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Data Evaluation Report on the Acute Toxicity of Rimon 10EC to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number {.....} EPA MRID Number 45638314

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D285479
	OECD Data Point	
	EPA MRID	45638314
	EPA Guideline	§72-1c

Test material: Rimon 10EC (formulated product) **Purity:** 9.1% (w:w) Novaluron
Common name: Novaluron (active ingredient)
Chemical name: IUPAC: 1,(3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea (a.i.)
CAS name: N-[[[3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]carbonyl]-2,6-difluorobenzamide (a.i.)
CAS No.: 116714-46-6 (a.i.)
Synonyms: "RIMON" 10 EC

Primary Reviewer: Rebecca Bryan **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/1/03

QC Reviewer: Christie E. Padova **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/1/03

Primary Reviewer: Bill Evans, Biologist **Date:** 11/6/03
OPP/EFED/ERB - I

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 124002

Date Evaluation Completed:

CITATION: Jenkins, C.A. 1998. "RIMON" 10EC: Acute toxicity to rainbow trout. Unpublished study performed by Huntingdon Life Sciences Ltd, Eye, Suffolk, England. Laboratory Project Identification No. MAK/462/982146. Study submitted by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel. Study initiated November 13, 1997 and completed October 29, 1998.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to Rimon 10EC [a formulated product containing 9.1% (w:w) Novaluron] at mean-measured concentrations of <detection limit (negative control), 2.62, 5.42, 11.0, 19.7, 43.1, and 90.3 mg/L under flow-through conditions. Nominal concentrations were 0 (negative control), 3.13, 6.25, 12.5, 25, 50, and 100 mg/L.

After 96 hours of exposure, there was 100% treatment-related mortality in the 90.3 mg/L treament group. There were no treatment-related mortalities in the control group, or in the ≤43.1 mg/L test groups. The 96-hour LC_{50} value (with 95% C.I.) is 62.4 (43.1-90.3) mg/L, which categories Rimon 10EC as slightly toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

In the ≥11.0 mg/L treatment groups, sublethal effects were observed throughout the exposure period and included hyperventilation, loss of coordination, coughing, darkened pigmentation, and/or reduced opercular movement. No sublethal effects were observed during the study in the control or the 2.62 and 5.42 mg/L treatment groups. The NOEC and LOEC levels were 5.42 and 11.0 mg/L, respectively.

This study is scientifically sound and fulfills guideline requirements for an acute toxicity study with the Rainbow trout [§72-1(c)]. This study is classified CORE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Juvenile, approximately 3 months old; mean fork length of 5.25 cm and mean wet weight of 1.65 g (n=10)
Test Type (Flow-through, Static, Static Renewal):	Flow-through

96-Hour
LC_{50}: 62.4 mg/L 95% C.I.: 43.1-90.3 mg/L
NOEC: 5.42 mg/L (based on sub-lethal effects)
LOEC: 11.0 mg/L (based on sub-lethal effects)
Endpoints affected: Mortality and sublethal effects
Most sensitive endpoint: Sublethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the Procedure 203 of the OECD Guidelines (1992), EC Methods Part C1 of the Annex Directive 92/69/EEC, and Series 72-1 of the US EPA Pesticide Assessment Guidelines (1982). Deviations from U.S. EPA FIFRA Guideline §72-1 included:

1. The test vessel (10 L) fill volume was less than recommended (15-30 L).

2. The dilution water was filtered, de-chlorinated, and softened tap water. The use of de-chlorinated tap water is generally not acceptable to EPA.

3. The water hardness (54-59 mg $CaCO_3$/L) was slightly greater than recommended (40-48 mg $CaCO_3$/L).

These deviations do not affect the acceptability or validity of the study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP Standards of the United Kingdom, EC Council Directive, OECD, U.S. EPA, and JMAFF (p. 3).

A. MATERIALS:

1. Test Material

Rimon 10 EC (formulated product)

Description: Clear liquid

Lot No./Batch No. : 960917

Purity: 9.1% (w:w) Novaluron

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0, 24, and 96 hours. Recoveries ranged from 77 to 96% of nominal concentrations (Table 1, p. 22).

