0.50939
I	I - II	-0.53385	-0.19182	0.15021
I	I - III	-0.36741	-0.05182	0.26377
I	I - solv	-0.35699	-0.03708	0.28283
II	II - I	-0.33748	0.16818	0.25511
II	II - cont	-0.14345	0.16818	0.47981
I	I - II	-0.70532	-0.36000	-0.01468
I	I - III	-0.53915	-0.22000	0.09915
I	I - solv	-0.52868	-0.20526	0.11816
II	II - I	-0.50939	-0.20417	0.10105
II	II - cont	-0.47981	-0.16818	0.14345

ISOXAFUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: MMGHT

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.014923  
Critical Value of T= 2.99831

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	I - solv	-0.06523	0.04567	0.15656
II	II - I	-0.06098	0.05600	0.18111
II	II - IV	-0.06911	0.05829	0.17272
II	II - I	0.01468	0.13189	0.24923
I	I - II	-0.15656	-0.04567	0.06523
I	I - cont	-0.10159	0.08652	0.11462
I	I - III	-0.11022	0.01033	0.13089
I	I - IV	-0.09683	0.01262	0.12206
I	I - solv	-0.02625	0.08623	0.19870

TRT Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit
cont - III	-0.16534	-0.05218	0.06098
cont - solv	-0.11462	-0.10159	0.10159
cont - II	-0.11983	0.00382	0.12646
cont - IV	-0.10564	0.00610	0.11785
cont - I	-0.03500	0.07971	0.19442
III - solv	-0.18111	-0.05600	0.06911
II - solv	-0.13089	-0.01033	0.11022
II - cont	-0.12646	-0.00382	0.11885
II - IV	-0.12154	0.00229	0.12611
II - I	-0.05061	0.07589	0.20240
IV - III	-0.17272	-0.05829	0.05615
IV - solv	-0.12206	-0.01262	0.09683
IV - cont	-0.11785	-0.00610	0.10544
IV - II	-0.12611	-0.00229	0.12154
IV - I	-0.04236	0.07361	0.18958
III - III	-0.24923	-0.13189	-0.01456
I - solv	-0.19870	-0.08623	0.02625
I - cont	-0.19442	-0.07971	0.03500
I - II	-0.20240	-0.07589	0.05061
I - IV	-0.18958	-0.07361	0.04236

ISOXAFLOTILE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: FLNGTH  
NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.116062  
Critical Value of Dunnett's T= 2.277

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT Comparison	Simultaneous	
	Lower Confidence Limit	Upper Confidence Limit
III - solv	-0.09954	0.15583
III - solv	-0.21908	0.01583
I - solv	-0.23716	0.00110
cont - solv	-0.26500	-0.03598
IV - solv	-0.43601	-0.20417

ISOXAFLOTILE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: MWGHT  
NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 114 MSE= 0.014923  
Critical Value of Dunnett's T= 2.277

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

Simultaneous Simultaneous

TRT Comparison	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit
III - solv	-0.03857	0.04567	0.12990
cont - solv	-0.08863	-0.00652	0.07560
II - solv	-0.10191	-0.01033	0.08124
IV - solv	-0.09575	-0.01262	0.07051
I - solv	-0.17166	-0.08623	-0.00079

ISOXAFLOTILE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Dependent Variable: FLNGTH					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	6	0.6908576	0.1151429	1.02	0.4185
Error	122	13.8339486	0.1133930		
Corrected Total	128	14.5248062			

R-Square C.V. Root MSE FLNGTH Mean  
0.047564 4.757865 0.3367 7.0775

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.6601736	0.1320347	1.16	0.3306
REP	1	0.0306839	0.0306839	0.27	0.6039
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.6825882	0.1365176	1.20	0.3114
REP	1	0.0306839	0.0306839	0.27	0.6039

ISOXAFLOTILE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Dependent Variable: MWGHT					
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	6	0.3935235	0.0655872	1.44	0.2055
Error	122	5.5639013	0.0456057		
Corrected Total	128	5.9574248			
R-Square		C.V.	Root MSE	MWGHT Mean	
0.066056		22.28490	0.2136	0.9583	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.3920642	0.0784128	1.72	0.1351
REP	1	0.0014593	0.0014593	0.03	0.8583
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.3904926	0.0780985	1.71	0.1367
REP	1	0.0014593	0.0014593	0.03	0.8583

Handwritten mark resembling a stylized 'f' or '4'.



ISOXAFLUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996  
General Linear Models Procedure  
Least Squares Means

TRT	FLNGTH LSMEAN	Pr >  T  1	H0: LSMEAN(i)=LSMEAN(j) 2	3	4	5	6
I	7.13645686	1	0.5069	0.2309	0.5248	0.1942	0.2361
II	7.20041970	2	0.5069	0.0777	0.2797	0.0590	0.0767
III	7.01987914	3	0.2309	0.0777	0.8290	0.9745	0.9910
IV	7.05000000	4	0.5248	0.2797	0.8290	0.8069	0.8354
cont	7.01677799	5	0.1942	0.0590	0.9745	0.8069	0.9652
solv	7.02102995	6	0.2361	0.0767	0.9910	0.8354	0.9652

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

ISOXAFLUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996  
General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: FLNGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 122 MSE= 0.113393  
Critical Value of T= 2.99410

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT Comparison	Simultaneous		Simultaneous	
	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
I - I	-0.22747	0.05862	0.34470	
I - IV	-0.26819	0.14565	0.53949	
I - III	-0.12775	-0.47292	0.47360	
I - cont	-0.10895	0.17713	0.46322	
II - solv	-0.12320	0.17747	0.47814	
I - II	-0.34470	-0.05862	0.22747	
I - IV	-0.31881	-0.08704	0.49289	
I - III	-0.17527	0.11431	0.40389	
I - cont	-0.15569	0.11852	0.39292	
II - solv	-0.17072	0.11886	0.40843	
IV - I	-0.55949	-0.14565	0.26819	
IV - II	-0.49289	-0.08704	0.31881	

IV - III	-0.38899	0.02727	0.44353
IV - cont	-0.37437	0.03148	0.43733
IV - solv	-0.38444	0.03182	0.44808
III - II	-0.47360	-0.17292	0.12775
III - IV	-0.40389	-0.11431	0.17527
III - cont	-0.44353	-0.02727	0.38899
III - solv	-0.28537	0.00421	0.29379
II - solv	-0.29945	0.00455	0.30854
cont - II	-0.46322	-0.17713	0.10895
cont - I	-0.39292	-0.11852	0.15589
cont - IV	-0.43733	-0.03148	0.37437
cont - III	-0.29379	-0.00421	0.28537
cont - solv	-0.28924	0.00034	0.28991
solv - II	-0.47814	-0.17747	0.12320
solv - I	-0.40843	-0.11886	0.17072
solv - IV	-0.44808	-0.03182	0.38444
solv - III	-0.30854	-0.00455	0.29945
solv - cont	-0.28991	-0.00034	0.28924

ISOXAFLUTOLE - MYSID CHRONIC TEST  
13:38 Tuesday, August 6, 1996  
General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: FLNGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 122 MSE= 0.045606  
Critical Value of T= 2.99410

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT Comparison	Simultaneous		Simultaneous	
	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
I - III	-0.18142	0.00222	0.18587	
I - IV	-0.24141	0.01397	0.27336	
I - solv	-0.15597	0.02768	0.21132	
I - II	-0.14225	0.03918	0.22061	
I - cont	-0.02662	0.14741	0.32143	
III - I	-0.18587	-0.00222	0.18142	
III - IV	-0.25024	0.01375	0.27774	
III - solv	-0.16733	0.02545	0.21824	
III - II	-0.15372	0.03696	0.22764	
III - cont	-0.03846	0.14519	0.32883	
IV - I	-0.27336	-0.01597	0.24141	
IV - III	-0.27774	-0.01375	0.25024	
IV - solv	-0.25228	0.01170	0.27569	
IV - II	-0.23924	0.02321	0.28566	
IV - cont	-0.12595	0.13144	0.38882	
I - I	-0.21132	-0.02768	0.15597	
I - III	-0.21824	-0.02545	0.16733	
I - IV	-0.27569	-0.01170	0.25228	
I - solv	-0.17918	0.01150	0.20218	
I - cont	-0.06392	0.11973	0.30338	
II - I	-0.22061	-0.03918	0.14225	

52

TRT	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit
II - III	-0.22764	-0.03696	0.15372
II - IV	-0.28566	-0.02321	0.23924
II - solv	-0.20218	-0.01150	0.17918
II - cont	-0.07320	0.10823	0.28966
cont - I	-0.32143	-0.14741	0.02662
cont - III	-0.32883	-0.14519	0.03846
cont - IV	-0.38882	-0.13144	0.12595
cont - solv	-0.30338	-0.11973	0.06392
cont - II	-0.28966	-0.10823	0.07320

ISOXAFLUTOLE - MYSID CHRONIC TEST

13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: FLNGTH

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 122 MSE= 0.113393  
Critical Value of Dunnett's T= 2.266

Comparisons significant at the 0.05 level are indicated by '\*\*\*'.

TRT	Simultaneous		Simultaneous	
	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
II - solv	-0.05012	0.17747	0.40506	
I - solv	-0.10034	0.11886	0.33805	
IV - solv	-0.28327	0.03182	0.34691	
III - solv	-0.22556	0.00455	0.23465	
cont - solv	-0.21886	0.00034	0.21953	

ISOXAFLUTOLE - MYSID CHRONIC TEST

13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: FMGHT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 122 MSE= 0.045606  
Critical Value of Dunnett's T= 2.266

Comparisons significant at the 0.05 level are indicated by '\*\*\*'.

TRT	Simultaneous		Simultaneous	
	Lower Confidence Limit	Difference Between Means	Upper Confidence Limit	
I - solv	-0.11133	0.02768	0.16669	
III - solv	-0.12048	0.02545	0.17139	
IV - solv	-0.18812	0.01170	0.21153	
II - solv	-0.15584	-0.01150	0.13283	
cont - solv	-0.25874	-0.11973	0.01928	

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure  
Class Level Information

Class	Levels	Values
REP	2	a b
TRT	6	I II III IV cont solv

Number of observations in data set = 148

Group Obs Dependent Variables

Group	Obs	Dependent Variables
1	121	MLNGTH MNGHT
2	129	FLNGTH FMGHT

NOTE: Variables in each group are consistent with respect to the presence or absence of missing values.

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2.1363799	0.1942164	1.72	0.0786
Error	109	12.3237854	0.1130623		
Corrected Total	120	14.4601653			
R-Square		C.V.	Root MSE	MLNGTH Mean	
	0.147742	4.746375	0.3362	7.0843	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0002638	0.0002638	0.00	0.9616
TRT	5	1.2288377	0.2457675	2.17	0.0622
REP*TRT	5	0.9072785	0.1814557	1.60	0.1648

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0040593	0.0040593	0.04	0.8501
TRT	5	0.8922446	0.1784489	1.58	0.1722
REP*TRT	5	0.9072785	0.1814557	1.60	0.1648

Tests of Hypotheses using the Type III MS for REP\*TRT as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.8922446	0.1784489	0.98	0.5071

General Linear Models Procedure

Dependent Variable: MNGHT	Sum of Squares	Mean Square	F Value	Pr > F	
Source	DF	Squares	Square	F Value	Pr > F

Handwritten mark: a stylized 'F' or 'ff' with a checkmark.

Model	11	0.2948553	0.0268050	1.83	0.0577
Error	109	1.5984968	0.0146651		
Corrected Total	120	1.8933521			
	R-Square	C.V.	Root MSE	MWGT Mean	
	0.155732	14.45644	0.1211	0.8377	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0178510	0.0178510	1.22	0.2723
TRT	5	0.1743000	0.0348600	2.38	0.0434
REP*TRT	5	0.1027043	0.0205409	1.40	0.2297
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0093918	0.0093918	0.64	0.4253
TRT	5	0.1656858	0.0331372	2.26	0.0535
REP*TRT	5	0.1027043	0.0205409	1.40	0.2297

Tests of Hypotheses using the Type III MS for REP\*TRT as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.1656858	0.0331372	1.61	0.3062

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996

General Linear Models Procedure  
Least Squares Means

Standard Errors and Probabilities calculated using the Type III MS for REP\*TRT as an Error term

TRT	MLNGTH	Pr >  T	H0: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
1	7.10055556	1	0.3783	0.8366	0.2711	0.8180	0.9791		
11	7.24444444	2	0.3783	0.4741	0.0872	0.2766	0.3686		
111	7.13030303	3	0.8366	0.4741	0.0872	0.0576	0.8478		
1111	6.93125000	4	0.2711	0.0872	0.2014	0.3469	0.2388		
cont	7.06818182	5	0.8180	0.2766	0.6576	0.3469	0.7862		
solv	7.10416667	6	0.9791	0.3686	0.8478	0.2388	0.7862		