Storage conditions of test chemicals: The test material was stored at 4°C in the dark.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: Approximately 3 months old

Weight at test initiation: 1.65 g (average of 10 fish)

Length at test initiation: 5.25 cm (average of 10 fish)

Source: Parkwood Trout Farm, Kent (reared at Gadewater Trout Farm, Hemel Hempstead from English eggs).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 96-hour static range-finding test was conducted at nominal concentrations of 0 (negative control), 1, 10, 100, and 1000 mg/L. The study author reported that

after 96 hours, the highest nominal exposure concentration at which no mortality had occurred was 10 mg/L and the lowest at which there was 100% mortality was 100 mg/L. The LC_{50} was approximately 32 mg/L. Recoveries of Rimon 10 EC were 81-113% of nominal values.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	14 days Same as test Trout crumb (TROUW UK (Ltd) Nutra Trout Fry 02), 1 to 4% of total fish wet-weight was provided daily, except during the 26.5 hours prior to testing. Mortality was 2 and 5% on acclimation days 7 and 14, respectively.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96 hours	*EPA/OECD requires: 96 hours*
Test conditions: static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Flow-through Intermittent-flow diluter N/A	The correct operation of the dosing apparatus was confirmed. The turnover rate was approximately 8.2 volume replacements/day. *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Test solutions were not aerated during the definitive test.	*EPA requires: no aeration; OECD permits aeration*

Parameter	Details	Remarks Criteria
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass 15L 10 L (13-cm depth)	The test vessel (10 L) fill volume was less than recommended (15-30 L). *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	Tap water was filtered through active carbon to remove chlorine and softened with reverse-osmosis treated tap water. Water was gently aerated prior to testing.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness	54-59 mg $CaCO_3$/L	The water hardness was slightly greater than recommended.
pH	7.4-7.8	
Dissolved oxygen	86-99%	***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1°C for estuarine/marine. OECD requires range of 21 - 25°C for bluegill and 13-17°C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Total Organic Carbon	0.8-0.9 mg/L	
Particulate Matter	Not specified	
Metals	See Appendix 2, p. 26	
Pesticides	Not detected	
Chlorine	Not reported	
Temperature	13.0-13.6°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, pH, and temperature were determined daily. Temperature was also continuously monitored in one control and one 100 mg/L test vessel. Water hardness was measured at test initiation and termination in the control and selected treatment groups.	

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative control), 3.13, 6.25, 12.5, 25, 50, and 100 mg/L Not detected (negative control), 2.62, 5.42, 11.0, 19.7, 43.1, and 90.3 mg/L	Mean measured concentrations calculated from 0, 24, and 96 hour test samples. *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	N/A	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*
Number of fish/replicates: negative control: solvent control: treated:	10 fish N/A 10 fish/level	Only one replicate was tested. *EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.20 g/L/day	*Static: ≤ 0.8 g/L at ≤ 17 °C, ≤ 0.5 g/L at > 17 °C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark, with a transition period.	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*

Parameter	Details	Remarks Criteria
Recovery of chemical Level of Quantitation Level of Detection	77-96% of nominal Not reported Not reported	Procedural recoveries are based on 96 hour test samples (Table 1, p. 22).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	0.25, 2, 3, 4, 24, 48, 72 and 96 hours of exposure	*EPA/OECD requires: minimally every 24 hours*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, there was 100% treatment-related mortality in the 90.3 mg/L treament group. There were no treatment-related mortalities in the control, and in the 2.62, 5.42, 11.0, 19.7, and 43.1 mg/L treatment groups.

Table 3: Effect of Rimon 10EC on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, mg/L, measured and (nominal conc.)	No. of fish at start of study	0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	10	0	0	0	0	0	0
2.62 (3.13)	10	0	0	0	0	0	0
5.42 (6.25)	10	0	0	0	0	0	0
11.0 (12.5)	10	0	0	0	0	0	0
19.7 (25)	10	0	0	0	0	0	0
43.1 (50)	10	0	0	1[a]	10	1[a]	10
90.3 (100)	10	0	0	9	90	10	100
NOEC (mortality)	43.1 mg/L						
LC_{50} (95% C.I.)	62.4 (43.1-90.3) mg/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] The one fish mortality in the 43.1 mg/L treatment groups was not considered treatment-related. The fish was damaged when trapped in the test vessel outlet.

B. NON-LETHAL TOXICITY ENDPOINTS:

In the ≥11.0 mg/L treatment groups, sublethal effects were observed throughout the exposure period and included hyperventilation, loss of coordination, coughing, darkened pigmentation, and/or reduced opercular movement. No sublethal effects were observed during the study in the control or the 2.62 and 5.42 mg/L treatment groups.