Standard Errors and Probabilities calculated using the Type III MS for REP\*TRT as an Error term

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996

TRT	MWGT	Pr >  T	H0: LSMEAN(i)=LSMEAN(j)	1	2	3	4	5	6
1	0.76238889	1	0.2302	0.0391	0.1800	0.1373	0.1085		
11	0.83083333	2	0.2302	0.2853	0.2853	0.9495	0.8301	0.7291	
111	0.89020202	3	0.0391	0.2853	0.2731	0.3255	0.3801	0.7574	
1111	0.83413462	4	0.1800	0.9495	0.2731	0.8694	0.7574	0.8836	
cont	0.84181818	5	0.1373	0.8301	0.3255	0.8694	0.7574	0.8836	
solv	0.84833333	6	0.1085	0.7291	0.3801	0.7574	0.8836		

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

General Linear Models Procedure  
Dunnnett's One-tailed T tests for variable: MLNGTH

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.  
Alpha= 0.05 Confidence= 0.95 df= 5 MSE= 0.181456  
Critical Value of Dunnnett's T= 3.015

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
11	- solv	-0.2668	0.1558	0.5785
111	- solv	-0.3730	0.0158	0.4046
1111	- solv	-0.3932	0.0011	0.3954
cont	- solv	-0.4150	-0.0360	0.3431
1111	- solv	-0.5879	-0.2042	0.1796

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996  
General Linear Models Procedure  
Dunnnett's One-tailed T tests for variable: MWGT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.  
Alpha= 0.05 Confidence= 0.95 df= 5 MSE= 0.020541  
Critical Value of Dunnnett's T= 3.015

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
1111	- solv	-0.08515	0.04567	0.17648
cont	- solv	-0.13404	-0.00652	0.12101
11	- solv	-0.15254	-0.01033	0.13188
111	- solv	-0.14172	-0.01262	0.11648
1	- solv	-0.21891	-0.08623	0.04645

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
13:38 Tuesday, August 6, 1996  
General Linear Models Procedure  
Dependent Variable: FLNGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1.1211719	0.1019247	0.89	0.5526
Error	117	13.4036343	0.1145610		
Corrected Total	128	14.5248062			
	R-Square	C.V.	Root MSE	FLNGTH Mean	

Handwritten mark: 27

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0082694	0.0082694	0.07	0.7887
TRT	5	0.6825882	0.1365176	1.19	0.3175
REP*TRT	5	0.4303143	0.0860629	0.75	0.5868
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0021742	0.0021742	0.02	0.8907
TRT	5	0.6914765	0.1382953	1.21	0.3101
REP*TRT	5	0.4303143	0.0860629	0.75	0.5868

Tests of Hypotheses using the Type III MS for REP\*TRT as an error term

Source DF Type III SS Mean Square F Value Pr > F

REP 1 0.0021742 0.0021742 0.02 0.8907

TRT 5 0.6914765 0.1382953 1.21 0.3101

REP\*TRT 5 0.4303143 0.0860629 0.75 0.5868

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
 13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dependent Variable: FLGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.8695274	0.0790479	1.82	0.0584
Error	117	5.0878974	0.0434863		
Corrected Total	128	5.9574248			
R-Square		C.V.	Root MSE	FLGHT Mean	
	0.145957	21.76092	0.2085	0.9583	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0030309	0.0030309	0.07	0.7922
TRT	5	0.3904926	0.0780985	1.80	0.1190
REP*TRT	5	0.4760039	0.0952008	2.19	0.0600
Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0093316	0.0093316	0.21	0.6441
TRT	5	0.4486850	0.0897370	2.06	0.0749
REP*TRT	5	0.4760039	0.0952008	2.19	0.0600

Tests of Hypotheses using the Type III MS for REP\*TRT as an error term

Source DF Type III SS Mean Square F Value Pr > F

REP 1 0.0093316 0.0093316 0.21 0.6441

TRT 5 0.4486850 0.0897370 2.06 0.0749

REP\*TRT 5 0.4760039 0.0952008 2.19 0.0600

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
 13:38 Tuesday, August 6, 1996

General Linear Models Procedure  
 Least Squares Means

Standard Errors and Probabilities calculated using the Type III MS for REP\*TRT as an Error term

TRT	FLNGHT	Pr >  T  H0: LSMEAN(I)=LSMEAN(J)	1	2	3	4	5	6
I	1.13791209	0.4478	0.2719	0.4902	0.1645	0.2441		
II	7.20833333	0.2719	0.1099	0.2510	0.0662	0.0992		
III	7.03290598	0.4478	0.1099	0.8938	0.1778	0.9388		
IV	7.05000000	0.4902	0.2510	0.8938	0.17340	0.8492		
cont	7.00750000	0.1645	0.0662	0.7778	0.7340	0.8399		
solv	7.02564103	0.2441	0.0992	0.9388	0.8492	0.8399		

Standard Errors and Probabilities calculated using the Type III MS for REP\*TRT as an Error term

TRT FLNGHT PR > |T| H0: LSMEAN(I)=LSMEAN(J)

LSMEAN I/J 1 2 3 4 5 6

I 1.00453297 1 0.9369 0.9660 0.8887 0.1304 0.7456

II 0.99704167 2 0.9369 0.9660 0.9362 0.1691 0.8169

III 1.00854701 3 0.9660 0.9084 0.9362 0.1417 0.7288

IV 0.98625000 4 0.8887 0.9362 0.8685 0.3303 0.2625

cont 0.85216667 5 0.1304 0.1691 0.1417 0.3303 0.2329

solv 0.97384615 6 0.7456 0.8169 0.7288 0.9265 0.2329

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
 13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: FLNGHT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 5 MSE= 0.086063  
 Critical Value of Dunnett's T= 2.996

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

TRT	Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I	- solv	-0.08461	0.17747	0.43955
II	- solv	-0.13356	0.11886	0.37127
IV	- solv	-0.33102	0.03182	0.39466
III	- solv	-0.26044	0.00455	0.26953
cont	- solv	-0.25208	0.00034	0.25275

ANALYSIS USING TRT\*REP INTERACTION AS THE ERROR TERM  
 13:38 Tuesday, August 6, 1996

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: FLGHT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 5 MSE= 0.095201  
 Critical Value of Dunnett's T= 2.996

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 5 MSE= 0.095201  
 Critical Value of Dunnett's T= 2.996

Comparisons significant at the 0.05 level are indicated by '\*\*\*\*'.

82

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
I - solv	-0.23780	0.02768	0.29315
III - solv	-0.25324	0.02545	0.30415
IV - solv	-0.36991	0.01170	0.39332
II - solv	-0.28715	-0.01150	0.26415
cont - solv	-0.38521	-0.11973	0.14575

9-3-96

MRID No. 439048-22

**DATA EVALUATION RECORD**  
**§ 72-1 -- ACUTE LC<sub>50</sub> TEST WITH A COLDWATER FISH**

1. **CHEMICAL:** Isoxaflutole **PC Code No.:** 123000

2. **TEST MATERIAL:** RPA 202248 **Purity:** 99.9%

3. **CITATION:**

**Author:** A. Mc Elligott  
**Title:** RPA 202248: Acute Toxicity (96 Hours) to Rainbow Trout (*Oncorhynchus mykiss*) Under Semi-Static Conditions

**Study Completion Date:** November 3, 1995

**Laboratory:** Rhône-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France

**Sponsor:** Rhône-Poulenc Agrochimie, Lyon, France

**Laboratory Report ID:** SA 95141

**MRID No.:** 439048-22

**DP Barcode:** D222982


4. **REVIEWED BY:** Max A. Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.,

**Signature:**  **Date:** 8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.,

**Signature:** P. Kosalwat **Date:** 8/8/96

5. **APPROVED BY:**

**Signature:**  **Date:** 9/3/96

6. **STUDY PARAMETERS:**

**Age or Size of Test Organism:** Average of 1.1 g  
**Definitive Test Duration:** 96 hours  
**Study Method:** Static renewal  
**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using a freshwater fish. The 96-hour LC<sub>50</sub> for rainbow trout exposed to RPA 202248 was >30.6 ppm, the solubility limit of this compound under the conditions of this test. The test material is classified, at worst, as slightly toxic to rainbow trout.

4.75

X 30

**Results Synopsis**

LC<sub>50</sub>: >30.6 ppm

95% C.I.: N/A

NOEC: 30.6 ppm

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY**

**A. Classification:** Core

**B. Rationale:** Although the highest test concentration was lower than 100 ppm and a more precise LC<sub>50</sub> was not obtained, the test was apparently conducted at the water solubility limit for this test material under the conditions of the test.

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

1. Tap water filtered through activated charcoal was used to prepare the test solutions. The guidelines discourage the use of dechlorinated water since removal of chlorine is rarely complete. However, no control mortality occurred in this test.
2. Each test concentration was 50% of the next highest concentration; 60% is recommended by the SEP.

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is the rainbow trout ( <i>Oncorhynchus mykiss</i> )	<i>Oncorhynchus mykiss</i>
<b><u>Mean Weight</u></b> 0.5-5.0 g	1.1 g
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	Mean (total length): 5.0 cm Range: 4.5 - 5.3 cm
<b><u>Supplier</u></b>	Pisciculture TALLET, Saumane, France

2 31

Guideline Criteria	Reported Information
All fish from same source?	Yes
All fish from the same year class?	Yes

### B. Source/Acclimation

Guideline Criteria	Reported Information
<b>Acclimation Period</b> Minimum 14 days	At least 14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<b>Feeding</b> No feeding during the study	Fish were not fed during the study
<b>Pretest Mortality</b> < 3% mortality 48 hours prior to testing	2.3% mortality in stock culture during the 7 days prior to testing.

### C. Test System

Guideline Criteria	Reported Information
<b>Source of dilution water</b> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Activated-charcoal filtered tap water diluted with deionized water
Does water support test animals without observable signs of stress?	Yes
<b>Water Temperature</b> 12°C	11.9 - 13.0°C
<b>pH</b> Prefer 7.2 to 7.6	6.7 - 7.7



Guideline Criteria	Reported Information
<p><b><u>Dissolved Oxygen</u></b>            Static: <math>\geq 60\%</math> during 1<sup>st</sup> 48 hrs            and <math>\geq 40\%</math> during 2<sup>nd</sup> 48 hrs,            flow-through: <math>\geq 60\%</math></p>	<p>&gt;74% for the entire test</p>
<p><b><u>Total Hardness</u></b>            Prefer 40 to 200 mg/L as CaCO<sub>3</sub></p>	<p>39-40 mg/L as CaCO<sub>3</sub>.</p>
<p><b><u>Test Aquaria</u></b>            1. <b><u>Material:</u></b>                Glass or stainless steel            2. <b><u>Size:</u></b>                Volume of 18.9 L (5 gal) or                30 x 60 x 30 cm            3. <b><u>Fill volume:</u></b>                15-30 L of solution</p>	<p>1. Glass            2. 30 L            3. 20 L</p>
<p><b><u>Type of Dilution System</u></b>            Must provide reproducible            supply of toxicant</p>	<p>N/A</p>
<p><b><u>Flow Rate</u></b>            Consistent flow rate of 5-10            vol/24 hours, meter systems            calibrated before study and            checked twice daily during            test period</p>	<p>N/A</p>
<p><b><u>Biomass Loading Rate</u></b>            Static: <math>\leq 0.8</math> g/L at <math>\leq 17^\circ\text{C}</math>,  <math>\leq 0.5</math> g/L at <math>&gt; 17^\circ\text{C}</math>; flow-            through: <math>\leq 1</math> g/L/day</p>	<p>0.56 g/L</p>
<p><b><u>Photoperiod</u></b>            16 hours light, 8 hours dark</p>	<p>16 hours light, 8 hours dark</p>
<p><b><u>Solvents</u></b>            Not to exceed 0.5 mL/L for            static tests or 0.1 mL/L for            flow-through tests</p>	<p>Dimethyl-formamide (0.1 mg/L)</p>

**D. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Range Finding Test</u></b> If LC<sub>50</sub> &gt;100 mg/L with 30 fish, then no definitive test is required.</p>	<p>Yes; 0.3, 1.0, 3.1, 10.0, and 32.0 mg/L. No mortality or adverse effects were observed at any concentration. The highest concentration (32.0 mg/L) was above the visual limit of aqueous solubility of the test compound.</p>
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	<p>Control, solvent control, 3.8, 7.5, 15, 30, and 60 mg/L.</p>
<p><b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers</p>	<p>20 per treatment level; 10 per replicate</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Yes</p>
<p><b>Biological observations made every 24 hours?</b></p>	<p>Yes</p>
<p><b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary &gt; 1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</p>	<p>Temperature was measured continuously in one test aquarium and every 24 hrs at each treatment level.  DO and pH were measured every 24 hrs at all dose levels.</p>
<p><b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used</p>	<p>Chemical analysis was performed on all fresh and old (48 hrs) test solutions and controls at test initiation and every 48 hrs.</p>