Table 4: Sub-lethal effects of Rimon 10EC on Rainbow trout (*Oncorhynchus mykiss*).

Treatment, mg/L, measured and (nominal conc.)	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
2.62 (3.13)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
5.42 (6.25)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
11.0 (12.5)	Hyperventilation-60%; Darkened pigmentation-30%	Hyperventilation-40%; Darkened pigmentation-30%	Hyperventilation-40%; Darkened pigmentation-60%; Coughing-10%	Darkened pigmentation-30%
19.7 (25)	Hyperventilation-100%	Hyperventilation-50%	Hyperventilation-60%; Darkened pigmentation-30%	Hyperventilation-50%; Darkened pigmentation-30%
43.1 (50)	Hyperventilation-100%; Darkened pigmentation-40%	Hyperventilation-100%; Darkened pigmentation-50%	Hyperventilation-100%; Darkened pigmentation-60%; Coughing-20%	Hyperventilation-100%; Darkened pigmentation-78%; Loss of Coordination-11%
90.3 (100)	Hyperventilation-100%; Loss of Coordination-100%; Coughing-100%; Darkened pigmentation-100%	Hyperventilation-50%; Loss of Coordination-100%; Darkened pigmentation-100%; Reduced opercular movement-50%	Hyperventilation-100%; Loss of Coordination-100%; Darkened pigmentation-100%	--
NOEC (sublethal)	5.42 mg/L			
LOEC (sublethal)	11.0 mg/L			
EC_{50}	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

–100% mortality.

C. REPORTED STATISTICS:

The 96-hour LC_{50} value and 95% confidence interval were estimated by non-linear interpolation and Binomial test, respectively. The values were derived using Stephan's computer program. The NOEC and LOEC were visually determined, based on observed treatment-related mortality or sublethal effects.

96-Hour
LC_{50}: 62.4 mg/L 95% C.I.: 43.1-90.3 mg/L
NOEC: 5.42 mg/L (based on sub-lethal effects)
LOEC: 11.0 mg/L (based on sub-lethal effects)
Endpoints affected: Mortality and sublethal effects
Most sensitive endpoint: Sublethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

The NOEC and LOEC for mortality and sublethal effects could be determined visually. The LC_{50} (with 95% C.I.) was determined using the binomial test via TOXANAL statistical software.

96-Hour
LC_{50}: 62.4 mg/L 95% C.I.: 43.1-90.3 mg/L
NOEC: 5.42 mg/L (based on sub-lethal effects)
LOEC: 11.0 mg/L (based on sub-lethal effects)
Endpoints affected: Mortality and sublethal effects
Most sensitive endpoint: Sublethal effects

E. STUDY DEFICIENCIES:

There were no deviations from FIFRA guideline §72-1 that affected the acceptability or validity of the study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those reported by the study author.

The 43.1 and 90.3 mg/L test solutions were off-white, opaque emulsions. A froth was observed on the surfaces of all treament solutions.

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1). This study is classified as CORE. Based on the results of this study, Rimon 10EC [a formulated product containing 9.1% (w:w) Novaluron] is categorized as slightly toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

96-Hour
LC_{50}: 62.4 mg/L 95% C.I.: 43.1-90.3 mg/L
NOEC: 5.42 mg/L (based on sub-lethal effects)
LOEC: 11.0 mg/L (based on sub-lethal effects)
Endpoints affected: Mortality and sublethal effects
Most sensitive endpoint: Sublethal effects

III. REFERENCES:

U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 72 Aquatic Organisms Testing $72-1 Acute toxicity test for freshwater aquatic fish, October 1982.

OECD Guidelines for Testing of Chemicals. "Fish, Acute Toxicity Test". Procedure 203, adopted 17 July 1992.

Official Journal of the European Communities. L383A. Part C: Methods for Determination of Ecotoxicity: C1. Acute toxicity for fish. Vol 35, 29 December 1992. ISSN 0378-6978.

Stephan, C.E. 1977. Methods for Calculating an LC_{50}. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634.

Stephan *et al.* 1982. A computer program for calculating an LC_{50}. US Environmental Protection Agency.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

90.3	10	10	100	9.765625E-02
43.1	10	0	0	9.765625E-02
19.7	10	0	0	9.765625E-02
11	10	0	0	9.765625E-02
5.42	10	0	0	9.765625E-02
2.62	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 43.1 AND 90.3 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 62.38535

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.