**12. REPORTED RESULTS:**

**A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	Analytical: 97-102% Test: 97-105% (14-99% for the highest treatment level)
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0% mortality
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

**Mortality**

Concentration (mg/L)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
Solvent	--	20	0	0	0	0
3.8	3.9	20	0	0	0	0
7.5	7.7	20	0	0	0	0
15.0	15.0	20	0	0	0	0
30.0	30.6	20	0	0	0	0
60.0	33.8	20	0	0	0	0

**Other Significant Results:** Undissolved particles were observed on the bottom of the 30 mg/L and 60 mg/L test aquaria. All particles had solubilized by the end of each 48-hour exposure period at the 30 mg/L level. Particles remained present in both replicates of the highest treatment

\$ 35

level (60 mg/L) throughout the test. Occasional signs of lethargy and pigmentation effects were observed in some fish at most of the test levels and controls.

#### B. Statistical Results

Method: Visual inspection

96-hr LC<sub>50</sub>: >33.8 mg/L                      95% C.I.: N/A  
Probit Slope: N/A                              NOEC: 33.8 mg/L

#### 13. VERIFICATION OF STATISTICAL RESULTS:

Method: Visual inspection

96-hr LC<sub>50</sub>: >30.6 mg/L                      95% C.I.: N/A  
Probit Slope: N/A                              NOEC: 30.6 mg/L

14. REVIEWER'S COMMENTS: Percent recovery of the test material at the highest treatment level ranged from 13.8 to 99.0%. Since insoluble fragments of test compound remained throughout the test period, the nominal concentration of 60.0 mg/L was obviously well above the limit of solubility for this compound under these test conditions. Undissolved test material was also present throughout the test period in the highest concentration. The 30.0 mg/L nominal concentration was approximately equal to the limit of solubility and was subsequently treated as the highest test level by the reviewer.

This study is scientifically sound and meets the guideline requirements for an acute freshwater fish toxicity test. The 96-hour LC<sub>50</sub> for rainbow trout exposed to RPA 202248 was >30.6 ppm, the limit of solubility for this compound under the conditions of this test. The test material is classified, at worst, as slightly toxic to rainbow trout. The NOEC was determined to be 30.6 ppm. This study is classified as **Core**.

**DATA EVALUATION RECORD**  
**§ 72-2 - ACUTE LC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE**

1. **CHEMICAL:** Isoxaflutole **PC Code No.:** 123000

2. **TEST MATERIAL:** RPA 202248 **Purity:** 99.9%

3. **CITATION:**

**Author:** A. Mc Elligott  
**Title:** RPA 202248: Acute Toxicity (48 Hours) to Daphnids (*Daphnia magna*) Under Semi-Static Conditions

**Study Completion Date:** November 30, 1995

**Laboratory:** Rhône-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France

**Sponsor:** Rhône-Poulenc Agrochimie, Lyon, France

**Laboratory Report ID:** SA 95142

**MRID No.:** 439048-23

**DP Barcode:** D222982

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.,

**Signature:**

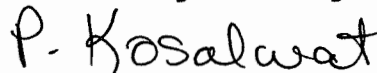


**Date:**

8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.,

**Signature:**

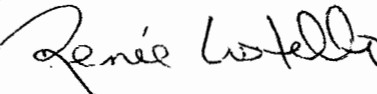


**Date:**

8/8/96

5. **APPROVED BY:**

**Signature:**



**Date:**

9/13/96

6. **STUDY PARAMETERS:**

**Age of Test Organism:** <24 hours

**Definitive Test Duration:** 48 hours

**Study Method:** Static renewal

**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates. The 96-hour LC<sub>50</sub> for *Daphnia magna* exposed to RPA 202248 was >59.6 ppm, the limit of solubility for this compound under the conditions of this test. The test material is classified, at worst, as slightly toxic to *Daphnia magna*.

**Results Synopsis**

EC<sub>50</sub>: >59.6 ppm

95% C.I.: N/A

NOEC: 59.6 ppm

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Core

**B. Rationale:** Although the highest test concentration was less than 100 ppm and a more precise EC<sub>50</sub> was not determined, the test was apparently conducted at the maximum water solubility of the test compound under the conditions of this test.

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:** No significant deviations.

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms:**

Guideline Criteria	Reported Information
<p><b><u>Species</u></b> Preferred species is <i>Daphnia magna</i></p>	<p><i>Daphnia magna</i></p>
<p><b>All organisms are approximately the same size and weight?</b></p>	<p>Not reported</p>
<p><b><u>Life Stage</u></b> Daphnids: 1<sup>st</sup> instar (&lt;24 h). Amphipods, stoneflies, and mayflies: 2<sup>nd</sup> instar. Midges: 2<sup>nd</sup> &amp; 3<sup>rd</sup> instar.</p>	<p>1<sup>st</sup> instar (&lt;24 h)</p>
<p><b><u>Supplier</u></b></p>	<p>In-house cultures</p>
<p><b>All organisms from the same source?</b></p>	<p>Yes</p>

**B. Source/Acclimation:**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> Minimum 7 days	N/A
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	Not reported
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A
<b><u>Feeding</u></b> No feeding during the study.	No feeding
<b><u>Pretest Mortality</u></b> No more than 3% mortality 48 hours prior to testing.	Not reported

**C. Test System:**

Guideline Criteria	Reported Information
<b><u>Source of dilution water</u></b> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	Reconstituted hard water prepared from a reverse-osmosed, de-ionized water which was also filtered through activated charcoal
<b>Does water support test animals without observable signs of stress?</b>	Yes
<b><u>Water Temperature</u></b> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	19.3 - 20.8°C
<b><u>pH</u></b> Prefer 7.2 to 7.6.	7.2 - 7.9

Guideline Criteria	Reported Information
<p><b><u>Dissolved Oxygen</u></b>            Static: <math>\geq 60\%</math> during 1<sup>st</sup> 48 h            and <math>\geq 40\%</math> during 2<sup>nd</sup> 48 h,            flow-through: <math>\geq 60\%</math>.</p>	>93% throughout test
<p><b><u>Total Hardness</u></b>            Prefer 40 to 200 mg/L as            CaCO<sub>3</sub>.</p>	170 - 172 mg/L as CaCO <sub>3</sub>
<p><b><u>Test Aquaria</u></b>            1. <b><u>Material:</u></b>                Glass or stainless steel.            2. <b><u>Size:</u></b>                250 mL (daphnids and                midges) or 3.9 L (1 gal).            3. <b><u>Fill volume:</u></b>                200 mL (daphnids and                midges) or 2-3 L.</p>	Glass  250 mL  200 mL
<p><b><u>Type of Dilution System</u></b>            Must provide reproducible            supply of toxicant.</p>	N/A
<p><b><u>Flow Rate</u></b>            Consistent flow rate of 5-10            vol/24 hours, meter systems            calibrated before study and            checked twice daily during            test period.</p>	N/A
<p><b><u>Biomass Loading Rate</u></b>            Static: <math>\leq 0.8</math> g/L at <math>\leq 17^\circ\text{C}</math>,  <math>\leq 0.5</math> g/L at <math>&gt; 17^\circ\text{C}</math>; flow-            through: <math>\leq 1</math> g/L/day.</p>	1 daphnid/20 mL
<p><b><u>Photoperiod</u></b>            16 hours light, 8 hours dark.</p>	16 hours light, 8 hours dark
<p><b><u>Solvents</u></b>            Not to exceed 0.5 mL/L for            static tests or 0.1 mL/L for            flow-through tests.</p>	Dimethyl formamide (0.1 mL/L)



**D. Test Design:**

Guideline Criteria	Reported Information
<p><b><u>Range Finding Test</u></b> If LC<sub>50</sub> &gt;100 mg/L, then no definitive test is required.</p>	<p>Yes; however, the concentration range was not reported. EC<sub>50</sub> was &gt;50 mg/L.</p>
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.</p>	<p>Control, solvent control, 3.8, 7.5, 15.0, 30.0, and 60.0 mg/L.</p>
<p><b><u>Number of Test Organisms</u></b> Minimum 20/level, may be divided among containers.</p>	<p>20 per treatment, 10 per replicate.</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Yes</p>
<p><b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary &gt; 1°C. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.</p>	<p>Temperature, DO, and pH were measured initially, at the end of 24 h, and at test termination (48-h) for each treatment level. Temperature was also measured continuously in a dilution water sample located in the test area.</p>
<p><b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used</p>	<p>Fresh and old test solutions were measured initially, after 24 hours, and at test termination for each treatment level and controls.</p>

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<b>Control Mortality</b> Static: ≤10% Flow-through: ≤5%	0% mortality
Percent Recovery of Chemical	Analytical: 97-102% Test: 97-111%
Raw data included?	Yes

**Mortality**

Concentration (mg/L)		Number of Daphnids	Cumulative Number Dead	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	<0.2	20	0	0
Solvent	<0.2	20	0	0
3.8	4.2	20	0	0
7.5	7.6	20	0	0
15.0	15.0	20	0	1
30.0	30.1	20	0	0
60.0	59.6	20	0	0

**Other Significant Results:** Undissolved particles were observed in test solutions at the 60 mg/L treatment level during each 24-hour cycle of exposure.

**B. Statistical Results**

Method: Visual inspection

48-hr EC<sub>50</sub>: >59.6 mg/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 59.6 mg/L

**13. VERIFICATION OF STATISTICAL RESULTS:**

Method: Visual inspection

48-hr EC<sub>50</sub>: >59.6 mg/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 59.6 mg/L

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates, and is classified as **Core**. The 96-hour LC<sub>50</sub> for *Daphnia magna* exposed to RPA 202248 was >59.6 ppm, the limit of solubility for this compound under the conditions of this test. The test material is classified, at worst, as slightly toxic to *Daphnia magna*. The NOEC was determined to be 59.6 ppm.

9-3-96

MRID No. 439048-24

**DATA EVALUATION RECORD**  
**§ 72-3 - ACUTE LC<sub>50</sub> TEST WITH AN ESTUARINE/MARINE SHRIMP**

1. **CHEMICAL:** Isoxaflutole **PC Code No.:** 123000

2. **TEST MATERIAL:** RPA 202248 **Purity:** 99.9%

3. **CITATION:**

**Author:** Maura K. Collins  
**Title:** RPA 202248 - Acute Toxicity to Mysids  
(*Mysidopsis bahia*) Under Static Renewal  
Conditions

**Study Completion Date:** December 22, 1995

**Laboratory:** Springborn Laboratories, Inc., Wareham,  
MA

**Sponsor:** Rhône-Poulenc Secteur Agro, Sophia  
Antipolis, France

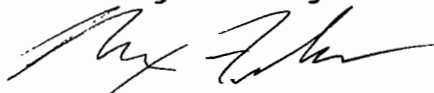
**Laboratory Report ID:** 95-12-6271

**MRID No.:** 439048-24

**DP Barcode:** D222982

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,  
KBN Engineering and Applied Sciences, Inc.

**Signature:**



**Date:**

8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist,  
KBN Engineering and Applied Sciences, Inc.

**Signature:**

P. Kosalwat

**Date:**

8/8/96

5. **APPROVED BY:**

**Signature:**

Renie Costello

**Date:**

9/3/96

6. **STUDY PARAMETERS:**

**Definitive Test Duration:** 96 hours

**Study Method:** Static Renewal

**Type of Concentrations:** Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements. The 96-hour LC<sub>50</sub> for mysid shrimp exposed to RPA 202248 was 3.6 ppm ai, which classifies RPA 202248 as moderately toxic to *Mysidopsis bahia*. The NOEC was determined to be 0.83 ppm ai.

4.75

44

**Results Synopsis**

LC<sub>50</sub>: 3.6 ppm ai

95% C.I.: 0.83 - 7.4 ppm ai

NOEC: 0.83 ppm ai

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Core

**B. Rationale:** N/A

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

1. The size of the test vessels (2.0 L) was less than recommended (3.9 L).
2. The temperature of the test solutions (24-26°C) was higher than recommended (22°C).

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes sp.</i>	<i>Mysidopsis bahia</i>
<b><u>Age</u></b> Juvenile, mysids should be ≤ 24 hours old	≤ 24 hours old (from protocol; p.34)
<b><u>Supplier</u></b>	Springborn Laboratories, Inc., Wareham, MA
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> minimum 10 days	N/A
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	None reported
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A
<b>Feeding</b> No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each.	Mysids were fed brine shrimp nauplii daily during the exposure period.
<b>Pretest Mortality</b> <3% mortality 48 hours prior to testing	Not reported

**C. Test System**

Guideline Criteria	Reported Information
<b>Source of dilution water</b> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Filtered seawater with the salinity adjusted to approximately 19 ‰ using well water.
<b>Does water support test animals without observable signs of stress?</b>	Yes
<b>Salinity</b> 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6‰	18-21 ‰
<b>Water Temperature</b> Approx. 22 ± 1 °C	24-26 °C

Guideline Criteria	Reported Information
<p><b>pH</b> 8.0-8.3 for marine (steno-haline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range &lt; 0.8</p>	7.2-7.7
<p><b>Dissolved Oxygen</b> Static: ≥ 60% during 1<sup>st</sup> 48 hrs and ≥ 40% during 2<sup>nd</sup> 48 hrs, Flow-through: ≥ 60%</p>	≥ 62% during 1 <sup>st</sup> 48 hrs and ≥ 51% during 2 <sup>nd</sup> 48 hrs
<p><b>Total Organic Carbon</b></p>	0.40 mg/L
<p><b>Test Aquaria</b>            1. <b>Material:</b>                Glass or stainless steel            2. <b>Size:</b>                19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp).            3. <b>Fill volume:</b>                15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.</p>	1. Glass 2. 2.0 L 3. Fill volume of 1 L.
<p><b>Type of Dilution System</b>            Must provide reproducible supply of toxicant</p>	N/A
<p><b>Flow Rate</b>            Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	N/A
<p><b>Biomass Loading Rate</b>            Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at &gt; 17°C; flow-through: ≤ 1 g/L/day</p>	1 mysid/100 ml
<p><b>Photoperiod</b>            16 hours light, 8 hours dark</p>	16 h light, 8 h dark

Guideline Criteria	Reported Information
<p><b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests</p>	<p>Solvent: acetone Maximum conc.: 0.25 mL/L</p>

#### D. Test Design

Guideline Criteria	Reported Information
<p><b>Range Finding Test</b> If <math>LC_{50} &gt; 100</math> mg/L with 30 shrimp, then no definitive test is required.</p>	<p>1.0, 10, 40 and 100 mg ai/L; undissolved test material was observed in 10, 40, and 100 mg ai/L solutions. These same concentration levels had 40, 70, and 70% mortality, respectively. No mortality was observed at the 1.0 mg ai/L level.</p>
<p><b>Nominal Concentrations of Definitive Test</b> Control &amp; 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.</p>	<p>Control, solvent control, 1.0, 5.2, 8.6, 14, 24 and 40 mg ai/L.</p>
<p><b>Number of Test Organisms</b> Minimum 20/level, may be divided among containers</p>	<p>20 per level, 10 per replicate</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Mysids impartially distributed by twos to each vessel.</p>
<p><b>Biological observations made every 24 hours?</b></p>	<p>Yes</p>
<p><b>Water Parameter Measurements</b></p> <ol style="list-style-type: none"> <li><b>Temperature</b> Measured constantly or, if water baths are used, every 6 hrs, may not vary <math>&gt; 1^{\circ}C</math></li> <li><b>DO and pH</b> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</li> </ol>	<ol style="list-style-type: none"> <li>Temperature measured continuously in the water bath and daily in each test vessel.</li> <li>DO and pH were measured daily in each test vessel.</li> </ol>



Guideline Criteria	Reported Information
<b>Chemical Analysis</b> needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, fresh and old test solutions were analyzed at test initiation, after 48 hours, and at termination.

## 12. REPORTED RESULTS:

### A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	75-87%
<b>Control Mortality</b> Not more than 10% of control organisms may die or show abnormal behavior.	0% in negative control and 5% in solvent control.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

### Mortality

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.51	20	0	0	0	0
Solvent	<0.51	20	0	0	0	1
1.0	0.83	20	0	0	0	0
5.2	4.5	20	0	0	0	12
8.6	7.4	20	0	3	14	20

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
14	11	20	0	6	17	19
24	20	20	0	9	17	19
40	33	20	3	11	17	20

**Other Significant Results:** Some undissolved test material was observed in the 11, 20, and 33 mg ai/L test solutions throughout the exposure period.

#### B. Statistical Results

Method: Nonlinear interpolation

96-hr LC<sub>50</sub>: 3.7 mg ai/L                      95% C.I.: 0.83 - 7.4 mg ai/L

Probit Slope: N/A                                      NOEC: 0.83 mg ai/L

#### 13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC <sub>50</sub> (C.I.)	3.6 (0.83 - 7.4) ppm ai
Moving Average Angle LC <sub>50</sub> (95% C.I.)	N/A
Probit LC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	N/A
NOEC	0.83 ppm ai

**14. REVIEWER'S COMMENTS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using *Mysidopsis bahia*. Based on mean measured concentrations, the 96-hour LC<sub>50</sub> was 3.6 ppm ai, which classifies RPA 202248 as moderately toxic to mysid shrimp. This study is classified as **Core**.

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

MAX FEKEN RPA 202248 MYSIDOPSIS 07-31-96

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.4	20	20	100	9.536742E-05
4.5	20	12	60.00001	25.17223
.83	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .83 AND 7.4 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 3.646719

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\*\*\*\*\*

9-3-96

MRID No. 439048-25

**DATA EVALUATION RECORD**  
**§ 72-1 -- ACUTE LC<sub>50</sub> TEST WITH A COLDWATER FISH**

1. **CHEMICAL:** Isoxaflutole **PC Code No.:** 123000

2. **TEST MATERIAL:** RPA 203328 **Purity:** 98.9%

3. **CITATION:**

**Author:** Mark W. Machado  
**Title:** RPA 203328 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions

**Study Completion Date:** June 22, 1995  
**Laboratory:** Springborn Laboratories, Inc., Wareham, MA

**Sponsor:** Rhône-Poulenc Ag Company, Research Triangle Park, NC

**Laboratory Report ID:** 95-5-5861

**MRID No.:** 439048-25

**DP Barcode:** D222982


4. **REVIEWED BY:** Max A. Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.,

**Signature:**  **Date:** 8/8/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.,

**Signature:** P. Kosalwat **Date:** 8/8/96

5. **APPROVED BY:**

**Signature:**  **Date:** 9/3/96

6. **STUDY PARAMETERS:**

**Age or Size of Test Organism:** Average of 0.59 g  
**Definitive Test Duration:** 96 hours  
**Study Method:** Flow-Through  
**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using a freshwater fish. The 96-hour LC<sub>50</sub> for rainbow trout exposed to RPA 203328 was 160 ppm ai, which classifies this compound as practically non-toxic to *Oncorhynchus mykiss*.

5.75

52

**Results Synopsis**

LC<sub>50</sub>: 160 ppm ai

95% C.I.: 130 - 210 ppm ai

NOEC: 130 ppm ai

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Core

**B. Rationale:** N/A

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

1. The pH of the test solutions (3.4 - 7.1) was lower than recommended (7.2 - 7.6).
2. The concentration of solvent in the solvent control and treatment solutions (0.5 mL/L) was greater than recommended (0.1 mL/L) for a flow-through test.
3. The size of the aquaria (39 x 20 x 25 cm) in this test was smaller than recommended (30 x 60 x 30 cm).

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is the rainbow trout ( <i>Oncorhynchus mykiss</i> )	<i>Oncorhynchus mykiss</i>
<b><u>Mean Weight</u></b> 0.5-5.0 g	0.59 g
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	Mean (total length): 41 mm Range: 35 - 45 mm
<b><u>Supplier</u></b>	Blue Stream Trout Hatchery, Barnstable, MA
<b>All fish from same source?</b>	Yes

Guideline Criteria	Reported Information
All fish from the same year class?	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	At least 14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study	Fish were not fed during the study
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	0.14% mortality in the test fish population during 48 hours prior to testing.

**C. Test System**

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Soft well water periodically sampled and analyzed for contaminants
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	11 - 13°C

Guideline Criteria	Reported Information
<p><b>pH</b> Prefer 7.2 to 7.6</p>	<p>Controls: 7.2 - 7.6 26 mg ai/L: 6.8 - 7.1 43 mg ai/L: 6.7 - 7.0 72 mg ai/L: 6.4 - 7.3 120 mg ai/L: 4.6 - 6.5 200 mg ai/L: 3.4 - 3.8</p>
<p><b>Dissolved Oxygen</b> Static: <math>\geq 60\%</math> during 1<sup>st</sup> 48 hrs and <math>\geq 40\%</math> during 2<sup>nd</sup> 48 hrs, flow-through: <math>\geq 60\%</math></p>	<p><math>\geq 80\%</math> for the entire test</p>
<p><b>Total Hardness</b> Prefer 40 to 200 mg/L as CaCO<sub>3</sub></p>	<p>32-38 mg/L as CaCO<sub>3</sub>.</p>
<p><b>Test Aquaria</b> 1. <b>Material:</b> Glass or stainless steel 2. <b>Size:</b> Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <b>Fill volume:</b> 15-30 L of solution</p>	<p>1. Glass 2. 39 x 20 x 25 cm 3. 11 L</p>
<p><b>Type of Dilution System</b> Must provide reproducible supply of toxicant</p>	<p>Intermittent-flow proportional diluter</p>
<p><b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	<p>6.9 volume replacements every 24 hours</p>
<p><b>Biomass Loading Rate</b> Static: <math>\leq 0.8</math> g/L at <math>\leq 17^\circ\text{C}</math>, <math>\leq 0.5</math> g/L at <math>&gt; 17^\circ\text{C}</math>; flow- through: <math>\leq 1</math> g/L/day</p>	<p>0.078 g/L</p>
<p><b>Photoperiod</b> 16 hours light, 8 hours dark</p>	<p>16 hours light, 8 hours dark</p>
<p><b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests</p>	<p>Acetone (0.5 mL/L)</p>

## D. Test Design

Guideline Criteria	Reported Information
<p><b><u>Range Finding Test</u></b> If LC<sub>50</sub> &gt;100 mg/L with 30 fish, then no definitive test is required.</p>	<p>Yes; 26, 43, 72, 120, and 200 mg ai/L. All fish died at the highest level (200 mg ai/L). No mortality or adverse effects were observed at any other concentration.</p>
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	<p>Control, solvent control, 26, 43, 72, 120, and 200 mg ai/L.</p>
<p><b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers</p>	<p>20 per treatment level; 10 per replicate</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Yes</p>
<p><b>Biological observations made every 24 hours?</b></p>	<p>Yes</p>
<p><b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary &gt; 1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</p>	<p>Temperature was measured continuously in one replicate of the control and every 24 hrs at each treatment level.  DO and pH were measured every 24 hrs at all dose levels.</p>
<p><b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used</p>	<p>Chemical analysis was performed on all test solutions and controls at test initiation and termination.</p>



**12. REPORTED RESULTS:****A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	101-118%
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0% mortality
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

**Mortality**

Concentration (mg ai/L)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<4.7	20	0	0	0	0
Solvent	<4.7	20	0	0	0	0
26	31	20	0	0	0	0
43	43	20	0	0	0	0
72	74	20	0	0	0	0
120	130	20	0	0	0	0
200	210	20	20	20	20	20

**Other Significant Results:** Complete mortality was observed at the highest treatment level (210 mg ai/L). No mortality or adverse effects were observed among fish exposed to the remaining treatment levels (31, 43, 74, and 130 mg ai/L) after 96 hours. The pH of the test solutions at the highest treatment level remained consistently low (3.4 - 3.8) throughout the test. A pH of <4.0 is considered lethal to

many freshwater fish according to EPA's "A review of the Red Book: Quality Criteria for Water" (April 1979).

**B. Statistical Results**

Method: Nonlinear interpolation

96-hr LC<sub>50</sub>: 160 mg ai/L                      95% C.I.: 130 - 210 mg ai/L

Probit Slope: N/A                              NOEC: 130 mg ai/L

**13. VERIFICATION OF STATISTICAL RESULTS:**

Method: Binomial test

96-hr LC<sub>50</sub>: 165 mg ai/L                      95% C.I.: 130 - 210 mg ai/L

Probit Slope: N/A                              NOEC: 130 mg ai/L

- 14. REVIEWER'S COMMENTS:** The mortality associated with the highest treatment level was probably a result of the extremely low pH measured in the replicate solutions and not directly due to the test material. However, no mortalities or treatment related effects were observed in fish at a concentration (130 mg ai/L) greater than the maximum concentration required (100 mg/L) for a fish acute toxicity test.

This study is scientifically sound and meets the guideline requirements for an acute freshwater fish toxicity test. The 96-hour LC<sub>50</sub> for rainbow trout exposed to RPA 203328 was 165 ppm ai, which classifies this compound as practically non-toxic to *Oncorhynchus mykiss*. The NOEC was determined to be 130 ppm ai. This study is classified as **Core**.

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
210	20	20	100	9.536742E-05
130	20	0	0	9.536742E-05
74	20	0	0	9.536742E-05
43	20	0	0	9.536742E-05
31	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 130 AND 210 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 165.227

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\*\*\*\*\*